ISSN 1948-9358 (online)

World Journal of *Diabetes*

World J Diabetes 2023 September 15; 14(9): 1330-1449

Published by Baishideng Publishing Group Inc

World Journal of Diabetes

Contents

Monthly Volume 14 Number 9 September 15, 2023

EDITORIAL

MINIREVIEWS

ORIGINAL ARTICLE

Basic Study

Retrospective Study

World Journal of Diabetes

Monthly Volume 14 Number 9 September 15, 2023

Contents

META-ANALYSIS

World Journal of Diabetes

Monthly Volume 14 Number 9 September 15, 2023

Contents

ABOUT COVER

Editorial Board Member of *World Journal of Diabetes*, Xiao-Dong Sun, MD, PhD, Chief Doctor, Chief Physician, Doctor, Professor, Department of Endocrinology and Metabolism, Clinical Research Center, Affiliated Hospital of Weifang Medical University, Weifang 261031, Shandong Province, China. xiaodong.sun@wfmc.edu.cn

AIMS AND SCOPE

The primary aim of *World Journal of Diabetes* (*WJD*, *World J Diabetes*) is to provide scholars and readers from various fields of diabetes with a platform to publish high-quality basic and clinical research articles and communicate their research findings online.

WJD mainly publishes articles reporting research results and findings obtained in the field of diabetes and covering a wide range of topics including risk factors for diabetes, diabetes complications, experimental diabetes mellitus, type 1 diabetes mellitus, type 2 diabetes mellitus, gestational diabetes, diabetic angiopathies, diabetic cardiomyopathies, diabetic coma, diabetic ketoacidosis, diabetic nephropathies, diabetic neuropathies, Donohue syndrome, fetal macrosomia, and prediabetic state.

INDEXING/ABSTRACTING

The *WJD* is now abstracted and indexed in Science Citation Index Expanded (SCIE, also known as SciSearch®), Current Contents/Clinical Medicine, Journal Citation Reports/Science Edition, PubMed, PubMed Central, Reference Citation Analysis, China National Knowledge Infrastructure, China Science and Technology Journal Database, and Superstar Journals Database. The 2023 Edition of Journal Citation Reports® cites the 2022 impact factor (IF) for *WJD* as 4.2; IF without journal self cites: 4.1; 5-year IF: 4.5; Journal Citation Indicator: 0.69; Ranking: 51 among 145 journals in endocrinology and metabolism; and Quartile category: Q2.

RESPONSIBLE EDITORS FOR THIS ISSUE

NAME OF JOURNAL
World Journal of Diabetes

ISSN
ISSN 1948-9358 (online)

LAUNCH DATE
June 15, 2010

FREQUENCY
Monthly

EDITORS-IN-CHIEF
Lu Cai, Md. Shahidul Islam, Michael Horowitz

EDITORIAL BOARD MEMBERS
https://www.wjgnet.com/1948-9358/editorialboard.htm

PUBLICATION DATE
September 15, 2023

INSTRUCTIONS TO AUTHORS
https://www.wjgnet.com/bpg/gerinfo/204

GUIDELINES FOR ETHICS DOCUMENTS
https://www.wjgnet.com/bpg/GerInfo/287

GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH
https://www.wjgnet.com/bpg/gerinfo/240

PUBLICATION ETHICS
https://www.wjgnet.com/bpg/GerInfo/288

PUBLICATION MISCONDUCT
https://www.wjgnet.com/bpg/gerinfo/208

ARTICLE PROCESSING CHARGE
https://www.wjgnet.com/bpg/gerinfo/242

STEPS FOR SUBMITTING MANUSCRIPTS
https://www.wjgnet.com/bpg/GerInfo/239

ONLINE SUBMISSION
https://www.f6publishing.com

World Journal of Diabetes

Submit a Manuscript: https://www.f6publishing.com

World J Diabetes 2023 September 15; 14(9): 1330-1333

DOI: 10.4239/wjd.v14.i9.1330

ISSN 1948-9358 (online)

EDITORIAL

Recent therapeutic targets for the prevention and management of diabetic complications

Md Shahidul Islam, Lu Cai, Michael Horowitz

Specialty type: Endocrinology and metabolism

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B, B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Rezus E, Romania; Sun XD, China

Received: August 16, 2023
Peer-review started: August 16, 2023
First decision: August 24, 2023
Revised: August 31, 2023
Accepted: September 5, 2023
Article in press: September 5, 2023
Published online: September 15, 2023

Md Shahidul Islam, Department of Biochemistry, School of Life Sciences, University of KwaZulu-Natal, Durban 4000, KwaZulu-Natal, South Africa

Lu Cai, Pediatric Research Institute, University of Louisville, Louisville, KY 40202, United States

Michael Horowitz, Department of Medicine, University of Adelaide, Adelaide 5005, Australia

Corresponding author: Md Shahidul Islam, BSc, MSc, PhD, Professor, Department of Biochemistry, School of Life Sciences, University of KwaZulu-Natal, University Road, Chiltern Hills, Durban 4000, KwaZulu-Natal, South Africa. islamd@ukzn.ac.za

Abstract

Diabetes and associated complications represent major global public health issues which are associated with impaired quality of life and premature death. Although some diabetic complications have decreased in the developed world, the majority are still prevalent, with an increasing trend in the developing world. Currently used therapies are mainly 'glucocentric', focusing on the optimization of glycemic control to prevent, delay or manage diabetes-associated complications- other common comorbidities, such as dyslipidemia and hypertension are often underestimated. Although a number of novel therapeutic approaches have been reported recently, some of them have not received comparable attention in relation to either further studies or potential clinical implementation. This editorial briefly discusses some recent therapeutic approaches to the prevention and management of diabetes and its associated complications, as well as potential directions for future research and development in this area.

Key Words: Diabetic complications; Oxidative stress; Phytochemicals; Zinc; Silent information regulator 1; FOXO; Micro RNA

Core Tip: 'Glucocentric' approaches are currently being used for the management of diabetes and its associated complications. This articles highlighted some recent therapeutic approaches for the management of diabetes and its associated complications such as the management of oxidative stress by using antioxidative phytochemicals, molecular cell signaling pathways *via* Silent information regulator 1 and FOXOs and micro RNAs.

Citation: Islam MS, Cai L, Horowitz M. Recent therapeutic targets for the prevention and management of diabetic complications. *World J Diabetes* 2023; 14(9): 1330-1333
URL: https://www.wjgnet.com/1948-9358/full/v14/i9/1330.htm
DOI: https://dx.doi.org/10.4239/wjd.v14.i9.1330

INTRODUCTION

Diabetes and associated complications are major global causes of premature mortality. A minimum of 50% of people who have type 2 diabetes face premature death from diabetes-associated cardiovascular diseases and some 10% from renal failure with a total of 3.8 million deaths per annum[1]. According to a recent review, the rates of myocardial infarction, stroke and limb amputation have decreased among people with diabetes with a concomitant decline in mortality. The majority of these data are, however, sourced from high income countries, when other diabetic complications such as nephropathy, retinopathy and cancers are well represented[2]. It has been reported that the prevalence of diabetic complications are much higher in low and middle-income countries with a range of 12%-16% for microvascular and 2%-6% for macrovascular complications[3].

Diabetes-associated microvascular complications occur frequently in individuals with diabetes, both their prevalence and severity are inversely proportional to the efficacy of management of hyperglycemia. At least 50% of diabetic patients have one or more diabetic complications in their lifetime and many have multiple complications. Microvascular complications such as, diabetic nephropathy, retinopathy, neuropathy and diabetic foot disease represent a major causes of morbidity, impaired quality of life and mortality and are more common than macrovascular complications, such as diabetic cardiomyopathy and peripheral vascular diseases[4]. While improved management of hyperglycemia represents a major approach to prevent or delay diabetic complications, currently available therapies are not consistent in maintaining optimum glycemic control, their efficacy in glucose lowering exhibits a substantial interindividual variation and their long-term use is associated with adverse effects[5]. Additional major challenges with currently available therapies include, but not limited to, optimizing the dose to control the blood glucose, blood pressure and lipids as well as self-management of diabetes and lifestyle[6]. Hence, there is a need for newer or alternative therapies not only for better glycemic control, but also for the management of blood pressure and blood lipids with an ultimate goal for the prevention of diabetes associated micro- and macro-vascular complications. The outcomes of recent, large cardiovascular prevention trials, initially mandated for regulatory purposes, have provided major insights into the need for a broader, rather than simply 'glucocentric', approach to therapy of type 2 diabetes. Both GLP-1 receptor agonist and SGLT-2 inhibitors are now used widely with the recognition that their beneficial effects include cardiovascular and renal protection[7]. A number of novel therapeutic approaches are currently being evaluated with the potential to improve the prevention and management of diabetic complications, some of which are highlighted below.

Oxidative stress is a major culprit for the induction of diabetic complications[8], since it causes endothelial dysfunction both in small and large vessels, not only by increasing the production of oxidative free radicals and advanced glycation end products, but also by the concomitant reduction of physiological antioxidative status. Over expression of the antioxidative enzyme, superoxide dismutase (SOD) in transgenic diabetic mice has been shown to prevent diabetic micro and macrovascular complications[9]. Accordingly, over expression of antioxidative enzymes, such SOD and catalase, may represent a therapeutic approach to the reduction of diabetic complications, however, the level of over expression also needs to be optimized in order to avoid additional complications[10].

Polyphenols, flavonoids, phenolic acids and zinc have recently been shown to have potent beneficial effects in relation to hyperglycemia, diabetes and its associated complications. Curcumin, the major bioactive compound of turmeric and its analogues, has anti-inflammatory, antioxidant, anti-tumor and epigenetics modulatory effects with potential efficacy against diabetic complications[11,12]. Depletion of zinc in diabetes increases oxidative stress while zinc supplementation has been shown to have a hypoglycemic, antioxidant effect and alleviates some diabetes-associated complications[13]. Resveratrol, a key bioactive compound derived from red grapes, has been shown to have number of benefits including on glycemic control and the management of diabetic complications[14]. Furthermore, nanotechnology or nano-formulations of polyphenols, flavonoids and phenolic acids has the potential to enhance solubility, and intestinal absorption, as well as bioavailability and, therapeutic efficacy in diabetes and its associated complications[15].

Silent information regulator 1 (SIRT1), a member of the sirtuins family when the sirtuins are NAD^+ dependent histone deacetylase. Apart from activating LKB1 mediated AMPK followed by PGCα, PPARα, eNOS pathways and inhibiting mTOR and NOX or NADPH oxidase pathways[16]; SIRT1, has been reported to regulate the activity of other proteins, such as forkhead box protein of class O or FOXO, which regulates oxidative stress resistance, insulin signaling and metabolism along with its other activities as a transcription factor[17]. Of the many FOXOs, FOXO1 is widely expressed in muscle, liver and pancreas and protects pancreatic β-cells from oxidative stress by increasing the expression of antioxidant genes[18]. On the other hand, there is evidence that FOXO3 can prevent atherosclerosis *via* inhibiting smooth

muscle cell proliferation and activation[19]. FOXO1 and FOXO3 are also involved in many other mechanisms of relevance to glucose metabolism, as well as diabetic complications. Hence, SIRT1 and FOXO1 and FOXO3 may also represent therapeutic targets for the management of diabetic complications.

Like many other molecular pathways, epigenetic factors, including histone modifications, DNA methylations and non-coding RNAs play a major role in the pathogenesis of diabetes and its complications[20]. Among many non-coding RNAs, some micro-RNAs have been shown to have a pivotal role in the management of diabetes and diabetic complications, particularly in relation to the diagnosis and prognosis of prevalent microvascular complications *e.g.* diabetic neuropathy. A number of microRNAs are involved in the signaling pathways of diabetic complications, which can be targeted for the early diagnosis and development of therapeutics for diabetic microvascular complications, particularly for diabetic neuropathy and diabetic foot disease[21].

CONCLUSION

In conclusion, although many other therapeutic targets are being investigated for the improved management of diabetes and its associated complications, the approach of reducing oxidative stress or increasing antioxidant status using antioxidant phytochemicals or bioactive compounds and mineral such as zinc; molecular metabolic pathways such as SIRT1 and FOXOs and micro RNAs represent important and novel approaches to the diagnosis, prevention and improved management of diabetic complications. We look forward to the outcomes of these ongoing studies, which will be facilitated by an effective collaboration between basic scientists, clinicians and pharma and, hopefully, their prompt translation to clinical practice.

FOOTNOTES

Author contributions: Islam MS conceptualized and drafted the initial manuscript and revised after receiving reviewers' comments; Cai L and Horowitz M revised and edited the original and revised manuscript before submission.

Conflict-of-interest statement: Islam MS, Cai L and Horowitz M have nothing to disclose.

Country/Territory of origin: South Africa

ORCID number: Md Shahidul Islam 0000-0003-0309-9491; Lu Cai 0000-0003-3048-1135; Michael Horowitz 0000-0002-0942-0306.

S-Editor: Fan JR
L-Editor: A
P-Editor: Fan JR

REFERENCES

1 **van Dieren S**, Beulens JW, van der Schouw YT, Grobbee DE, Neal B. The global burden of diabetes and its complications: an emerging pandemic. *Eur J Cardiovasc Prev Rehabil* 2010; **17** (Suppl 1): S3-S8 [PMID: 20489418 DOI: 10.1097/01.hjr.0000368191.86614.5a]
2 **Harding JL**, Pavkov ME, Magliano DJ, Shaw JE, Gregg EW. Global trends in diabetes complications: a review of current evidence. *Diabetologia* 2019; **62**: 3-16 [PMID: 30171279 DOI: 10.1007/s00125-018-4711-2]
3 **Aikaeli F**, Njim T, Gissing S, Moyo F, Alam U, Mfinanga SG, Okebe J, Ramaiya K, Webb EL, Jaffar S, Garrib A. Prevalence of microvascular and macrovascular complications of diabetes in newly diagnosed type 2 diabetes in low-and-middle-income countries: A systematic review and meta-analysis. *PLOS Glob Public Health* 2022; **2**: e0000599 [PMID: 36962416 DOI: 10.1371/journal.pgph.0000599]
4 **Forbes JM**, Cooper ME. Mechanisms of diabetic complications. *Physiol Rev* 2013; **93**: 137-188 [PMID: 23303908 DOI: 10.1152/physrev.00045.2011]
5 **Grover A**, Sharma K, Gautam S, Gulati M, Singh SK. Diabetes and Its Complications: Therapies Available, Anticipated and Aspired. *Curr Diabetes Rev* 2021; **17**: 397-420 [PMID: 33143627 DOI: 10.2174/1573399816666201103144231]
6 **Blonde L**. Current challenges in diabetes management. *Clin Cornerstone* 2005; **7** (Suppl 3): S6-17 [PMID: 16545737 DOI: 10.1016/s1098-3597(05)80084-5]
7 **Brown E**, Heerspink HJL, Cuthbertson DJ, Wilding JPH. SGLT2 inhibitors and GLP-1 receptor agonists: established and emerging indications. *Lancet* 2021; **398**: 262-276 [PMID: 34216571 DOI: 10.1016/S0140-6736(21)00536-5]
8 **Darenskaya MA**, Kolesnikova LI, Kolesnikov SI. Oxidative Stress: Pathogenetic Role in Diabetes Mellitus and Its Complications and Therapeutic Approaches to Correction. *Bull Exp Biol Med* 2021; **171**: 179-189 [PMID: 34173093 DOI: 10.1007/s10517-021-05191-7]
9 **Giacco F**, Brownlee M. Oxidative stress and diabetic complications. *Circ Res* 2010; **107**: 1058-1070 [PMID: 21030723 DOI: 10.1161/CIRCRESAHA.110.223545]

10 **Tan Y**, Zhang Z, Zheng C, Wintergerst KA, Keller BB, Cai L. Mechanisms of diabetic cardiomyopathy and potential therapeutic strategies: preclinical and clinical evidence. *Nat Rev Cardiol* 2020; **17**: 585-607 [PMID: 32080423 DOI: 10.1038/s41569-020-0339-2]

11 **Parsamanesh N**, Moossavi M, Bahrami A, Butler AE, Sahebkar A. Therapeutic potential of curcumin in diabetic complications. *Pharmacol Res* 2018; **136**: 181-193 [PMID: 30219581 DOI: 10.1016/j.phrs.2018.09.012]

12 **Tang C**, Liu Y, Liu S, Yang C, Chen L, Tang F, Wang F, Zhan L, Deng H, Zhou W, Lin Y, Yuan X. Curcumin and Its Analogs as Potential Epigenetic Modulators: Prevention of Diabetes and Its Complications. *Pharmacology* 2022; **107**: 1-13 [PMID: 34915505 DOI: 10.1159/000520311]

13 **Barman S**, Srinivasan K. Diabetes and zinc dyshomeostasis: Can zinc supplementation mitigate diabetic complications? *Crit Rev Food Sci Nutr* 2022; **62**: 1046-1061 [PMID: 33938330 DOI: 10.1080/10408398.2020.1833178]

14 **Huang DD**, Shi G, Jiang Y, Yao C, Zhu C. A review on the potential of Resveratrol in prevention and therapy of diabetes and diabetic complications. *Biomed Pharmacother* 2020; **125**: 109767 [PMID: 32058210 DOI: 10.1016/j.biopha.2019.109767]

15 **Agarawal K**, Anant Kulkarni Y, Wairkar S. Nanoformulations of flavonoids for diabetes and microvascular diabetic complications. *Drug Deliv Transl Res* 2023; **13**: 18-36 [PMID: 35637334 DOI: 10.1007/s13346-022-01174-x]

16 **Meng T**, Qin W, Liu B. SIRT1 Antagonizes Oxidative Stress in Diabetic Vascular Complication. *Front Endocrinol (Lausanne)* 2020; **11**: 568861 [PMID: 33304318 DOI: 10.3389/fendo.2020.568861]

17 **Jalgaonkar MP**, Parmar UM, Kulkarni YA, Oza MJ. SIRT1-FOXOs activity regulates diabetic complications. *Pharmacol Res* 2022; **175**: 106014 [PMID: 34856334 DOI: 10.1016/j.phrs.2021.106014]

18 **Kandula V**, Kosuru R, Li H, Yan D, Zhu Q, Lian Q, Ge RS, Xia Z, Irwin MG. Forkhead box transcription factor 1: role in the pathogenesis of diabetic cardiomyopathy. *Cardiovasc Diabetol* 2016; **15**: 44 [PMID: 26956801 DOI: 10.1186/s12933-016-0361-1]

19 **Wang Y**, Zhou Y, Graves DT. FOXO transcription factors: their clinical significance and regulation. *Biomed Res Int* 2014; **2014**: 925350 [PMID: 24864265 DOI: 10.1155/2014/925350]

20 **Kaur P**, Kotru S, Singh S, Munshi A. miRNA signatures in diabetic retinopathy and nephropathy: delineating underlying mechanisms. *J Physiol Biochem* 2022; **78**: 19-37 [PMID: 35098434 DOI: 10.1007/s13105-021-00867-0]

21 **Kaur P**, Kotru S, Singh S, Munshi A. Role of miRNAs in diabetic neuropathy: mechanisms and possible interventions. *Mol Neurobiol* 2022; **59**: 1836-1849 [PMID: 35023058 DOI: 10.1007/s12035-021-02662-w]

World Journal of Diabetes

Submit a Manuscript: https://www.f6publishing.com

World J Diabetes 2023 September 15; 14(9): 1334-1340

DOI: 10.4239/wjd.v14.i9.1334

ISSN 1948-9358 (online)

MINIREVIEWS

MicroRNA-155 mediates endogenous angiotensin II type 1 receptor regulation: implications for innovative type 2 diabetes mellitus management

Konstantinos I Papadopoulos, Alexandra Papadopoulou, Tar-Choon Aw

Specialty type: Endocrinology and metabolism

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): A
Grade B (Very good): B, B
Grade C (Good): C, C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Cai L, United States; Cen LS, China; Moreno-Gómez-Toledano R, Spain; Papadopoulos VP, Greece

Received: April 14, 2023
Peer-review started: April 14, 2023
First decision: June 13, 2023
Revised: June 18, 2023
Accepted: July 13, 2023
Article in press: July 13, 2023
Published online: September 15, 2023

Konstantinos I Papadopoulos, Department of R&D, THAI StemLife, Bangkok 10310, Thailand

Alexandra Papadopoulou, Occupational and Environmental Health Services, Feelgood Lund, Lund 223-63, Skåne, Sweden

Tar-Choon Aw, Department of Laboratory Medicine, Changi General Hospital, Singapore 529889, Singapore, Singapore

Tar-Choon Aw, Department of Medicine, National University of Singapore, Singapore 119228, Singapore, Singapore

Corresponding author: Konstantinos I Papadopoulos, MD, PhD, Chief Physician, Director, Department of R&D, THAI StemLife, 566/3 THAI StemLife bldg., Soi Ramkhamhaeng 39 (Thepleela 1), Prachaouthit Rd., Wangthonglang, Bangkok 10310, Thailand.
kostas@thaistemlife.co.th

Abstract

Type 2 diabetes mellitus (T2DM) is a lifelong condition and a threat to human health. Thorough understanding of its pathogenesis is acutely needed in order to devise innovative, preventative, and potentially curative pharmacological interventions. MicroRNAs (miRNA), are small, non-coding, one-stranded RNA molecules, that can target and silence around 60% of all human genes through translational repression. MiR-155 is an ancient, evolutionarily well-conserved miRNA, with distinct expression profiles and multifunctionality, and a target repertoire of over 241 genes involved in numerous physiological and pathological processes including hematopoietic lineage differentiation, immunity, inflammation, viral infections, cancer, cardiovascular conditions, and particularly diabetes mellitus. MiR-155 Levels are progressively reduced in aging, obesity, sarcopenia, and T2DM. Thus, the loss of coordinated repression of multiple miR-155 targets acting as negative regulators, such as *C/EBPβ*, *HDAC4*, and *SOCS1* impacts insulin signaling, deteriorating glucose homeostasis, and causing insulin resistance (IR). Moreover, deranged regulation of the renin angiotensin aldosterone system (RAAS) through loss of Angiotensin II Type 1 receptor downregulation, and negated repression of *ETS-1*, results in unopposed detrimental Angiotensin II effects, further promoting IR. Finally, loss of *BACH1* and *SOCS1* repression abolishes cytoprotective, anti-oxidant, anti-apoptotic, and anti-inflam-

matory cellular pathways, and promotes β-cell loss. In contrast to RAAS inhibitor treatments that further decrease already reduced miR-155 Levels, strategies to increase an ailing miR-155 production in T2DM, *e.g.*, the use of metformin, mineralocorticoid receptor blockers (spironolactone, eplerenone, finerenone), and verapamil, alone or in various combinations, represent current treatment options. In the future, direct tissue delivery of miRNA analogs is likely.

Key Words: Angiotensin II; Angiotensin II type 1 receptor; Arginase 2; L-type calcium channel; Mineralocorticoid receptor; MiRNA-155; Renin-angiotensin aldosterone system; Type 1/2 diabetes mellitus; Verapamil

Core Tip: MicroRNAs (miRNA) are small, non-coding, one-stranded RNA molecules that can target and silence over 60% of human genes thereby effectively regulating huge genetic networks. MiRNAs are abundantly found in every human cell and their production is tightly controlled. They play critical roles in regulating almost every cellular pathway, numerous human diseases, and have been linked to the development of diabetes mellitus (DM) and the regulation of blood pressure. In this minireview, we comment on crucial miR-155 effects in type 2 DM (T2DM). Deeper mechanistical understanding of this miRNA's permeating action may lead to innovative therapeutic approaches in T2DM.

Citation: Papadopoulos KI, Papadopoulou A, Aw TC. MicroRNA-155 mediates endogenous angiotensin II type 1 receptor regulation: implications for innovative type 2 diabetes mellitus management. *World J Diabetes* 2023; 14(9): 1334-1340
URL: https://www.wjgnet.com/1948-9358/full/v14/i9/1334.htm
DOI: https://dx.doi.org/10.4239/wjd.v14.i9.1334

INTRODUCTION

Diabetes mellitus (DM), until recently considered a lifelong and irreversible condition, is a devastating burden to over half a billion people worldwide[1]. The overwhelming majority of diabetic patients-over 90%-suffer from Type 2 DM (T2DM) caused by an intricate interaction between lifestyle and genetics that through insulin resistance (IR) lead to metabolic syndrome, pre-diabetes, failure of insulin-secreting pancreatic β-cells and ultimately overt disease[2,3]. Uncontrolled T2DM eventually progresses to a myriad of severe health complications [among which cardiovascular disease (CVD), chronic renal failure, and hypertension (HT)], and to an early death[1]. The syndemic of coronavirus disease 2019 and T2DM has affirmed the latter's lethal effect[4]. Ominous future predictions estimate the number of DM-afflicted individuals to be over 800 million by 2045, up from the current 500 million[1]. Increased understanding of the T2DM pathogenesis is, therefore, acutely needed in order to devise innovative, preventative and potentially curative pharmacological interventions[5].

The pathophysiological role of the renin angiotensin aldosterone system (RAAS) and its major effector, Angiotensin II (Ang II) through the Ang II Type 1 receptor (AT1R), in the development of IR in T2DM have long been recognized[6]. Furthermore, convincing evidence exists advocating the use of RAAS inhibition, ACE inhibitors (ACEi) or AT1 receptor antagonists/blockers (ARB), in patients with T2DM, not only for proteinuria and HT, but also as a means to improve IR and glucose homeostasis[6].

MicroRNAs (miRNAs or miRs) are small (21-25 nucleotides), non-coding RNAs, able to translationally repress and downregulate gene expression[7]. Present abundantly in all human cells, miRNAs are endogenously biosynthesized through a strictly regulated process that will ultimately result in a mature miRNA, with a 2-8 nucleotide long seed sequence in its 5'untranslated region (UTR), that will bind to a target messenger RNA (mRNA). If the miRNA seed sequence binds perfectly to the corresponding 3'UTR of a specific mRNA, the latter will be recruited to be degraded by an RNA silencing complex. If the binding is incomplete, mRNA translational machinery will be blocked, thereby inhibiting protein translational efficiency, and repressing (silencing) gene expression[7]. As a specific miRNA can target multiple mRNA molecules, and equally, a single mRNA molecule can bind to multiple miRNAs, the host can modulate response feedback, through regulatory gene networks, in a concerted effort to control diverse aspects of cellular processes[7]. In this minireview, we present additional miRNA- modulated pathways that can modulate AT1R and Ang II effects that are of importance for the pathogenesis of IR, T2DM, and the development of cardiovascular and renal diabetic complications.

MiR-155 is of particular interest as it is intricately involved both in the pathogenesis of DM and the regulation of AT1R and Ang II effects (Figure 1)[6,8-12]. First identified in 1997, miR-155 is a highly conserved and ancient miRNA primarily expressed in the thymus and spleen. It exhibits unique expression profiles and multifunctionality but is minimally detected under normal physiological conditions[13]. With a target repertoire of over 241 genes, miR-155 plays critical roles in various physiological and pathological processes, such as hematopoietic cell line differentiation, inflammation, immunity (especially viral and parasitic infections), cancer, cardiovascular conditions, and notably, DM (Table 1)[5,8,12-25].

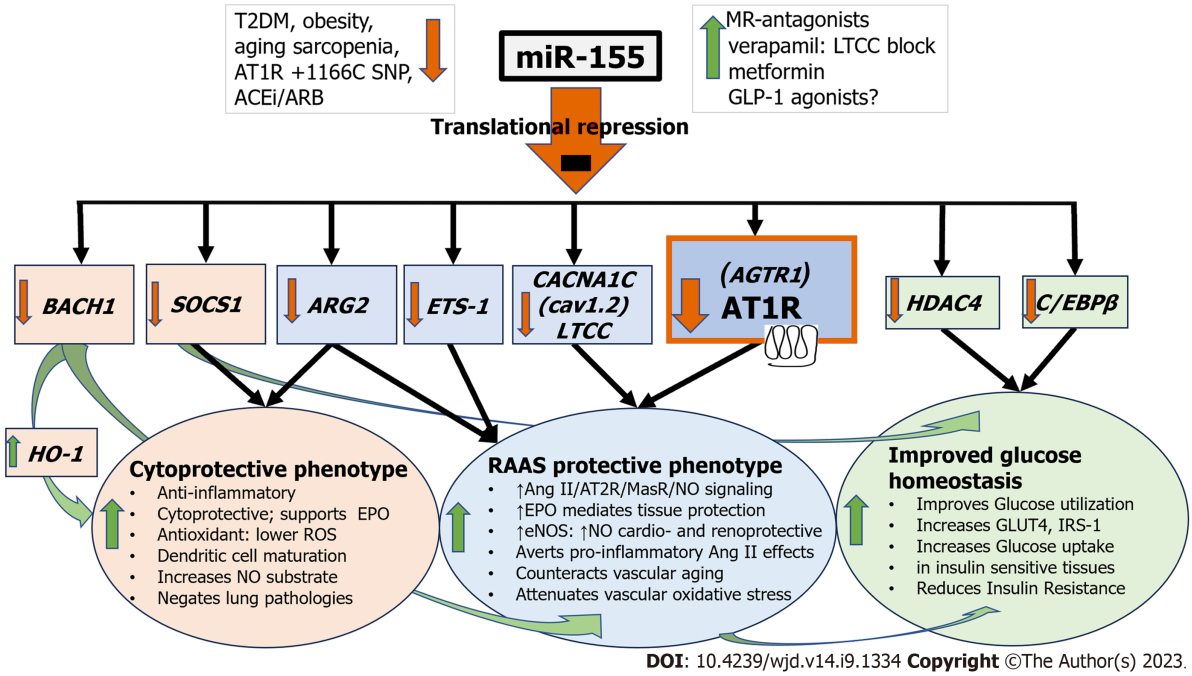
Table 1 Direct gene targets of microRNA-155 relevant to type 2 diabetes mellitus

Gene symbol	Full gene name	Action
AGTR1	*Angiotensin II type 1 receptor gene*	Repressed translation downregulates gene expression mediating endogenous AT1R antagonism[9,10,21]. Human *in-vitro* and *in-vivo* studies
ARG2	*Arginase-2*	Repressed translation prevents L-arginine depletion, supports dendritic cell maturation, and negates lung pathologies[22,23]. Human and mouse *in-vitro* and *in-vivo* studies
BACH1	*BTB and CNC homology 1, basic leucine zipper transcription factor 1*	Translational repression of *BACH1* leads to potent anti-inflammatory, cytoprotective, antioxidant programs through Heme Oxygenase-1[12]. Review of human *in-vitro* and *in-vivo* studies
C/EBPβ	*CCAAT/enhancer-binding protein β*	Repression downregulates *Pyruvate Kinase 4* (*PDK4*) gene expression and negatively regulates Pyruvate kinase complex (PDC) activity, thereby improving glucose utilization [16]. Mouse *in-vitro* and human *in-vivo* studies
ETS-1	*E26 Transformation-specific Sequence-1*	Translational repression averts Ang II effects involving gene regulation of vascular remodeling, angiogenesis, and inflammation[9,10,24]. Review of human *in-vitro* and *in-vivo* studies. Mouse *in-vitro* and *in-vivo* studies
HDAC4	*Histone deacetylase 4*	Its repression increases GLUT4 and enhances glucose uptake in insulin-sensitive tissues, *i.e.*, skeletal muscle [16]. Mouse *in-vitro* and human *in-vivo* studies
CACNA1C (Cav1.2)	*L-type calcium channel subunit, LTCC*	As a subunit of the L-type calcium channel, this pro-constrictive gene contributes to influx of calcium in vascular smooth muscle cells and reactive oxygen species production, thereby mediating the important components of vascular aging: Vasoconstriction and vascular oxidative stress[21]. Human *in-vitro* and *in-vivo* studies
SOCS1	*Suppressor of cytokine signaling 1*	Repression prevents the degradation of IRS-1 (Insulin Receptor Substrate-1) protein that mediates the effect of insulin in muscle, liver, and adipose tissue. Supports the JAK2/Y343/STAT5 pathway through which the protective effects of EPO against ischemic injury are mediated[16,25]. Human *in-vivo* study. Mouse *in-vitro* and *in-vivo* study

AT1R: Angiotensin II Type 1 receptor; Ang II: Angiotensin II; LTCC: L-type calcium channel; EPO: Erythropoietin; ROS: Reactive oxygen species; JAK2: Janus kinase 2; STAT5: Signal transducer and activator of transcription 5.

In T2DM, miR-155 Levels in plasma, peripheral blood cells, platelets, and urine are significantly and consistently decreased, with surprising congruence between different ethnicities[8]. Ranging from obesity to IR to diabetic complications in T2DM, miR-155 Levels are progressively reduced[8,14,15,17]. MiR-155's underlying molecular mechanism in enhancing insulin signaling, improving glucose homeostasis, and alleviating IR in T2DM, occurs partly through the coordinated repression of multiple negative regulators, such as *CCAAT/enhancer-binding protein β* (*C/EBPβ*), *Histone Deacetylase 4* (*HDAC4*), and *Suppressor of cytokine signaling 1* (*SOCS1*) (Table 1)[16]. MiR-155-mediated *C/EBPβ* repression downregulates *Pyruvate Kinase 4* (*PDK4*) gene expression and negatively regulates Pyruvate kinase complex activity, thereby improving glucose utilization[16]. *HDAC4* repression increases GLUT4 and enhances glucose uptake in insulin-sensitive tissues, *i.e.*, skeletal muscle, while *SOCS1* repression prevents the degradation of Insulin Receptor Substrate-1 (IRS-1) protein that mediates the effect of insulin in muscle, liver, and adipose tissue (Figure 1 and Table 1)[16].

Aging, obesity, sarcopenia, chronic RAAS activation, and IR, invariably predate the development of T2DM[26]. Shared miRNA signatures have been reported, highlighting the central role of miR-155 in the common pathogenesis of those conditions (Figure 1)[8,14,26]. One particularly important observation is the activation of the classical RAAS axis arm that involves Ang II/AT1R signaling in aging skeletal muscle and white adipose tissue (WAT), both fundamentally involved in T2DM pathogenesis[26,27]. In WAT, a chronically activated RAAS axis increases lipogenesis and reduces lipolysis, while in the aging skeletal musculature RAAS hyperactivity promotes protein degradation, and sarcopenia, altogether ultimately leading to oxidative stress, inflammation, fat accumulation, muscle atrophy, and IR[26,27]. In addition, RAAS's protective arm, involving Ang 1-7/AT2R/MasR signaling, is inhibited at the same time, further augmenting an unfavorable AT1R/AT2R imbalance[26,27]. MiR-155, acting as a master regulator, is the key player in chronic RAAS/Ang II/AT1R activation, thereby, intricately associated with the development of IR[6]. Through its repression of the *AGTR1* (the gene that codes for the AT1R) miR-155 regulates the homeostasis of the AT1R receptor, its membrane presence, and thus the biological activity of Ang II (Table 1)[9,10,28-30]. Moreover, its regulation of the *E26 Transformation-specific Sequence-1 (ETS-1)* averts several detrimental vascular Ang II effects involving gene regulation of inflammation, proliferation, remodeling, fibrosis, and angiogenesis (Table 1)[9,13,24]. Furthermore, its repressive effects on *Arginase-2* (*ARG2)* prevent the depletion of l-arginine, the obligate substrate of endothelial nitric oxide (NO) synthase (eNOS), improving substrate availability and further increasing NO-production and NO-bioavailability that further support NO-dependent cardio- and renoprotection in T2DM (Table 1)[13,23]. From the sum of these actions, it is thus evident that the reported loss of miR-155 in T2DM has profound effects leading to persistent RAAS hyperactivity through chronic Ang II stimulation of the AT1R, thereby exerting its detrimental, pro-oxidant, pro-fibrotic, proliferative, and pro- inflammatory actions (Figure 1 and Table 1). Additional miR-155 effects through repressive actions on *BTB and CNC homology 1, basic leucine zipper transcription factor 1* (*BACH1*) and *SOCS1*, synergistically enhance cytoprotective, anti-oxidant, anti-apoptotic, and anti-inflammatory cellular pathways and promote a protective cellular milieu, which is subsequently lost



DOI: 10.4239/wjd.v14.i9.1334

Figure 1 Schematic depiction of coordinated repression of multiple miR-155 targets relevant for T2DM. Translational repression of *AGTR1*, *ARG2*, *CACNA1C*, and *ETS-1* reshapes RAAS towards cardio-, vasculo-, and renoprotective phenotypes. *BACH1* and *SOCS1* repression promotes cytoprotective phenotypes and preserves β-cell function. *C/EBPβ*, *HDAC4*, and *SOCS1* repression improves glucose homeostasis, enhances insulin signaling, and reverses insulin resistance. Aging, obesity, sarcopenia, AT1R 1169C SNP, and ACEi/ARB treatment negatively impact miR-155 levels and/or function while MR antagonists, metformin, GLP-1 agonists, and verapamil exert beneficial effects. Red arrows or lines represent downregulation, lower Level, inhibition, repression. Green arrows or lines represent increased Level or stimulatory/beneficial action. ACEi: Angiotensin-converting enzyme inhibitors; *AGTR1*: Angiotensin II type 1 receptor gene; Ang II: Angiotensin II; ARB: Angiotensin II type 1 receptor blockers; *ARG2*: Arginase 2; AT1/2R: Angiotensin II type 1/2 receptor; *BACH1*: BTB and CNC homology 1, basic leucine zipper transcription factor 1; *CACNA1C (Cav1.2)*: L-type calcium channel subunit; *C/EBPβ*: CCAAT/enhancer-binding protein β; eNOS: Endothelial nitric oxide synthetase; EPO: Erythropoietin; *ETS-1*: E26 Transformation-specific Sequence-1; GLP-1: Glucagon-like peptide 1; GLUT4: Glucose transporter type 4; HO-1: Heme oxygenase 1; *HDAC4*: Histone Deacetylase 4; IRS-1: Insulin receptor substrate-1; LTCC: L-type Calcium Channel; MasR: Mas Receptor; MicroRNA-155: MiR-155; MR: Mineralocorticoid receptor; NO: Nitric oxide; RAAS: Renin-Angiotensin Aldosterone System; ROS: Reactive oxygen species; *SOCS1*: Suppressor of cytokine signaling 1; SNP: Single nucleotide polymorphism; T2DM: Type 2 Diabetes Mellitus.

following miR-155 downregulation (Table 1)[12,13]. Genetic variants that perturb miR-155's action (such as in carriers of AT1R + 1166C-allele) or that increase its synthesis (such as in trisomy 21 and the rs767649 polymorphism of miR-155) biochemically and molecularly demonstrate this central significance of miR in a plethora of DM-associated pathological conditions[11,18,31-33]. Moreover, clinical data in obese individuals demonstrate that miR-155 Levels correlate with improved insulin sensitivity post-bariatric surgery and are critical in mediating the effects of endurance exercise[34,35].

While miR-155 is consistently reduced in serum and tissues in T2DM, it is reported to be upregulated in Type 1 DM (T1DM), highlighting T1DM's autoimmune pathogenesis and miR's crucial and differential role in autoimmunity and innate and adaptive immunity[8,36]. However, even if robustly elevated in newly diagnosed T1DM, miR-155 strikingly diminishes within 5 years of diagnosis[32].

AT1R substrate modulation (ACEi) and/or receptor inhibition (ARBs) may improve glucose homeostasis[6]. However, strategies to increase an ailing miR-155 production in T2DM could prove to be a more appropriate course of action (Figure 1). Metformin with ACEi/ARB improves HbA1c goals[6]. Metformin and the newer Glucagon Like Peptide 1 (GLP-1) analogs have been shown to repress *SOCS1* and *3* and increase IRS-1[37]. Metformin mediates miR-155 increases that repress *SOCS1* and reduce NF-κB (nuclear factor κB), thereby disrupting NF-κB-mediated high-fat induced inflammatory effects in T2DM[38,39]. The clinical effects of GLP-1 analogs on miR-155 in humans are, to date, unknown, and additional research is needed, but miR-155 has been shown to promote GLP-1 production in the murine pancreas[40]. Moreover, in the resistance vessels of aging humans, elevated expression of mineralocorticoid receptor (MR) is accompanied by a decrease in miR-155 Levels and an upregulation of miR-155 targets such as the *CACNA1C* (Cav1.2) gene [a subunit of the L-type calcium channel (LTCC)], and the *AGTR1* gene. These alterations in gene expression play a role in promoting vasoconstriction and oxidative stress in aging mice (Table 1)[21]. MR inhibition reverses and reinstates the significantly low basal serum miR-155 Levels in the aging blood vessels and blocks two interactive steps involving LTCC and *AGTR1* that underlie the pathogenesis of HT[13]. A correlation between improved blood pressure response to therapy with MR antagonists and changes in miR-155 Levels in older individuals has been reported[21]. Moreover, the use of MR-antagonists (spironolactone, eplerenone, finerenone) has shown renal and cardiovascular benefits in T2DM[41-43]. LTCC blockade *per se*, through verapamil alone, or in combination with MR antagonists/metformin, will offer additional therapeutic options in T2DM[44]. Besides improved blood pressure regulation and cardio-renal protection, verapamil demonstrates additional benefits while avoiding many of the common adverse effects associated with ACEi/ARB[45]. Verapamil's mode of action is of particular interest in diabetes[46]. Apart from being present in cardiomyocytes,

LTCCs are also present in pancreatic β-cells and participate in insulin homeostasis[47]. In the heart and the pancreas, effective pharmacological LTCC blockage can inhibit the expression of pro-apoptotic thioredoxin-interacting protein, a significant contributor to pancreatic β-cell dysfunction and a key gene regulated in response to hyperglycemia, thereby promoting the survival and proper functioning of β-cells and improving glucose homeostasis[46,48]. Verapamil has, thus, the potential not only to enhance β-cell survival and function, but also improve and even prevent overt diabetes of both types[48,49]. In a recent study, verapamil combined with metformin, significantly improved glycemic control in T2DM [49]. Finally, a drawback in the use of monotherapy as ACEi/ARBs (in conditions that already are associated with low miR-155 Levels) is that they significantly further decrease already reduced miR-155 Levels[50,51]. RAAS inhibition could, thus, theoretically deprive T2DM patients of additional miR-155-engendered favorable immunological and cytoprotective effects and potentially explain ACEi's modest and ARBs' non-existent effects in preventing CVD or improving glycemic indices in DM and HT (Figure 1)[13,50-53].

CONCLUSION

The data presented above strongly support the role of miR-155 as a major player in the pathogenesis of T2DM and complications, by triggering IR and β-cell loss as well as through RAAS modulatory effects (Figure 1)[5,8]. Large multicenter trials are required to establish this role of miRNA as a reliable biomarker and potential therapeutic target in DM. Then, as increased mechanistic knowledge regarding miR-155 becomes available, novel miRNA-modulating approaches with miR-155 as a target are likely in T2DM. Even though these therapeutic modalities are still in their infancy and might yet be far from the clinic, research must address this knowledge gap in order to devise how to effectively deliver specific, synthetic miRNA mimics (T2DM, aging, obesity, sarcopenia) or inhibitors-antagomiRs (T1DM, cancer), to a specific tissue, in the diabetic patient, as miR-155 actions are tissue-sensitive[54]. In addition, a better understanding is needed on how several miRNAs work synergistically on the same mRNA targets and how miRNA networks function. As disease-specific miRNA expression pattern is ubiquitous in all related tissues, it can prove challenging in a complex disease like DM to accomplish precise delivery to certain tissues/organs and avoid adverse off-target effects in others[5].

ACKNOWLEDGEMENTS

We are thankful to James T A Marshall for his invaluable editorial assistance.

FOOTNOTES

Author contributions: Papadopoulos KI conceived and conceptualized the argumentation, designed the layout, drafted the initial manuscript, and reviewed and revised the manuscript; Papadopoulou A performed the literature search, extracted vital information, contributed to the synthesis of the minireview, and reviewed and revised the manuscript; Aw TC coordinated and supervised literature search, made substantial and direct intellectual contributions and critically reviewed the manuscript for important intellectual content; All authors approved the submitted final manuscript and agree to be accountable for all aspects of the work.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Country/Territory of origin: Thailand

ORCID number: Konstantinos I Papadopoulos 0000-0003-0041-7853; Alexandra Papadopoulou 0000-0002-7488-8271; Tar-Choon Aw 0000-0002-7814-8836.

S-Editor: Li L
L-Editor: A
P-Editor: Ji MX

REFERENCES

1 **Magliano DJ**, Boyko EJ; IDF Diabetes Atlas 10th edition scientific committee. IDF Diabetes Atlas [Internet]. 10th ed. Brussels: International Diabetes Federation; 2021 [PMID: 35914061]
2 **Taylor R**, Al-Mrabeh A, Sattar N. Understanding the mechanisms of reversal of type 2 diabetes. *Lancet Diabetes Endocrinol* 2019; **7**: 726-736

[PMID: 31097391 DOI: 10.1016/S2213-8587(19)30076-2]

3 **Cai L**, Wheeler E, Kerrison ND, Luan J, Deloukas P, Franks PW, Amiano P, Ardanaz E, Bonet C, Fagherazzi G, Groop LC, Kaaks R, Huerta JM, Masala G, Nilsson PM, Overvad K, Pala V, Panico S, Rodriguez-Barranco M, Rolandsson O, Sacerdote C, Schulze MB, Spijkerman AMW, Tjonneland A, Tumino R, van der Schouw YT, Sharp SJ, Forouhi NG, Riboli E, McCarthy MI, Barroso I, Langenberg C, Wareham NJ. Genome-wide association analysis of type 2 diabetes in the EPIC-InterAct study. *Sci Data* 2020; **7**: 393 [PMID: 33188205 DOI: 10.1038/s41597-020-00716-7]

4 **Papadopoulos KI**, Sutheesophon W, Aw TC. Too hard to die: Exercise training mediates specific and immediate SARS-CoV-2 protection. *World J Virol* 2022; **11**: 98-103 [PMID: 35433336 DOI: 10.5501/wjv.v11.i2.98]

5 **Palihaderu P**, Mendis B, Premarathne J, Dias W, Yeap SK, Ho WY, Dissanayake AS, Rajapakse IH, Karunanayake P, Senarath U, Satharasinghe DA. Therapeutic Potential of miRNAs for Type 2 Diabetes Mellitus: An Overview. *Epigenet Insights* 2022; **15**: 25168657221130041 [PMID: 36262691 DOI: 10.1177/25168657221130041]

6 **Lopez DL**, Casillas OE, Jaramillo HJ, Romero-Garcia T, Vazquez-Jimenez JG. AT1 receptor downregulation: A mechanism for improving glucose homeostasis. *World J Diabetes* 2023; **14**: 170-178 [PMID: 37035227 DOI: 10.4239/wjd.v14.i3.170]

7 **Papadopoulos KI**, Wattanaarsakit P, Prasongchean W, Narain R. 10 - Gene therapies in clinical trials. In: Narain R, editor. Polymers and Nanomaterials for Gene Therapy. Cambridge: Woodhead Publishing, 2016: 231-256 [DOI: 10.1016/B978-0-08-100520-0.00010-2]

8 **Jankauskas SS**, Gambardella J, Sardu C, Lombardi A, Santulli G. Functional Role of miR-155 in the Pathogenesis of Diabetes Mellitus and Its Complications. *Noncoding RNA* 2021; **7** [PMID: 34287359 DOI: 10.3390/ncrna7030039]

9 **Zhu N**, Zhang D, Chen S, Liu X, Lin L, Huang X, Guo Z, Liu J, Wang Y, Yuan W, Qin Y. Endothelial enriched microRNAs regulate angiotensin II-induced endothelial inflammation and migration. *Atherosclerosis* 2011; **215**: 286-293 [PMID: 21310411 DOI: 10.1016/j.atherosclerosis.2010.12.024]

10 **Elton TS**, Selemon H, Elton SM, Parinandi NL. Regulation of the MIR155 host gene in physiological and pathological processes. *Gene* 2013; **532**: 1-12 [PMID: 23246696 DOI: 10.1016/j.gene.2012.12.009]

11 **Sethupathy P**, Borel C, Gagnebin M, Grant GR, Deutsch S, Elton TS, Hatzigeorgiou AG, Antonarakis SE. Human microRNA-155 on chromosome 21 differentially interacts with its polymorphic target in the AGTR1 3' untranslated region: a mechanism for functional single-nucleotide polymorphisms related to phenotypes. *Am J Hum Genet* 2007; **81**: 405-413 [PMID: 17668390 DOI: 10.1086/519979]

12 **Faraoni I**, Antonetti FR, Cardone J, Bonmassar E. miR-155 gene: a typical multifunctional microRNA. *Biochim Biophys Acta* 2009; **1792**: 497-505 [PMID: 19268705 DOI: 10.1016/j.bbadis.2009.02.013]

13 **Papadopoulos KI**, Papadopoulou A, Aw TC. Beauty and the beast: host microRNA-155 *versus* SARS-CoV-2. *Hum Cell* 2023; **36**: 908-922 [PMID: 36847920 DOI: 10.1007/s13577-023-00867-w]

14 **Huang S**, Xiang C, Song Y. Identification of the shared gene signatures and pathways between sarcopenia and type 2 diabetes mellitus. *PLoS One* 2022; **17**: e0265221 [PMID: 35271662 DOI: 10.1371/journal.pone.0265221]

15 **Mahdavi R**, Ghorbani S, Alipoor B, Panahi G, Khodabandehloo H, Esfahani EN, Razi F, Meshkani R. Decreased Serum Level of miR-155 is Associated with Obesity and its Related Metabolic Traits. *Clin Lab* 2018; **64**: 77-84 [PMID: 29479888 DOI: 10.7754/Clin.Lab.2017.170618]

16 **Lin X**, Qin Y, Jia J, Lin T, Lin X, Chen L, Zeng H, Han Y, Wu L, Huang S, Wang M, Xie R, Liang L, Liu Y, Liu R, Zhang T, Li J, Wang S, Sun P, Huang W, Yao K, Xu K, Du T, Xiao D. MiR-155 Enhances Insulin Sensitivity by Coordinated Regulation of Multiple Genes in Mice. *PLoS Genet* 2016; **12**: e1006308 [PMID: 27711113 DOI: 10.1371/journal.pgen.1006308]

17 **Corral-Fernández NE**, Salgado-Bustamante M, Martínez-Leija ME, Cortez-Espinosa N, García-Hernández MH, Reynaga-Hernández E, Quezada-Calvillo R, Portales-Pérez DP. Dysregulated miR-155 expression in peripheral blood mononuclear cells from patients with type 2 diabetes. *Exp Clin Endocrinol Diabetes* 2013; **121**: 347-353 [PMID: 23616185 DOI: 10.1055/s-0033-1341516]

18 **Latini A**, Spallone V, D'Amato C, Novelli G, Borgiani P, Ciccacci C. A common polymorphism in MIR155 gene promoter region is associated with a lower risk to develop type 2 diabetes. *Acta Diabetol* 2019; **56**: 717-718 [PMID: 30631931 DOI: 10.1007/s00592-018-1277-x]

19 **Zhiping L**, Chunli L, Shanna Z. Study on the correlation between PPARγ, Aβ1-42, miR-155 and the occurrence and development of diabetes. *Cell Mol Biol (Noisy-le-grand)* 2022; **67**: 214-221 [PMID: 35809285 DOI: 10.14715/cmb/2021.67.4.24]

20 **Chatzopoulou F**, Kyritsis KA, Papagiannopoulos CI, Galatou E, Mittas N, Theodoroula NF, Papazoglou AS, Karagiannidis E, Chatzidimitriou M, Papa A, Sianos G, Angelis L, Chatzidimitriou D, Vizirianakis IS. Dissecting miRNA-Gene Networks to Map Clinical Utility Roads of Pharmacogenomics-Guided Therapeutic Decisions in Cardiovascular Precision Medicine. *Cells* 2022; **11** [PMID: 35203258 DOI: 10.3390/cells11040607]

21 **DuPont JJ**, McCurley A, Davel AP, McCarthy J, Bender SB, Hong K, Yang Y, Yoo JK, Aronovitz M, Baur WE, Christou DD, Hill MA, Jaffe IZ. Vascular mineralocorticoid receptor regulates microRNA-155 to promote vasoconstriction and rising blood pressure with aging. *JCI Insight* 2016; **1**: e88942 [PMID: 27683672 DOI: 10.1172/jci.insight.88942]

22 **Dunand-Sauthier I**, Irla M, Carnesecchi S, Seguín-Estévez Q, Vejnar CE, Zdobnov EM, Santiago-Raber ML, Reith W. Repression of arginase-2 expression in dendritic cells by microRNA-155 is critical for promoting T cell proliferation. *J Immunol* 2014; **193**: 1690-1700 [PMID: 25009204 DOI: 10.4049/jimmunol.1301913]

23 **Gambardella J**, Khondkar W, Morelli MB, Wang X, Santulli G, Trimarco V. Arginine and Endothelial Function. *Biomedicines* 2020; **8** [PMID: 32781796 DOI: 10.3390/biomedicines8080277]

24 **Zhan Y**, Brown C, Maynard E, Anshelevich A, Ni W, Ho IC, Oettgen P. Ets-1 is a critical regulator of Ang II-mediated vascular inflammation and remodeling. *J Clin Invest* 2005; **115**: 2508-2516 [PMID: 16138193 DOI: 10.1172/JCI24403]

25 **Breggia AC**, Wojchowski DM, Himmelfarb J. JAK2/Y343/STAT5 signaling axis is required for erythropoietin-mediated protection against ischemic injury in primary renal tubular epithelial cells. *Am J Physiol Renal Physiol* 2008; **295**: F1689-F1695 [PMID: 18815218 DOI: 10.1152/ajprenal.90333.2008]

26 **Shou J**, Chen PJ, Xiao WH. Mechanism of increased risk of insulin resistance in aging skeletal muscle. *Diabetol Metab Syndr* 2020; **12**: 14 [PMID: 32082422 DOI: 10.1186/s13098-020-0523-x]

27 **Frantz EDC**, Prodel E, Braz ID, Giori IG, Bargut TCL, Magliano DC, Nobrega ACL. Modulation of the renin-angiotensin system in white adipose tissue and skeletal muscle: focus on exercise training. *Clin Sci (Lond)* 2018; **132**: 1487-1507 [PMID: 30037837 DOI: 10.1042/CS20180276]

28 **Zheng L**, Xu CC, Chen WD, Shen WL, Ruan CC, Zhu LM, Zhu DL, Gao PJ. MicroRNA-155 regulates angiotensin II type 1 receptor expression and phenotypic differentiation in vascular adventitial fibroblasts. *Biochem Biophys Res Commun* 2010; **400**: 483-488 [PMID: 20735984 DOI: 10.1016/j.bbrc.2010.08.067]

29 **Cheng W**, Liu T, Jiang F, Liu C, Zhao X, Gao Y, Wang H, Liu Z. microRNA-155 regulates angiotensin II type 1 receptor expression in

umbilical vein endothelial cells from severely pre-eclamptic pregnant women. *Int J Mol Med* 2011; **27**: 393-399 [PMID: 21234519 DOI: 10.3892/ijmm.2011.598]

30 **Yang Y**, Zhou Y, Cao Z, Tong XZ, Xie HQ, Luo T, Hua XP, Wang HQ. miR-155 functions downstream of angiotensin II receptor subtype 1 and calcineurin to regulate cardiac hypertrophy. *Exp Ther Med* 2016; **12**: 1556-1562 [PMID: 27588076 DOI: 10.3892/etm.2016.3506]

31 **Izmailova O**, Shlykova O, Vatsenko A, Ivashchenko D, Dudchenko M, Koval T, Kaidashev I. Allele C (rs5186) of at1r is associated with the severity of COVID-19 in the Ukrainian population. *Infect Genet Evol* 2022; **98**: 105227 [PMID: 35091110 DOI: 10.1016/j.meegid.2022.105227]

32 **Assmann TS**, Recamonde-Mendoza M, Puñales M, Tschiedel B, Canani LH, Crispim D. MicroRNA expression profile in plasma from type 1 diabetic patients: Case-control study and bioinformatic analysis. *Diabetes Res Clin Pract* 2018; **141**: 35-46 [PMID: 29679626 DOI: 10.1016/j.diabres.2018.03.044]

33 **Assmann TS**, Duarte GCK, Brondani LA, de Freitas PHO, Martins ÉM, Canani LH, Crispim D. Polymorphisms in genes encoding miR-155 and miR-146a are associated with protection to type 1 diabetes mellitus. *Acta Diabetol* 2017; **54**: 433-441 [PMID: 28101643 DOI: 10.1007/s00592-016-0961-y]

34 **Hubal MJ**, Nadler EP, Ferrante SC, Barberio MD, Suh JH, Wang J, Dohm GL, Pories WJ, Mietus-Snyder M, Freishtat RJ. Circulating adipocyte-derived exosomal MicroRNAs associated with decreased insulin resistance after gastric bypass. *Obesity (Silver Spring)* 2017; **25**: 102-110 [PMID: 27883272 DOI: 10.1002/oby.21709]

35 **Soplinska A**, Zareba L, Wicik Z, Eyileten C, Jakubik D, Siller-Matula JM, De Rosa S, Malek LA, Postula M. MicroRNAs as Biomarkers of Systemic Changes in Response to Endurance Exercise-A Comprehensive Review. *Diagnostics (Basel)* 2020; **10** [PMID: 33066215 DOI: 10.3390/diagnostics10100813]

36 **Xu WD**, Feng SY, Huang AF. Role of miR-155 in inflammatory autoimmune diseases: a comprehensive review. *Inflamm Res* 2022; **71**: 1501-1517 [PMID: 36308539 DOI: 10.1007/s00011-022-01643-6]

37 **Akarsu E**, Sayiner ZA, Balcı SO, Demirel C, Bozdag Z, Korkmaz M, Yılmaz I. Effects of antidiabetics and exercise therapy on suppressors of cytokine signaling-1, suppressors of cytokine signaling-3, and insulin receptor substrate-1 molecules in diabetes and obesity. *Rev Assoc Med Bras (1992)* 2023; **69**: 112-118 [PMID: 36629649 DOI: 10.1590/1806-9282.20220856]

38 **Zhou Y**, Ma XY, Han JY, Yang M, Lv C, Shao Y, Wang YL, Kang JY, Wang QY. Metformin regulates inflammation and fibrosis in diabetic kidney disease through TNC/TLR4/NF-κB/miR-155-5p inflammatory loop. *World J Diabetes* 2021; **12**: 19-46 [PMID: 33520106 DOI: 10.4239/wjd.v12.i1.19]

39 **Gou L**, Liu G, Ma R, Regmi A, Zeng T, Zheng J, Zhong X, Chen L. High fat-induced inflammation in vascular endothelium can be improved by Abelmoschus esculentus and metformin *via* increasing the expressions of miR-146a and miR-155. *Nutr Metab (Lond)* 2020; **17**: 35 [PMID: 32467714 DOI: 10.1186/s12986-020-00459-7]

40 **Zhu M**, Wei Y, Geißler C, Abschlag K, Corbalán Campos J, Hristov M, Möllmann J, Lehrke M, Karshovska E, Schober A. Hyperlipidemia-Induced MicroRNA-155-5p Improves β-Cell Function by Targeting Mafb. *Diabetes* 2017; **66**: 3072-3084 [PMID: 28970282 DOI: 10.2337/db17-0313]

41 **Bakris GL**, Agarwal R, Anker SD, Pitt B, Ruilope LM, Rossing P, Kolkhof P, Nowack C, Schloemer P, Joseph A, Filippatos G; FIDELIO-DKD Investigators. Effect of Finerenone on Chronic Kidney Disease Outcomes in Type 2 Diabetes. *N Engl J Med* 2020; **383**: 2219-2229 [PMID: 33264825 DOI: 10.1056/NEJMoa2025845]

42 **Hu H**, Zhao X, Jin X, Wang S, Liang W, Cong X. Efficacy and safety of eplerenone treatment for patients with diabetic nephropathy: A meta-analysis. *PLoS One* 2022; **17**: e0265642 [PMID: 35324976 DOI: 10.1371/journal.pone.0265642]

43 **Lin M**, Heizati M, Wang L, Nurula M, Yang Z, Wang Z, Abudoyreyimu R, Wu Z, Li N. A systematic review and meta-analysis of effects of spironolactone on blood pressure, glucose, lipids, renal function, fibrosis and inflammation in patients with hypertension and diabetes. *Blood Press* 2021; **30**: 145-153 [PMID: 33682538 DOI: 10.1080/08037051.2021.1880881]

44 **McTavish D**, Sorkin EM. Verapamil. An updated review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in hypertension. *Drugs* 1989; **38**: 19-76 [PMID: 2670511 DOI: 10.2165/00003495-198938010-00003]

45 **Lido P**, Romanello D, Tesauro M, Bei A, Perrone MA, Palazzetti D, Noce A, Di Lullo L, Calò L, Cice G. Verapamil: prevention and treatment of cardio-renal syndromes in diabetic hypertensive patients? *Eur Rev Med Pharmacol Sci* 2022; **26**: 1524-1534 [PMID: 35302215 DOI: 10.26355/eurrev_202203_28217]

46 **Xu G**, Chen J, Jing G, Shalev A. Preventing β-cell loss and diabetes with calcium channel blockers. *Diabetes* 2012; **61**: 848-856 [PMID: 22442301 DOI: 10.2337/db11-0955]

47 **Borowiec AM**, Właszczuk A, Olakowska E, Lewin-Kowalik J. TXNIP inhibition in the treatment of diabetes. Verapamil as a novel therapeutic modality in diabetic patients. *Med Pharm Rep* 2022; **95**: 243-250 [PMID: 36060506 DOI: 10.15386/mpr-2187]

48 **Forlenza GP**, McVean J, Beck RW, Bauza C, Bailey R, Buckingham B, DiMeglio LA, Sherr JL, Clements M, Neyman A, Evans-Molina C, Sims EK, Messer LH, Ekhlaspour L, McDonough R, Van Name M, Rojas D, Beasley S, DuBose S, Kollman C, Moran A; CLVer Study Group. Effect of Verapamil on Pancreatic Beta Cell Function in Newly Diagnosed Pediatric Type 1 Diabetes: A Randomized Clinical Trial. *JAMA* 2023; **329**: 990-999 [PMID: 36826844 DOI: 10.1001/jama.2023.2064]

49 **Wang CY**, Huang KC, Lu CW, Chu CH, Huang CN, Chen HS, Lee IT, Chen JF, Chen CC, Chen CS, Hsieh CH, Tien KJ, Chien HY, Huang YY, Hsu JP, Shane GT, Chang AC, Wu YC, Sheu WH. A Randomized Controlled Trial of R-Form Verapamil Added to Ongoing Metformin Therapy in Patients with Type 2 Diabetes. *J Clin Endocrinol Metab* 2022; **107**: e4063-e4071 [PMID: 35917580 DOI: 10.1210/clinem/dgac436]

50 **Weber M**, Baker MB, Patel RS, Quyyumi AA, Bao G, Searles CD. MicroRNA Expression Profile in CAD Patients and the Impact of ACEI/ARB. *Cardiol Res Pract* 2011; **2011**: 532915 [PMID: 21785714 DOI: 10.4061/2011/532915]

51 **Zhu GF**, Yang LX, Guo RW, Liu H, Shi YK, Ye JS, Yang ZH. microRNA-155 is inversely associated with severity of coronary stenotic lesions calculated by the Gensini score. *Coron Artery Dis* 2014; **25**: 304-310 [PMID: 24525789 DOI: 10.1097/MCA.0000000000000088]

52 **Fuchs FD**, DiNicolantonio JJ. Angiotensin receptor blockers for prevention of cardiovascular disease: where does the evidence stand? *Open Heart* 2015; **2**: e000236 [PMID: 25893104 DOI: 10.1136/openhrt-2014-000236]

53 **Yao J**, Fan S, Shi X, Gong X, Zhao J, Fan G. Angiotensin-converting enzyme inhibitors *versus* angiotensin II receptor blockers on insulin sensitivity in hypertensive patients: A meta-analysis of randomized controlled trials. *PLoS One* 2021; **16**: e0253492 [PMID: 34234365 DOI: 10.1371/journal.pone.0253492]

54 **Mahesh G**, Biswas R. MicroRNA-155: A Master Regulator of Inflammation. *J Interferon Cytokine Res* 2019; **39**: 321-330 [PMID: 30998423 DOI: 10.1089/jir.2018.0155]

World Journal of Diabetes

Submit a Manuscript: https://www.f6publishing.com

World J Diabetes 2023 September 15; 14(9): 1341-1348

DOI: 10.4239/wjd.v14.i9.1341

ISSN 1948-9358 (online)

MINIREVIEWS

Hypothesis that alpha-amylase evokes regulatory mechanisms originating in the pancreas, gut and circulation, which govern glucose/insulin homeostasis

Stefan G Pierzynowski, Christine Stier, Kateryna Pierzynowska

Specialty type: Endocrinology and metabolism

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification
Grade A (Excellent): A
Grade B (Very good): B, B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Gong N, China; Shuang W, China; Horowitz M, Australia

Received: April 24, 2023
Peer-review started: April 24, 2023
First decision: June 14, 2023
Revised: June 28, 2023
Accepted: August 2, 2023
Article in press: August 2, 2023
Published online: September 15, 2023

Stefan G Pierzynowski, Department of Medical Biology, Institute of Rural Health, Lublin 20090, Poland

Stefan G Pierzynowski, Kateryna Pierzynowska, Department of Biology, Lund University, Lund 22362, Sweden

Stefan G Pierzynowski, Kateryna Pierzynowska, Anara AB, Trelleborg 23132, Sweden

Christine Stier, Department of General, Visceral, Transplant, Vascular, and Pediatric Surgery and Division of Endocrinology, University Hospital Würzburg, Würzburg 97080, Germany

Christine Stier, Department of Surgical Endoscopy, Sana Hospital, Huerth 50354, Germany

Kateryna Pierzynowska, Department of Animal Physiology, The Kielanowski Institute of Animal Physiology and Nutrition, Jablonna 05110, Poland

Kateryna Pierzynowska, Anagram Therapeutics, Inc, Framingham, MA 01701, United States

Corresponding author: Kateryna Pierzynowska, DSc, PhD, Researcher, Department of Biology, Lund University, Sölvegatan 35, Lund 22362, Sweden. katerina.goncharova@biol.lu.se

Abstract

The anti-incretin theory involving the abolishment of diabetes type (DT) II by some of methods used in bariatric surgery, first appeared during the early years of the XXI century and considers the existence of anti-incretin substances. However, to date no exogenous or endogenous anti-incretins have been found. Our concept of the acini-islet-acinar axis assumes that insulin intra-pancreatically stimulates alpha-amylase synthesis ("halo phenomenon") and in turn, alpha-amylase reciprocally inhibits insulin production, thus making alpha-amylase a candidate for being an anti-incretin. Additionally, gut as well as plasma alpha-amylase, of pancreatic and other origins, inhibits the appearance of dietary glucose in the blood, lowering the glucose peak after iv or oral glucose loading. This effect of alpha-amylase can be interpreted as an insulin down regulatory mechanism, possibly limiting the depletion of pancreatic beta cells and preventing their failure. Clinical observations agree with the above statements, where patients with high blood alpha-amylase concentrations are seldom obese and seldom develop DT2. Obese-DT2, as well as DT1 patients, usually develop exo-

crine pancreatic insufficiency (EPI) and vice versa. Ultimately, DT2 patients develop DT1, when the pancreatic beta cells are exhausted and insulin production ceases. Studies on biliopancreatic diversion (BPD) and on BPD with duodenal switch, a type of bariatric surgery, as well as studies on EPI pigs, allow us to observe and investigate the above-mentioned phenomena of intra-pancreatic interactions.

Key Words: Pancreas; Alpha-amylase; Acini-islet-acinar axis; Hyperglycaemia; Bariatrics; Insulin; Incretins; Glucose-dependent insulinotropic polypeptide; Glucagon-like peptide-1; Exocrine pancreatic insufficiency; Pancreatic enzyme therapy; Diabetes

Core Tip: The concept of the acini-islet-acinar axis postulates that insulin stimulates amylase synthesis and amylase in turn inhibits insulin production. This regulation is of particular significance with regards to postoperative glucose regulation after bariatric bypass surgery. The interaction of salivary amylase at the alimentary limb and pancreatic amylase, exclusively at the common channel, results in an immediate metabolic effect that leads to a significant reduction in incretin-induced hyperinsulinaemia and a marked improvement in glucose action on insulin production. As a result, both pancreatic beta cells and acinar cells are effectively protected against depletion.

Citation: Pierzynowski SG, Stier C, Pierzynowska K. Hypothesis that alpha-amylase evokes regulatory mechanisms originating in the pancreas, gut and circulation, which govern glucose/insulin homeostasis. *World J Diabetes* 2023; 14(9): 1341-1348
URL: https://www.wjgnet.com/1948-9358/full/v14/i9/1341.htm
DOI: https://dx.doi.org/10.4239/wjd.v14.i9.1341

INTRODUCTION

Studies on the dependency of pancreatic enzyme synthesis on insulin release started 60 years ago and resulted in the discovery of the so-called "halo phenomenon"[1-5]. For several years we have explored the 'reverse arm' of regulation; namely the dependency of insulin release on pancreatic enzyme secretion and the role of the interaction between these parameters on glucose metabolism[6]. Our studies indicate the local and peripheral dependency of insulin secretion on pancreatic alpha-amylase. Alpha-amylase, beyond its digestive functions, actively participates in glucose metabolism, both *via* inhibition of glucose absorption from the gut[7,8] and by lowering insulin release[9].

It should be mentioned that other pancreatic-like enzymes, such as proteinases and lipases of microbial origin, were tested with respect to their effect on insulin secretion and glucose utilization. We have shown that lipase does not affect the afore mentioned parameters, while proteinase decreases the insulin sensitivity index and stimulates insulin release[6].

The above-described alpha-amylase-dependent inhibition of glucose absorption, through its storage or metabolism in the gut tissues could reduce insulin release, making the secretion adequate to glucose levels entering the circulation. Thus, there appears to be some sort of intra-pancreatic down regulation effect of alpha-amylase on insulin secretion and/or synthesis. Insulin stimulates the secretion of alpha-amylase and other pancreatic enzymes, while alpha-amylase inhibits insulin release[10]. In fact, chronic hyperinsulinemia could lead not only to insulin resistance, but also to the destruction of pancreatic acinar cells, with the further development of exocrine pancreatic insufficiency (EPI)[11].

The above observations were confirmed in several *in vitro* and *in vivo* studies in our lab[9,10,12-14]. Both the alpha-amylase-insulin reciprocal acini-islet-acinar (AIA) local interactions, as well as the peripheral interactions originating in the gut and blood, which attenuate and downregulate insulin secretion, belong to the postulated new type of regulations where alpha-amylase exhibits a hormonal-like action. In 1976, Arnesjö and Lundquist[15] demonstrated a decrease in insulin release and the improvement of glucose tolerance in rats with long-term CCK-PZ stimulated pancreatic enzymes secretion[15]. We noted the stronger suppression of insulin release after pancreatin administration in the meal glucose tolerance test (44%), when compared to the intravenous glucose tolerance test (31%)[13]. In the same year, Lindqvist *et al*[16] observed a decrease in levels of active glucagon-like peptide-1 (GLP-1) after Roux-and-Y gastric bypass (RYGB) surgery in pigs[16], and in early 2018 our group showed a consequential increase in plasma amylase activity in the EPI porcine model[14]. We recognized that the effects of amylase on the regulation of insulin secretion are para-hormonal and considering all our observations, we thought it would be suitable to locate the anti-incretin action of amylase in the same place where incretins *e.g.*, GLP-1 usually act.

Reciprocal AIA regulations are likely species- and individual-dependent, thus they are in some way immanent. We recognize that dietary factors *e.g.*, carbohydrates (glucose), acting from the duodenum, stimulate insulin and alpha-amylase secretion in a species-specific manner and in individuals' adequate amount in islands and acini surrounding them. Previous studies have shown that an iv infusion of glucose does not stimulate the secretion of pancreatic alpha-amylase[17]. Considering the observed counteraction of alpha-amylase to glucose-dependent insulinotropic polypeptide (GIP)-1 we used the term anti-incretin to describe metabolic effects of alpha-amylase. This "anti-incretin" hypothesis needs to be confirmed by future studies, with specific focus on enteral-insular regulatory mechanisms. In fact,

comparative studies on the exclusive effect of pancreatic alpha-amylase on insulin secretion, stimulated by oral or intravenous glucose administration, have not yet been carried out. The effect of alpha-amylase on the secretion of incretins (GLP-1 and GIP) has also not yet been assessed to date.

Incretin-dependent regulation of insulin secretion is coupled with the intake of "quick" energy in the form of carbohydrates and thus could be recognized as temporal. These regulatory mechanisms need to be abruptly inhibited after the digestion of simple, 'easily-digested' carbohydrates in order to prevent the overproduction of insulin by beta cells and thus beta cell exhaustion. The overlooked alpha-amylase dependent downregulation of insulin levels protects both pancreatic beta and acinar cells from metabolic failure.

BILIOPANCREATIC DIVERSION SURGERY REVEALS THE INHIBITORY EFFECT OF ALPHA-AMYLASE ON INSULIN SECRETION

The clinical efficacy of bariatric surgery has encouraged scientific investigation of the gut as a major endocrine organ. Manipulation of gastrointestinal anatomy, through bariatric surgery, has profound effects. Even though these procedures were designed to restrict food intake and increase nutrient malabsorption, evidence suggests that the contribution of the above-mentioned factors to weight loss is minimal. Instead, these interventions reduce body weight gain by decreasing hunger, increasing satiation during a meal, changing food preferences and energy expenditure. GLP-1 analogues, currently used as a treatment for diabetes type 2 (DT2) or slimming injections, are now recognized as alternatives to bariatric surgery in terms of their weight loss and anti-diabetic effects. However, not much is known about their safety with regards to beta cell function in the long run, since the primary target of GLP-1 analogues is the stimulation of insulin release[18].

It is worth noticing that RYGB and sleeve gastrectomy (SG)[19-21] (Figure 1) have very similar outcomes both in short-term excessive weight loss and long term total weight loss, as well as in type II diabetes resolution and both procedures are superior when compared to the adjustable gastric banding[22-24]. At the same time, the greatest diabetes remission was observed for patients undergoing biliopancreatic diversion with duodenal switch (BPD-DS) (Figure 2[25,26]) (95.1% resolved), followed by gastric bypass (80.3%), gastroplasty (79.7%), and then laparoscopic adjustable gastric banding (56.7%) The same pattern was observed even for an excessive weight loss and total body weight loss in the long term perspective[27-29]. However, despite better outcomes in terms of obesity and obesity-related comorbidities, BPD-DS counts only for ca 2% of bariatric surgeries performed worldwide due to increased risk of complications and development of malnutrition[30].

We describe the possible pathways of alpha-amylase-dependent AIA regulation of insulin secretion and their integration with food-related "dietary" incretin regulation of insulin secretion, which are usually eliminated or ameliorated by successful bariatric surgery, using the example of BPD or BPD-DS surgery (Figure 3). Breaking contact between the duodenal mucosa and the digesta coming from the stomach through BPD/BPD-DS surgery, in a broad sense interrupts the enteral-insular axis and thus the resulting decrease in insulin secretion could be due to other mechanisms-it is obvious. However, alpha-amylase still flows into the duodenum as a result of stimulation by gastro-pancreatic reflexes and by the attenuated (yet still existing) reflexes which originate in the alimentary limb (stimulated by digesta). Thus, the inhibitory effect of alpha-amylase on insulin secretion can result in the enhanced elimination of incretins.

It seems that BPD/BPD-DS bariatric surgery followed by immediate and long-term remission of DT2[22-24,27-30] has allowed us, for the first time, to observe and reveal these alpha-amylase-dependent inhibitory effects on insulin secretion and glucose metabolism. BPD/BPD-DS surgery prominently highlights the role of pancreatic alpha-amylase in the down regulation of insulin secretion, not only by means of elimination of the duodenal incretin effects on insulin release.

As shown in Figures 3, the remission of DT2 is related to the elimination of the action of incretins from the duodenum in BPD-SD patients. In these patients, insulin production is mainly dependent on blood glucose levels and its local (in pancreas) down regulation by alpha-amylase and in the diverted duodenum, as was proven in a pig model[13].

Attenuation of incretin release, due to the absence of contact between the chyme and the gastro-duodenal mucosa in the biliopancreatic limb after BPD-SD surgery, results in lower insulin release. At the same time, the passage of food to the alimentary limb is responsible for the slight stimulation of pancreatic enzyme secretion. Since alpha-amylase from the pancreatic juice which is being secreted into the biliopancreatic limb does not interact with its' starch substrate, the other functions of alpha-amylase, over and above that of starch digestion, *e.g.*, inhibitory effect on insulin secretion, can be highlighted. Whole or partially digested alpha-amylase attenuates glucose absorption and improves the metabolism and/or storage of glucose in the gut tissues[13]. One should bear in mind that alpha-amylase, as well as the other pancreatic enzymes being digested in the BP limb, could serve as a source of different types of biologically active peptides which could affect insulin secretion, as has been proven for alpha-amylase-derived peptides[13]. At the same time, alpha-amylase from the acini surrounding the pancreatic islets can additionally slow down insulin release, which can be recognized as the inhibitory action of alpha-amylase on insulin release. These events are most probably responsible for the elimination of DT2, following BPD/BPD-DS bariatric surgery.

To highlight the efficacy of classical RYGB bariatric surgery, in terms of DT2 remission, one should consider that the residual secretion of stomach/gastric juice is probably the most important factor which could influence the overall success of bariatric metabolic surgery. Lack of stomach juice entering the biliopancreatic limb-which is what happens in patients after BPD/BPD-DS surgery-eliminates the release of most incretins (GIP, GLP-1), thus insulin secretion is stimulated to a much lower extent. The above is not true for other types of bariatric surgery where small amounts of gastric juice still enter the duodenum, thus stimulating incretin release. The role of alpha-amylase in AIA regulations[10,13] has been experimentally proven both in the pig model of BPD[13], with complete isolation of the stomach from the

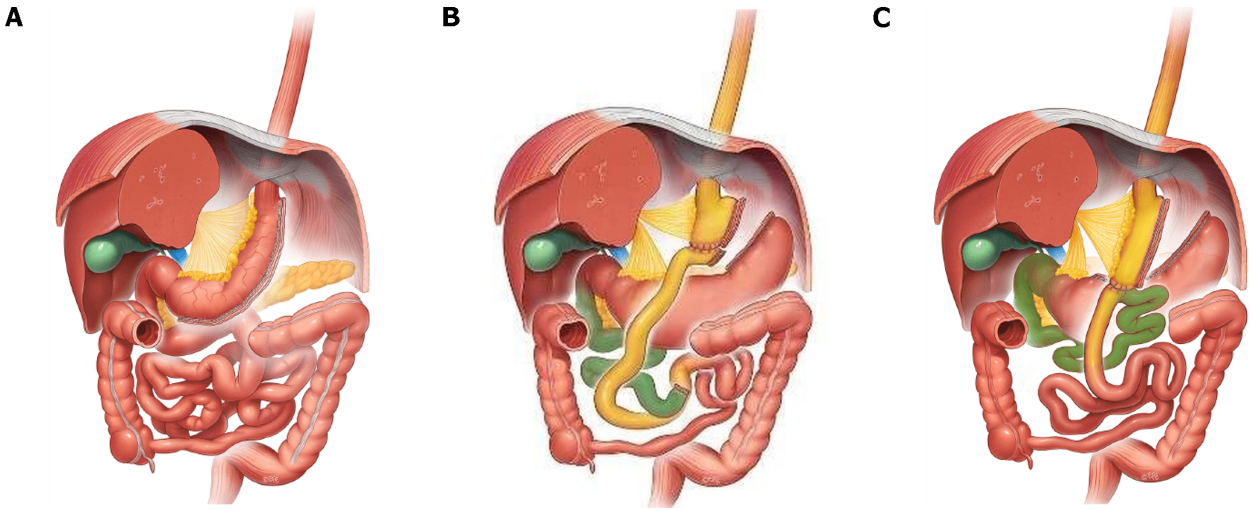


Figure 1 Main types of bariatric surgery, from left to right. A: Sleeve gastrectomy; B: Roux-en-Y gastric bypass; C: One-anastomosis gastric bypass. Citation: IFSO. Sleeve Gastrectomy. [cited 20 December 2022]. Available from: https://www.ifso.com/sleeve-gastrectomy/[19]; IFSO. Roux-en-Y Gastric Bypass. [cited 20 December 2022]. Available from: https://www.ifso.com/roux-en-y-gastric-bypass/[20]; IFSO. One Anastomosis Gastric Bypass. [cited 20 December 2022]. Available from: https://www.ifso.com/alternative-intestinal-procedures/[21]. Copyright© Dr. Levent Efe, courtesy of IFSO, 2022.

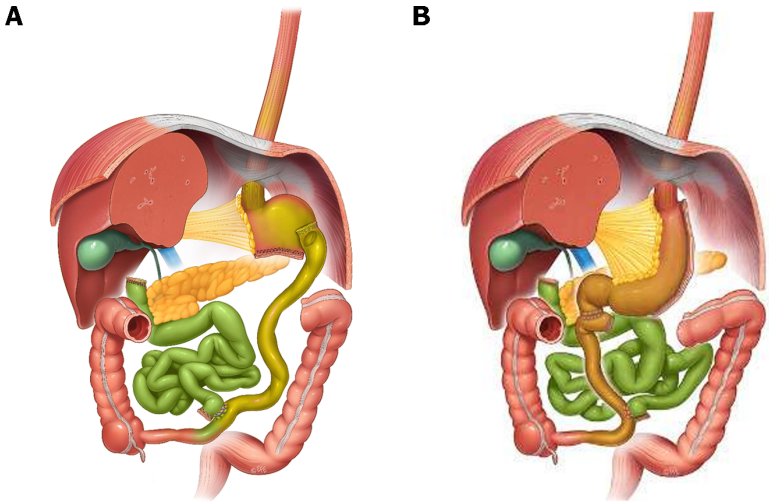
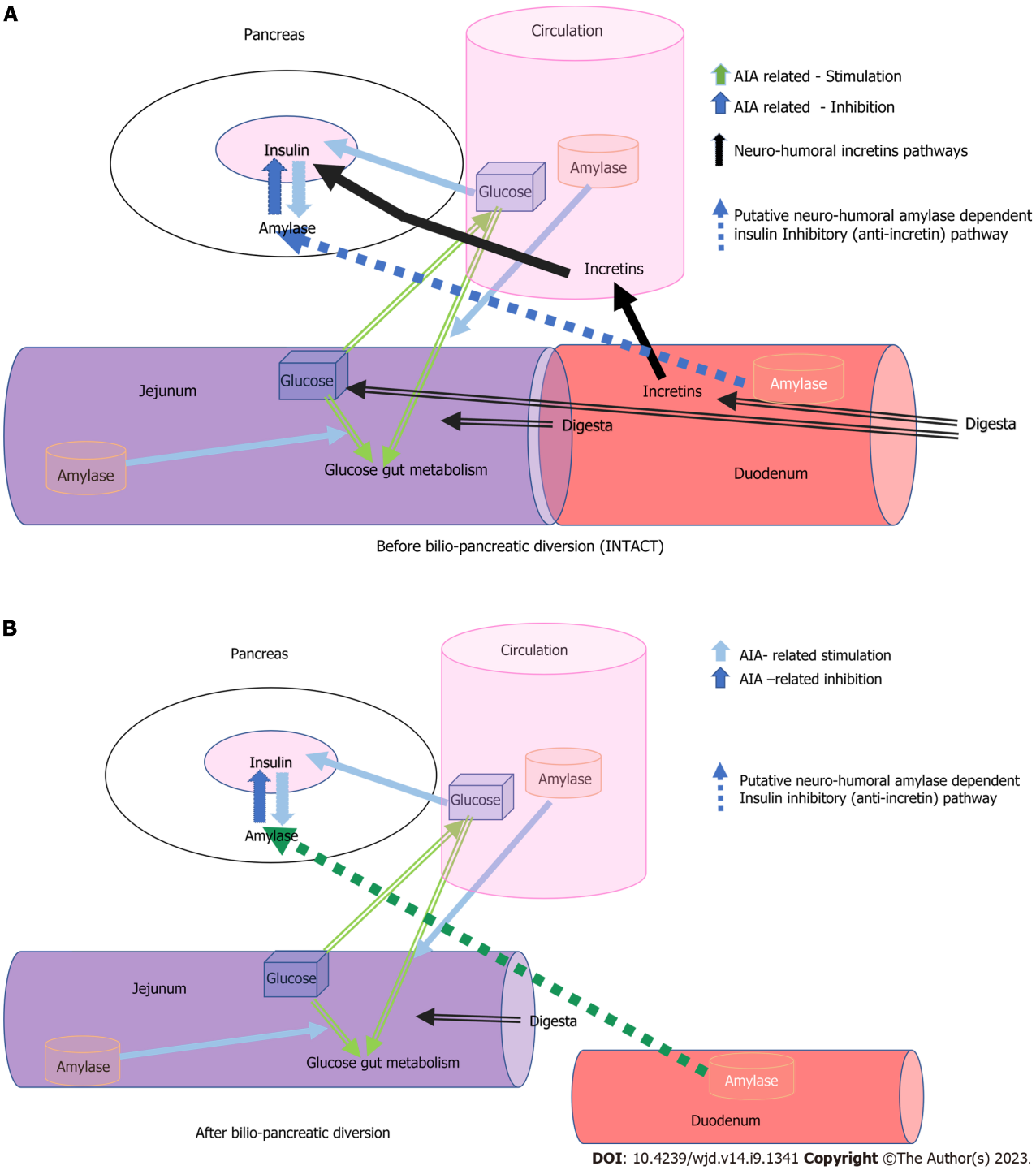


Figure 2 Forms of biliopancreatic diversions, from left to right. A: Biliopancreatic diversion (BPD); B: BPD with duodenal switch. Citation: IFSO. Biliopancreatic Diversion (BPD). [cited 20 December 2022]. Available from: https://www.ifso.com/bilio-pancreatic-diversion/[25]; IFSO. Biliopancreatic Diversion with Duodenal Switch. [cited 20 December 2022]. Available from: https://www.ifso.com/bilio-pancreatic-diversion-with-duodenal-switch/[26]. Copyright© Dr. Levent Efe, courtesy of IFSO, 2022.

biliopancreatic limb, and in *in vitro* experiments, where alpha-amylase directly inhibited insulin release from an insulinoma cell line, BRIN-11[9].

Importantly, the pharmacological duodenal inhibition of the actions of incretins, through the use of GLY-200 polymer [31], which mimics bariatric (metabolic) surgery, is being studied in a clinical trial. Mechanical duodenal exclusion, realized *via* endoscopy, is now being tested in clinics and has produced very good results to date with respect to the remission of DT2[32]. Both strategies assume the exclusion of incretin action and the mechanical prevention of contact between the duodenal mucosa and digesta from the stomach (duodenum isolation), thus allowing AIA pancreatic reflexes, including the anti-incretin action of alpha-amylase, to be realized to the full extent.

Pancreatic alpha-amylase, which is synthesized in the acinar cells and then appears in the pancreatic juice, also leaks into the periinsular interstitial fluid, downregulating insulin secretion. Additionally, digesta entering the alimentary arm (proximal jejunum) stimulates reflexes responsible for insulin and enzyme secretion to a lower extent, since in the proximal jejunum the number of incretins and other gastrointestinal hormone receptors is markedly decreased, when compared to the duodenum.



DOI: 10.4239/wjd.v14.i9.1341

Figure 3 A schematic view of acini-islet-acinar- and alpha-amylase-dependent inhibitory pathway involved in the regulation of glucose metabolism before and after Biliopancreatic diversion surgery. A: Before biliopancreatic diversion surgery; B: After biliopancreatic diversion surgery. Incretin-dependent, quick stimulatory pathways (black) and acini-islet-acinar–dependent intrapancreatic inhibitory pathway and downregulating alpha-amylase dependent pathways, originating in the duodenum, of insulin secretion (orange and green respectively). AIA: Acini-islet-acinar.

PANCREATIC ENZYME THERAPY AMELIORATES EPI-DEPENDENT HYPERINSULINEMIA

EPI is frequently diagnosed in both DT1 and DT2 patients[11]. Our own studies on streptozotocin (STZ)-induced diabetes [9] and on EPI[12] pig models have clearly shown that both iv administration of alpha-amylase of microbial origin (Amano, Japan) and the oral administration of pig pancreatic enzymes (enteric coated pancrelipase-Abbot Healthcare Products Ltd, Southampton, United Kingdom), lowers insulin and glucose levels during an iv-glucose tolerance test (IvGTT). However, responses to oral glucose loading are very weak compared to that observed following intravenous loading of a similar amount of glucose. Moreover, studies on healthy pigs[6] have shown that different pancreatic-like enzymes of microbial origin (Sigma-Aldrich), after oral administration, affect insulin release differently; alpha-amylase lowers insulin release, while protease enhances insulin release during an IvGTT and lipase has no effect on insulin

release. The above-mentioned studies on EPI and STZ pigs have allowed us to explore the potency of the alpha-amylase-induced, long-lasting AIA regulations of glucose homeostasis, revealing at the same time the protective/downregulating effects of enteral and blood alpha-amylase on the survival of pancreatic beta cells.

Enteral or parenteral alpha-amylase, of microbial or porcine origin, lowers blood levels of insulin and glucose during IvGTT and oral glucose tolerance tests. Undoubtfully, in this scenario, high levels of alpha-amylase in the gut and blood should be treated as a factor which protects pancreatic beta cells from metabolic failure. Nowadays more and more reports about the negative correlation between high plasma amylase activity and the development of metabolic diseases have become available in the literature[33,34].

The "anti-incretin" theory of metabolic surgery mode of action was presented by Rubino *et al*[35-37] in the beginning of century. The authors considered the gut as a primary organ involved in the regulation of metabolism and describe the balance of "anti-incretins" as a crucial factor which regulates glucose metabolism and could be impaired in metabolic disorders. In their comments to the article by Lindqvist *et al*[38] describing an increase in b-cell mass after RYGB surgery, Rubino and Amiel[37] point out that proliferation is not always beneficial and in fact, could be due to impaired "incretin/anti-incretin" balance[37]. The same research group demonstrated an improvement in insulin sensitivity in diabetic rats after duodenal-jejunal bypass surgery, without changes in incretin and insulin secretion[39] and revealed the anti-incretin effect of orally administered glucose[40].

However, no endogenic "anti-incretin" factor have been recognized until now what is *conditio sine qua non* to complete anti-incretin hypotheses. At the same time, the number of reports confirming the alternative, anti-incretin-like role of alpha-amylase, has been increasing over the last few years. For example, the active role of alpha-amylase of duodenal origin in the regulation of enterocytes' proliferation was shown by Date *et al*[41,42]; there are reports about antiproliferative effects of alpha-amylase in cancer cell lines[43,44]; alpha-amylase is currently even being considered as one of the main energy regulators in the brain[45]. Although almost all tissues express their own amylase and the extra-digestive roles of amylase have become more and more obvious, the possible role of amylase in the regulation of glucose metabolism is still ignored and overlooked.

The overarching question is how are alpha-amylase signals from the gut lumen and from the blood transferred to pancreatic beta and acinar cells? Due to the nature of the portal vascular system in the pancreas[46,47], local peri-islet insulin levels are very high compared to systemic levels. Our supraphysiological doses of iv infused alpha-amylase were much higher than physiological alpha-amylase levels and thus could possibly have increased the alpha-amylase concentration in the peri-islet circulation to a level which inhibited insulin release[12]. The inhibitory action of alpha-amylase on insulin from the gut lumen requires the existence of a gut-pancreas, alpha-amylase-dependent reflex, which ends in the peri-islet interstitial tissue. The more physiological mechanism, however, would be the direct action of alpha-amylase or alpha-amylase-derived peptides on gut glucose absorption[9,13] or on its storage and metabolism in the gut.

To confirm this intriguing postulation, it is necessary to perform *in vivo* studies on direct infusion of alpha-amylase alone and/or as a mixture with other pancreatic enzymes, to the artery suppling the pancreas, during iv glucose loading. The expected inhibition of insulin release during such an experiment will finally confirm the role of alpha-amylase in the protection of beta cells from the overproduction of insulin.

CONCLUSION

In summary, we suggest that bariatric BPD/BPD-DS surgery (which is actually seldom performed due to the development of severe EPI syndrome) highlights the never before described alpha-amylase-induced, anti-incretin-like regulation of glucose metabolism, which protects the pancreatic beta cells from exhaustion and subsequent failure. BPD/BPD-DS surgery eliminates the incretin-dependent vicious cycle driven from the duodenum in obese diabetic patients. Food stimulates the synthesis of insulin and alpha-amylase in parallel. Alpha-amylase synthesis is extraordinarily stimulated by insulin-halo phenomenon. In turn, alpha-amylase, through its ability to inhibit glucose absorption and direct downregulation of insulin release[9] limits hyperglycemia and hyperinsulinemia to a certain degree.

ACKNOWLEDGEMENTS

The authors would like to express their thanks to Dr. Janine Donaldson for her constructive feedback and valuable input with regards to the editing of the manuscript.

FOOTNOTES

Author contributions: Pierzynowski SG conceived and wrote the manuscript; Stier C and Pierzynowska K wrote the manuscript and prepared the figures.

Conflict-of-interest statement: Authors declare no competing interests.

Country/Territory of origin: Sweden

ORCID number: Stefan G Pierzynowski 0000-0002-0308-3214; Kateryna Pierzynowska 0000-0002-3290-339X.

S-Editor: Fan JR
L-Editor: A
P-Editor: Chen YX

REFERENCES

1 **Hellman B**, Wallgren A, Petersson B. Cytological characteristics of the exocrine pancreatic cells with regard to their position in relation to the islets of Langerhans. A study in normal and obese-hyperglycaemic mice. *Acta Endocrinol (Copenh)* 1962; **39**: 465-473 [PMID: 13906279 DOI: 10.1530/acta.0.0390465]
2 **Henderson JR**, Daniel PM, Fraser PA. The pancreas as a single organ: the influence of the endocrine upon the exocrine part of the gland. *Gut* 1981; **22**: 158-167 [PMID: 6111521 DOI: 10.1136/gut.22.2.158]
3 **Pierzynowski S**, Barej W. The dependence of exocrine pancreatic secretion on insulin in sheep. *Q J Exp Physiol* 1984; **69**: 35-39 [PMID: 6201945 DOI: 10.1113/expphysiol.1984.sp002793]
4 **Williams JA**, Goldfine ID. The insulin-pancreatic acinar axis. *Diabetes* 1985; **34**: 980-986 [PMID: 2412919 DOI: 10.2337/diab.34.10.980]
5 **Pierzynowski SG**, Podgurniak P, Mikołajczyk M, Szczesny W. Insulin and the parasympathetic dependence of pancreatic juice secretion in healthy and alloxan diabetic sheep. *Q J Exp Physiol* 1986; **71**: 401-407 [PMID: 3532163 DOI: 10.1113/expphysiol.1986.sp002998]
6 **Pierzynowski SG**, Goncharova K, Woliński J, Prykhodko O, Weström B, Lozinska L. Enteral pancreatic-like enzymes of microbial origin affect insulin release during an intravenous glucose tolerance test. *J Diabetes Met* 2016; **7**: 681
7 **Asanuma-Date K**, Hirano Y, Le N, Sano K, Kawasaki N, Hashii N, Hiruta Y, Nakayama K, Umemura M, Ishikawa K, Sakagami H, Ogawa H. Functional regulation of sugar assimilation by N-glycan-specific interaction of pancreatic α-amylase with glycoproteins of duodenal brush border membrane. *J Biol Chem* 2012; **287**: 23104-23118 [PMID: 22584580 DOI: 10.1074/jbc.M111.314658]
8 **Date K**, Satoh A, Iida K, Ogawa H. Pancreatic α-Amylase Controls Glucose Assimilation by Duodenal Retrieval through N-Glycan-specific Binding, Endocytosis, and Degradation. *J Biol Chem* 2015; **290**: 17439-17450 [PMID: 26023238 DOI: 10.1074/jbc.M114.594937]
9 **Pierzynowska K**, Oredsson S, Pierzynowski S. Amylase-Dependent Regulation of Glucose Metabolism and Insulin/Glucagon Secretion in the Streptozotocin-Induced Diabetic Pig Model and in a Rat Pancreatic Beta-Cell Line, BRIN-BD11. *J Diabetes Res* 2020; **2020**: 2148740 [PMID: 33294459 DOI: 10.1155/2020/2148740]
10 **Pierzynowski SG**, Gregory PC, Filip R, Woliński J, Pierzynowska KG. Glucose homeostasis dependency on acini-islet-acinar (AIA) axis communication: a new possible pathophysiological hypothesis regarding diabetes mellitus. *Nutr Diabetes* 2018; **8**: 55 [PMID: 30293998 DOI: 10.1038/s41387-018-0062-9]
11 **Radlinger B**, Ramoser G, Kaser S. Exocrine Pancreatic Insufficiency in Type 1 and Type 2 Diabetes. *Curr Diab Rep* 2020; **20**: 18 [PMID: 32239341 DOI: 10.1007/s11892-020-01304-0]
12 **Lozinska L**, Weström B, Prykhodko O, Lindqvist A, Wierup N, Ahrén B, Szwiec K, Pierzynowski SG. Decreased insulin secretion and glucose clearance in exocrine pancreas-insufficient pigs. *Exp Physiol* 2016; **101**: 100-112 [PMID: 26663041 DOI: 10.1113/EP085431]
13 **Pierzynowski SG**, Goncharova K, Gregory PC, Weström B, Podpryatov SE, Podpriatov SS, Woliński J, Repich H, Wierup N, Lozinska L. Experiments suggesting extra-digestive effects of enteral pancreatic amylase and its peptides on glucose homeostasis in a pig model. *Sci Rep* 2017; **7**: 8628 [PMID: 28819193 DOI: 10.1038/s41598-017-07387-2]
14 **Pierzynowska KG**, Lozinska L, Woliński J, Pierzynowski S. The inverse relationship between blood amylase and insulin levels in pigs during development, bariatric surgery, and intravenous infusion of amylase. *PLoS One* 2018; **13**: e0198672 [PMID: 29874296 DOI: 10.1371/journal.pone.0198672]
15 **Arnesjö II**, Lundquist I. Effects on exocrine and endocrine rat pancreas of long-term administration of CCK-PZ (cholecystokinin-pancreozymin) or synthetic octapeptide - CCK-PZ. *Scand J Gastroenterol* 1976; **11**: 529-535 [PMID: 785590]
16 **Lindqvist A**, Ekelund M, Pierzynowski S, Groop L, Hedenbro J, Wierup N. Gastric bypass in the pig increases GIP levels and decreases active GLP-1 levels. *Peptides* 2017; **90**: 78-82 [PMID: 28242256 DOI: 10.1016/j.peptides.2017.02.009]
17 **O'Keefe SJ**, Lemmer ER, Ogden JM, Winter T. The influence of intravenous infusions of glucose and amino acids of pancreatic enzyme and mucosal protein synthesis in human subjects. *JPEN J Parenter Enteral Nutr* 1998; **22**: 253-258 [PMID: 9739026 DOI: 10.1177/0148607198022005253]
18 **Kapitza C**, Dahl K, Jacobsen JB, Axelsen MB, Flint A. Effects of semaglutide on beta cell function and glycaemic control in participants with type 2 diabetes: a randomised, double-blind, placebo-controlled trial. *Diabetologia* 2017; **60**: 1390-1399 [PMID: 28526920 DOI: 10.1007/s00125-017-4289-0]
19 **IFSO**. Sleeve Gastrectomy. [cited 20 December 2022]. Available from: https://www.ifso.com/sleeve-gastrectomy/
20 **IFSO**. Roux-en-Y Gastric Bypass. [cited 20 December 2022]. Available from: https://www.ifso.com/roux-en-y-gastric-bypass/
21 **IFSO**. One Anastomosis Gastric Bypass. [cited 20 December 2022]. Available from: https://www.ifso.com/alternative-intestinal-procedures/
22 **Colquitt JL**, Pickett K, Loveman E, Frampton GK. Surgery for weight loss in adults. *Cochrane Database Syst Rev* 2014; **2014**: CD003641 [PMID: 25105982 DOI: 10.1002/14651858.CD003641.pub4]
23 **Kang JH**, Le QA. Effectiveness of bariatric surgical procedures: A systematic review and network meta-analysis of randomized controlled trials. *Medicine (Baltimore)* 2017; **96**: e8632 [PMID: 29145284 DOI: 10.1097/MD.0000000000008632]
24 **Sudan R**, Jain-Spangler K. Tailoring Bariatric Surgery: Sleeve Gastrectomy, Roux-en-Y Gastric Bypass and Biliopancreatic Diversion with Duodenal Switch. *J Laparoendosc Adv Surg Tech A* 2018; **28**: 956-961 [PMID: 30059264 DOI: 10.1089/lap.2018.0397]

25 **IFSO**. Biliopancreatic Diversion (BPD). [cited 20 December 2022]. Available from: https://www.ifso.com/bilio-pancreatic-diversion/

26 **IFSO**. Biliopancreatic Diversion with Duodenal Switch. [cited 20 December 2022]. Available from: https://www.ifso.com/bilio-pancreatic-diversion-with-duodenal-switch/

27 **Buchwald H**, Estok R, Fahrbach K, Banel D, Jensen MD, Pories WJ, Bantle JP, Sledge I. Weight and type 2 diabetes after bariatric surgery: systematic review and meta-analysis. *Am J Med* 2009; **122**: 248-256.e5 [PMID: 19272486 DOI: 10.1016/j.amjmed.2008.09.041]

28 **Frenken M**, Kemmet O, Frenken M, Röhrig I, Fischer L, Hellinger A. Long-term Remission of Type 2 Diabetes and Patient Survival After Biliopancreatic Diversion with Duodenal Switch. *Obes Surg* 2022; **32**: 3340-3350 [PMID: 35939221 DOI: 10.1007/s11695-022-06223-5]

29 **Kapeluto JE**, Tchernof A, Masckauchan D, Biron S, Marceau S, Hould FS, Lebel S, Lescelleur O, Julien F, Biertho L. Ten-year remission rates in insulin-treated type 2 diabetes after biliopancreatic diversion with duodenal switch. *Surg Obes Relat Dis* 2020; **16**: 1701-1712 [PMID: 32800734 DOI: 10.1016/j.soard.2020.06.052]

30 **Buchwald H**, Oien DM. Metabolic/bariatric surgery worldwide 2011. *Obes Surg* 2013; **23**: 427-436 [PMID: 23338049 DOI: 10.1007/s11695-012-0864-0]

31 **Gluscend**. Mimicking the effect of bariatric surgery with an oral pill. [cited 28 November 2022]. Available from: https://glyscend.com/

32 **Maselli DB**, Abusaleh R, Trad KS, Lopez-Nava G, Turró R, Abu Dayyeh BK. Feasibility of a single endoscopic plication procedure for the treatment of gastroesophageal reflux disease and obesity. *Endoscopy* 2022; **54**: E203-E204 [PMID: 33979860 DOI: 10.1055/a-1481-7844]

33 **Ko J**, Cho J, Petrov MS. Low serum amylase, lipase, and trypsin as biomarkers of metabolic disorders: A systematic review and meta-analysis. *Diabetes Res Clin Pract* 2020; **159**: 107974 [PMID: 31811884 DOI: 10.1016/j.diabres.2019.107974]

34 **Nakajima K**. Low serum amylase and obesity, diabetes and metabolic syndrome: A novel interpretation. *World J Diabetes* 2016; **7**: 112-121 [PMID: 27022442 DOI: 10.4239/wjd.v7.i6.112]

35 **Rubino F**, Gagner M. Potential of surgery for curing type 2 diabetes mellitus. *Ann Surg* 2002; **236**: 554-559 [PMID: 12409659 DOI: 10.1097/00000658-200211000-00003]

36 **Rubino F**. Is type 2 diabetes an operable intestinal disease? A provocative yet reasonable hypothesis. *Diabetes Care* 2008; **31** Suppl 2: S290-S296 [PMID: 18227499 DOI: 10.2337/dc08-s271]

37 **Rubino F**, Amiel SA. Is the gut the "sweet spot" for the treatment of diabetes? *Diabetes* 2014; **63**: 2225-2228 [PMID: 24962924 DOI: 10.2337/db14-0402]

38 **Lindqvist A**, Spégel P, Ekelund M, Garcia Vaz E, Pierzynowski S, Gomez MF, Mulder H, Hedenbro J, Groop L, Wierup N. Gastric bypass improves β-cell function and increases β-cell mass in a porcine model. *Diabetes* 2014; **63**: 1665-1671 [PMID: 24487021 DOI: 10.2337/db13-0969]

39 **Salinari S**, le Roux CW, Bertuzzi A, Rubino F, Mingrone G. Duodenal-jejunal bypass and jejunectomy improve insulin sensitivity in Goto-Kakizaki diabetic rats without changes in incretins or insulin secretion. *Diabetes* 2014; **63**: 1069-1078 [PMID: 24241532 DOI: 10.2337/db13-0856]

40 **Salinari S**, Mingrone G, Bertuzzi A, Previti E, Capristo E, Rubino F. Downregulation of Insulin Sensitivity After Oral Glucose Administration: Evidence for the Anti-Incretin Effect. *Diabetes* 2017; **66**: 2756-2763 [PMID: 28851712 DOI: 10.2337/db17-0234]

41 **Date K**, Yamazaki T, Toyoda Y, Hoshi K, Ogawa H. α-Amylase expressed in human small intestinal epithelial cells is essential for cell proliferation and differentiation. *J Cell Biochem* 2020; **121**: 1238-1249 [PMID: 31478242 DOI: 10.1002/jcb.29357]

42 **Date K**. Regulatory Functions of α-Amylase in the Small Intestine Other than Starch Digestion: α-Glucosidase Activity, Glucose Absorption, Cell Proliferation, and Differentiation [Internet]. New Insights Into Metabolic Syndrome. IntechOpen; 2021. [cited 20 December 2022]. Available from: https://www.intechopen.com/chapters/72321

43 **Fedrowitz M**, Hass R, Bertram C, Löscher W. Salivary α-amylase exhibits antiproliferative effects in primary cell cultures of rat mammary epithelial cells and human breast cancer cells. *J Exp Clin Cancer Res* 2011; **30**: 102 [PMID: 22027017 DOI: 10.1186/1756-9966-30-102]

44 **Pierzynowska K**, Thomasson S, Oredsson S. Alpha-Amylase Inhibits Cell Proliferation and Glucose Uptake in Human Neuroblastoma Cell Lines. *Biomed Res Int* 2022; **2022**: 4271358 [PMID: 35924266 DOI: 10.1155/2022/4271358]

45 **Byman E**, Schultz N; Netherlands Brain Bank, Fex M, Wennström M. Brain alpha-amylase: a novel energy regulator important in Alzheimer disease? *Brain Pathol* 2018; **28**: 920-932 [PMID: 29485701 DOI: 10.1111/bpa.12597]

46 **Lifson N**, Kramlinger KG, Mayrand RR, Lender EJ. Blood flow to the rabbit pancreas with special reference to the islets of Langerhans. *Gastroenterology* 1980; **79**: 466-473 [PMID: 7000613]

47 **Lifson N**, Lassa CV, Dixit PK. Relation between blood flow and morphology in islet organ of rat pancreas. *Am J Physiol* 1985; **249**: E43-E48 [PMID: 2409813 DOI: 10.1152/ajpendo.1985.249.1.E43]

World Journal of Diabetes

Submit a Manuscript: https://www.f6publishing.com

World J Diabetes 2023 September 15; 14(9): 1349-1368

DOI: 10.4239/wjd.v14.i9.1349

ISSN 1948-9358 (online)

ORIGINAL ARTICLE

Basic Study

Genipin relieves diabetic retinopathy by down-regulation of advanced glycation end products *via* the mitochondrial metabolism related signaling pathway

Ke-Xin Sun, Yan-Yi Chen, Zhen Li, Shi-Jie Zheng, Wen-Juan Wan, Yan Ji, Ke Hu

Specialty type: Medicine, research and experimental

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B, B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Salceda R, Mexico; Wagner KD, France; Cai L, United States

Received: March 3, 2023
Peer-review started: March 3, 2023
First decision: April 26, 2023
Revised: May 4, 2023
Accepted: August 7, 2023
Article in press: August 7, 2023
Published online: September 15, 2023

Ke-Xin Sun, Yan-Yi Chen, Shi-Jie Zheng, Wen-Juan Wan, Yan Ji, Ke Hu, Department of Ophthalmology, The First Affiliated Hospital of Chongqing Medical University, Chongqing 400016, China

Zhen Li, Department of Ophthalmology, The People's Hospital of Leshan, Leshan 400000, Sichuan Province, China

Corresponding author: Ke Hu, Doctor, MD, Chief Doctor, Doctor, Professor, Department of Ophthalmology, The First Affiliated Hospital of Chongqing Medical University, No. 1 Youyi Road, Yuzhong District, Chongqing 400016, China. 42222@qq.com

Abstract

BACKGROUND

Glycation is an important step in aging and oxidative stress, which can lead to endothelial dysfunction and cause severe damage to the eyes or kidneys of diabetics. Inhibition of the formation of advanced glycation end products (AGEs) and their cell toxicity can be a useful therapeutic strategy in the prevention of diabetic retinopathy (DR). Gardenia jasminoides Ellis (GJE) fruit is a selective inhibitor of AGEs. Genipin is an active compound of GJE fruit, which can be employed to treat diabetes.

AIM

To confirm the effect of genipin, a vital component of GJE fruit, in preventing human retinal microvascular endothelial cells (hRMECs) from AGEs damage in DR, to investigate the effect of genipin in the down-regulation of AGEs expression, and to explore the role of the CHGA/UCP2/glucose transporter 1 (GLUT1) signal pathway in this process.

METHODS

In vitro, cell viability was tested to determine the effects of different doses of glucose and genipin in hRMECs. Cell Counting Kit-8 (CCK-8), colony formation assay, flow cytometry, immunofluorescence, wound healing assay, transwell assay, and tube-forming assay were used to detect the effect of genipin on hRMECs cultured in high glucose conditions. *In vivo*, streptozotocin (STZ) induced mice were used, and genipin was administered by intraocular injection (IOI). To explore the effect and mechanism of genipin in diabetic-induced retinal

dysfunction, reactive oxygen species (ROS), mitochondrial membrane potential (MMP), and 2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) amino]-2-deoxy-d-glucose (2-NBDG) assays were performed to explore energy metabolism and oxidative stress damage in high glucose-induced hRMECs and STZ mouse retinas. Immunofluorescence and Western blot were used to investigate the expression of inflammatory cytokines [vascular endothelial growth factor (VEGF), SCG3, tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1β, IL-18, and nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing 3 (NLRP3)]. The protein expression of the receptor of AGEs (RAGE) and the mitochondria-related signal molecules CHGA, GLUT1, and UCP2 in high glucose-induced hRMECs and STZ mouse retinas were measured and compared with the genipin-treated group.

RESULTS

The results of CCK-8 and colony formation assay showed that genipin promoted cell viability in high glucose (30 mmol/L D-Glucose)-induced hRMECs, especially at a 0.4 μmol/L dose for 7 d. Flow cytometry results showed that high glucose can increase apoptosis rate by 30%, and genipin alleviated cell apoptosis in AGEs-induced hRMECs. A high glucose environment promoted ATP, ROS, MMP, and 2-NBDG levels, while genipin inhibited these phenotypic abnormalities in AGEs-induced hRMECs. Furthermore, genipin remarkably reduced the levels of the pro-inflammatory cytokines TNF-α, IL-1β, IL-18, and NLRP3 and impeded the expression of VEGF and SCG3 in AGEs-damaged hRMECs. These results showed that genipin can reverse high glucose induced damage with regard to cell proliferation and apoptosis *in vitro*, while reducing energy metabolism, oxidative stress, and inflammatory injury caused by high glucose. In addition, ROS levels and glucose uptake levels were higher in the retina from the untreated eye than in the genipin-treated eye of STZ mice. The expression of inflammatory cytokines and pathway protein in the untreated eye compared with the genipin-treated eye was significantly increased, as measured by Western blot. These results showed that IOI of genipin reduced the expression of CHGA, UCP2, and GLUT1, maintained the retinal structure, and decreased ROS, glucose uptake, and inflammation levels *in vivo*. In addition, we found that SCG3 expression might have a higher sensitivity in DR than VEGF as a diagnostic marker at the protein level.

CONCLUSION

Our study suggested that genipin ameliorates AGEs-induced hRMECs proliferation, apoptosis, energy metabolism, oxidative stress, and inflammatory injury, partially *via* the CHGA/UCP2/GLUT1 pathway. Control of advanced glycation by IOI of genipin may represent a strategy to prevent severe retinopathy and vision loss.

Key Words: Genipin; Human retinal microvascular endothelial cells; Angiogenesis; Vascularization; Secretogranin III; Diabetic retinopathy

Core Tip: The formation of advanced glycation end products (AGEs) has been widely validated in pathological changes of diabetic retinopathy (DR). A new vital compound in Gardenia jasminoides Ellis fruit, genipin, can be used to treat DR and decrease AGEs. Genipin ameliorated AGEs-induced human retinal microvascular endothelial cell proliferation, apoptosis, energy metabolism, oxidative stress, and inflammatory injury, partially *via* the CHGA/UCP2/glucose transporter 1 pathway. Control of AGEs by intraocular injection of genipin may represent a strategy to prevent severe retinopathy and vision loss. Here, we confirmed the effectiveness of genipin to treat DR both *in vivo* and *in vitro*, and explored its related molecular mechanism.

Citation: Sun KX, Chen YY, Li Z, Zheng SJ, Wan WJ, Ji Y, Hu K. Genipin relieves diabetic retinopathy by down-regulation of advanced glycation end products *via* the mitochondrial metabolism related signaling pathway. *World J Diabetes* 2023; 14(9): 1349-1368

URL: https://www.wjgnet.com/1948-9358/full/v14/i9/1349.htm

DOI: https://dx.doi.org/10.4239/wjd.v14.i9.1349

INTRODUCTION

The number of patients with diabetes mellitus (DM) has increased from less than 110 million in 1980 to approximately 420 million in 2015 worldwide and is expected to reach 642 million in 2040[1]. The estimated overall prevalence of DM is 10.9% among Chinese citizens based on national-wide surveillance[2]. Diabetic retinopathy (DR) is the most common microvascular complication of DM and is a vital reason for blindness in citizens aged over 55 years[3]. Some new treatments, such as intravitreal vascular endothelial growth factor (VEGF) inhibitors or steroid hormones, have been introduced for DR[4]. However, up to 50% of the patients failed to respond to such agents. Despite being an inherently destructive procedure, laser photocoagulation remains the mainstay therapy for people with proliferative DR (PDR). This

necessitates other effective methods for DR treatment. In the pathogenesis of DR, the retinal circulation is damaged by microvascular lesions. If this change is not controlled in the early stages, continual progression leads to retinal detachment and vision loss[5]. The primary pathogenesis of DR involves angiogenesis, chronic inflammation, and oxidative stress. The retinal capillary endothelium is composed of endothelial cells, pericytes, and the basement membrane. Neovascularization of the retinal surface is the primary stage of PDR[6]. It is also the most important factor in retinal detachment. Endothelial dysfunction leads to angiogenesis, which involves the proliferation, migration, and formation of tubes and is central to the development of vascular complications[7]. Angiogenesis requires energy support such as adenosine triphosphate (ATP) or glucose. Damage to human retinal microvascular endothelial cells (hRMECs) in DR arises from metabolic abnormalities in glucose metabolism, which are principally caused by advanced glycation end products (AGEs).

Glycation is an important step in aging and oxidative stress[8]. Persistently elevated glucose concentrations lead to rapid and intensive glycation reactions. Both acute and chronic hyperglycemia can enhance AGEs production[9], which results in endothelial dysfunction and causes severe damage to the eyes or kidneys of diabetics. Inhibition of AGEs and its cell toxicity can be a useful strategy for the prevention of DR[10]. According to previous studies, reducing AGEs receptor/ligand interaction or breaking established AGEs crosslinks prevent AGEs formation[11].

Mitochondrial dysfunction and oxidative stress are largely involved in aging, cancer, age-related neurodegenerative disorder, and metabolic syndrome[12]. Mitochondrial dysfunction may predispose to the development of DM with the accompanying risk of developing DR or may contribute directly to diabetic metabolic dysregulation and thereby increase the risk of late diabetic complications including retinopathy[13,14]. The relation between mitochondrial dysfunction and diabetic eye complications has two elements: (1) Mitochondrial diseases may predispose to the development of DM and such type of diabetes may be accompanied with an increased risk of developing DR; and (2) metabolic dysregulation in DM may increase the risk of development of DR through a disturbance in mitochondrial function[13,14]. A hypothesis has recently been proposed that the metabolic dysfunction in diabetic patients induces the synthesis of a number of reactive oxygen species (ROS) that are normally eliminated in the mitochondria[13]. In addition, enhanced ROS may contribute to the induction of autophagy in the retina. Another mechanism of mitochondrial dysfunction leading to DR is the formation of free radicals[14-17]. These reactive compounds may contribute to a switch in the metabolism, *e.g.*, by changing the activity of glyceraldehyde-3-phosphate dehydrogenase and the polyol pathway and activating protein kinase C, and consequent development of DR[13].

Genipin (Figure 1A), one of the principal bioactive components extracted from the Gardenia jasminoides Ellis (GJE, Chinese herbal name "Zhizi"), has multiple bioactivities, such as anti-inflammation, antitumor, antidepression, and the protection of hippocampal neurons from the toxicity of Alzheimer's amyloid-β[18]. GJE, as a selective inhibitor of AGEs, prevents the development of diabetic vascular complications in experimental animal models; however, its possible role and molecular mechanism in the relief of DM symptoms are unknown[19]. To explore these, we searched the traditional Chinese medicine systems pharmacology database and analysis platform (TCMSP) (https://old.tcmsp-e.com/tcmsp.php) using the keyword "Zhizi". Genipin was the third most active constituent of Zhizi (Table 1). Other active constituents of Zhizi include quercetin and lutein. Recent studies demonstrated that quercetin inhibited the overexpression of TLR4 and NF-κB p65, and reduced the expression of VEGF and soluble intercellular adhesion molecule-1, thus exerting therapeutic effects in DR[20,21]. Many basic and clinical studies have reported that lutein has anti-oxidative and anti-inflammatory properties in the eye, suggesting its beneficial effects in protection and alleviation of ocular diseases such as age-related macular degeneration (AMD), DR, retinopathy of prematurity, myopia, and cataract[22,23]. However, some publications report that genipin, but not quercetin or lutein, can influence AGEs. In the present study, we administered streptozotocin (STZ)-treated mice by intraocular injection (IOI) of genipin to investigate whether genipin could attenuate the development of DR in experimental models through AGEs inhibition[24].

MATERIALS AND METHODS

Reagents

Genipin (99%, Figure 1) was purchased from Selleck (Shanghai, China). D-glucose (PB180418) and the complete endothelial cell medium of hRMECs (CM-H130) were purchased from Procell (Wuhan, Hunan Province, China). Trypsin-ethylenediamine tetraacetic acid (EDTA) solution (S310JV), Dulbecco's modified Eagle's medium (L170KJ), minimum essential medium (L510KJ), penicillin-streptomycin solution (S110JV), and phosphate buffer solution (B320KJ) were purchased from BasalMedia (Shanghai, China). Cell Counting Kit-8 (BS350A) and 4% paraformaldehyde fix solution (BL539A) were purchased from Biosharp (Hefei, Anhui Province, China). Endothelial cell medium (1001), endothelial cell growth supplement (1052), fetal bovine serum (0025), and penicillin and streptomycin solution (0503) were purchased from ScienCell (Shanghai, China).

Animals and ethical statement

All animal procedures were conducted in accordance with the China Animal Welfare Legislation. We purchased 4-wk-old C57/BL6 male or female mice (3-8 g, n = 60) from the Animal Ethics Committee of Chongqing Medical University (Yuzhong, Chongqing, China). They were housed in a light-temperature automatically controlled room with free access to food and water. One female mouse and four male mice were housed in a single cage. All animal experimental protocols were approved by the Institutional Animal Care and Use Committee of Chongqing Medical University (2022-K45). All experiments were performed in accordance with the Guidelines and Regulations for the Care and Use of Laboratory Animals by the Chongqing Medical University (Yuzhong, Chongqing, China) and the Association for

Table 1 Active components of Gardenia jasminoides Ellis

MolID	Active component	OB (%)	DL	BBB
MOL009038	GBGB	45.58	0.83	-5.43
MOL001652	1H-2,6-dioxacyclopent(cd)inden-1-one, 4-((acetyloxy)methyl)-5-(beta-D-glucopyranosyloxy)-2a,4a,5,7b-tetrahydro-, (2aS-(2aalpha,5alpha,7balpha))-	26.43	0.71	-2.00
MOL001648	Genipin	26.06	0.10	-0.98
MOL000098	Quercetin	46.43	0.28	-0.77
MOL000422	Kaempferol	41.88	0.24	-0.55
MOL007245	3-Methylkempferol	60.16	0.26	-0.49
MOL001406	Crocetin	35.30	0.26	-0.83
MOL000551	Hederagenol	22.42	0.74	-0.51
MOL003515	(3S,4S,4aR,6aR,6bS,8aS,12aS,14aR,14bR)-3-hydroxy-4,6a,6b,11,11,14b-hexamethyl-1,2,3,4a,5,6,7,8,9,10,12,12a,14,14a-tetradecahydropicene-4,8a-dicarboxylic acid	27.21	0.72	-0.68
MOL013377	Lutein	22.59	0.55	-0.99
MOL009548	Desacetyl asperulosidic acid_qt	32.49	0.10	-1.85
MOL004560	SHANZHISIDE_qt	117.77	0.10	-1.44
MOL001667	Deacetyl asperuloside acid_qt	62.46	0.11	-1.62
MOL007148	Shanzhiside methyl ester_qt	109.77	0.12	-3.18
MOL004555	GARDENOSIDE_qt	52.77	0.12	-0.91
MOL007994	Ilexoside A_qt	22.43	0.74	-0.33

Oral bioavailability ≥ 20%, drug like ≥ 0.1, and brain-blood barrier ≤ -0.30. OB: Oral bioavailability; DL: Drug like; BBB: Brain-blood barrier.

Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research.

STZ-induced diabetic mice and intraperitoneal injections

STZ-induced hyperglycemia is a widely used DM model. At the beginning of the experiment, the mice were fed a high-sugar and high-fat diet (rodent diet with 60% calories from fat; XTHF60, XieTong Scientific Diets, Wuhan, China) for 70 d (20-35 g, n = 60). Using 1 mL syringes and 25 G needles, intraperitoneal injection (IPI) of freshly prepared STZ solution at 40 mg/kg (1.0 mL/100 g of citrate buffer, pH 4.5) was performed. This step was repeated on days 2 and 3 after fasting [25]. We used similar forage and feeding management practices. Fasting blood glucose (FBG) levels were measured with a Precision Plus blood glucose meter 14 d after STZ IPI. Mice with FBG levels > 11.1 mmol/L were considered diabetic.

Drug treatment

In the genipin-treated group, IOI of genipin [genipin dissolved in penicillin-streptomycin solution (PBS)] at a dose of 10 mmol/L was administered to the right eye (OD) (0.4 μL/eye) of mice after 3 mo of STZ administration. The same volume of PBS was administered to the left eye (OS) of the same mouse. Mice were maintained on a high-sugar and high-fat diet with free access to water under standard conditions and were assessed once every 2 wk and 1 mo for their body weight and FBG, respectively. All animals were killed at the end of the third time of IOI administration. Both eyes were collected and frozen at -80 °C or fixed in 4% paraformaldehyde fix solution for further experiments.

Hematoxylin and eosin staining

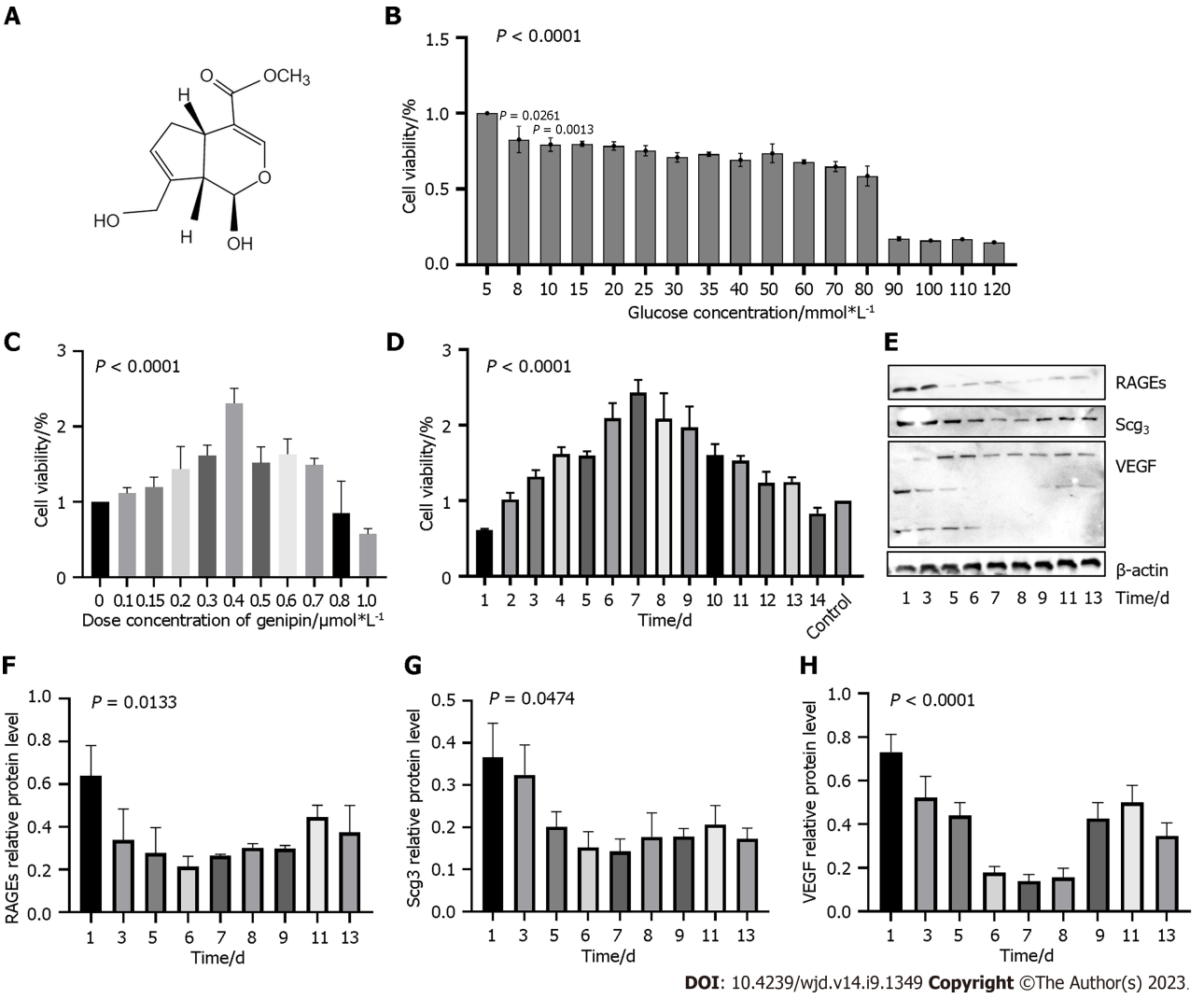
The eyes of the mice were fixed overnight with 4% paraformaldehyde fix solution and embedded in paraffin. Coronal sections (5 mm thick) were dewaxed and stained with hematoxylin and eosin stain (H&E). The wound areas of each sample were observed under a Leica DM2000 microscope (Leica, Wetzlar, Germany).

Cell culture

hRMECs (BNCC 358978) were purchased from BeNa Culture Collection (Beijing, China). The cells were plated in T_{25} flasks and incubated in a humidified incubator with 5% CO_2 at 37 °C.

Cell counting kit-8 assay

hRMEC pellet was resuspended in 100 μL of endothelial cell medium (ECM) without FBS, incubated in 96-well plates for 1 d, and treated with various concentrations of D-glucose. Cell viability was assessed using the Cell Counting Kit-8 (CCK-8). We added CCK-8 (10%) with a culture medium of 110 μL was added to each well and incubated for 2.5 h (37 °C, 5%



DOI: 10.4239/wjd.v14.i9.1349

Figure 1 Optimization of dose of genipin and high glucose in human retinal microvascular endothelial cells. A: Chemical structure of genipin; B and C: Cell viability of human retinal microvascular endothelial cells (hRMECs) treated with different concentrations of glucose and genipin; D: Cell viability of hRMECs treated with 30 mmol/L glucose and 0.4 μmol/L genipin for different durations; E-I: RAGE, SCG3, and vascular endothelial growth factor protein expression in cells treated with genipin for different durations. VEGF: Vascular endothelial growth factor.

CO_2). Absorbance at 450 nm and 600 nm was measured using a microplate reader (Thermo Fisher). The cell inhibition rate (I%) was calculated as $(A_{control}-A_{treated})/A_{control} \times 100\%$.

Drug preparation

hRMEC pellet was resuspended in 100 mL of ECM supplemented with 30 mmol/L of D-glucose (HG), incubated in 96-well plates for 1 d, and then treated with various concentrations of genipin. After 7 d of treatment, cell viability was assessed using CCK-8 assay.

Flow cytometry

Apoptosis was detected using an Annexin V-FITC Apoptosis Detection Kit (Beyotime) according to the manufacturer's instructions (CytoFLEX, United States). Cells (2×10^6) were seeded in 6-well plates and incubated with a high concentration of glucose and genipin for 1 wk. They were collected after digestion with trypsin-EDTA (Canada). After washing with PBS twice, the supernatant was discarded. The pellet was resuspended using ice-cold 70% ethanol (30 min at 4 °C) and incubated at 37 °C for 30 min. Next, 500 μL of 1 × binding buffer was added, and the cells were stained with 5 μL of annexin V-FITC and 5 μL of propidine iodide in the dark at 4 °C for 20 min, followed by flow cytometry analysis.

Western blot analysis

Cells were lysed with ice-cold radioimmunoprecipitation assay (RIPA) lysis buffer (Beyotime) and centrifuged at 12000 r/min for 15 min at 4 °C, and the supernatant was collected. Protein concentrations in the supernatant were measured using the BCA protein assay kit (ThermoFisher, Shanghai, China). Proteins (40 μg) were resolved by SDS-PAGE and transferred onto polyvinylidene difluoride membranes. The membranes were blocked with 5% BSA in TBST (1 × Tris buffered saline, 0.1% TWEEN 20) for 2 h at room temperature. The blocked membranes were immunoblotted with rabbit anti-UCP2

(A4178, ABclonal, Wuhan, Hubei Province, China), rabbit anti-glucose transporter 1 (GLUT1)/SLC2A1 (A11208, ABclonal), rabbit anti-CHGA (A1668, ABclonal), rabbit anti-AGER (A13264, ABclonal), rabbit anti-SCG3 (A7799, ABclonal), and rabbit anti-VEGF (A12303, ABclonal) antibodies overnight at 4 °C. The membranes were then washed and probed with secondary antibody (horseradish peroxidase-labeled goat anti-rabbit IgG; A0208, Beyotime) for 2 h at room temperature. Chemiluminescence detection was performed using a SuperSignal West Atto chemiluminescence detection kit (Thermo Fisher, Shanghai, China) according to the manufacturer's instructions.

Immunofluorescence

hRMECs were seeded in 24-well plates with a cover glass (REF.10212424C, Shitai, Jiangsu, China) at a final cell density of 5.0×10^4 cells/mL. In the genipin group, 4 mmol/L genipin was added. When the cell confluence reached approximately 50%, the cells were transferred to the FBS-free and antibiotic-free medium and cultured for 1 d. They were washed with PBS three times. The cells were fixed in 4% paraformaldehyde fix solution (P0099, Beyotime) for 30 min and blocked for 0.5 h using goat serum (C0265, Beyotime). Then, the membranes were washed and immunoblotted with primary antibodies overnight at 4 °C, followed by probing with Alexa Fluor 488-labeled Goat Anti-Rabbit IgG (H + L) (A0423, Beyotime) as secondary antibody for 2 h at room temperature. 2-(4-amidinophenyl)-6-indolecarbamidine dihydrochloride staining solution (C1005, Beyotime) was used for counterstaining. Antifade mounting medium (P0126, Beyotime) was used to reduce the luminescence delay. All pictures were obtained using a Leica fluorescence microscope (DMR, Deerfield, IL, United States).

Colony formation assay

hRMECs were seeded into 6-well plates, and the number of cells was adjusted to approximately 2000 cells/well. After allowing cells to attach the plates, ECM with 20% FBS was added. After 2 wk, the cells were fixed in 4% paraformaldehyde fix solution for 0.5 h and stained with crystal violet (Beyotime Institute of Biotechnology, Shanghai, China) for 15 min. Each well with > 50 cells was photographed and observed under a phase-contrast microscope.

Wound healing assay

hRMECs were seeded in 6-well plates at a final cell density of 2.0×10^5 cells/mL. After treatment for 5 d, the confluent cell monolayer was scraped with a sterile 200 μL tip to create a scratch across the center of the circle when the hRMECs confluence reached > 95%. The wound areas of each sample were observed under a microscope and evaluated using ImageJ software (Media Cybernetics, Silver Springs, MD, United States) at 0 h, 12 h, 24 h, 48 h, and 72 h.

Transwell assay

We used 8 μm sized Transwell BD Matrigel chambers (Costar 3470, Corning, NY, United States) to measure cell migration. hRMECs were seeded into the upper chamber with 100 μL of serum-free ECM, and the number of cells was adjusted to approximately 2000 cells/well. The lower chamber contained 600 μL ECM with 20% of FBS. After 30 h of culturing, the chambers were removed and fixed with 4% paraformaldehyde fix solution for 0.5 h. The cells were stained with crystal violet for 15 min. Medical Cotton Stickers (Sanhe, Sichuan Province, China) were used to remove non-migratory cells from the upper chamber. Photographs were captured by using a microscope. Five fields with evenly distributed cells were examined.

Tube formation assay

To analyze the influence of high glucose and genipin on the tube-forming activity of hRMECs, we plated 50 μL Matrigel (BioCoat Matrigel 356234, Corning, NY, United States) onto a cold 96-well plate. hRMECs were added into each well at a cell density of 4.0×10^5. After 6 h, images were captured under a microscope.

ATP concentration determination

hRMECs were plated onto 6-well plates and grown until they reached confluence. The cells were starved in an endothelial basal medium (EBM) without serum for 1 d. They were collected after transfection and washed twice with PBS. The cells were collected into 1.5 mL Eppendorf tubes, and the ATP concentration was measured using an ATP fluorometric assay kit (S0026, Beyotime).

ROS and mitochondrial membrane potential assay

hRMECs were plated onto 6-well plates and grown until they reached approximately 60% confluence. The cells were starved in an EBM medium without serum for 1 d. The ROS Assay Kit (S0033, Beyotime) and Apoptosis Detection Kit with Mito-Tracker Red CMXRos and annexin V-FITC (C1071S, Beyotime) were used to measure the ROS and mitochondrial membrane potential (MMP) changes.

Glucose uptake assay

hRMECs were plated onto 6-well plates and grown until they reached confluence. The cells were starved in an EBM medium without serum for 1 d. D-glucose analog (100 mmol/L; 2-NBDG) assay (HY-116215, MCE, Shanghai, China) was performed to measure the D-glucose transported into the hRMECs. Photographs were captured under a fluorescence microscope.

Cytokine analysis

Pro-inflammatory cytokines [tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1β, IL-18, and NLRP3] and other hallmark proteins of DR (VEGF and SCG3) were assayed by Western blot and immunofluorescence as described previously.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics 25 (IBM Corp., Armonk, NY, United States) and GraphPad Prism 8 (GraphPad Software, LLC). All experiments were repeated at least three times independently. All data are presented as the mean ± SEM. Independent sample *t*-test was used for comparing variables with a normal distribution. One-way analysis of variance and multiple comparisons for trends were used for comparing continuous variables. The chi-square test (or Fisher's exact test, when appropriate) was used to analyze dichotomous variables. Pearson's correlation coefficient was used to determine the relationship between variables. A *P* value less than 0.05 was considered statistically significant.

RESULTS

Effects of genipin on hRMECs viability and proliferation

First, we investigated the effects of high glucose and genipin on hRMEC viability and cytotoxicity using CCK-8 assay. Figure 1B depicts that 30 mmol/L D-glucose could decrease approximately 30% of the cell viability by approximately 30%, and cell viability decreased by < 30% until high glucose reached 90 mmol/L. Thus, 30 mmol/L of D-glucose was used for subsequent experiments. After treatment with different doses of genipin (0.1 μmol/L to 1.0 μmol/L) in a 30 mmol/L glucose medium, we detected cell proliferation by CCK-8 assay. The administration of 0.4 μmol/L genipin exerted a notable effect on hRMECs proliferation (Figure 1C). However, genipin at 0.8 μmol/L remarkably inhibited cell proliferation ($P < 0.001$). The optimal effective dose of high glucose was 30 mmol/L to 50 mmol/L and that of genipin was approximately 0.3-0.8 μmol/L; these doses did not exert any cytotoxic effects on the cells.

Next, we explored the optimal action time of genipin by CCK-8 assay (Figure 1D). Compared with normal glucose medium without genipin, treatment with 0.4 μM genipin for 4 d exerted a notable effect on high glucose-induced HRMECs ($P < 0.001$) (Figure 1D). In hRMECs cultured in ECM containing 30 mmol/L glucose, we observed the highest cell viability after 7 d of treatment with 0.4 μmol/L genipin (Figure 1D). We verified these results using Western blot (Figure 1E-H). Based on these results, treatment with 30 mmol/L high glucose and 0.4 μmol/L genipin for 7 d was used for the subsequent experiments.

Genipin protects hRMECs from high-glucose damage with regard to cell proliferation, apoptosis, angiogenesis, energy metabolism, oxidative stress, and inflammatory injury in vitro

Action of glucose and genipin on hRMECs proliferation, apoptosis, and angiogenesis: CCK-8 assay demonstrated that genipin, particularly 0.4 μM genipin for 7 d, increased the viability of hRMECs in high-glucose ECM. The colony formation results supported this finding (Figure 2A). Results of flow cytometry (Figure 2B) suggested that the number of apoptotic hRMECs increased after the stimulation with 30 mmol/L glucose (61.05%), compared with normal medium stimulation (30.30%). However, the cell apoptosis ratio decreased after genipin treatment, and particularly, early cell apoptosis decreased from 33.98% to 11.07%.

To explore the effects of genipin on cell migration, we performed scratch wound healing and transwell migration assays. High glucose decelerated the closure of hRMECs, and genipin could restore this change (Figure 2C). Furthermore, high glucose levels increased the number of migrated cells, and genipin restored this change (Figure 2D).

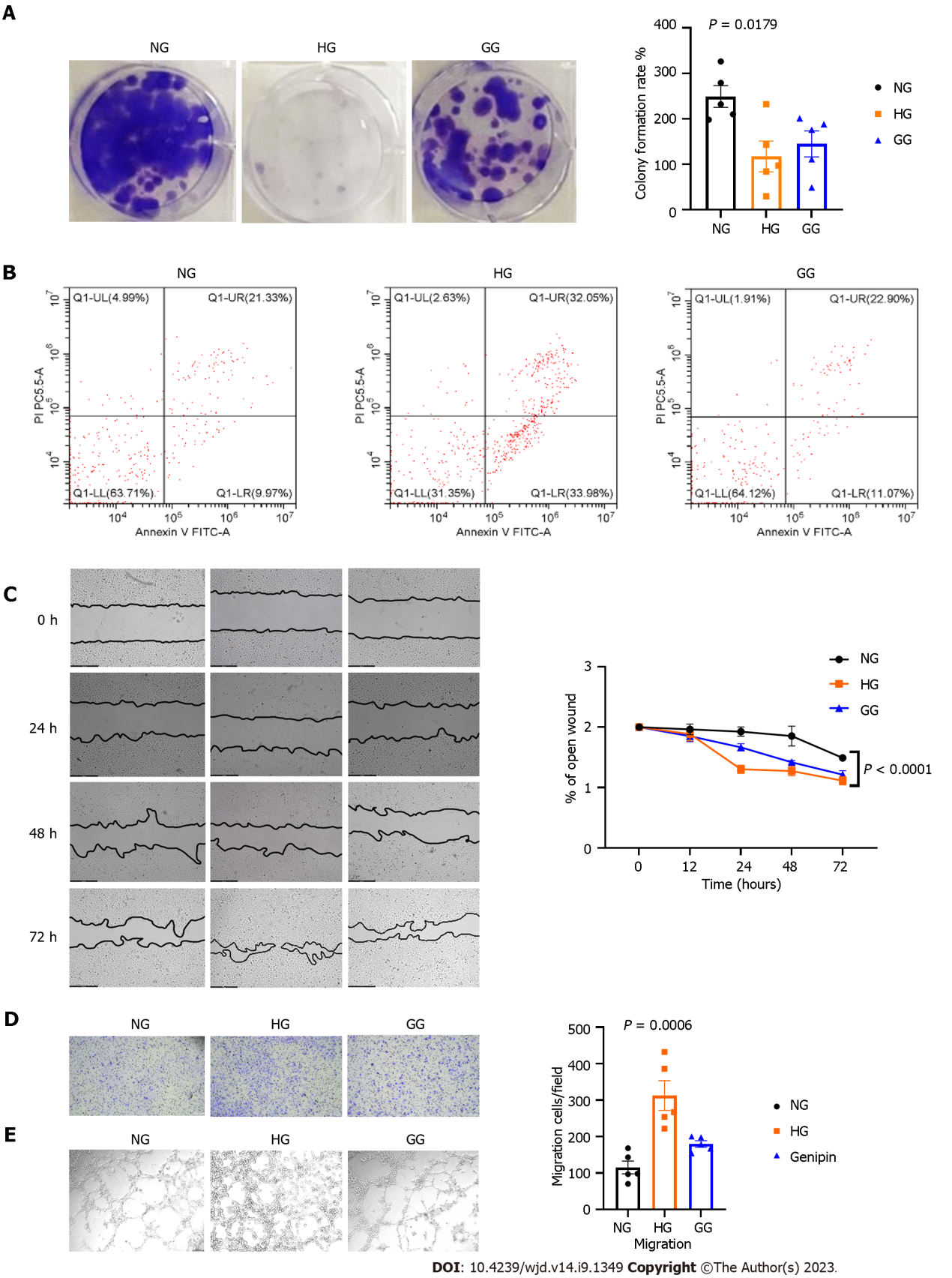
Then, we analyzed the tube-forming capacity of hRMECs in high glucose, with or without genipin. High glucose significantly increased the number of newly formed tubes, while genipin reversed this change (Figure 2E).

Action of glucose and genipin on intracellular ATP levels and MMP: Hyperglycemia in patients with DM is associated with abnormally elevated cellular glucose levels. ATP, as the primary energy storage molecule, is central to cell survival, proliferation, and migration. Increased cellular glucose levels alter glucose metabolism and influence intracellular ATP levels by inhibiting complexes I and II[26]. Subsequently, we measured the intracellular ATP levels and observed that high glucose increased the ATP level by 60%, and genipin could reduce this level by approximately 20% (Figure 3A).

Next, we explored the mitochondrial changes in DR. MMP increased after high glucose stimulation (Figure 3B and C), indicating that DR produced more ATP, which promotes the formation of mitochondria[27]. Simultaneously, these mitochondrial changes may lead to increased ROS accumulation.

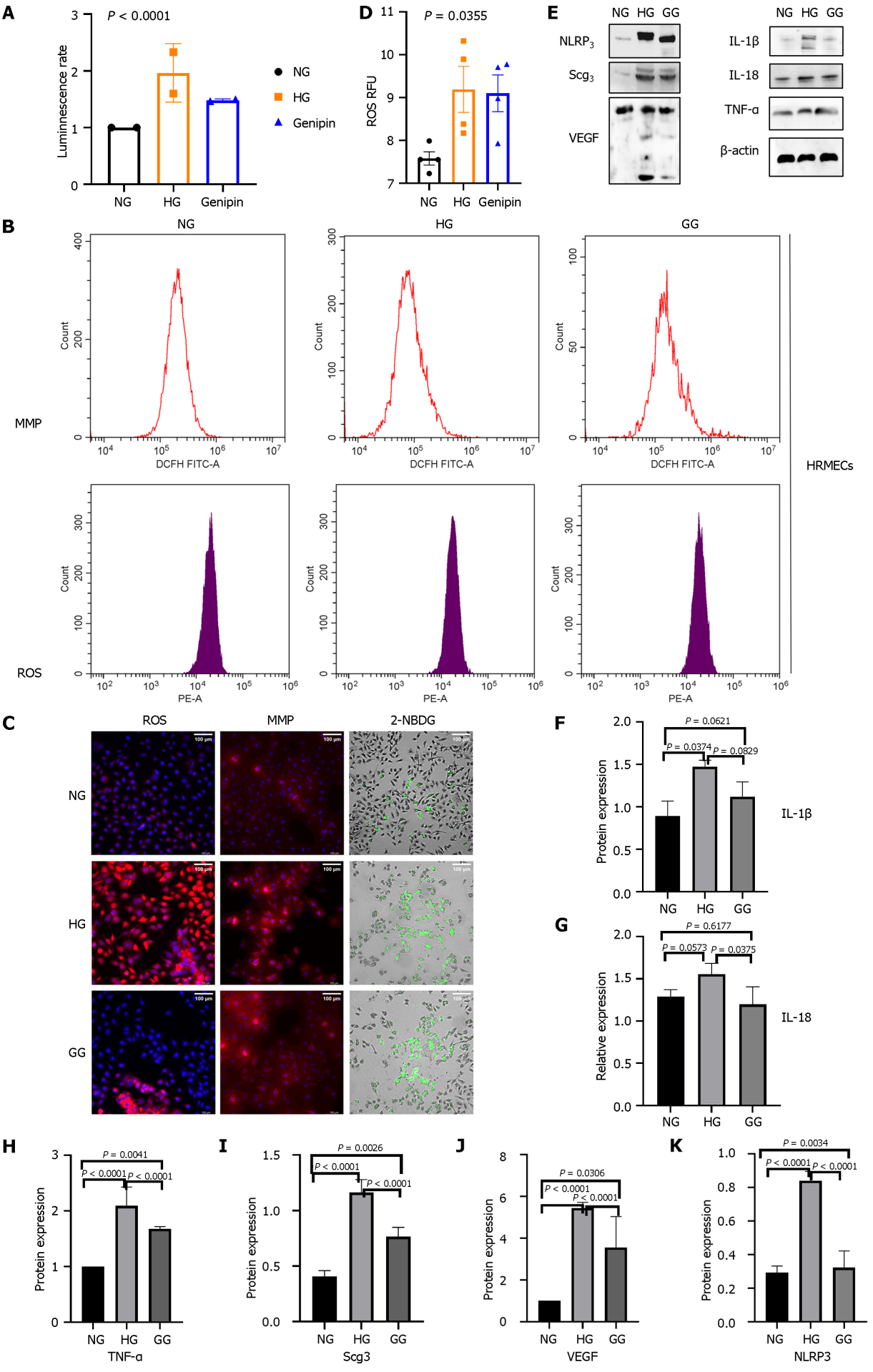
Action of glucose and genipin on intracellular oxidative stress levels: To confirm the oxidative stress damage caused by high glucose levels, we measured intracellular ROS production. High glucose increased the fluorescence significantly; however, this increase was alleviated by genipin (Figure 3B-D).

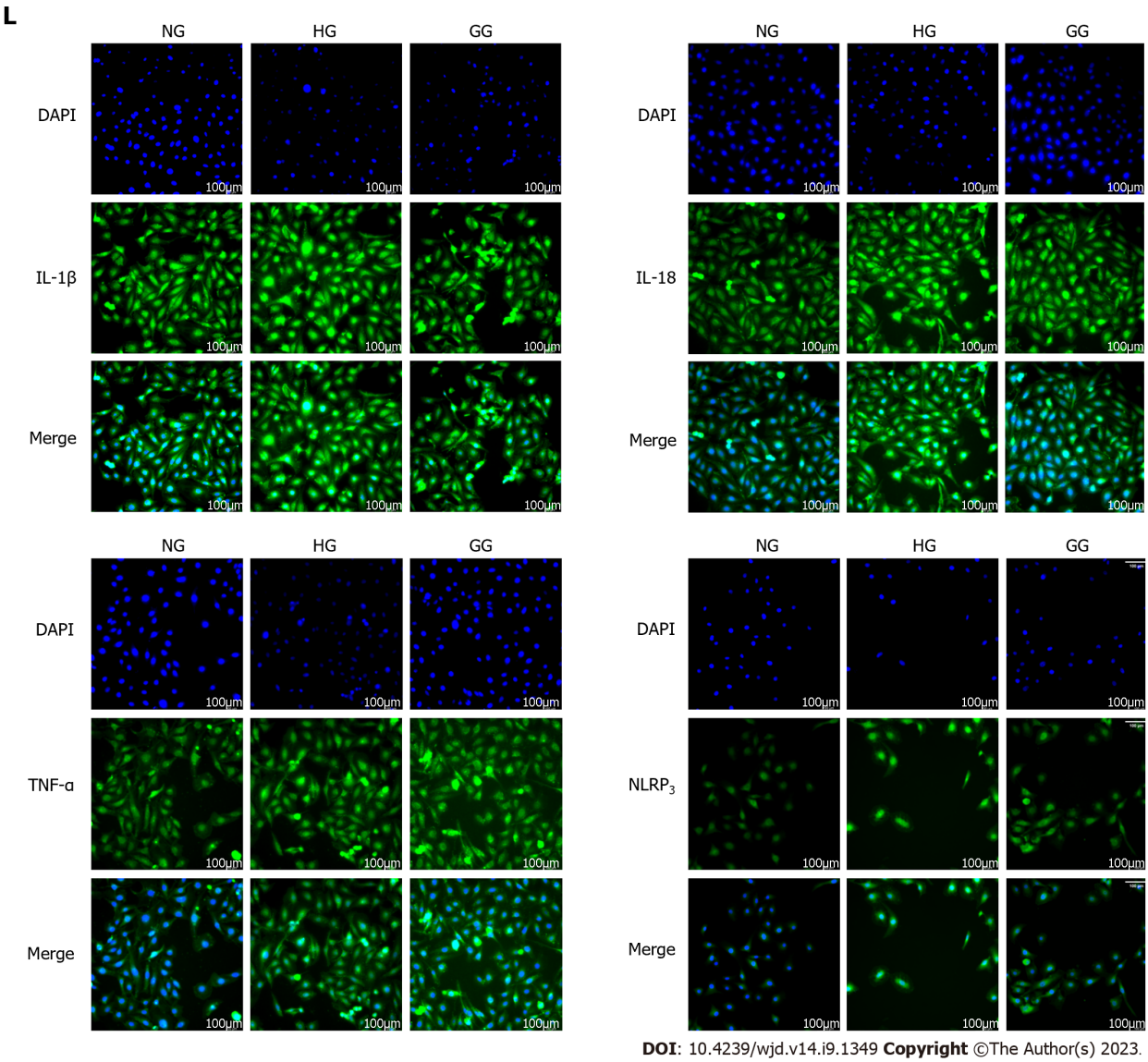
Action of glucose and genipin on intracellular glucose metabolism levels: NBDG is a fluorescence-labeled 2-deoxy-glucose analog that works as a tracer for evaluating cellular glucose metabolism[28]. High glucose increased the fluorescence, and genipin might reverse the effects.



DOI: 10.4239/wjd.v14.i9.1349 **Copyright** ©The Author(s) 2023.

Figure 2 Genipin protects from advanced glycation-induced human retinal microvascular endothelial cell proliferation, apoptosis, and angiogenesis. A and B: Genipin protects human retinal microvascular endothelial cell (hRMECs) from high glucose-induced damage with regard to cell proliferation as revealed by colony formation assay and flow cytometry; C-E: Wounding healing, migration and tube formation of hRMECs treated with 30 mmol/L glucose and 0.4 μmol/L genipin. NG: 5 mmol/L glucose containing ECM-treated hRMECs group; HG: 30 mmol/L glucose containing ECM-treated hRMECs group; GG: 0.4 μM genipin + 30 mmol/L glucose containing ECM-treated hRMECs.


A
P < 0.0001
Luminescence rate
NG
HG
Genipin
D
P = 0.0355
ROS RFU
E
NG HG GG
NLRP3
Scg3
VEGF
IL-1β
IL-18
TNF-α
β-actin
B
MMP
ROS
HRMECs
Count
DCFH FITC-A
PE-A
C
ROS
MMP
2-NBDG
F
P = 0.0621
P = 0.0374
P = 0.0829
Protein expression
IL-1β
G
P = 0.6177
P = 0.0573
P = 0.0375
Relative expression
IL-18
H
P = 0.0041
P < 0.0001
TNF-α
I
P = 0.0026
P < 0.0001
Scg3
J
P = 0.0306
P < 0.0001
VEGF
K
P = 0.0034
P < 0.0001
NLRP3



DOI: 10.4239/wjd.v14.i9.1349

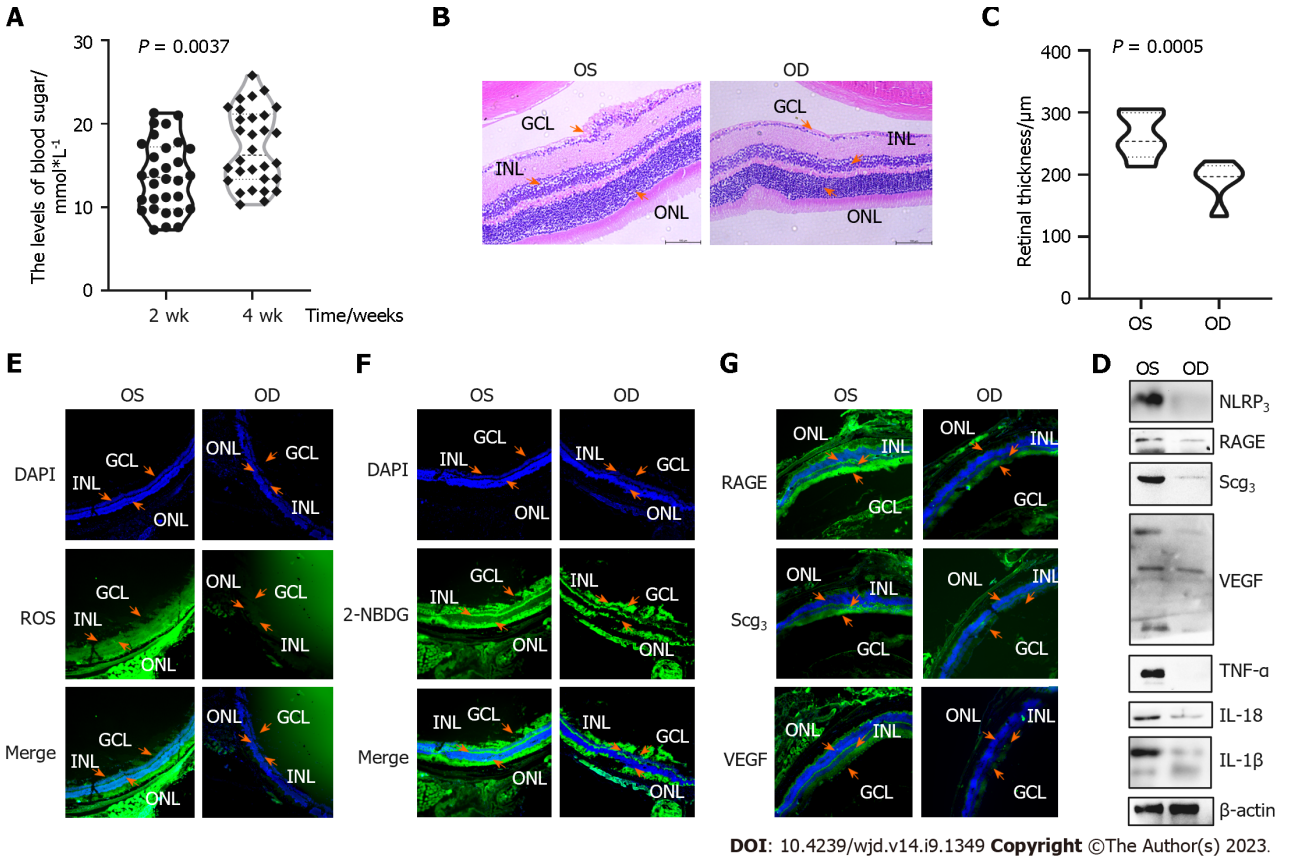
Figure 3 Genipin protects human retinal microvascular endothelial cells from high glucose damage with regard to energy metabolism, oxidative stress, and inflammatory injury *in vitro*. A: ATP levels among normal glucose, high glucose, and genipin-treated human retinal microvascular endothelial cells (hRMECs); B-D: Reactive oxygen species, mitochondrial membrane potential, and 2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) amino]-2-deoxy-d-glucose levels in hRMECs treated with high glucose and genipin; E-L: Western blot and immunofluorescence to measure the expression of inflammatory factors expression. IL: Interleukin; TNF: Tumor necrosis factor-alpha; NLRP3: Nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing 3; VEGF: Vascular endothelial growth factor; ROS: Reactive oxygen species; MMP: Mitochondrial membrane potential; 2-NBDG: 2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) amino]-2-deoxy-d-glucose; hRMECs: Human retinal microvascular endothelial cells. NG: 5 mmol/L glucose containing ECM-treated hRMECs group; HG: 30 mmol/L glucose containing ECM-treated hRMECs group; GG: 0.4 μM genipin + 30 mmol/L glucose containing ECM-treated hRMECs.

Action of glucose and genipin on inflammation: To evaluate the effects of genipin on inflammation following HG stimulation, we performed Western blot and Immunofluorescence to detect the protein levels of inflammatory cytokines (TNF-α, IL-1β, IL-18, and NLRP3) and DR biomarkers (VEGF and SCG3) in hRMECs inclubated with high glucose (Figures 3E-L and 5D). High glucose significantly increased the protein expression of TNF-α, IL-1β, IL-18, and NLRP3, and DR biomarkers. Interestingly, genipin down-regulated the expression of these proteins. Therefore, genipin may alleviate the damage caused by inflammation. Next, we will investigate the mechanisms underlying this effect.

Genipin IOI protects the retina of STZ mice from high-glucose damage in vivo

After continuous STZ IPI for 3 d, the blood glucose levels were > 7.5 mmol/L in 2 wk and > 11.1 mmol/L in 1 mo in C57 mice.

DM-induced neuronal loss was assessed by measuring the retinal thickness at 3 mo after DM induction using retinal sections from untreated eyes (OS) and genipin-injected eyes (Figure 4B and C). At 3 mo after DR induction, H&E staining was performed to assess the structural morphology of the retinal cells. We measured four spots of the retina; the thickness of genipin-treated retina (190.70 μm ± 58.32 μm) was lesser than that of DR-untreated retina (262.10 μm ± 55.52 μm, P = 0.045). Representative microphotographs demonstrate a disorder of the entire retina, vacuolization, and dissolution of the



DOI: 10.4239/wjd.v14.i9.1349

Figure 4 Genipin reduces retinal oxidant stress, glucose metabolism, and inflammation in streptozotocin-induced mice. A: Blood sugar levels of mice induced with streptozotocin (STZ) for 2 wk and 4 wk; B: Hematoxylin and eosin staining of STZ mouse retina; C: Retinal thickness in different eyes of STZ mice; D: Inflammatory factor expression measured by Western blot; E and F: Reactive oxygen species and 2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) amino]-2-deoxy-d-glucose levels in STZ mice; G and F: RAGE, SCG3, and vascular endothelial growth factor expression in STZ mice. IL: Interleukin; TNF: Tumor necrosis factor-alpha; $NLRP_3$: Nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing 3; VEGF: Vascular endothelial growth factor; ROS: Reactive oxygen species; MMP: Mitochondrial membrane potential; 2-NBDG: 2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) amino]-2-deoxy-d-glucose; OS: Left eye, without genipin-treated; OD: Right eye, genipin-treated; GCL: Ganglion cell layer; INL: Inner nuclear layer; ONL: Outer nuclear layer.

ganglion cell layer in the left eye.

Genipin reduces retinal oxidant stress, glucose metabolism, and inflammation in STZ mice

We performed ROS and 2-NBDG assays to measure oxidative stress and glucose metabolism in the frozen sections. Genipin-treated cells had significantly increased oxidant stress and glucose metabolism. SCG3 and VEGF expression increased in the untreated group.

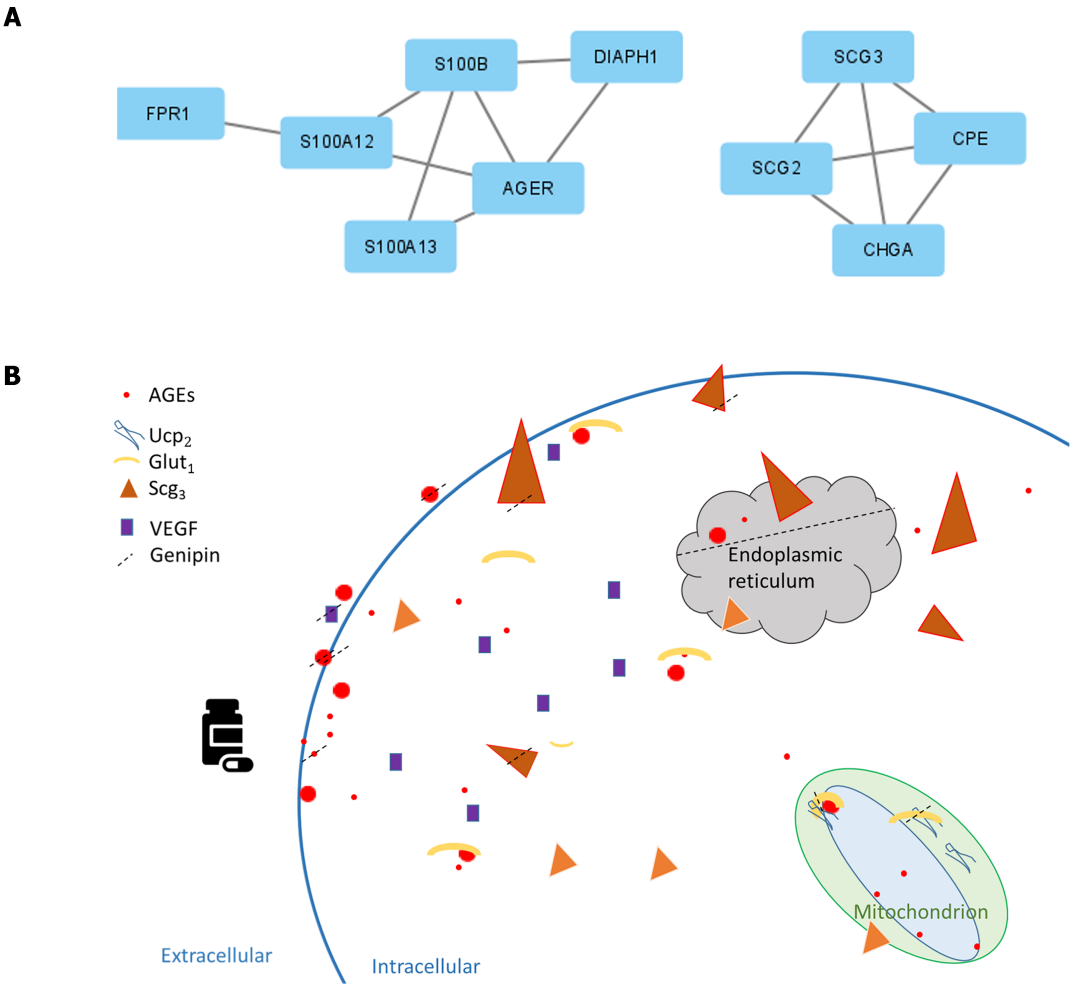
In addition, the protein levels of inflammatory cytokines (TNF-α, IL-1β, IL-18, and NLRP3) and DR biomarkers (VEGF and SCG3) were measured by Western blot. Genipin protected the cells from inflammation.

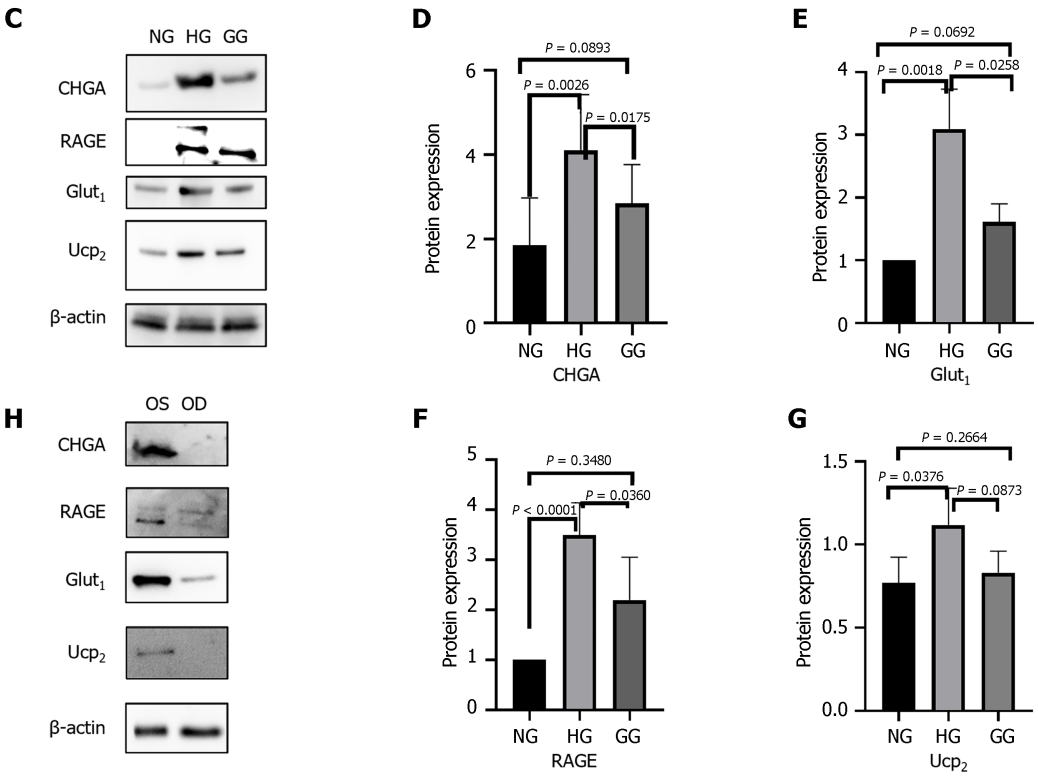
Mechanism of genipin to relives retinal endothelial damage induced by high glucose

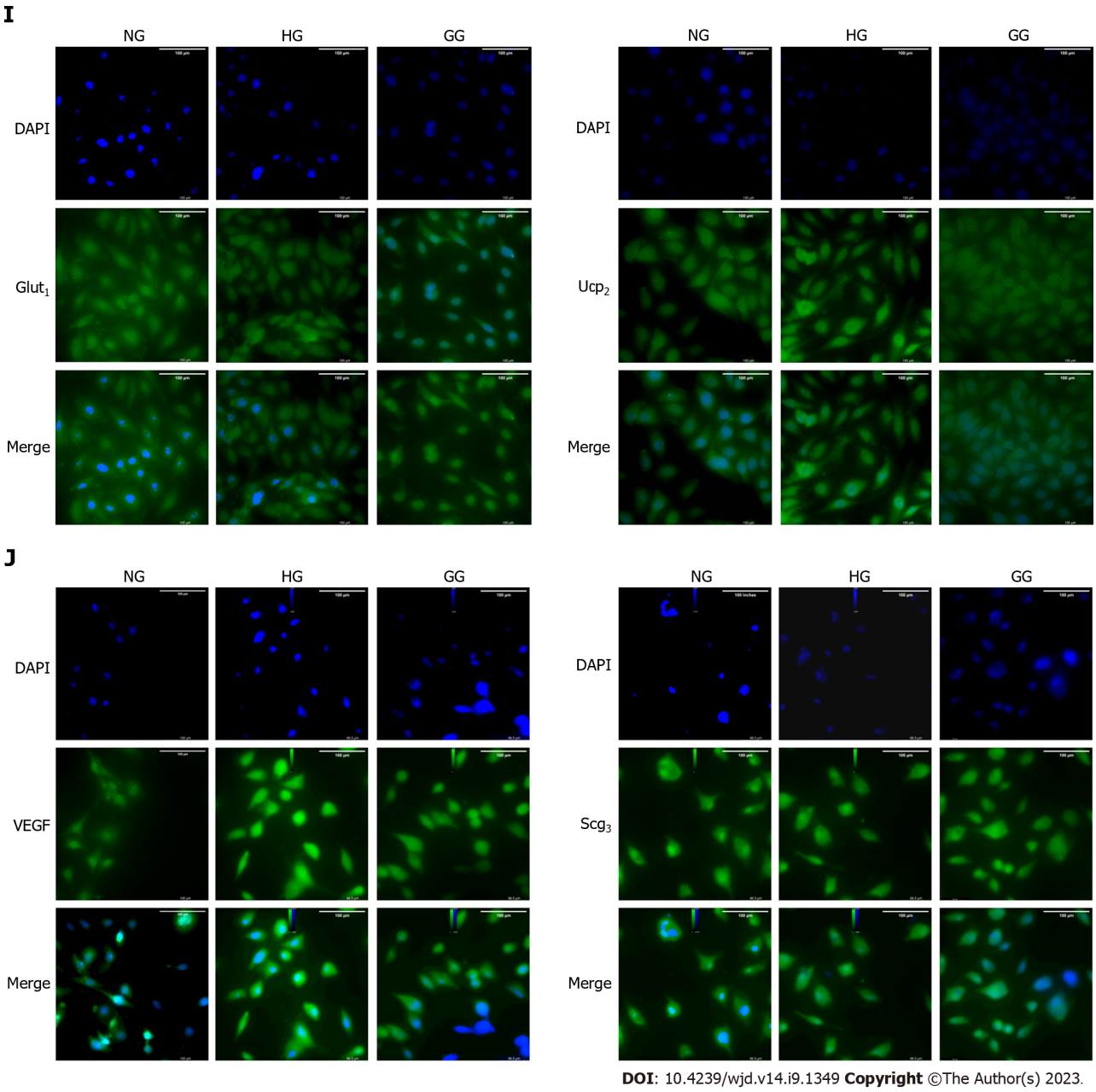
UCP2/GLUT1 pathway is involved in the protective effect of genipin on hRMECs (Figure 5): The decrease in glucose uptake in genipin-treated cells was mediated by the reduction of GLUT1 expression, as demonstrated by Western blot (Figure 5C and D). To investigate the glycation process that occurred after glucose, we explored the AGE-receptor interactions in both cells and tissues, which showed that genipin treatment decreased AGEs.

We used STRING (Version: 11.5, https://cn.string-db.org/cgi/input) to analyze the relationships among "RAGE", "SCG3", "UCP2", "GLUT1", and "VEGF" (Figure 5A). GLUT1 may be influenced by UCP2 expression in oxygen-induced retinopathy (OIR) at both the mRNA and protein levels, whereas elevated circulating CHGA can lead to UCP2 overexpression in the hypertensive phenotype[9]. Next, we performed Western blot and immunofluorescence to verify the expression of these proteins. The protein expression of these three proteins was increased in high-glucose conditions, and genipin could restore such change.

Genpin relives retinal endothelial damage in high-glucose conditions by influencing energy metabolism, oxidative stress, and inflammatory injury: We identified 33 and 4328 DR-related genes using GeneCards (https://www.genecards.org/) and OMIM (https://omim.org/), respectively, with the keywords "DR" or "diabetic retinopathy". Next, we used STRING to analyze the relationships among these genes (Figure 6A); Gene Ontology (GO)/Kyoto Encyclopedia of Genes and Genomes function enrichment analyses revealed that AGE-RAGE plays a vital role in DR (Figure 6B). Restraining AGEs and alleviating toxicity may be a novel way to control DR.


A
S100B
DIAPH1
FPR1
S100A12
AGER
S100A13
SCG3
CPE
SCG2
CHGA
B
AGEs
Ucp2
Glut1
Scg3
VEGF
Genipin
Endoplasmic reticulum
Mitochondrion
Extracellular
Intracellular


C
NG HG GG
CHGA
RAGE
Glut1
Ucp2
β-actin
D
P = 0.0893
P = 0.0026
P = 0.0175
Protein expression
NG HG GG
CHGA
E
P = 0.0692
P = 0.0018
P = 0.0258
Protein expression
NG HG GG
Glut1
H
OS OD
CHGA
RAGE
Glut1
Ucp2
β-actin
F
P = 0.3480
P = 0.0360
P < 0.0001
Protein expression
NG HG GG
RAGE
G
P = 0.2664
P = 0.0376
P = 0.0873
Protein expression
NG HG GG
Ucp2



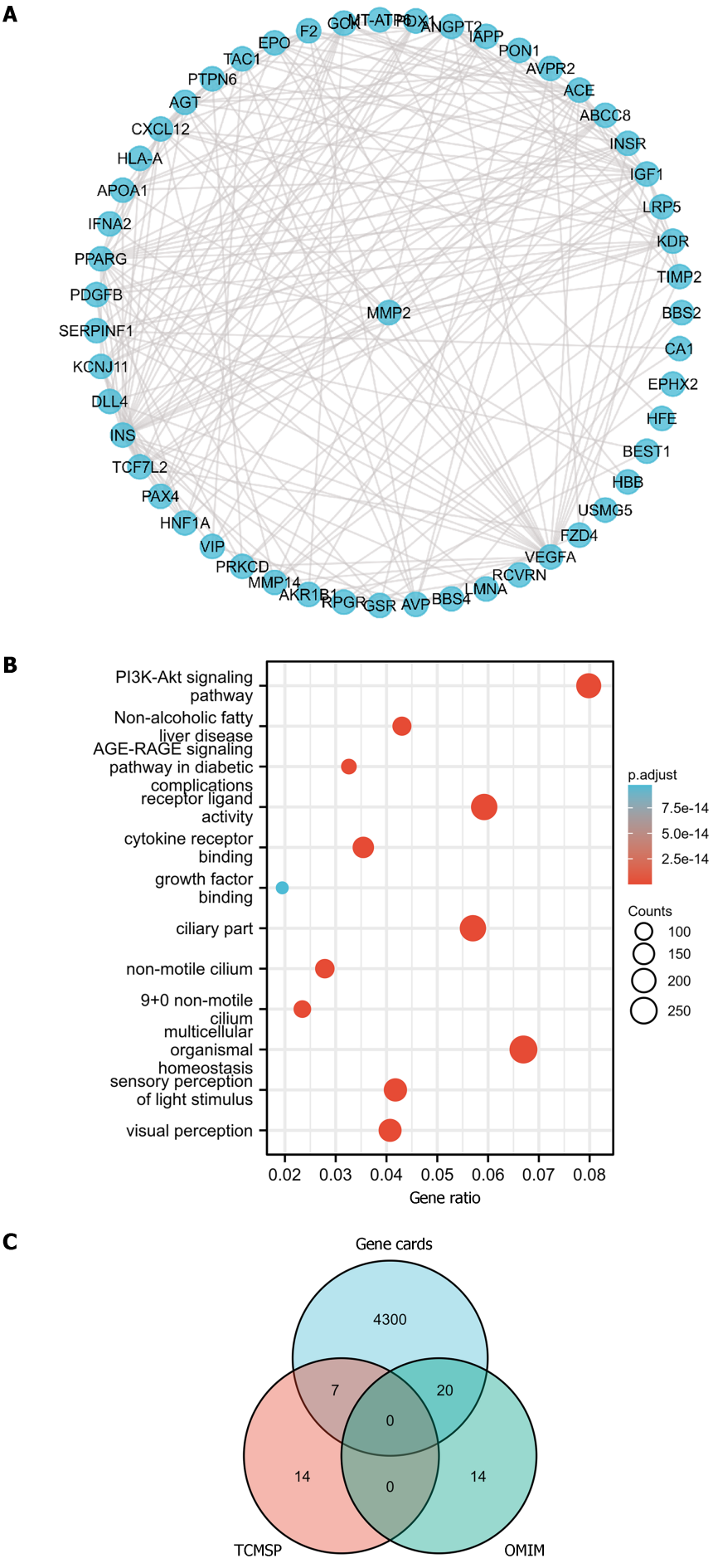
DOI: 10.4239/wjd.v14.i9.1349

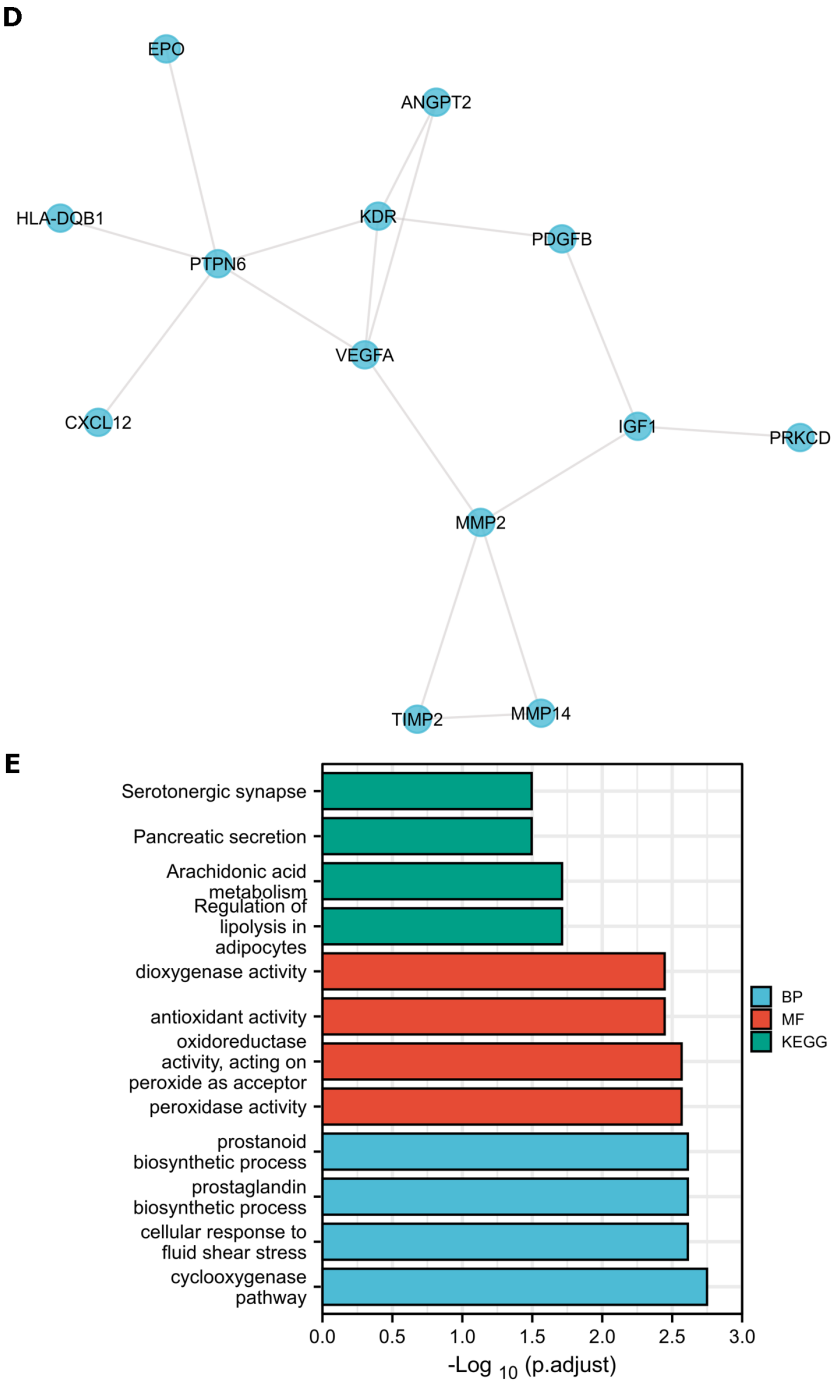
Figure 5 Mechanism of genipin to relive retinal endothelial damage caused by high glucose. A and B: Schematic summary of the results; C-I: Western blot and immunofluorescence (IF) analysis of expression of proteins related to CHGA/UCP2/GLUT1; J: RAGE, SCG3, and vascular endothelial growth factor expression detected by IF.

We used TCMSP to identify 21 genes possibly targeted by genipin. UniProt (https://www.uniprot.org/) was used to assess and convert these gene names. A Wayne diagram was plotted to display the intersection of the genipin target and DR-related genes (Figure 6C and D). To determine the available phenotypic abnormalities, we used GO function enrichment and pathway enrichment analyses to explore the relationships among genes that were mixed by DR-related and genipin targets. Finally, oxidoreductase activity and arachidonic acid metabolism demonstrated distinct correlations (Figure 6E), indicating that genipin may influence ROS and inflammation as two vital phenotypic abnormalities in DR.

DISCUSSION

Vision loss in DR principally occurs in either diabetic macular edema (DME) or PDR[29]. DR is classified based on visible ophthalmologic changes and manifestations of retinal neovascularization[30]. Therefore, the early diagnosis and treatment of DR cover the complete range of retinopathies and are complex. Fortunately, regardless of the type of DR, abnormal AGE accumulation has the same basic reason.


A
MT-ATP6
ANGPT2
IAPP
PON1
AVPR2
ACE
ABCC8
INSR
IGF1
LRP5
KDR
TIMP2
BBS2
CA1
EPHX2
HFE
BEST1
HBB
USMG5
FZD4
VEGFA
RCVRN
LMNA
BBS4
AVP
GSR
RPGR
AKR1B1
MMP14
PRKCD
VIP
HNF1A
PAX4
TCF7L2
INS
DLL4
KCNJ11
SERPINF1
PDGFB
PPARG
IFNA2
APOA1
HLA-A
CXCL12
AGT
PTPN6
TAC1
EPO
F2
MMP2
B
PI3K-Akt signaling pathway
Non-alcoholic fatty liver disease
AGE-RAGE signaling pathway in diabetic complications
receptor ligand activity
cytokine receptor binding
growth factor binding
ciliary part
non-motile cilium
9+0 non-motile cilium
multicellular organismal homeostasis
sensory perception of light stimulus
visual perception
0.02
0.03
0.04
0.05
0.06
0.07
0.08
Gene ratio
p.adjust
7.5e-14
5.0e-14
2.5e-14
Counts
100
150
200
250
C
Gene cards
4300
7
20
0
14
0
14
TCMSP
OMIM



DOI: 10.4239/wjd.v14.i9.1349 **Copyright** ©The Author(s) 2023.

Figure 6 Pathway enrichment analysis. A: Prediction of diabetic retinopathy (DR)-related genes; B: Pathway enrichment analysis of DR-related genes; C and D: Intersection of genipin target and DR-related genes; E: Pathway enrichment analysis of genes which are mixed by DR-related and genipin targets. EPO: Erythropoietin; BP: Biological process-pathways and larger processes made up of the activities of multiple gene products; MF: Molecular Function-molecular activities of gene products; KEGG: Kyoto Encyclopedia of Genes and Genomes.

DR has a mixed pathogenesis. It involves cell cycle regulation, apoptosis signal transduction, oxidative stress response, protein biosynthesis, carbohydrate metabolism, and other important proteins and proteases. The ROS assay demonstrated a high level of oxidative stress response in both cells in the high glucose group and STZ mice without treatment. Carbohydrate metabolism and mitochondrial injury in the high glucose group led to increased ATP levels and cell apoptosis. Glucose uptake was higher in the high glucose group than in the control group, which can be validated by GLUT1 expression. Genipin, an aglycone derived from the iridoid glycoside, is also used as an anti-tumor drug in oral squamous cell carcinoma, hepatocellular carcinoma, and gastric cancer *via* the signal transducer and activator of the transcription-3 pathway and upregulated MMP genes[31,32]; the relationships between genipin and glucose was rarely reported. We explained whether genipin could change the GLUT family in different cell types. This finding may help explain how genipin influences tumors and apoptosis in various diseases. VEGF is a classical biomarker of DR, and it is widely used to treat inflammation[32-35]. NLRP3 inflammasomes are involved in the production and persistence of

inflammation in diabetic nephropathy[36-38]. We observed that high glucose stimulation could up-regulate IL-1β, IL-18, TNF-α and NLRP3, but not IL-6.

Upon exposure to sugar, proteins, lipids, and nucleic acids are oxidized and rearrange to form stable products, which can further undergo crosslinking, thus yielding AGEs[39]. AGEs are associated with a series of diseases such as Alzheimer's disease, skin senescence, face splash capillary ectasia, and pathologies such as AMD, diabetic keratopathy, and DR. AGEs are reactive intermediates of chronic hyperglycemia with proteins, lipids, and nucleic acids *in vivo*, leading to the accumulation of pro-inflammatory cytokines and ROS. Furthermore, they damage the vasculature, eye, heart, and kidneys in patients with DM.

Consequently, we focused on glucose metabolism and AGEs production in hRMECs and addressed the hypothesis that high-glucose medium affects glucose uptake and mitochondrial metabolism, leading to intravitreal neovascularization in DR. UCP2, a mitochondrial carrier protein, can increase the cellular sensitivity to glucose and promote cellular glucose uptake by mediating proton leak across the inner mitochondrial membrane[40]. Genipin is a standard UCP2-inhibiting agent in various experimental models of ROS generation and mitochondrial activity[41]. Most tumor growth and proliferation processes involve excessive glucose uptake mediated by GLUTs[42]. The retina takes up glucose, principally through GLUT1[43]. Genipin promotes physiological retinal vascular development and improves endothelial function by inhibiting the UCP2/GLUT1 pathway in premature retinopathy.

Thus, we focused on the effects and molecular mechanisms of genipin on the proliferation, apoptosis, angiogenesis, energy metabolism, oxidative stress, and inflammation of AGEs-induced hRMECs.

Genipin have been used to treat several diseases due to its choleretic, anti-inflammatory, antioxidant, anti-apoptotic, autophagy-inducing, anti-necroptotic, and anti-pyroptotic properties[41,44-47]. Simultaneously, genipin is an aglycone of an iridoid glycoside termed geniposide. Moreover, it is a commonly used biological agent, *e.g.*, as a cross-linker, because it can be better tolerated by cells. Despite its benefits and good biocompatibility, genipin is still unpopular in classical medicine because of its unclear mechanism of action. We analyzed the role of genipin as an important element of Zhizi against AGEs accumulation in DR. First, we evaluated the intracellular concentration of AGEs by assessing the expression level of RAGE. RAGE expression increased significantly with high glucose stimulation, and genipin decreased this increase. High glucose stimulation increased apoptosis, ROS, ATP, mitochondrial formation, glucose metabolism, and inflammation. High glucose could increase the accumulation of AGEs *via* pathways that can be affected by genipin and finally decrease GLUT1 expression. UCP2, as a binding site for genipin, decreases GLUT1 expression at both the mRNA and protein levels[40]. Then, we tried to connect RAGE and DR biomarkers with these pathways. We identified RAGE and DR biomarkers in these pathways. We analyzed the relationships between these five proteins and identified the "CHGA" protein. Elevated CHGA levels result in UCP2 overexpression in hypertension[48]. We evaluated these changes in hRMECs treated with high glucose and STZ mouse retinas and observed increased protein expression of CHGA, UCP2, and GLUT1. Hence, high glucose leads to the addition of AGEs, increases CHGA expression, and promotes UCP2 and GLUT1 expression, which influences cell proliferation, apoptosis, oxides, cellular energy metabolism, and inflammation. Genipin can inhibit this CHGA/UCP2/GLUT1 pathway not only by controlling UCP2 but also through other mechanisms.

Genipin is the third most active constituent of Zhizi (Table 1). The oral bioavailability of genipin is 26.06% and its topological polar surface area is 75.99, which suggests that genipin is preferentially administered by injection than orally. However, genipin is principally used as an oral formulation in previous research, whereas injection formulations have been less investigated. In addition, genipin is an extremely good natural cross-linking agent that contributes to sustained-release or delayed-release joint use with other drugs[49]. AGEs are a type of cross-linker that combines sugars and proteins and can also reduce this step by linking with proteins by physical methods. Furthermore, genipin is widely used in the clinic to treat corneal or scleral diseases[46,49].

Ocular drug delivery is difficult because of the lacrimal fluid-eye barrier and the retina-blood barrier[50]. Retinal diseases, such as retinal detachment, AMD, and DR, require an intravitreal injection to inhibit the formation of ocular neovascularization. Such common drugs include anti-VEGF medications and hormones. Our previous report describes the use of an AGE inhibitor that can penetrate the vitreous humor and treat retinal disease in STZ mice. We used the most effective dose of genipin in hRMECs for injection (C = m × V). The vitreous volume of mice was approximately 10 μL, and the maximum injection volume was 1 μL. We used 4 μmol/eye (5 mmol/L × 0.8 μL/eye, 10 mmol/L × 0.4 μL/eye) and 8 μmol/eye (10 mmol/L × 0.8 μL/eye), three different doses for IOI, and the results did not have a measurable difference. Finally, we selected the minimal volume for subsequent experiments.

Eventually, we identified the biomarkers of DR. Previous studies have reported on VEGF, monocyte chemoattractant protein-1, TNF-α, transforming growth factor-β, and nicotinamide adenine dinucleotide phosphate oxidase as the markers of inflammation. L-citrulline, indoleacetic acid, chenodeoxycholic acid, and eicosapentaenoic acid have been reported as markers of metabolism[51]. SCG3 is an angiogenic factor restricted to pathological conditions in DR[52]. Jiao *et al*[53] demonstrated that the concentration of SCG3 in the vitreous increased in 77 patients with DR. LeBlanc *et al*[54] reported that SCG3 antibodies alleviated retinal vascular leakage in diabetic mice with high efficacy. Furthermore, we assessed SCG3 expression in cells and tissues. Our results implied that SCG3 may be another biomarker of DR, which displayed a similar growing tendency in high glycogen to VEGF expression but with a higher sensitivity.

However, this study had several limitations. First, how the CHGA/UCP2/GLUT1 pathway is regulated by AGEs-RAGE binding requires further research. Some studies have demonstrated that they may form a GLUT1-UCP2 complex attached to the mitochondrial membrane; nonetheless, some details are still unknown. Second, we did not explain whether different doses of AGEs influence the severity of DR. Consequently, we added quantitative exogenous AGEs and observed changes in the cells and tissues. Third, the IOI group comprised limited doses *in vivo*. Next, we will add more dose groups injected with perfect genipin drug transduction *in vivo* and evaluate clinical medicine. Finally, we determined that SCG3 may replace VEGF in evaluating DR; however, the function of SCG3 in angiogenesis is unclear.

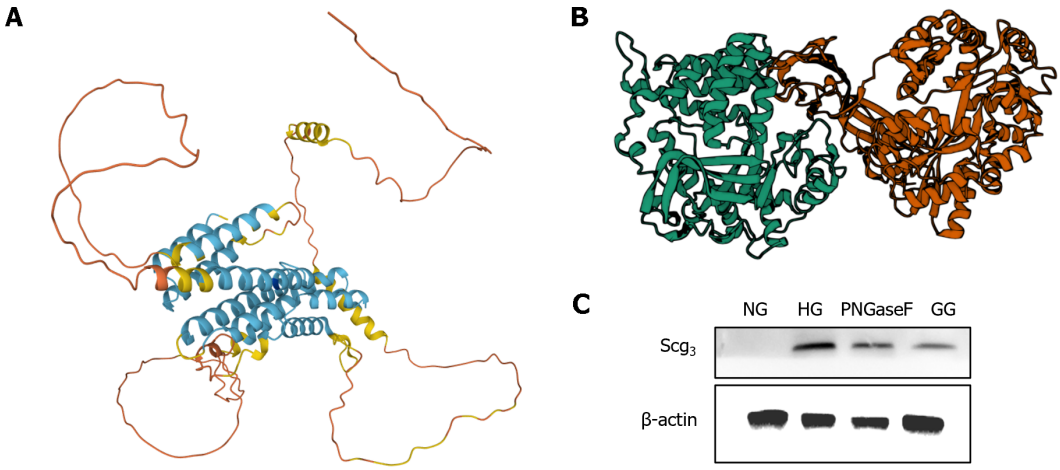
Our future work will focus on the changes in SCG3 during abnormal physiological processes. Some researchers have mentioned that SCG3 is central to tumors, apoptosis, and other vital processes[55]. Togayachi *et al*[56] demonstrated that SCG3 is activated by adding N-glycosylation to change short-form SgIII into a long form in small-cell lung carcinoma. This biological process may be related with the GLUT family. To further explore the relevant mechanisms, we used UniProt (https://www.uniprot.org/) to determine the structure of SCG3 and explained the potential target site of glycosylation (Figure 7A, Table 2). The ZDOCK score for RAGE and SCG3 was 1277.337, which means that RAGE can be linked with SCG3 easily. There are totally three positions of RAGE associated with SCG3 (at sites 216, 231, and 359; Figure 7B). PNGase F, used to split SCG3, decreased SCG3 protein expression (Figure 7C).

CONCLUSION

Overall, apoptosis, angiogenesis, proliferation, ROS production, carbohydrate metabolism, and inflammation can reflect DR damage both *in vivo* and *in vitro*. Genipin is useful against AGEs, and its protective role in DR has been confirmed. This action may occur *via* AGEs-RAGE binding to control the CHGA/UCP2/GLUT1 pathway. SCG3 is a novel and sensitive biomarker for DR.

Table 2 Features for signal, chain, modified residue, and glycosylation in SCG3

Type	Position	Description
Signal	1-19	-
Chain	20-468	Secretogranin-3
Modified residue	37	Phosphoserine
Glycosylation1	216	O-linked (GalNAcan) threonine
Glycosylation 2	231	O-linked (GalNAcan) threonine
Glycosylation 3	359	O-linked (GalNAcan) threonine
Modified residue	362	Phosphoserine



DOI: 10.4239/wjd.v14.i9.1349

Figure 7 SCG3 plays a vital role in diabetic retinopathy *via* glycosylation. A: Structure of SCG3; B: Molecular docking simulation with RAGE (green, left) and SCG3 (orange, right); C: PNGase F cleavaged Scg_3. NG: 5 mmol/l glucose containing ECM-treated human retinal microvascular endothelial cells (hRMECs) group; HG: 30 mmol/L glucose containing ECM-treated hRMECs group; GG: 0.4 μM genipin + 30 mmol/L glucose containing ECM-treated hRMECs.

ARTICLE HIGHLIGHTS

Research background

Diabetic retinopathy (DR) is a serious and common complication of diabetes. Advanced glycation end products (AGEs) are a group of reversible and poisonous products formed by nonenzymatic glycation of glucose with protein and lipids under hyperglycemia conditions. Both acute and chronic hyperglycemia can enhance AGEs production, which results in endothelial dysfunction and causes severe damage to diabetic retina. Some traditional Chinese herb like Gardenia jasminoides Ellis (GJE) fruit is a selective inhibitor of AGEs.

Research motivation
To confirm the effect of genipin, a vital component of GJE fruit, in preventing human retinal microvascular endothelial cells (hRMECs) from AGEs damage in DR and explored its mechanism.

Research objectives
To demonstrate whether genipin could lessen the development of DR in experimental models (4-wk-old C57/BL6 mice) through AGEs inhibition.

Research methods
Cell Counting Kit-8 (CCK-8) assay, colony formation assay, flow cytometry, immunofluorescence, wound healing assay, transwell assay, and tube-forming assay were used to detect the effect of genipin on hRMECs *in vivo*. Streptozotocin induced mice were used to explore retinal dysfunction with DM *in vitro*. Reactive oxygen species (ROS), mitochondrial membrane potential (MMP), and 2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) amino]-2-deoxy-d-glucose assays were used to evaluate energy metabolism and oxidative stress damage in high glucose-induced hRMECs and STZ mouse retinas. Immunofluorescence and Western blot were used to investigate the expression of inflammatory cytokines (VEGF, SCG3, TNF-α, IL-1β, IL-18, and NLRP3).

Research results
Our study confirmed that genipin ameliorated AGEs-induced hRMECs proliferation, apoptosis, energy metabolism, oxidative stress, and inflammatory injury, partially *via* the CHGA/UCP2/glucose transporter 1 pathway. Control of AGEs by IOI of genipin may represent a strategy to prevent severed retinopathy and vision loss.

Research conclusions
Our study suggested that intraocular injection of genipin can ameliorate AGEs to control DR. Control of AGEs and using principal bioactive components extracted from herb by intraocular injection may represent strategies to prevent DR.

Research perspectives
Our future work will focus on the changes in SCG3 during abnormal physiological processes.

FOOTNOTES

Author contributions: Sun KX and Hu K were involved in design and conduct of the study, and preparation of the manuscript; Sun KX, Chen YY, and Li Z participated in the collection of the data; Zheng SJ, Wan WJ, Ji Y, and Hu K participated in the management of this program; all authors have read and approved the final manuscript.

Supported by the National Natural Science Foundation of China, No. 81870650, No. 81570832, and No. 81900885; Science and Technology Program Chongqing, No. 2018GDRC008 and No. XKTS049.

Institutional animal care and use committee statement: All animal experimental protocols were reviewed and approved by the Institutional Animal Care and Use Committee of Chongqing Medical University (No. 2022-K45).

Conflict-of-interest statement: None of the authors have any financial/conflicting interests to disclose.

Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author Professor Ke Hu at 42222@qq.com.

ARRIVE guidelines statement: The authors have read the ARRIVE Guidelines, and the manuscript was prepared and revised according to the ARRIVE Guidelines.

Country/Territory of origin: China

ORCID number: Ke-Xin Sun 0000-0002-4103-2792; Yan Ji 0000-0003-3914-7256; Ke Hu 0000-0002-8055-389X.

S-Editor: Chen YL
L-Editor: Wang TQ
P-Editor: Chen YX

REFERENCES

1 **Simó-Servat O**, Hernández C, Simó R. Diabetic Retinopathy in the Context of Patients with Diabetes. *Ophthalmic Res* 2019; **62**: 211-217 [PMID: 31129667 DOI: 10.1159/000499541]
2 **Leley SP**, Ciulla TA, Bhatwadekar AD. Diabetic Retinopathy in the Aging Population: A Perspective of Pathogenesis and Treatment. *Clin Interv Aging* 2021; **16**: 1367-1378 [PMID: 34290499 DOI: 10.2147/CIA.S297494]
3 **Pelikánová T**. [Diabetic retinopathy in the Czech National Diabetes Programme 2012-2022]. *Vnitr Lek* 2013; **59**: 214-217 [PMID: 23713191]
4 **Chua J**, Lim CXY, Wong TY, Sabanayagam C. Diabetic Retinopathy in the Asia-Pacific. *Asia Pac J Ophthalmol (Phila)* 2018; **7**: 3-16 [PMID: 29376231 DOI: 10.22608/APO.2017511]
5 **Pan CW**, Wang S, Wang P, Xu CL, Song E. Diabetic retinopathy and health-related quality of life among Chinese with known type 2 diabetes mellitus. *Qual Life Res* 2018; **27**: 2087-2093 [PMID: 29740784 DOI: 10.1007/s11136-018-1876-6]
6 **Lin Z**, Wang Y, Li D, Wen L, Zhai G, Ding XX, Zang DX, Wang FH, Liang YB. Higher prevalence of diabetic retinopathy among female Chinese diabetic patients with metabolic syndrome. *Jpn J Ophthalmol* 2022; **66**: 102-109 [PMID: 34655347 DOI: 10.1007/s10384-021-00877-x]
7 **Hattori Y**, Hashizume K, Nakajima K, Nishimura Y, Naka M, Miyanaga K. The effect of long-term treatment with sulindac on the progression of diabetic retinopathy. *Curr Med Res Opin* 2007; **23**: 1913-1917 [PMID: 17621415 DOI: 10.1185/030079907X218770]
8 **Ellis MP**, Lent-Schochet D, Lo T, Yiu G. Emerging Concepts in the Treatment of Diabetic Retinopathy. *Curr Diab Rep* 2019; **19**: 137 [PMID: 31748965 DOI: 10.1007/s11892-019-1276-5]
9 **Angermann R**, Rauchegger T, Nowosielski Y, Casazza M, Bilgeri A, Ulmer H, Zehetner C. Treatment compliance and adherence among patients with diabetic retinopathy and age-related macular degeneration treated by anti-vascular endothelial growth factor under universal health coverage. *Graefes Arch Clin Exp Ophthalmol* 2019; **257**: 2119-2125 [PMID: 31286206 DOI: 10.1007/s00417-019-04414-y]
10 **Wang L**, Wu Q, Wang RQ, Wang RZ, Wang J. Protection of leukemia inhibitory factor against high-glucose-induced human retinal endothelial cell dysfunction. *Arch Physiol Biochem* 2023; **129**: 33-40 [PMID: 32658632 DOI: 10.1080/13813455.2020.1792506]
11 **Arcadu F**, Benmansour F, Maunz A, Willis JR, Prunotto M, Haskova Z. Deep learning algorithm for patient-level prediction of diabetic retinopathy (DR) response to vascular endothelial growth factor (VEGF) inhibition. *Invest Ophthalmol & Vis Sci* 2019; **60**: 2806 [DOI: 10.1038/s41746-019-0172-3]
12 **Bhatti JS**, Bhatti GK, Reddy PH. Mitochondrial dysfunction and oxidative stress in metabolic disorders - A step towards mitochondria based therapeutic strategies. *Biochim Biophys Acta Mol Basis Dis* 2017; **1863**: 1066-1077 [PMID: 27836629 DOI: 10.1016/j.bbadis.2016.11.010]
13 **Bek T**. Mitochondrial dysfunction and diabetic retinopathy. *Mitochondrion* 2017; **36**: 4-6 [PMID: 27456429 DOI: 10.1016/j.mito.2016.07.011]
14 **Kowluru RA**, Mishra M. Oxidative stress, mitochondrial damage and diabetic retinopathy. *Biochim Biophys Acta* 2015; **1852**: 2474-2483 [PMID: 26248057 DOI: 10.1016/j.bbadis.2015.08.001]
15 **Kersten HM**, Roxburgh RH, Danesh-Meyer HV. Ophthalmic manifestations of inherited neurodegenerative disorders. *Nat Rev Neurol* 2014; **10**: 349-362 [PMID: 24840976 DOI: 10.1038/nrneurol.2014.79]
16 **Te BC**, Goh BS. Case series of tuberculous otitis media: Spectrum of clinical presentation and outcome. *Acta Otorrinolaringol Esp (Engl Ed)* 2022; **73**: 123-129 [PMID: 35397821 DOI: 10.1016/j.otoeng.2020.09.006]
17 **Conlon TA**, Hawkes CP, Brady JJ, Murphy NP. The presentation of congenital adrenal hyperplasia in an unscreened population. *J Pediatr Endocrinol Metab* 2021; **34**: 1123-1129 [PMID: 34167176 DOI: 10.1515/jpem-2021-0123]
18 **Shi ZH**, Han XY, Yao MD, Liu C, Jiang Q, Yan B. Differential MicroRNA Expression Pattern in Endothelial Progenitor Cells During Diabetic Retinopathy. *Front Cell Dev Biol* 2021; **9**: 773050 [PMID: 34977023 DOI: 10.3389/fcell.2021.773050]
19 **Malhotra NA**, Greenlee TE, Iyer AI, Conti TF, Chen AX, Singh RP. Racial, Ethnic, and Insurance-Based Disparities Upon Initiation of Anti-Vascular Endothelial Growth Factor Therapy for Diabetic Macular Edema in the US. *Ophthalmology* 2021; **128**: 1438-1447 [PMID: 33716048 DOI: 10.1016/j.ophtha.2021.03.010]
20 **Yan L**, Vaghari-Tabari M, Malakoti F, Moein S, Qujeq D, Yousefi B, Asemi Z. Quercetin: an effective polyphenol in alleviating diabetes and diabetic complications. *Crit Rev Food Sci Nutr* 2022; 1-24 [PMID: 35468007 DOI: 10.1080/10408398.2022.2067825]
21 **Chai GR**, Liu S, Yang HW, Chen XL. Quercetin protects against diabetic retinopathy in rats by inducing heme oxygenase-1 expression. *Neural Regen Res* 2021; **16**: 1344-1350 [PMID: 33318415 DOI: 10.4103/1673-5374.301027]
22 **Bungau S**, Abdel-Daim MM, Tit DM, Ghanem E, Sato S, Maruyama-Inoue M, Yamane S, Kadonosono K. Health Benefits of Polyphenols and Carotenoids in Age-Related Eye Diseases. *Oxid Med Cell Longev* 2019; **2019**: 9783429 [PMID: 30891116 DOI: 10.1155/2019/9783429]
23 **Li LH**, Lee JC, Leung HH, Lam WC, Fu Z, Lo ACY. Lutein Supplementation for Eye Diseases. *Nutrients* 2020; **12** [PMID: 32526861 DOI: 10.3390/nu12061721]
24 **Noma H**, Funatsu H, Yamashita H, Kitano S, Mishima HK, Hori S. Regulation of angiogenesis in diabetic retinopathy: possible balance between vascular endothelial growth factor and endostatin. *Arch Ophthalmol* 2002; **120**: 1075-1080 [PMID: 12149062 DOI: 10.1001/archopht.120.8.1075]
25 **Furman BL**. Streptozotocin-Induced Diabetic Models in Mice and Rats. *Curr Protoc* 2021; **1**: e78 [PMID: 33905609 DOI: 10.1002/cpz1.78]
26 **Wu Q**, Liu H, Zhou M. Fangchinoline Ameliorates Diabetic Retinopathy by Inhibiting Receptor for Advanced Glycation End-Products (RAGE)-Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells (NF-κB) Pathway in Streptozotocin (STZ)-Induced Diabetic Rats. *Med Sci Monit* 2019; **25**: 1113-1121 [PMID: 30739905 DOI: 10.12659/MSM.912927]
27 **Ahmad Fadzil MH**, Izhar LI, Nugroho H, Nugroho HA. Analysis of retinal fundus images for grading of diabetic retinopathy severity. *Med Biol Eng Comput* 2011; **49**: 693-700 [PMID: 21271293 DOI: 10.1007/s11517-011-0734-2]
28 **Ahmad Fadzil M**, Ngah NF, George TM, Izhar LI, Nugroho H, Adi Nugroho H. Analysis of foveal avascular zone in colour fundus images for grading of diabetic retinopathy severity. *Annu Int Conf IEEE Eng Med Biol Soc* 2010; **2010**: 5632-5635 [PMID: 21097305 DOI: 10.1109/IEMBS.2010.5628041]
29 **Lee JH**, Cho YS, Jung KH, Park JW, Lee KH. Genipin enhances the antitumor effect of elesclomol in A549 lung cancer cells by blocking uncoupling protein-2 and stimulating reactive oxygen species production. *Oncol Lett* 2020; **20**: 374 [PMID: 33154772 DOI: 10.3892/ol.2020.12237]
30 **Wang GF**, Wu SY, Rao JJ, Lü L, Xu W, Pang JX, Liu ZQ, Wu SG, Zhang JJ. Genipin inhibits endothelial exocytosis *via* nitric oxide in cultured human umbilical vein endothelial cells. *Acta Pharmacol Sin* 2009; **30**: 589-596 [PMID: 19349964 DOI: 10.1038/aps.2009.31]
31 **Cao H**, Feng Q, Xu W, Li X, Kang Z, Ren Y, Du L. Genipin induced apoptosis associated with activation of the c-Jun NH2-terminal kinase

and p53 protein in HeLa cells. *Biol Pharm Bull* 2010; **33**: 1343-1348 [PMID: 20686229 DOI: 10.1248/bpb.33.1343]

32 **Shindo S**, Hosokawa Y, Hosokawa I, Ozaki K, Matsuo T. Genipin inhibits MMP-1 and MMP-3 release from TNF-a-stimulated human periodontal ligament cells. *Biochimie* 2014; **107** Pt B: 391-395 [PMID: 25457105 DOI: 10.1016/j.biochi.2014.10.008]

33 **Kaštelan S**, Orešković I, Bišćan F, Kaštelan H, Gverović Antunica A. Inflammatory and angiogenic biomarkers in diabetic retinopathy. *Biochem Med (Zagreb)* 2020; **30**: 030502 [PMID: 32774120 DOI: 10.11613/BM.2020.030502]

34 **Semeraro F**, Morescalchi F, Cancarini A, Russo A, Rezzola S, Costagliola C. Diabetic retinopathy, a vascular and inflammatory disease: Therapeutic implications. *Diabetes Metab* 2019; **45**: 517-527 [PMID: 31005756 DOI: 10.1016/j.diabet.2019.04.002]

35 **Sheemar A**, Soni D, Takkar B, Basu S, Venkatesh P. Inflammatory mediators in diabetic retinopathy: Deriving clinicopathological correlations for potential targeted therapy. *Indian J Ophthalmol* 2021; **69**: 3035-3049 [PMID: 34708739 DOI: 10.4103/ijo.IJO_1326_21]

36 **Zou W**, Luo S, Zhang Z, Cheng L, Huang X, Ding N, Pan Y, Wu Z. ASK1/p38mediated NLRP3 inflammasome signaling pathway contributes to aberrant retinal angiogenesis in diabetic retinopathy. *Int J Mol Med* 2021; **47**: 732-740 [PMID: 33416127 DOI: 10.3892/ijmm.2020.4833]

37 **Ge K**, Wang Y, Li P, Li M, Zhang W, Dan H, Hu X, Zhou J, Yang Q, Wang J, Song Z. Down-expression of the NLRP3 inflammasome delays the progression of diabetic retinopathy. *Microvasc Res* 2022; **139**: 104265 [PMID: 34662588 DOI: 10.1016/j.mvr.2021.104265]

38 **Hao J**, Zhang H, Yu J, Chen X, Yang L. Methylene Blue Attenuates Diabetic Retinopathy by Inhibiting NLRP3 Inflammasome Activation in STZ-Induced Diabetic Rats. *Ocul Immunol Inflamm* 2019; **27**: 836-843 [PMID: 29608341 DOI: 10.1080/09273948.2018.1450516]

39 **Li Z**, Han Y, Ji Y, Sun K, Chen Y, Hu K. The effect of a-Lipoic acid (ALA) on oxidative stress, inflammation, and apoptosis in high glucose-induced human corneal epithelial cells. *Graefes Arch Clin Exp Ophthalmol* 2023; **261**: 735-748 [PMID: 36058948 DOI: 10.1007/s00417-022-05784-6]

40 **Han X**, Kong J, Hartnett ME, Wang H. Enhancing Retinal Endothelial Glycolysis by Inhibiting UCP2 Promotes Physiologic Retinal Vascular Development in a Model of Retinopathy of Prematurity. *Invest Ophthalmol Vis Sci* 2019; **60**: 1604-1613 [PMID: 30995317 DOI: 10.1167/iovs.19-26553]

41 **Cho YS**. Genipin, an Inhibitor of UCP2 as a Promising New Anticancer Agent: A Review of the Literature. *Int J Mol Sci* 2022; **23** [PMID: 35628447 DOI: 10.3390/ijms23105637]

42 **Bernardo AF**, Cortez E, Neves FA, Vieira AK, Soares Vde M, Rodrigues-Cunha AC, Andrade DC, Thole AA, Gabriel-Costa D, Brum PC, Moura AS, Garcia-Souza ÉP. Overnutrition during lactation leads to impairment in insulin signaling, up-regulation of GLUT1 and increased mitochondrial carbohydrate oxidation in heart of weaned mice. *J Nutr Biochem* 2016; **29**: 124-132 [PMID: 26608021 DOI: 10.1016/j.jnutbio.2015.09.021]

43 **Brandi J**, Cecconi D, Cordani M, Torrens-Mas M, Pacchiana R, Dalla Pozza E, Butera G, Manfredi M, Marengo E, Oliver J, Roca P, Dando I, Donadelli M. The antioxidant uncoupling protein 2 stimulates hnRNPA2/B1, GLUT1 and PKM2 expression and sensitizes pancreas cancer cells to glycolysis inhibition. *Free Radic Biol Med* 2016; **101**: 305-316 [PMID: 27989750 DOI: 10.1016/j.freeradbiomed.2016.10.499]

44 **Shanmugam MK**, Shen H, Tang FR, Arfuso F, Rajesh M, Wang L, Kumar AP, Bian J, Goh BC, Bishayee A, Sethi G. Potential role of genipin in cancer therapy. *Pharmacol Res* 2018; **133**: 195-200 [PMID: 29758279 DOI: 10.1016/j.phrs.2018.05.007]

45 **Koudouna E**, Huertas-Bello M, Rodriguez CN, Consuelo Henao S, Navarrete ML, Avila MY. Genipin in an Ex Vivo Corneal Model of Bacterial and Fungal Keratitis. *Transl Vis Sci Technol* 2021; **10**: 31 [PMID: 34436544 DOI: 10.1167/tvst.10.9.31]

46 **Donovan C**, Koudouna E, Margo CE, Avila MY, Espana EM. Genipin Delays Corneal Stromal Enzymatic Digestion. *Transl Vis Sci Technol* 2021; **10**: 25 [PMID: 34424287 DOI: 10.1167/tvst.10.9.25]

47 **Fan X**, Lin L, Cui B, Zhao T, Mao L, Song Y, Wang X, Feng H, Qingxiang Y, Zhang J, Jiang K, Cao X, Wang B, Sun C. Therapeutic potential of genipin in various acute liver injury, fulminant hepatitis, NAFLD and other non-cancer liver diseases: More friend than foe. *Pharmacol Res* 2020; **159**: 104945 [PMID: 32454225 DOI: 10.1016/j.phrs.2020.104945]

48 **Mir SA**, Li Y, Story JD, Bal S, Awdishu L, Street AA, Mehta RL, Singh P, Vaingankar SM. Mice overexpressing chromogranin A display hypergranulogenic adrenal glands with attenuated ATP levels contributing to the hypertensive phenotype. *J Hypertens* 2018; **36**: 1115-1128 [PMID: 29389743 DOI: 10.1097/HJH.0000000000001678]

49 **Teimouri S**, Kasapis S. Morphology of genipin-crosslinked BSA networks yields a measurable effect on the controlled release of vitamin B6. *Food Chem* 2020; **314**: 126204 [PMID: 31978719 DOI: 10.1016/j.foodchem.2020.126204]

50 **Sun K**, Hu K. Preparation and Characterization of Tacrolimus-Loaded SLNs in situ Gel for Ocular Drug Delivery for the Treatment of Immune Conjunctivitis. *Drug Des Devel Ther* 2021; **15**: 141-150 [PMID: 33469266 DOI: 10.2147/DDDT.S287721]

51 **Anton N**, Dragoi EN, Tarcoveanu F, Ciuntu RE, Lisa C, Curteanu S, Doroftei B, Ciuntu BM, Chiselita D, Bogdanici CM. Assessing Changes in Diabetic Retinopathy Caused by Diabetes Mellitus and Glaucoma Using Support Vector Machines in Combination with Differential Evolution Algorithm. *Appl Sci* 2021; **11**: 3944 [DOI: 10.3390/app11093944]

52 **Li W**, Webster KA, LeBlanc ME, Tian H. Secretogranin III: a diabetic retinopathy-selective angiogenic factor. *Cell Mol Life Sci* 2018; **75**: 635-647 [PMID: 28856381 DOI: 10.1007/s00018-017-2635-5]

53 **Jiao MF**, Sun S, Wang MM, Dong LJ, Hu BJ, Liu JP, Li W, Li XR. [The observation of secretogranin III in the peripheral blood and vitreous of patients with diabetic retinopathy]. *Zhonghua Yan Ke Za Zhi* 2020; **56**: 933-937 [PMID: 33342120 DOI: 10.3760/cma.j.cn112142-20200410-00260]

54 **LeBlanc ME**, Wang W, Chen X, Caberoy NB, Guo F, Shen C, Ji Y, Tian H, Wang H, Chen R, Li W. Secretogranin III as a disease-associated ligand for antiangiogenic therapy of diabetic retinopathy. *J Exp Med* 2017; **214**: 1029-1047 [PMID: 28330905 DOI: 10.1084/jem.20161802]

55 **LeBlanc ME**, Wang W, Ji Y, Tian H, Liu D, Zhang X, Li W. Secretogranin III as a novel target for the therapy of choroidal neovascularization. *Exp Eye Res* 2019; **181**: 120-126 [PMID: 30633921 DOI: 10.1016/j.exer.2019.01.009]

56 **Togayachi A**, Iwaki J, Kaji H, Matsuzaki H, Kuno A, Hirao Y, Nomura M, Noguchi M, Ikehara Y, Narimatsu H. Glycobiomarker, Fucosylated Short-Form Secretogranin III Levels Are Increased in Serum of Patients with Small Cell Lung Carcinoma. *J Proteome Res* 2017; **16**: 4495-4505 [PMID: 28949141 DOI: 10.1021/acs.jproteome.7b00484]

World Journal of Diabetes

Submit a Manuscript: https://www.f6publishing.com

World J Diabetes 2023 September 15; 14(9): 1369-1384

DOI: 10.4239/wjd.v14.i9.1369

ISSN 1948-9358 (online)

ORIGINAL ARTICLE

Basic Study

XB130 inhibits healing of diabetic skin ulcers through the PI3K/Akt signalling pathway

Xin-Lin Zhu, Dong-Ying Hu, Zhao-Xiang Zeng, Wei-Wei Jiang, Tian-Yang Chen, Tian-Cheng Chen, Wan-Qing Liao, Wen-Zhi Lei, Wen-Jie Fang, Wei-Hua Pan

Specialty type: Endocrinology and metabolism

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Lee CG, United States; Mohamed M, United States

Received: May 24, 2023
Peer-review started: May 24, 2023
First decision: June 12, 2023
Revised: July 6, 2023
Accepted: August 2, 2023
Article in press: August 2, 2023
Published online: September 15, 2023

Xin-Lin Zhu, Dong-Ying Hu, Wei-Wei Jiang, Tian-Yang Chen, Tian-Cheng Chen, Wan-Qing Liao, Wen-Zhi Lei, Wen-Jie Fang, Wei-Hua Pan, Department of Dermatology, Shanghai Key Laboratory of Medical Mycology, Changzheng Hospital, Naval Medical University, Shanghai 200003, China

Zhao-Xiang Zeng, Department of Vascular Surgery, Department of Vascular Surgery, Changhai Hospital, Navy Medical University, Shanghai 20003, China

Corresponding author: Wei-Hua Pan, PhD, Chief Doctor, Department of Dermatology, Shanghai Key Laboratory of Medical Mycology, Changzheng Hospital, Naval Medical University, No. 415 Fengyang Road, Shanghai 200003, China. panweihua9@sina.com

Abstract

BACKGROUND

Diabetic skin ulcers, a significant global healthcare burden, are mainly caused by the inhibition of cell proliferation and impaired angiogenesis. XB130 is an adaptor protein that regulates cell proliferation and migration. However, the role of XB130 in the development of diabetic skin ulcers remains unclear.

AIM

To investigate whether XB130 can regulate the inhibition of proliferation and vascular damage induced by high glucose. Additionally, we aim to determine whether XB130 is involved in the healing process of diabetic skin ulcers, along with its molecular mechanisms.

METHODS

We conducted RNA-sequencing analysis to identify the key genes involved in diabetic skin ulcers. We investigated the effects of XB130 on wound healing using histological analyses. In addition, we used reverse transcription-quantitative polymerase chain reaction, Western blot, terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling staining, immunofluorescence, wound healing, and tubule formation experiments to investigate their effects on cellular processes in human umbilical vein endothelial cells (HUVECs) stimulated with high glucose. Finally, we performed functional analysis to elucidate the molecular mechanisms underlying diabetic skin ulcers.

RESULTS

RNA-sequencing analysis showed that the expression of XB130 was up-regulated in the tissues of diabetic skin ulcers. Knockdown of XB130 promoted the healing of skin wounds in mice, leading to an accelerated wound healing process and shortened wound healing time. At the cellular level, knockdown of XB130 alleviated high glucose-induced inhibition of cell proliferation and angiogenic impairment in HUVECs. Inhibition of the PI3K/Akt pathway removed the proliferative effects and endothelial protection mediated by XB130.

CONCLUSION

The findings of this study indicated that the expression of XB130 is up-regulated in high glucose-stimulated diabetic skin ulcers and HUVECs. Knockdown of XB130 promotes cell proliferation and angiogenesis *via* the PI3K/Akt signalling pathway, which accelerates the healing of diabetic skin ulcers.

Key Words: XB130; Diabetes mellitus; Diabetic skin ulcers; PI3K/Akt signalling pathway

Core Tip: The role of XB130 in the occurrence and development of diabetic skin ulcers healing is unclear. This study showed that the expression of XB130 was up-regulated in tissues of diabetic skin ulcers and human umbilical vein endothelial cells (HUVECs) stimulated by high glucose. Knockdown of XB130 promote the healing of skin wounds in mice, leading to an accelerated wound healing process and shortened wound healing time and alleviated hyperglycemia-induced cell proliferation inhibition and angiogenic impairment in HUVECs.

Citation: Zhu XL, Hu DY, Zeng ZX, Jiang WW, Chen TY, Chen TC, Liao WQ, Lei WZ, Fang WJ, Pan WH. XB130 inhibits healing of diabetic skin ulcers through the PI3K/Akt signalling pathway. *World J Diabetes* 2023; 14(9): 1369-1384
URL: https://www.wjgnet.com/1948-9358/full/v14/i9/1369.htm
DOI: https://dx.doi.org/10.4239/wjd.v14.i9.1369

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disease in which the body does not produce enough insulin or reacts abnormally to the hormone, leading to typically high blood sugar levels. In 2021, more than 37 million Americans have diabetes. Of those, 28.7 million have been diagnosed and 8.5 million are undiagnosed[1]. Impaired wound healing often results in severe skin ulcers[2]; a high percentage (15%-27%) of diabetic foot skin ulcer cases require lower extremity amputation owing to treatment failure[3]. Therefore, developing effective strategies to prevent and treat cutaneous wounds in patients with diabetes is crucial.

Impaired healing of diabetic wounds is characterised by significant deficits in cellular proliferation and migration, and a notable decrease in protein synthesis[4,5]. These impairments result in delayed re-epithelialisation, angiogenesis, and granulation tissue formation[6]. Numerous molecular pathways play crucial roles in cell proliferation and protein synthesis during the wound healing process[7]. Laplante *et al*[8] discovered that mTOR signalling could effectively treat diabetic skin damage by promoting cell proliferation, inhibiting skin cell apoptosis, and accelerating epithelial regeneration.

XB130 is a bridging protein that serves as the mediator of multiple tyrosine kinases, playing an important role in regulating cell proliferation, survival, migration, and invasion[9,10]. As a tumor suppressor in carcinogen-induced skin tumorigenesis, knockout of XB130 significantly up-regulated epidermal tumor cell proliferation[11]. XB130 also found in stomach, oesophagus, and thyroid epithelial cells, and has been studied as a signal transduction protein[12-14]. The expression of XB130 were up-regulated in cholangiocarcinoma, non-small cell-lung cancer, prostate cancer, esophageal squamous cell carcinoma and gastric cancer, regulating XB130 expression may inhibit the development and progression of tumors[12,13,15-17]. However, whether XB130 regulate wound healing in patients with diabetes and the underlying molecular mechanisms have not been reported.

In this study, we first confirmed that the expression of XB130 was up-regulation in tissues of diabetic skin ulcer mice. Knockdown of XB130 can accelerate the healing process and shorten the healing time of skin wound in mice. At the cellular level, down-regulation of XB130 alleviated hyperglycaemia-induced inhibition of cell proliferation and angiogenic impairment in human umbilical vein endothelial cells (HUVECs). Mechanistically, down-regulation of XB130 accelerates healing of diabetic skin ulcers by promoting cell proliferation and angiogenesis through the PI3K/Akt signalling pathway.

MATERIALS AND METHODS

Animals

C57BL/6 mice (20-25 g, 6 wk old) were obtained from Beijing Weitong Lihua Experimental Animal Technology, China. The mice were provided with free access to food and water; the temperature and relative humidity of the room were maintained at 20 °C-24 °C and 40%-60%, respectively. The mice were allowed to acclimatize to their surroundings for 1 wk prior to the experiment.

Diabetes induction and wound creation

Diabetes was induced in mice by injecting them with streptozotocin (STZ; 40 mg/kg) for 5 consecutive days. The control group received an equivalent dose of citrate buffer solution[18]. Diabetic status was determined in mice if their blood glucose levels exceeded 250 mg/dL following STZ injection. Both diabetic and normal mice were anaesthetised with 2% isoflurane. Full-thickness excisional wounds measuring 8 mm in diameter were created on the dorsal skin of the mice.

Adenovirus

Adenovirus knockdown of XB130 targeting sequence and its corresponding control were constructed by ABM (Nanjing, China). Mice were given tail vein injections of adenovirus at the concentration of 5×10^{11} genome-equivalents five days before surgery.

Haematoxylin & eosin and Masson staining

On day 14 post-treatment, the mice were euthanised and their wound tissues were fixed in 4% paraformaldehyde and embedded in paraffin. Haematoxylin & eosin (HE) and Masson staining were performed using kits from Solarbio (China) and Sigma (United States), respectively, following the manufacturer's protocols. Images were captured using a Ci-L microscope (Nikon, Japan) and analysed using the Image J software (version 1.8.0).

Cell culture and transfection

HUVECs were sourced from Guangzhou Saliai Stemcell Co., Ltd. And cultured using EGM-2 BulletKit (Lonza). Subconfluent cells from passages five to seven were used for the experiments. Before cell culture procedures, stock media were replaced with phenol red-free low-glucose DMEM (Gibco, United States) supplemented with 1% calf serum (Gibco, CA, United States) and left for 12 h. HUVECs were then exposed to EGM-2 supplemented with either normal glucose (NG, 5.5 mmol/L) or high glucose (HG, 33 mmol/L) for 72 h, while using D-mannitol as an osmotic control for the HG condition. The knockdown plasmid of XB130 and its corresponding control plasmid were obtained from Guangdong Ruibo Biotechnology Co., Ltd. Transfection efficiency was confirmed by reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and Western blot.

Transferase-mediated dUTP nick end labeling assay

Transferase-mediated dUTP nick end labeling (TUNEL) staining was performed according to the instruction of TUNEL apoptosis detection kit (Promega, United States). The cells were seeded onto 24-well plates and incubated at 37 °C for 12 h. After staining, cell apoptosis was counted by photographing. The apoptosis cells were brown considered as the TUNEL-positive cells. Apoptotic rate was evaluated.

Tubular formation assay

Upon completion of the experimental protocol, HUVECs were stained with calcein (Corning, United States). The stained cells were then replated onto Matrigel-precoated 24-well plates containing growth-factor reduced Matrigel (150 μL/well) and incubated at 37 °C for 12 h. To assess capillary-like tubule formation, a computer-assisted microscope was used to detect tube-like structures that were at least four times longer than their widths.

Wound healing assay

To evaluate cell migration, a scratch assay was performed as described previously[19]. Following overnight incubation, the cells were seeded onto a 3.5-cm-diameter dish and allowed to form a confluent monolayer. Subsequently, a wound was created by scratching with a 200-mL pipette tip. The wounded cell monolayers were then imaged using a CoolSNAP HQ CCD (Nippon Roper Japan) at 0 h and 24 h post-wounding.

Immunofluorescence staining

Well-grown HUVECs were fixed with 4.0% paraformaldehyde in phosphate-buffered saline for 10 min and permeabilised with 0.5% Triton for 15 min. Antibodies against PCNA, Ki67, Bcl2, Bax, and Cleaved caspase-3 obtained from Abcam (United States) were then used to incubate the cells overnight, followed by a 1 h incubation with or without Alexa Fluor 488-conjugated anti-mouse IgG secondary antibody (Abcam, United States) at room temperature. Nuclei were labelled with the fluorescent dye DAPI after 5 min of incubation. The cells were then observed under a confocal microscope (Leica, Germany) under each experimental condition.

RT-qPCR

Total RNA was extracted from skin tissues or HUVECs using TRIzol reagent (Invitrogen, United States) and reverse-transcribed into cDNA using a Tiangen Biotechnology (China) kit. RT-qPCR was conducted with β-Actin as an internal

Table 1 Primer sequences

Gene	Forward (5'→3')	Reverse (5'→3')
XB130	AAGCAGCAGCTCTGATGAGG	GGTCTGGAAGGCTCTTCTGA
β-Actin	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT

Table 2 Antibodies used in this study

Gene	Brand	Provenance
XB130	Abcam	Rabbit
Cleaved-caspase-3	Abcam	Rabbit
Bcl2	Abcam	Rabbit
Bax	Abcam	Rabbit
Ki67	Abcam	Rabbit
PCNA	Abcam	Rabbit
AKT	Abcam	Rabbit
p-AKT	Abcam	Rabbit
p85α	Abcam	Rabbit
p-p85α	Abcam	Rabbit
β-Actin	Sigma	Mouse

control, using 2 × SYBR Green QPCR Master Mix (Shanghai Dongsheng Biotechnology, China). The relative gene expression was determined using the $2^{-\Delta\Delta Ct}$ method, and the primer sequences are provided in Table 1.

Western blot

The protein from cell or tissue was extracted by RIPA and PMSF (Shanghai Life Mode Engineering, Shanghai, China), and the concentration of protein was determined by BCA kit (Shanghai Dongsheng Biotechnology, shanghai, China). The PVDF membrane (0.22 μm, Millipore ISEQ00010, United States) was then incubated with primary antibodies (Table 2) overnight at 4 °C, followed by incubation with HRP-conjugated secondary antibodies (Abcam, United States). Protein bands were detected using Prime Western Blot Detection Reagent (Cytiva, United Kingdom). A ChemiDoc MP imaging system (Tanon 4800, China) was used to detect chemiluminescence, and the ImageJ software was used to analyse the grey values of the bands.

Statistical analysis

Experimental data were analysed using GraphPad Prism 9.0, SPSS 24.0, and R software 4.2.0. Statistical tests were chosen based on the distribution and variance homogeneity of the data. For normally distributed and homogeneous variance data, *t*-tests were used to compare two groups. For multiple group comparisons Dunnett's-T3 test was used based on the distribution and variance. Data are presented as mean ± SD, and statistical significance was set at $P < 0.05$.

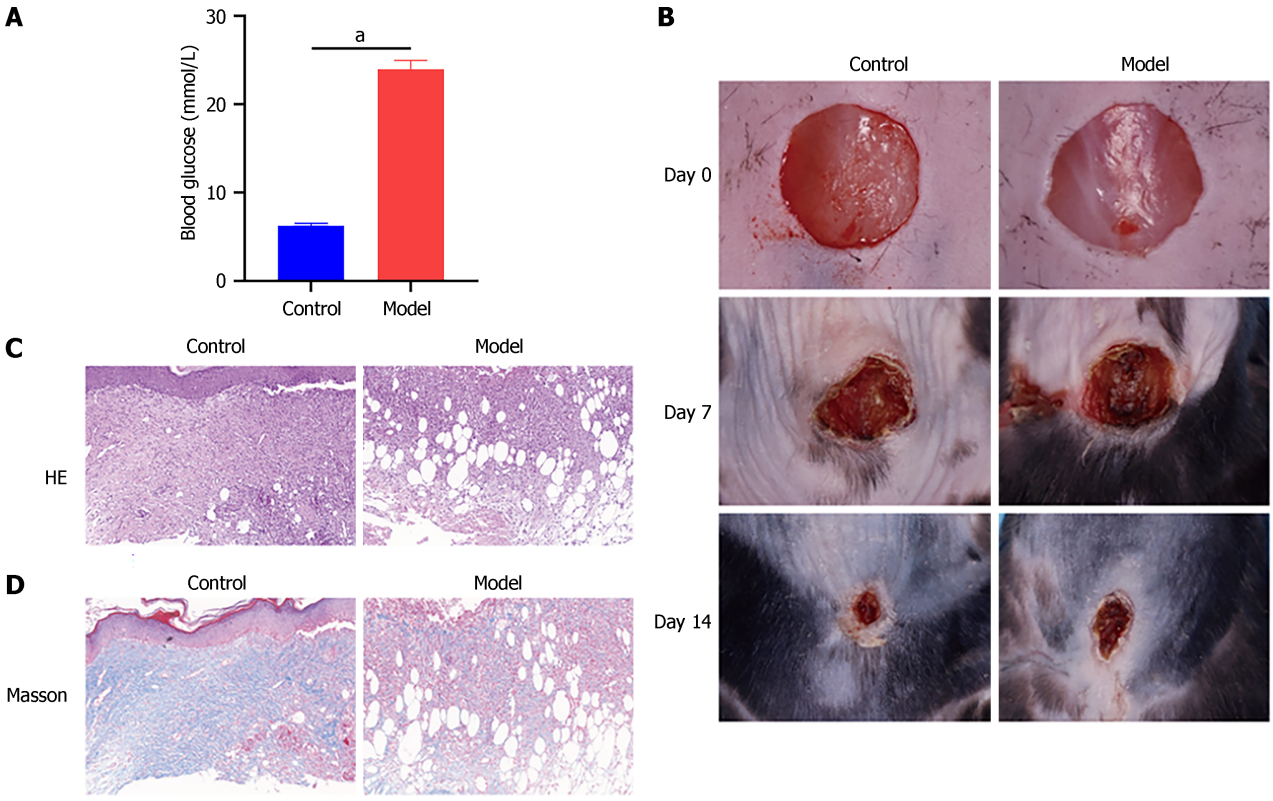
RESULTS

Construction of a diabetic mice model

The toxicity of STZ to pancreatic cells makes it a popular choice for inducing diabetes mellitus in mice and rats[20]. After administering STZ for 5 consecutive days, the model group showed significantly elevated fasting blood glucose levels compared with the control group (Figure 1A). As shown in Figure 1B, the model group exhibited slower wound healing time and rate. Tissue samples from the model and control groups were collected on day 14 for HE and Masson staining. The wounds of the model mice showed severe tissue damage, disorganised tissue granulation, and reduced collagen deposition (Figure 1C and D).

HG inhibited proliferation and tubule formation in HUVECs

To simulate the HG environment during diabetic wound healing, we incubated HUVECs in an HG medium. Immunofluorescence staining for Bax and Cleaved caspase-3 in HG-treated HUVECs was higher than that in the NG group, whereas staining for PCNA, Ki67, and Bcl2 was lower (Figure 2A). The results of immunofluorescence analysis were confirmed by Western blot analysis (Supplementary Figure 1A and B). Moreover, HG treatment significantly impaired



DOI: 10.4239/wjd.v14.i9.1369

Figure 1 Establishment of diabetic model using streptozotocin. A: The blood glucose level of mice, control (n = 10) and model (n = 10); B: Representative skin wound images from control and model mice at day 0 d, 7 d, and 14 d; C and D: HE and Masson staining of skin wound in each group. ^{a}P < 0.0001, compared to the control group.

HUVEC migration and tube-forming activity (Figure 2B and C). We used the TUNEL assay to assess apoptosis in different groups and revealed an increased number of TUNEL-positive cells in the HG group (Figure 2D).

HG led to increased expression of XB130 in vivo and in vitro

To investigate the genes involved in diabetic wound healing, RNA sequencing was performed on the ulcer tissues from control and model mice group. Prior to differential expression analysis, we conducted background correction, normalisation, and gene filtering. Total 1547 differentially expressed genes (DEGs) were yielded (Figure 3A-C). We employed the STRING database for protein-protein interaction (PPI) network analysis to better understand the interactions between DEGs. Using the CytoHubba plug-in in Cytoscape, we identified hub genes based on their degree, betweenness, and closeness centrality, and found XB130 to be the most significant hub gene (Figure 3D).

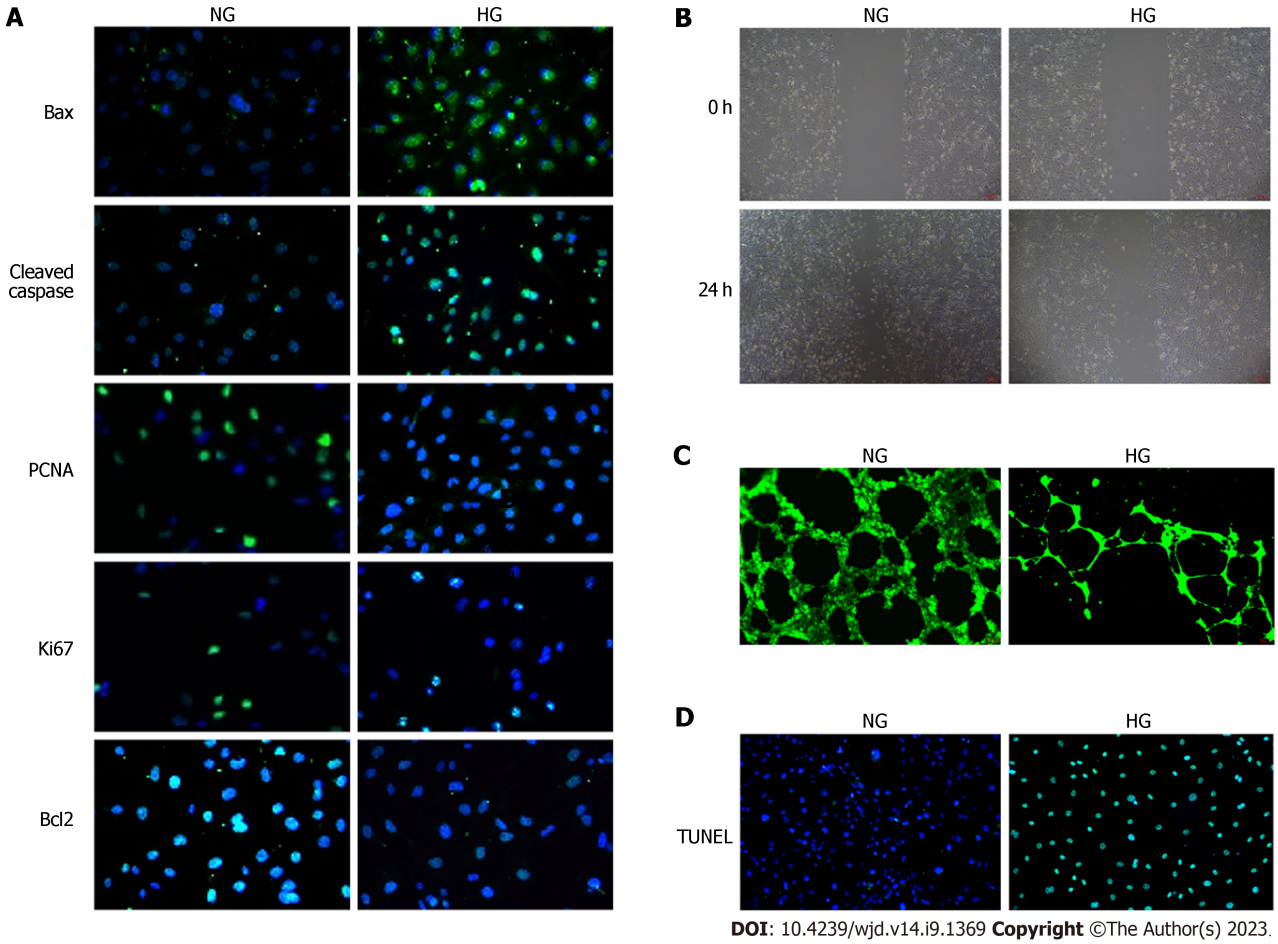
To confirm the accuracy of the microarray results, we assessed XB130 mRNA and protein levels using RT-qPCR and Western blot. Compared with the control group, the mRNA level of XB130 was significantly increased in the model group (Figure 4A). HG also resulted in increased mRNA expression of XB130 in HUVECs (Figure 4D). Western blot analysis validated the RT-qPCR results (Figure 4B, C, E, and F).

Down-regulation of XB130 promoted wound healing in diabetic mice

Next, we established a mouse model knockdown XB130 (XB130-KD) in diabetic mice by injection of adenovirus vectors carrying short hairpin RNA targeting XB130. The XB130-KD group showed a significant decrease in protein expression of XB130 compared with the vector group (Figure 5A and B). Subsequently, we evaluated the wound healing process and found that knocking down XB130 Led to a shorter wound healing time and a higher wound healing rate (Figure 5C). Furthermore, histological analysis revealed that knocking down XB130 resulted in better granulation formation, collagen deposition, and denser alignment compared with the model group (Figure 5D).

Down-regulation of XB130 attenuated HG-induced inhibitory effects on proliferation and tubule formation in vitro

We evaluated the potential function of XB130 in HUVECs. After transfection, the expression of XB130 was significantly reduced in the XB130-KD group (Figure 6A and B). Immunofluorescence results showed that XB130 down-expression had a restraining effect on cell apoptosis and promoted cell proliferation in HUVECs (Figure 6C). These findings were further confirmed by Western blot analysis (Supplementary Figure 2A and B). Additionally, the TUNEL assay indicated that knocking down of XB130 reduced HG-induced apoptosis in HUVECs (Supplementary Figure 2C). Furthermore, XB130 down-expression counteracted the negative impact of hyperglycaemia on the migration and tube-forming activity of HUVECs (Figure 6D-E).



DOI: 10.4239/wjd.v14.i9.1369

Figure 2 High glucose inhibited proliferation and tubule formation of human umbilical vein endothelial cells. A: Immunofluorescence staining of Bax, cleaved caspase-3, PCNA, Ki67, and Bcl2 in human umbilical vein endothelial cells (HUVECs); B: Wound healing assay in HUVECs, scale bars = 200 μm; C: Capillary-like tubule formation, scale bars = 200 μm; D: Transferase-mediated dUTP nick end labeling assay.

HG inhibited the PI3K/Akt signalling pathway in vivo and in vivo

To investigate the mechanism underlying delayed wound healing in mice patients with diabetes, we conducted a functional enrichment analysis of the previously obtained hub genes. Gene Ontology (GO) analysis revealed that the hub genes were involved in the of cell proliferation, differentiation, and tube morphogenesis (Figure 7A). Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis indicated that these genes were predominantly associated with the PI3K/Akt, Ras, p53, and NF-kappa B signalling pathways. Notably, the PI3K/Akt signalling pathway had the highest number of differentially expressed genes (Figure 7B).

To validate this finding, we performed Western blot analysis and confirmed that the ratio of p-AKT to AKT was significantly decreased in diabetic mice (Figure 7C and D). Similar to results *in vivo*, HG levels inherited the PI3K/Akt signalling pathway in HUVECs (Figure 7E and F).

Down-regulation of XB130 accelerated wound healing in diabetic mice via the PI3K/Akt signalling pathway

To investigate the effect of XB130 on wound healing *via* this pathway, diabetic mice with reduced XB130 expression were treated with LY294002, an inhibitor of the PI3K/Akt signalling pathway. Western blot analysis revealed that knocking down XB130 stimulated the Akt phosphorylation, however, the AKT phosphorylation was suppressed by LY294002 treatment (Figure 8A and B). Additionally, wound healing time was significantly longer in the LY294002 group than in the XB130-KD group (Figure 8C). Histological analysis showed that LY294002 treatment decreased collagen deposition in the wound area and angiogenesis in the granulation tissue (Figure 8D).

Down-regulation of XB130 reversed HG-induced inhibitory effects on proliferation and tubule formation via the PI3K/Akt signalling pathway

Western blot analysis showed that knocking down XB130 stimulated the PI3K/Akt signalling pathway, which was subsequently inhibited by LY294002 in HUVECs (Figure 9A and B). In LY294002-treated HUVECs, the protective effect of knocking down XB130 against HG was lost, resulting in increased apoptosis and decreased proliferation (Figure 9C). Western blot analysis yielded similar results (Supplementary Figure 3A and B). The TUNEL assay further demonstrated that LY294002 reversed the inhibitory effect of knocking down XB130 on HUVEC apoptosis (Supplementary Figure 3C). Moreover, in LY294002-treated HUVECs, the effects of knocking down XB130 on the pro-migration and tube-forming

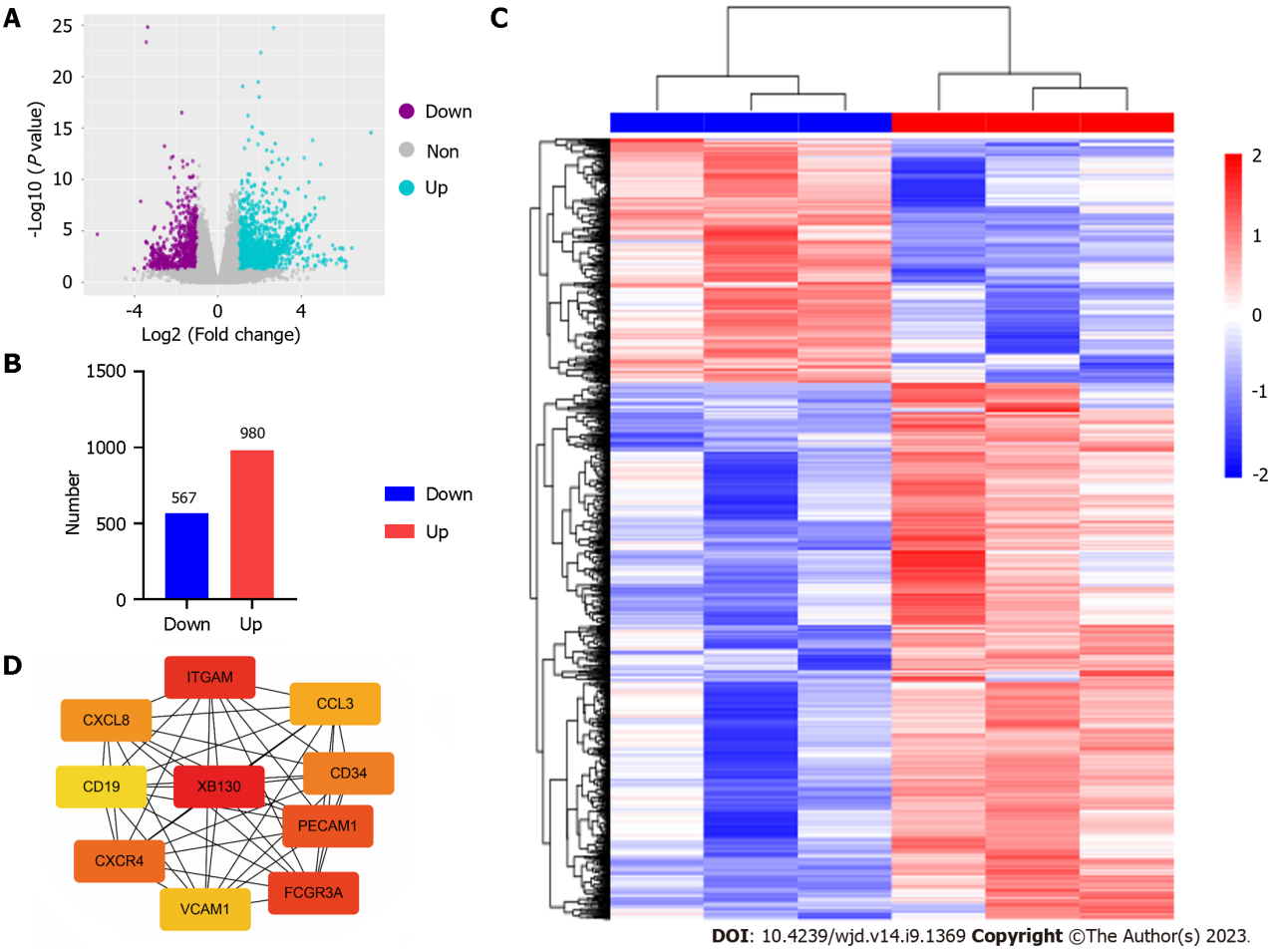


Figure 3 Differential expression genes identification and hub genes identification. A: Volcano plot; B: Histogram; C: Heatmap; D: Top 10 hub genes obtained from protein-protein interaction network.

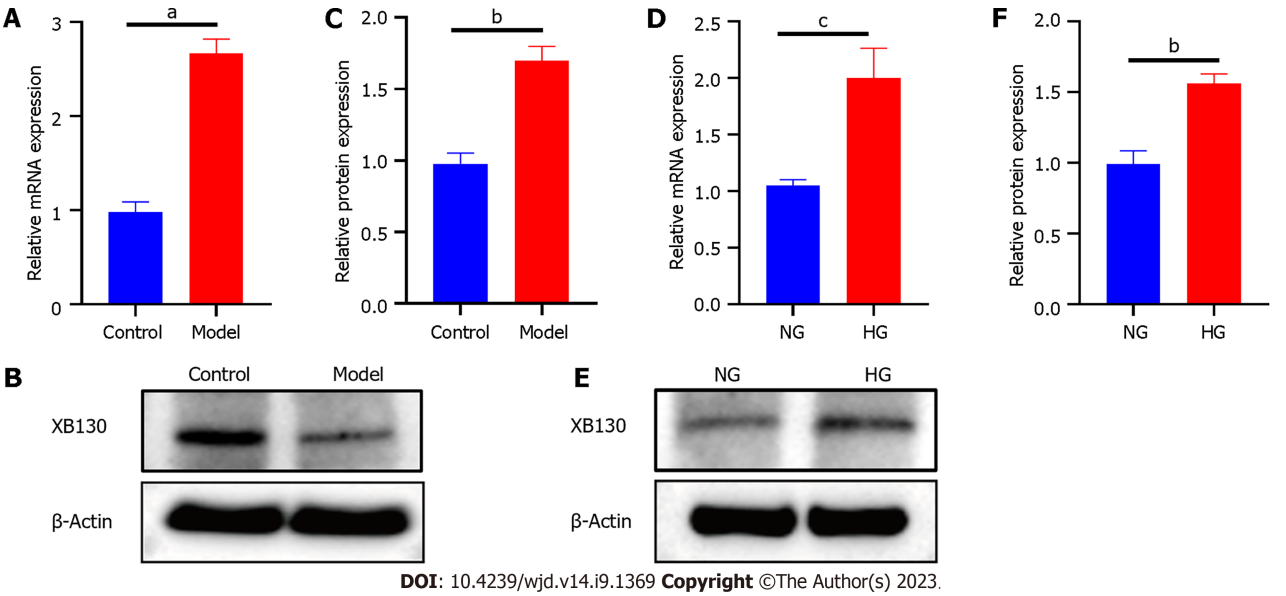
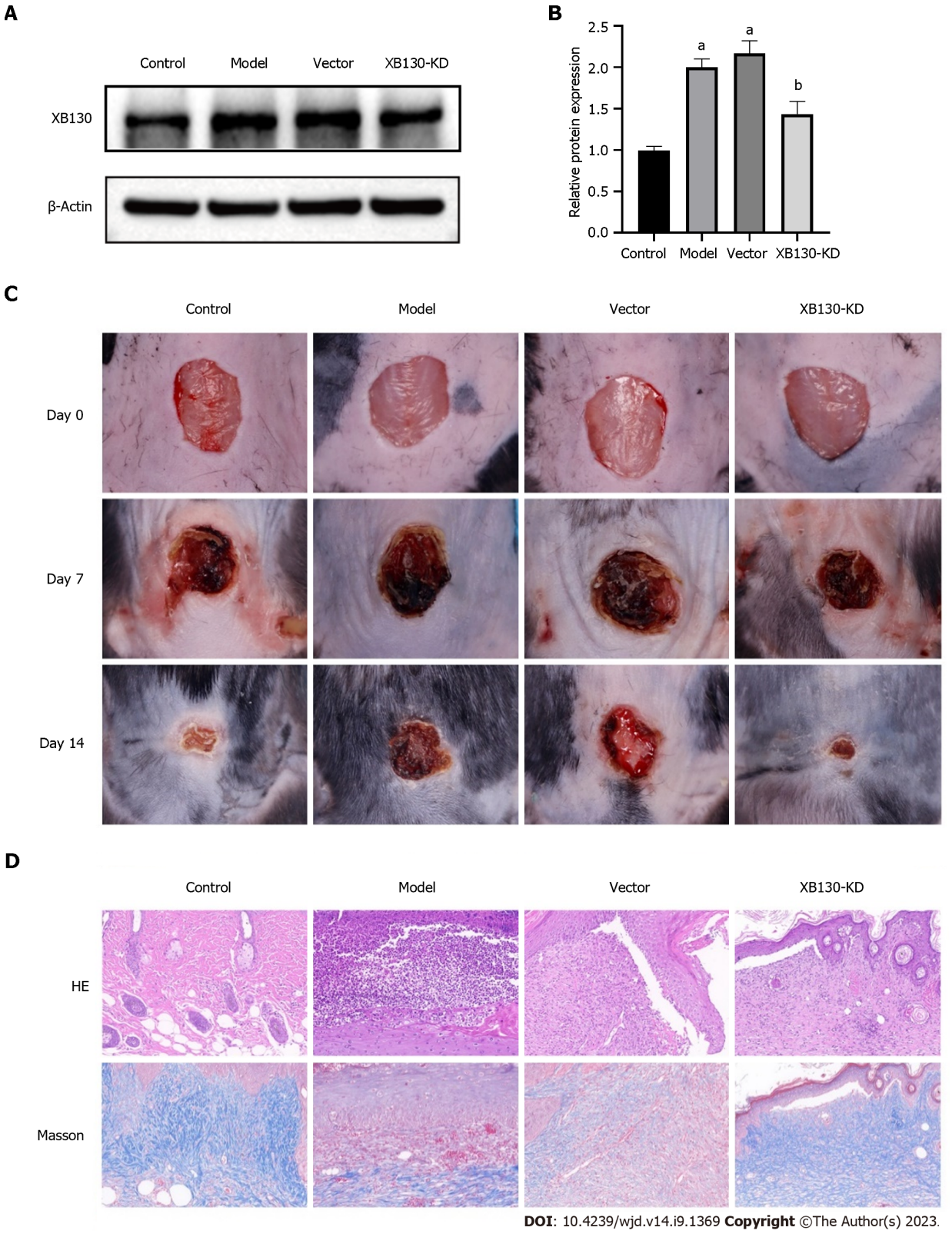
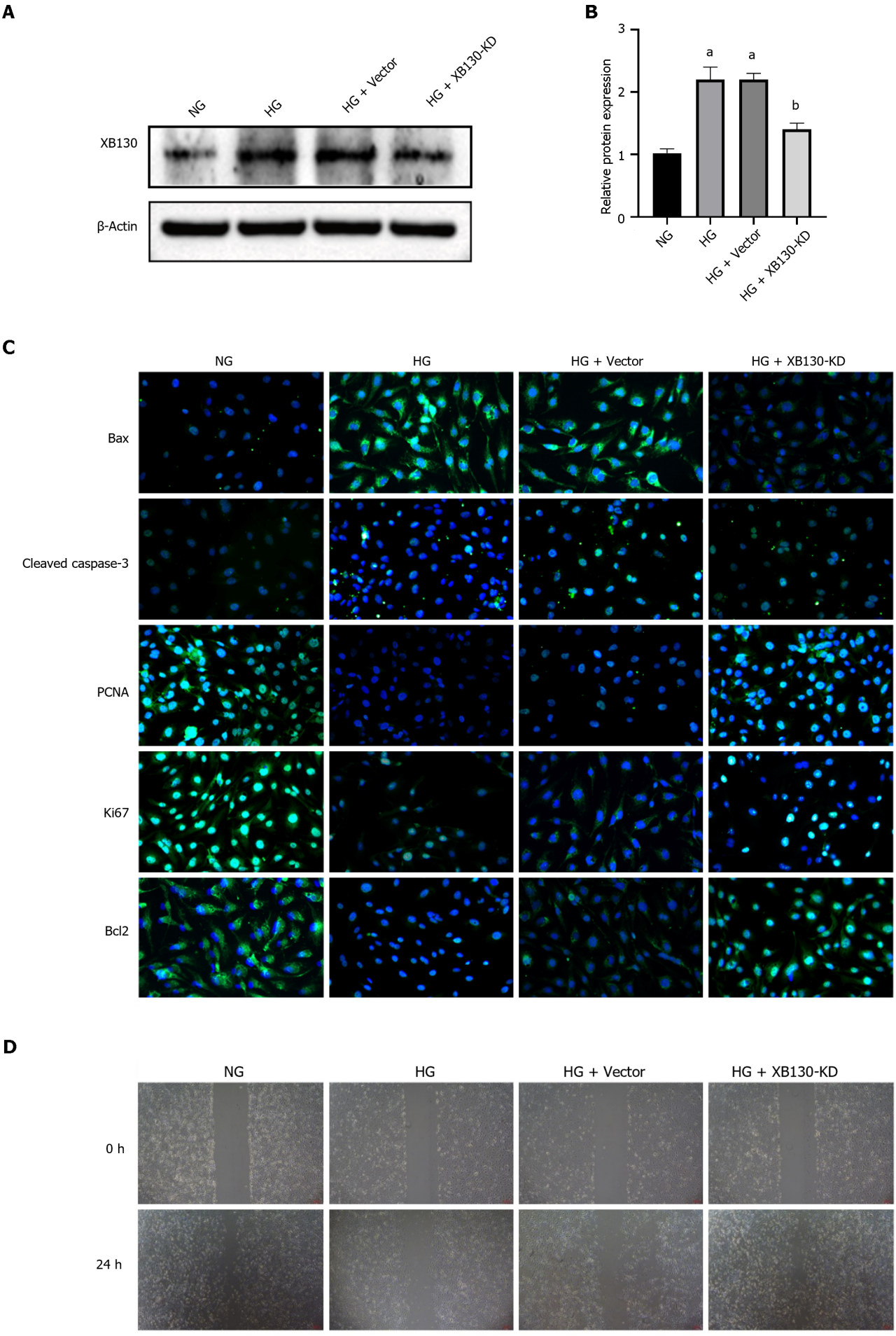


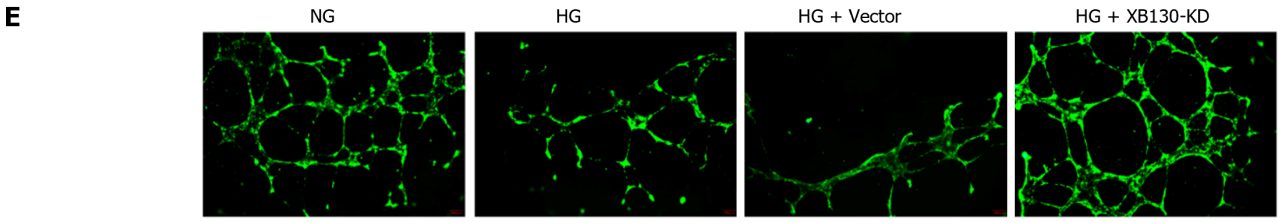
Figure 4 High glucose environment increased the expression of XB130. A: Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis of XB130 mRNA expression in mice; B: Western blot analysis of XB130 protein expression in mice; C: Quantification of Western blot results; D: RT-qPCR analysis of XB130 mRNA expression in human umbilical vein endothelial cells (HUVECs); E: Western blot analysis of XB130 protein expression in HUVECs; F: Quantification of Western blot results. $^{c}P < 0.05$, $^{b}P < 0.01$, $^{a}P < 0.001$, compared to the control or NG group.



DOI: 10.4239/wjd.v14.i9.1369

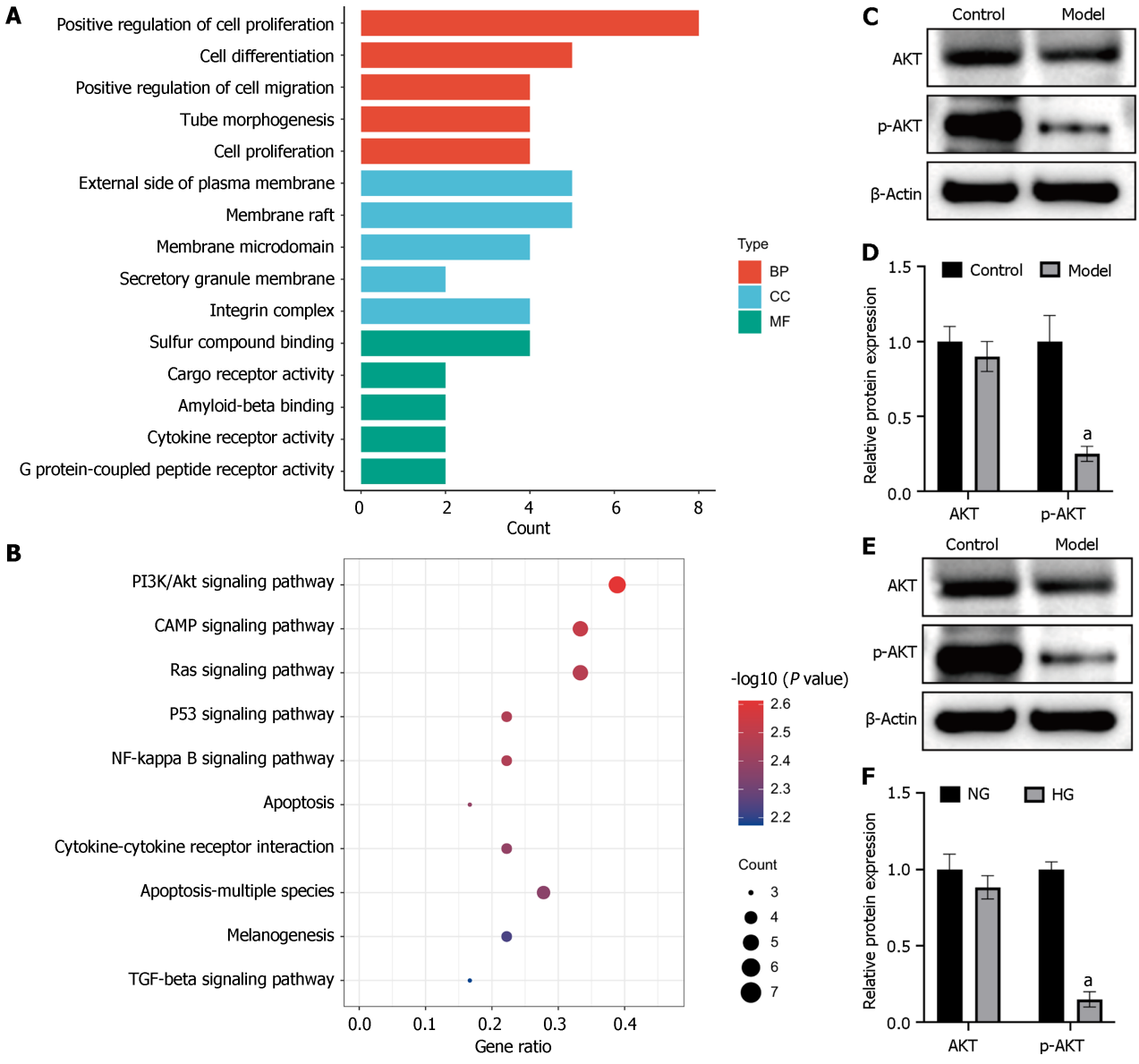
Figure 5 Knock-down of XB130 accelerated wound healing in diabetic mice. A: Western blot analysis of XB130 protein expression in each group; B: Quantification of Western blot results; C: Representative skin wound images from control and model mice at day 0 d, 7 d, 14 d, control (n = 10), model (n = 10), Vector (n = 10), and XB130-KD (n = 10); D: HE and Masson staining of skin wound in each group. $^{a}P < 0.001$, compared to the Control group; $^{b}P < 0.01$, compared to the Vector group.


A
NG
HG
HG + Vector
HG + XB130-KD
XB130
β-Actin
B
Relative protein expression
a
a
b
C
Bax
Cleaved caspase-3
PCNA
Ki67
Bcl2
D
0 h
24 h



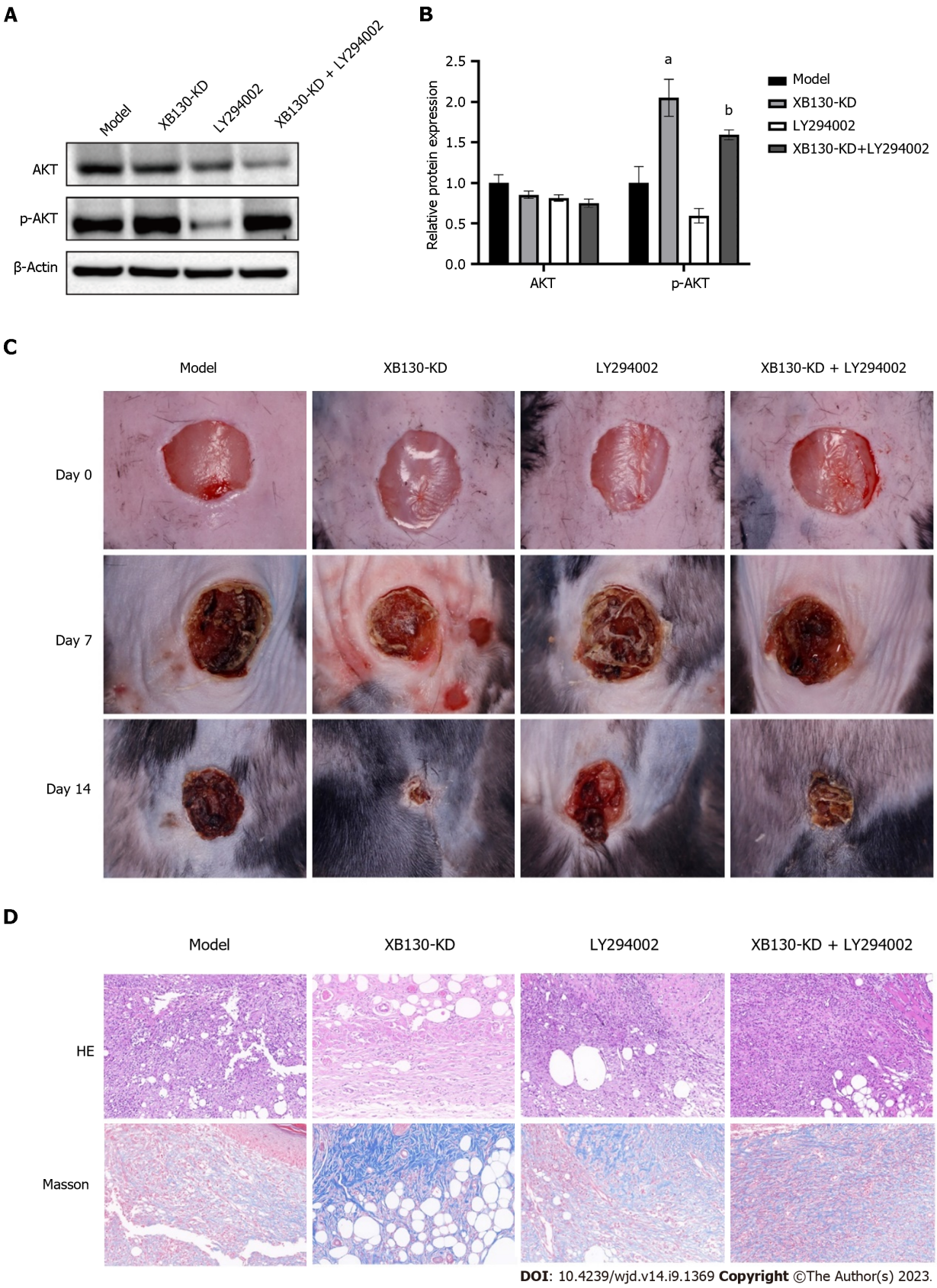
DOI: 10.4239/wjd.v14.i9.1369 **Copyright** ©The Author(s) 2023.

Figure 6 Knockdown of XB130 attenuated hyperglycemia-induced inhibitory effects on proliferation and tubule formation of human umbilical vein endothelial cells. A: Western blot analysis of XB130 protein expression in human umbilical vein endothelial cells; B: Quantification of Western blot results; C: Immunofluorescence staining of Bax, cleaved caspase-3, PCNA, Ki67, and Bcl2 in each group; D: Wound healing assay in each group, scale bars = 200 μm; E: Capillary-like tubule formation, scale bars = 200 μm. [a]*P* < 0.001, compared to the high glucose group; [b]*P* < 0.001, compared to the HG + Vector group.



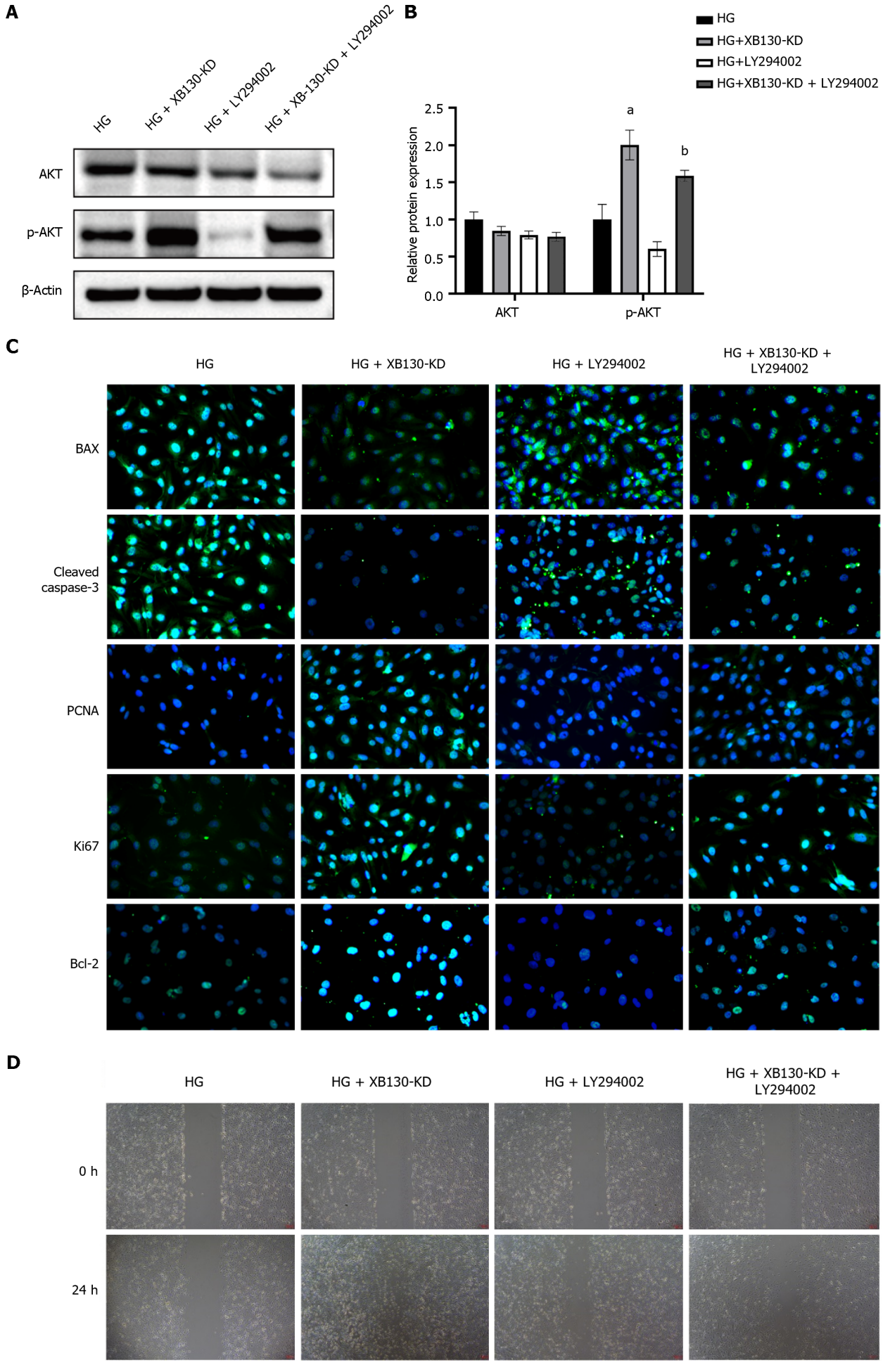
DOI: 10.4239/wjd.v14.i9.1369 **Copyright** ©The Author(s) 2023.

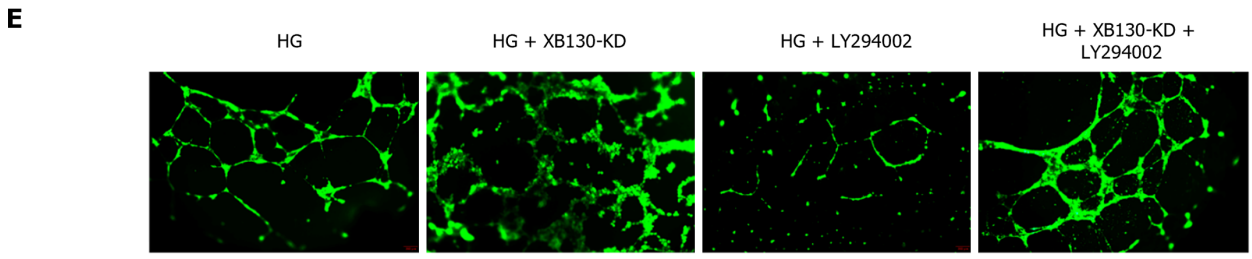
Figure 7 High glucose inhibited PI3K/Akt signalling pathway *in vivo* and *in vitro*. A: Barplot of Gene Ontology enrichment analysis; B: Dotplot of Kyoto Encyclopedia of Genes and Genomes pathway analysis; C: Western blot analysis of AKT and p-AKT expression in mice; D: Quantification of Western blot results; E: Western blot analysis of AKT and p-AKT expression in human umbilical vein endothelial cells (HUVECs); F: Quantification of Western blot results. [a]*P* < 0.01, compared to the control or NG group.



DOI: 10.4239/wjd.v14.i9.1369

Figure 8 Knock-down of XB130 accelerated wound healing in diabetic mice *via* PI3K/Akt signalling pathway. A: Western blot analysis of AKT and p-AKT expression in each group; B: Quantification of Western blot results; C: Representative skin wound images from control and model mice at day 0, 7, and 14, model (n = 10), XB130-KD (n = 10), LY294002 (n = 10), XB130-KD + LY294002 (n = 10); D: HE and Masson staining of skin wound in each group. [a]P < 0.01, compared to the control group; [b]P < 0.05, compared to the XB130-KD group.


A
HG
HG + XB130-KD
HG + LY294002
HG + XB-130-KD + LY294002
AKT
p-AKT
β-Actin
B
HG
HG+XB130-KD
HG+LY294002
HG+XB130-KD + LY294002
Relative protein expression
2.5
2.0
1.5
1.0
0.5
0.0
a
b
AKT
p-AKT
C
HG
HG + XB130-KD
HG + LY294002
HG + XB130-KD + LY294002
BAX
Cleaved caspase-3
PCNA
Ki67
Bcl-2
D
HG
HG + XB130-KD
HG + LY294002
HG + XB130-KD + LY294002
0 h
24 h



DOI: 10.4239/wjd.v14.i9.1369 **Copyright** ©The Author(s) 2023.

Figure 9 Knock-down of XB130 attenuated hyperglycemia-induced inhibitory effects on human umbilical vein endothelial cells *via* PI3K/Akt signalling pathway. A: Western blot analysis of AKT and p-AKT expression in human umbilical vein endothelial cells; B: quantification of Western blot results; C: Immunofluorescence staining of Bax, cleaved caspase-3, PCNA, Ki67, and Bcl2 in each group; D: Wound healing assay in each group, scale bars = 200 μm; E: Capillary-like tubule formation, scale bars = 200 μm. $^{a}P < 0.01$, compared to the high glucose group; $^{b}P < 0.05$, compared to the HG + XB130-KD group.

activities against HG impairment were removed (Figure 9D and E).

DISCUSSION

Diabetic skin ulcers, resulting from both internal and local pathological changes caused by diabetes, are a serious complication that can lead to amputation, despite therapeutic management[21,22]. The process of wound healing is complex, dynamic, and orderly, and is characterised by cell proliferation and migration[23,24]. In this study, we chose STZ, a well-established method for chemically inducing diabetes, to construct diabetic mice as an experimental model [18]. HUVECs were cultured *in vitro* in a high-glucose medium to mimic diabetic conditions. Our findings demonstrated that a high-glucose environment prolongs wound healing by inhibiting cell proliferation and tubule formation.

Aberrant gene expression is associated with various pathological conditions, including diabetic skin ulcers[25]. However, key driver genes that trigger and exacerbate this condition are not fully understood. We performed RNA sequencing the ulcer tissues from control and model mice group and identified 980 upregulated and 567 downregulated genes. Moreover, by performing PPI network analysis, we identified ten hub genes associated with diabetic skin ulcers (XB130, ITGAM, FCGR3A, PECAM1, CXCR4, CD34, CXCL8, CCL3, VCA11, and CD19). Among them, XB130 had the highest score based on topological algorithms. The expression of XB130 was up-regulated in HG induced diabetic skin ulcers tissue and HUVECs using RT-qPCR and Western blot.

XB130 is vital for regulating signal transduction and affects the cell cycle, proliferation, survival, and migration[14,16,17]. Although limited research has explored the relationship between XB130 and diabetic wound healing, our study aimed to address this knowledge gap by creating a model of XB130-downexpressed diabetic mice. Our findings indicate that knockdown of XB130 can enhance the healing of diabetic wounds, as evidenced by shorter epithelialisation time and rapid wound contraction. Moreover, histological analysis revealed that knockdown of XB130 not only improved diabetic wound healing, but also significantly promoted angiogenesis. To investigate the mechanism and function of XB130, we establish a knockdown of XB130 with HUVCEs. Our results revealed that the expression of PCNA, Ki67 and Bcl2 were significantly increased, while decreasing the expression of Bax and Cleaved caspase-3 in XB130-KD HUVECs treated with HG. Furthermore, knockdown of XB130 enhanced the migration and tube-forming activities of HUVECs. These findings indicate that knockdown of XB130 promotes diabetic wound healing by enhancing cell proliferation and tubule formation.

To investigate how HG environment affects wound healing, functional analysis of hub genes was performed. GO analysis revealed that the hub genes were enriched in cell proliferation, differentiation, migration, apoptosis, and tube morphogenesis. These results confirmed that HG levels hindered wound healing by affecting these cellular processes. Meanwhile, KEGG pathway analysis showed that these genes were involved in PI3K/Akt, Ras, p53, and NF-kappa B signalling pathways, and in apoptosis. Among these, the PI3K/Akt signalling pathway was the most important, with seven enriched genes. Our findings further prove that HG conditions reduce the ratio of p-AKT/AKT, leading to inhibition of the PI3K/Akt pathway.

The PI3K/AKT pathway plays a pivotal role in regulating cell proliferation and metabolism and is involved in the progression of various diseases[26-29]. When RTKs and GPCRs are activated, PI3K phosphorylates phosphatidylinositol 3,4-diphosphate, resulting in the production of PIP3. PIP3 activates AKT, which in turn phosphorylates and activates the mTORC complexes. In turn, activated mTORC complexes promote cell proliferation by phosphorylating and activating matrix metalloproteinase[30-32]. Our results indicate that knockdown of XB130 increases the levels of phosphorylated AKT. LY294002 and Wortmannin are commonly used inhibitors of the PI3K/Akt pathway. Our findings indicate that LY294002 delays wound healing in diabetic mice. Furthermore, inhibition of the PI3K/Akt signalling pathway reverses the negative effects of XB130 on proliferation and tubule formation in HUVECs.

CONCLUSION

This study shows that knockdown of XB130 can prevent high glucose-induced inhibition of proliferation and angiogenic impairment through the PI3K/Akt pathway. Based on our results, decreasing the expression of XB130 could serve as a promising therapeutic approach to accelerate the healing of diabetic skin ulcers.

ARTICLE HIGHLIGHTS

Research background

Diabetic skin ulcers are mainly caused by the inhibition of cell proliferation and impaired angiogenesis, a high percentage (15%-27%) of diabetic foot skin ulcer cases require lower extremity amputation owing to treatment failure. XB130 is an adaptor protein that regulates cell proliferation and migration.

Research motivation

To explore the role of XB130 in the development of diabetic skin ulcers.

Research objectives

To investigate whether XB130 can regulate the inhibition of proliferation and vascular damage induced by high glucose. Additionally, we aim to determine whether XB130 is involved in the healing process of diabetic skin ulcers, along with its molecular mechanisms.

Research methods

We conducted RNA-sequencing analysis to identify the key genes. The RT-qPCR, Western blot, TUNEL staining, immunofluorescence, wound healing, and tubule formation experiments were used to investigate their effects on cellular processes in human umbilical vein endothelial cells (HUVECs) stimulated with high glucose. Finally, we performed functional analysis to elucidate the molecular mechanisms underlying diabetic skin ulcers.

Research results

RNA-sequencing analysis showed that the expression of XB130 was up-regulated in the tissues of diabetic skin ulcers. Knockdown of XB130 promoted the healing of skin wounds in mice, leading to an accelerated wound healing process and shortened wound healing time. At the cellular level, knockdown of XB130 alleviated high glucose-induced inhibition of cell proliferation and angiogenic impairment in HUVECs. Inhibition of the PI3K/Akt pathway removed the proliferative effects and endothelial protection mediated by XB130.

Research conclusions

The expression of XB130 is up-regulated in high glucose-stimulated diabetic skin ulcers and HUVECs. Knockdown of XB130 promotes cell proliferation and angiogenesis *via* the PI3K/Akt signalling pathway, which accelerates the healing of diabetic skin ulcers.

Research perspectives

Decreasing the expression of XB130 could serve as a promising therapeutic approach to accelerate the healing of diabetic skin ulcers.

FOOTNOTES

Author contributions: Pan WH, Liao WQ, Fang WJ, Lei WZ, and Zhu XL contributed to conceptualization; Zhu XL, Zeng ZX, and Jiang WW contributed to data curation; Zhu XL, Jiang WW, Zeng ZX, Hu DY, Chen TY, Chen TC, Liao WQ, Lei WZ, Fang WJ, and Pan WH contributed to investigation; Zhu XL, Zeng ZX, Jiang WW, Fang WJ, and Pan WH contributed to methodology; Pan WH contributed to project administration; Pan WH, Fang WJ, and Lei WZ contributed to supervision; Zeng ZX, Hu DY, and Zhu XL contributed to visualization; Zhu XL contributed to writing-original draft preparation; Liao WQ, Fang WJ, Pan WH, and Zhu XL contributed to writing-review and editing; Zhu XL, Hu DY, and Zeng ZX contribute equally to this paper.

Supported by the National Natural Science Foundation of China, No. 82272355; and Shanghai Science and Technology Committee, No. 21410750500.

Institutional animal care and use committee statement: All procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee of the Naval Medical University Medical Ethics Committee (Protocol No: 2020YffiPZ-102).

Conflict-of-interest statement: All the authors have declared no conflict of interest.

Data sharing statement: All data are provided in this study, and raw data can be requested to the corresponding author.

ARRIVE guidelines statement: The authors have read the ARRIVE Guidelines, and the manuscript was prepared and revised according to the ARRIVE Guidelines.

Country/Territory of origin: China

ORCID number: Wei-Hua Pan 0000-0001-7381-5188.

S-Editor: Chen YL
L-Editor: A
P-Editor: Chen YX

REFERENCES

1 **Azevedo FF**, Cantarutti TA, Remiro PFR, Barbieri B, Azoubel RA, Nagahara MHT, Moraes ÂM, Lima MHM. Histological and Molecular Evidence of the Positive Performance of Glycerol-Plasticized Chitosan-Alginate Membranes on Skin Lesions of Hyperglycemic Mice. *Polymers (Basel)* 2022; **14** [PMID: 36365748 DOI: 10.3390/polym14214754]
2 **Yue C**, Guo Z, Luo Y, Yuan J, Wan X, Mo Z. c-Jun Overexpression Accelerates Wound Healing in Diabetic Rats by Human Umbilical Cord-Derived Mesenchymal Stem Cells. *Stem Cells Int* 2020; **2020**: 7430968 [PMID: 32399050 DOI: 10.1155/2020/7430968]
3 **Situm M**, Kolić M, Redzepi G, Antolić S. [Chronic wounds as a public health problem]. *Acta Med Croatica* 2014; **68** Suppl 1: 5-7 [PMID: 25326983]
4 **Huang H**, Cui W, Qiu W, Zhu M, Zhao R, Zeng D, Dong C, Wang X, Guo W, Xing W, Li X, Li L, Tan Y, Wu X, Chen L, Fu X, Luo D, Xu X. Impaired wound healing results from the dysfunction of the Akt/mTOR pathway in diabetic rats. *J Dermatol Sci* 2015; **79**: 241-251 [PMID: 26091964 DOI: 10.1016/j.jdermsci.2015.06.002]
5 **Wagner T**, Traxler D, Simader E, Beer L, Narzt MS, Gruber F, Madlener S, Laggner M, Erb M, Vorstandlechner V, Gugerell A, Radtke C, Gnecchi M, Peterbauer A, Gschwandtner M, Tschachler E, Keibl C, Slezak P, Ankersmit HJ, Mildner M. Different pro-angiogenic potential of γ-irradiated PBMC-derived secretome and its subfractions. *Sci Rep* 2018; **8**: 18016 [PMID: 30573762 DOI: 10.1038/s41598-018-36928-6]
6 **Singh K**, Sinha M, Pal D, Tabasum S, Gnyawali SC, Khona D, Sarkar S, Mohanty SK, Soto-Gonzalez F, Khanna S, Roy S, Sen CK. Cutaneous Epithelial to Mesenchymal Transition Activator ZEB1 Regulates Wound Angiogenesis and Closure in a Glycemic Status-Dependent Manner. *Diabetes* 2019; **68**: 2175-2190 [PMID: 31439646 DOI: 10.2337/db19-0202]
7 **Goren I**, Müller E, Schiefelbein D, Gutwein P, Seitz O, Pfeilschifter J, Frank S. Akt1 controls insulin-driven VEGF biosynthesis from keratinocytes: implications for normal and diabetes-impaired skin repair in mice. *J Invest Dermatol* 2009; **129**: 752-764 [PMID: 18668138 DOI: 10.1038/jid.2008.230]
8 **Laplante M**, Sabatini DM. mTOR signaling in growth control and disease. *Cell* 2012; **149**: 274-293 [PMID: 22500797 DOI: 10.1016/j.cell.2012.03.017]
9 **Moodley S**, Hui Bai X, Kapus A, Yang B, Liu M. XB130/Tks5 scaffold protein interaction regulates Src-mediated cell proliferation and survival. *Mol Biol Cell* 2015; **26**: 4492-4502 [PMID: 26446840 DOI: 10.1091/mbc.E15-07-0483]
10 **Lodyga M**, Bai XH, Kapus A, Liu M. Adaptor protein XB130 is a Rac-controlled component of lamellipodia that regulates cell motility and invasion. *J Cell Sci* 2010; **123**: 4156-4169 [PMID: 21084565 DOI: 10.1242/jcs.071050]
11 **Cho HR**, Wang Y, Bai X, Xiang YY, Lu C, Post A, Al Habeeb A, Liu M. XB130 deficiency enhances carcinogen-induced skin tumorigenesis. *Carcinogenesis* 2019; **40**: 1363-1375 [PMID: 30820526 DOI: 10.1093/carcin/bgz042]
12 **Shi M**, Huang W, Lin L, Zheng D, Zuo Q, Wang L, Wang N, Wu Y, Liao Y, Liao W. Silencing of XB130 is associated with both the prognosis and chemosensitivity of gastric cancer. *PLoS One* 2012; **7**: e41660 [PMID: 22927913 DOI: 10.1371/journal.pone.0041660]
13 **Shiozaki A**, Kosuga T, Ichikawa D, Komatsu S, Fujiwara H, Okamoto K, Iitaka D, Nakashima S, Shimizu H, Ishimoto T, Kitagawa M, Nakou Y, Kishimoto M, Liu M, Otsuji E. XB130 as an independent prognostic factor in human esophageal squamous cell carcinoma. *Ann Surg Oncol* 2013; **20**: 3140-3150 [PMID: 22805860 DOI: 10.1245/s10434-012-2474-4]
14 **Li GM**, Liang CJ, Zhang DX, Zhang LJ, Wu JX, Xu YC. XB130 Knockdown Inhibits the Proliferation, Invasiveness, and Metastasis of Hepatocellular Carcinoma Cells and Sensitizes them to TRAIL-Induced Apoptosis. *Chin Med J (Engl)* 2018; **131**: 2320-2331 [PMID: 30246718 DOI: 10.4103/0366-6999.241800]
15 **Poosekeaw P**, Pairojkul C, Sripa B, Sa Ngiamwibool P, Iamsaard S, Sakonsinsiri C, Thanan R, Ungarreevittaya P. Adaptor protein XB130 regulates the aggressiveness of cholangiocarcinoma. *PLoS One* 2021; **16**: e0259075 [PMID: 34780466 DOI: 10.1371/journal.pone.0259075]
16 **Wang Q**, Yang G, Jiang Y, Luo M, Li C, Zhao Y, Xie Y, Song K, Zhou J. XB130, regulated by miR-203, miR-219, and miR-4782-3p, mediates the proliferation and metastasis of non-small-cell lung cancer cells. *Mol Carcinog* 2020; **59**: 557-568 [PMID: 32159887 DOI: 10.1002/mc.23180]
17 **Chen B**, Liao M, Wei Q, Liu F, Zeng Q, Wang W, Liu J, Hou J, Yu X. XB130 is overexpressed in prostate cancer and involved in cell growth and invasion. *Oncotarget* 2016; **7**: 59377-59387 [PMID: 27509056 DOI: 10.18632/oncotarget.11074]
18 **Furman BL**. Streptozotocin-Induced Diabetic Models in Mice and Rats. *Curr Protoc* 2021; **1**: e78 [PMID: 33905609 DOI: 10.1002/cpz1.78]
19 **Das A**, Huang GX, Bonkowski MS, Longchamp A, Li C, Schultz MB, Kim LJ, Osborne B, Joshi S, Lu Y, Treviño-Villarreal JH, Kang MJ, Hung TT, Lee B, Williams EO, Igarashi M, Mitchell JR, Wu LE, Turner N, Arany Z, Guarente L, Sinclair DA. Impairment of an Endothelial NAD(+)-H(2)S Signaling Network Is a Reversible Cause of Vascular Aging. *Cell* 2018; **173**: 74-89.e20 [PMID: 29570999 DOI: 10.1016/j.cell.2018.02.008]

20 **Qaed E**, Wang J, Almoiliqy M, Song Y, Liu W, Chu P, Alademi S, Alademi M, Li H, Alshwmi M, Al-Azab M, Ahsan A, Mahdi S, Han G, Niu M, Ali A, Shopit A, Wang H, Li X, Qaid A, Ma X, Li T, Peng J, Ma J, Zhang J, Tang Z. Phosphocreatine Improves Cardiac Dysfunction by Normalizing Mitochondrial Respiratory Function through JAK2/STAT3 Signaling Pathway In Vivo and In Vitro. *Oxid Med Cell Longev* 2019; **2019**: 6521218 [PMID: 31885809 DOI: 10.1155/2019/6521218]

21 **Tang Y**, Zhang MJ, Hellmann J, Kosuri M, Bhatnagar A, Spite M. Proresolution therapy for the treatment of delayed healing of diabetic wounds. *Diabetes* 2013; **62**: 618-627 [PMID: 23043160 DOI: 10.2337/db12-0684]

22 **Kuai L**, Fei XY, Xing JQ, Zhang JT, Zhao KQ, Ze K, Li X, Li B. An Efficacy Predictive Method for Diabetic Ulcers Based on Higher-Order Markov Chain-Set Pair Analysis. *Evid Based Complement Alternat Med* 2020; **2020**: 5091671 [PMID: 32617110 DOI: 10.1155/2020/5091671]

23 **Wang D**, Chen H, Lei L, Chen J, Gao J, Liu J, Li Q, Xie Y, Hu Y, Ni Y. Biofabricated macrophage and fibroblast membranes synergistically promote skin wound healing. *Bioeng Transl Med* 2022; **7**: e10344 [PMID: 36176601 DOI: 10.1002/btm2.10344]

24 **Vaid B**, Chopra BS, Raut S, Sagar A, Badmalia MD, Ashish, Khatri N. Antioxidant and Wound Healing Property of Gelsolin in 3T3-L1 Cells. *Oxid Med Cell Longev* 2020; **2020**: 4045365 [PMID: 32104532 DOI: 10.1155/2020/4045365]

25 **Qian L**, Xia Z, Zhang M, Han Q, Hu D, Qi S, Xing D, Chen Y, Zhao X. Integrated Bioinformatics-Based Identification of Potential Diagnostic Biomarkers Associated with Diabetic Foot Ulcer Development. *J Diabetes Res* 2021; **2021**: 5445349 [PMID: 34513999 DOI: 10.1155/2021/5445349]

26 **Brognard J**, Clark AS, Ni Y, Dennis PA. Akt/protein kinase B is constitutively active in non-small cell lung cancer cells and promotes cellular survival and resistance to chemotherapy and radiation. *Cancer Res* 2001; **61**: 3986-3997 [PMID: 11358816]

27 **Lu H**, Kang F. Down-regulating NEAT1 inhibited the viability and vasculogenic mimicry formation of sinonasal squamous cell carcinoma cells *via* miR-195-5p/VEGFA axis. *Biosci Rep* 2020; **40** [PMID: 33146672 DOI: 10.1042/BSR20201373]

28 **Wu Y**, Li Y, Jiang T, Yuan Y, Li R, Xu Z, Zhong X, Jia G, Liu Y, Xie L, Xu K, Zhang H, Li X, Xiao J. Reduction of cellular stress is essential for Fibroblast growth factor 1 treatment for diabetic nephropathy. *J Cell Mol Med* 2018; **22**: 6294-6303 [PMID: 30320493 DOI: 10.1111/jcmm.13921]

29 **Yudushkin I**. Getting the Akt Together: Guiding Intracellular Akt Activity by PI3K. *Biomolecules* 2019; **9** [PMID: 30781447 DOI: 10.3390/biom9020067]

30 **Janku F**, Yap TA, Meric-Bernstam F. Targeting the PI3K pathway in cancer: are we making headway? *Nat Rev Clin Oncol* 2018; **15**: 273-291 [PMID: 29508857 DOI: 10.1038/nrclinonc.2018.28]

31 **Wu YJ**, Neoh CA, Tsao CY, Su JH, Li HH. Sinulariolide Suppresses Human Hepatocellular Carcinoma Cell Migration and Invasion by Inhibiting Matrix Metalloproteinase-2/-9 through MAPKs and PI3K/Akt Signaling Pathways. *Int J Mol Sci* 2015; **16**: 16469-16482 [PMID: 26204832 DOI: 10.3390/ijms160716469]

32 **Rodríguez-Cortez VC**, Del Pino-Molina L, Rodríguez-Ubreva J, Ciudad L, Gómez-Cabrero D, Company C, Urquiza JM, Tegnér J, Rodríguez-Gallego C, López-Granados E, Ballestar E. Monozygotic twins discordant for common variable immunodeficiency reveal impaired DNA demethylation during naïve-to-memory B-cell transition. *Nat Commun* 2015; **6**: 7335 [PMID: 26081581 DOI: 10.1038/ncomms8335]

World Journal of Diabetes

Submit a Manuscript: https://www.f6publishing.com

World J Diabetes 2023 September 15; 14(9): 1385-1392

DOI: 10.4239/wjd.v14.i9.1385

ISSN 1948-9358 (online)

ORIGINAL ARTICLE

Retrospective Study

Effects of paricalcitol combined with hemodiafiltration on bone-metabolism-related indexes in patients with diabetic nephropathy and chronic renal failure

Xiao-Ying Ma, Yu-Ping Sheng, Xing-Meng Yang, Hao-Ran Zhang, Fu-Yun Sun

Specialty type: Urology and nephrology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C, C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Kramer JR, United States; Negera WG, Germany; Horowitz M, Australia

Received: May 4, 2023
Peer-review started: May 4, 2023
First decision: May 15, 2023
Revised: May 25, 2023
Accepted: August 7, 2023
Article in press: August 7, 2023
Published online: September 15, 2023

Xiao-Ying Ma, Yu-Ping Sheng, Xing-Meng Yang, Hao-Ran Zhang, Fu-Yun Sun, Department of Nephrology, Cangzhou Central Hospital, Cangzhou 061000, Hebei Province, China

Corresponding author: Fu-Yun Sun, MM, Professor, Department of Nephrology, Cangzhou Central Hospital, No. 16 Xinhua West Road, Yunhe District, Cangzhou 061000, Hebei Province, China. 17734498766@163.com

Abstract

BACKGROUND

Diabetic nephropathy (DN) is frequently seen in the development of diabetes mellitus, and its pathogenic factors are complicated. Its current treatment is controversial, and there is a lack of a relevant efficacy prediction model.

AIM

To determine the effects of paricalcitol combined with hemodiafiltration on bone-metabolism-related indexes in patients with DN and chronic renal failure (CRF), and to construct an efficacy prediction model.

METHODS

We retrospectively analyzed 422 patients with DN and CRF treated in Cangzhou Central Hospital between May 2020 and May 2022. We selected 94 patients who met the inclusion and exclusion criteria. Patients were assigned to a dialysis group (n = 45) and a joint group (n = 49) in relation to therapeutic regimen. The clinical efficacy of the two groups was compared after treatment. The changes in laboratory indexes after treatment were evaluated, and the two groups were compared for the incidence of adverse reactions. The predictive value of laboratory indexes on the clinical efficacy on patients was analyzed.

RESULTS

The dialysis group showed a notably worse improvement in clinical efficacy than the joint group (P = 0.017). After treatment, the joint group showed notably lower serum levels of serum creatinine, uric acid (UA) and blood urea nitrogen (BUN) than the dialysis group (P < 0.05). After treatment, the joint group had lower serum levels of phosphorus, procollagen type I amino-terminal propeptide (PINP) and intact parathyroid hormone than the dialysis group, but a higher calcium level (P < 0.001). Both groups had a similar incidence of adverse reactions (P >

0.05). According to least absolute shrinkage and selection operator regression analysis, UA, BUN, phosphorus and PINP were related to treatment efficacy. According to further comparison, the non-improvement group had higher risk scores than the improvement group ($P < 0.0001$), and the area under the curve of the risk score in efficacy prediction was 0.945.

CONCLUSION

For treatment of CRF and DN, combined paricalcitol and hemodiafiltration can deliver higher clinical efficacy and improve the bone metabolism of patients, with good safety.

Key Words: Paricalcitol; Hemodiafiltration; Diabetic nephropathy; Chronic renal failure; Serum calcium; Serum phosphorus; Intact Paricalcitol hormone

Core Tip: This study confirmed that paricalcitol combined with hemodiafiltration can effectively improve the condition of patients with diabetic nephropathy (DN) and chronic renal failure (CRF) and alleviate calcium–phosphorus metabolism disorder. This study has also successfully constructed a predictive model. It provides a new reference for evaluating the efficacy of treatment of combined DN and CRF.

Citation: Ma XY, Sheng YP, Yang XM, Zhang HR, Sun FY. Effects of paricalcitol combined with hemodiafiltration on bone-metabolism-related indexes in patients with diabetic nephropathy and chronic renal failure. *World J Diabetes* 2023; 14(9): 1385-1392
URL: https://www.wjgnet.com/1948-9358/full/v14/i9/1385.htm
DOI: https://dx.doi.org/10.4239/wjd.v14.i9.1385

INTRODUCTION

Improvement of socioeconomic and living standards has resulted in an increase in the global prevalence of diabetes mellitus (DM), bringing an increasing incidence of diabetic nephropathy (DN)[1]. DN is one of the most frequent causes of chronic renal failure (CRF) worldwide, and 30%–40% of DM patients develop DN[2]. The pathogenesis of DN is complex, and is bound up with abnormal balance in the body, including hemodynamics, metabolic disorder, inflammation and fibrosis[3]. The imbalance between renal injury and renal protection factors is the primary cause of disease progression[4]. DN has a heavy burden on the global economy and a high mortality risk in DM patients.

DN is a most common complication in DM patients and a common cause of CRF[5]. Approximately 30%-40% of the patients with DM will develop DN, leading to CRF[6,7]. When DN progresses to the advanced stage, the patients may need dialysis or kidney transplantation to help maintain renal function and quality of life[8]. Hemodiafiltration is an effective method of treatment for CRF, which can prolong the survival of patients[9]. However, patients receiving hemodialysis often have abnormal serum calcium and phosphorus, and adverse reactions such as joint calcification, bone pain, arrhythmia, pruritus and platelet insufficiency[10].

Paricalcitol is a vitamin D analog that is used to treat secondary hyperparicalcitolism triggered by renal insufficiency [11]. Paricalcitol can promote intestinal absorption of calcium and inhibit secretion and differentiation of Paricalcitol cells by binding to vitamin D receptor, thus reducing the synthesis and secretion of Paricalcitol hormone (PTH)[12]. Paricalcitol can also alleviate kidney damage triggered by inflammation and apoptosis, thus helping to protect kidney function[13].

However, there is controversy about the impact of paricalcitol combined with hemodiafiltration on serum calcium, phosphorus and intact PTH (iPTH) in patients with both DN and CRF. Accordingly, this study aimed to provide a basis for clinical treatment of comorbid DN and CRF.

MATERIALS AND METHODS

Patient characteristics

We retrospectively analyzed 422 patients with both DN and CRF treated in Cangzhou Central Hospital between May 2020 and May 2022. The patients were screened according to the following criteria. Inclusion criteria: patients who met the diagnostic criteria of DN[14] and were not allergic to the drugs used in this study. Exclusion criteria: patients with primary nephropathy or secondary DM; patients comorbid severe dysfunction of the heart, liver or brain; patients with proteinuria due to other reasons; and patients who had received angiotensin-converting enzyme inhibitors or diuretics before admission. According to the above standards, 94 patients with DN and CRF were selected. The patients were assigned to a dialysis group ($n = 45$) and a joint group ($n = 49$) according to therapeutic regimen. This study was carried out with approval of the Medical Ethics Committee of Cangzhou Central Hospital.

Therapeutic regimen

Both groups received symptomatic treatment, including correction of fluid and electrolyte imbalance, anti-allergy, anti-hemolysis and correction of acid poisoning. The dialysis group was treated by hemodiafiltration through an Fx80 dialyzer (Fresenius, Germany) , with blood flow of 200–300 mL/min, and dialysis time of 4 h (3 times/wk). Twelve sessions were taken as a course of dialysis treatment, and two courses of treatment were conducted. The joint group was treated with paricalcitol while being treated with hemodiafiltration. Paricalcitol was injected at a dose of 0.04–0.1 μg/kg (2.8–7.0 μg) each time, and the dose was adjusted according to the serum iPTH level. The frequency of administration was no more than once every other day, and it was administered for eight continuous weeks. During treatment, serum calcium, phosphorus and iPTH were detected every 2 wk, and the dose was adjusted according to the test results.

Clinical data collection

The clinical data were collected through the medical record system of our hospital and included: age, gender, body mass index, course of renal decompensation, history of hypertension, history of hyperlipidemia, and history of smoking and alcoholism. Laboratory indexes included renal-function-related indexes [serum creatinine (SCr), uric acid (UA) and urea nitrogen (BUN)], and bone-metabolism-related indexes [serum phosphorus, serum calcium, procollagen type I N-terminal propeptide (PINP) and iiPTH]. Improvement of clinical efficacy in patients after treatment and the incidence of adverse reactions during treatment were evaluated.

Evaluation criteria of clinical efficacy

Markedly effective: After treatment, the related symptoms of the patients subsided, and the levels of renal-function-related indexes such as SCr, UA and BUN decreased by over 30%. Effective: After treatment, the related symptoms were relieved, and the levels of SCr, UA and BUN decreased by 5%–30%. Ineffective: The related symptoms and condition of the patients were not notably alleviated, and there was even some aggravation of disease, with the indexes such as SCr, UA and BUN decreased by < 5%, or even increased. Total effective rate = (number of cases with markedly effectively treatment + number of cases with effective treatment]/total number of cases × 100%.

Outcome measures

Primary outcome measures: The clinical efficacy on the two groups was compared. The changes in laboratory indexes in patients after treatment were evaluated. Secondary outcome measures: The clinical data of the two groups were compared. The adverse reactions of the two groups were also compared. The predictive value of laboratory indexes on the clinical efficacy on patients was analyzed.

Statistical analysis

This study used *R* language 4.1.1 software (*R* Foundation for Statistical Computing, Vienna, Austria) for data reduction and data analysis, and established a model. The predictors of non-zero coefficient were screened by least absolute shrinkage and selection operator (LASSO) regression, and the nomogram was drawn by *R* (R3.5.3) software package and rms package. The consistency index (C-index) was calculated by rms package, and its clinical value was verified by the receiver operating characteristic curve. We used Graph Pad Prism 8.0 (La Jolla, CA, USA) for visualization of data. $P < 0.05$ implies a notable difference.

RESULTS

Comparison of clinical data

According to inter-group comparison of clinical data, the dialysis and joint groups were similar ($P > 0.05$, Table 1).

Evaluation of clinical efficacy

The dialysis group showed a significantly lower improvement in clinical efficacy than the joint group ($P = 0.017$, Table 2).

Changes in renal-function-related indexes

Before treatment, SCr, UA and BUN levels were similar in the dialysis and joint groups ($P > 0.05$). After treatment, SCr, UA and BUN levels in both groups decreased significantly ($P < 0.05$), with greater decreases in the joint group than in the dialysis group ($P < 0.05$, Figure 1).

Changes in bone-metabolism-related indexes

Before treatment, the levels of phosphorus, calcium, PINP and iPTH did not differ significantly between the dialysis and joint groups ($P > 0.05$). After treatment, phosphorus, PINP and iPTH in both groups decreased significantly ($P < 0.0001$), while calcium increased significantly. After treatment, the joint group had significantly lower serum levels of phosphorus, PINP and iPTH than the dialysis group, and a significantly higher Ca level ($P < 0.001$, Figure 2).

Occurrence of adverse reactions

The incidence of adverse reactions was similar in the joint and dialysis groups ($P > 0.05$, Table 3).

Table 1 Clinical data

Factors	Dialysis group (n = 45)	Joint group (n = 49)	χ^2 value	P value
Age (yr)			2.042	0.153
> 60	25	20		
≤ 60	20	29		
Gender			0.641	0.423
Male	22	28		
Female	23	21		
BMI (kg/m^2)			0.300	0.583
> 25	15	19		
≤ 25	30	30		
Course of renal decompensation (yr)			0.526	0.468
> 2	29	35		
≤ 2	16	14		
History of hypertension			0.512	0.474
Yes	12	10		
No	33	39		
History of hyperlipidemia			0.566	0.451
Yes	8	6		
No	37	43		
History of smoking			0.641	0.423
Yes	22	28		
No	23	21		
History of alcoholism			0.222	0.637
Yes	6	5		
No	39	44		

BMI: Body mass index.

Table 2 Efficacy evaluation, n (%)

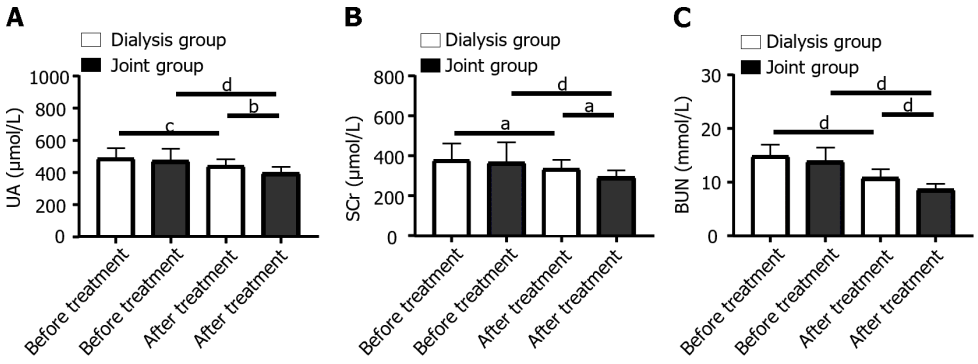
Group	Markedly effective	Effective	Ineffective	Total effective rate
Dialysis group (n = 45)	25 (55.56)	8 (17.78)	12 (26.66)	33 (73.34)
Joint group (n = 49)	33 (67.35)	12 (24.48)	4 (8.16)	45 (91.84)
χ^2 value				5.686
P value				0.017

Value of laboratory indexes in predicting efficacy

The value of laboratory indexes for predicting treatment efficacy was determined using LASSO regression. Patients with markedly effective treatment and those with effective treatment were assigned to an improvement group (n = 78), while patients with ineffective treatment were assigned to a non-improvement group (n = 16). Through LASSO regression analysis, UA, BUN, phosphorus and PINP were related to treatment efficacy (Figure 3). A predictive equation was constructed according to the risk coefficient: = -0.0056463379 × UA + 0.1334320595 × BUN + 0.9356101817 × P + -0.0002297355 × PINP. The non-improvement group had higher risk scores than the improvement group (Figure 4A, P < 0.0001), and the area under the curve of risk score in forecasting efficacy was 0.945 (Figure 4B).

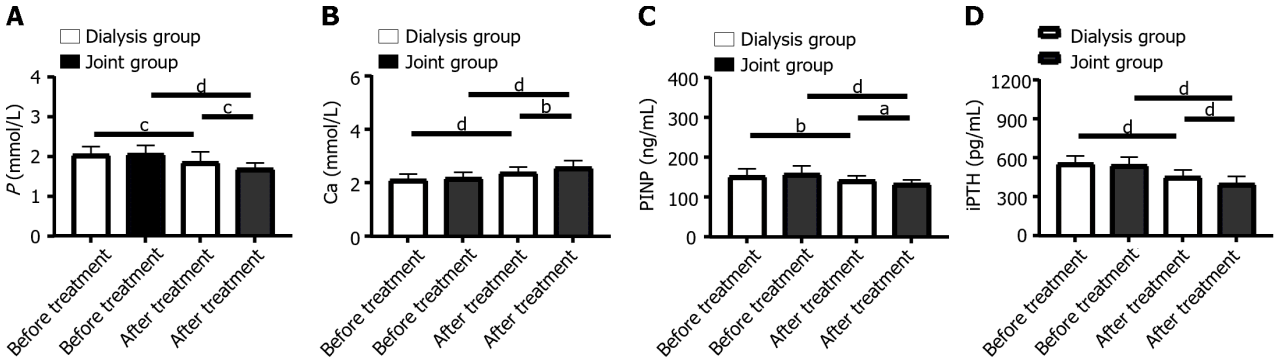
Table 3 Adverse reactions, *n* (%)

Group	Nausea and vomiting	Loss of appetite	Phlebitis	Gastrointestinal reaction	Rash	Total incidence rate
Dialysis group (n = 45)	2 (4.44)	2 (4.44)	1 (2.22)	2 (4.44)	1 (2.22)	8 (17.78)
Joint group (n = 49)	1 (2.04)	1 (2.04)	2 (4.08)	1 (2.04)	1 (2.04)	6 (12.24)
χ^2 value						0.566
P value						0.451



DOI: 10.4239/wjd.v14.i9.1385 **Copyright** ©The Author(s) 2023.

Figure 1 Changes in renal-function-related indexes in the patients before and after treatment. A: Comparison of uric acid changes in the two groups before and after treatment; B: Comparison of serum creatinine changes in the two groups before and after treatment; C: Comparison of blood urea nitrogen changes in the two groups before and after treatment. $^{a}P < 0.05$, $^{b}P < 0.01$ $^{c}P < 0.001$, $^{d}P < 0.0001$. SCr: Serum creatinine; UA: Uric acid; BUN: Blood urea nitrogen.



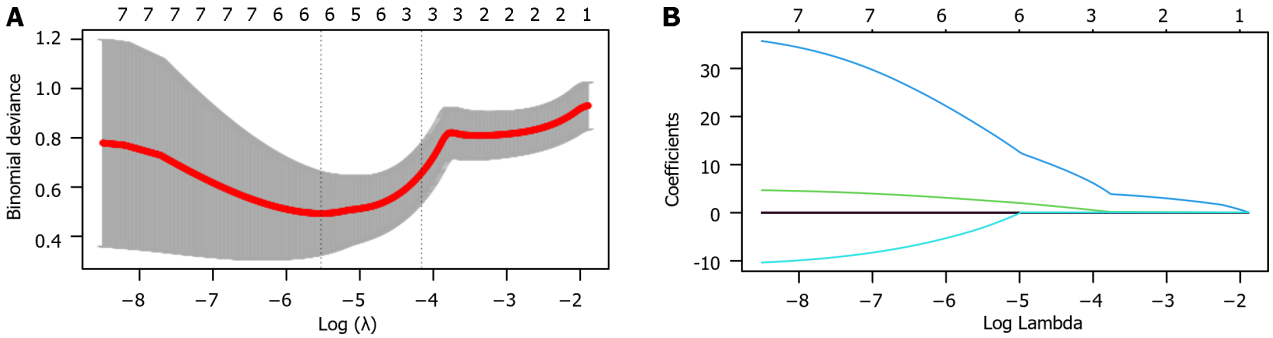
DOI: 10.4239/wjd.v14.i9.1385 **Copyright** ©The Author(s) 2023.

Figure 2 Changes in bone-metabolism-related indexes in patients before and after treatment. A: Comparison of phosphorus changes before and after treatment; B: Comparison of calcium changes before and after treatment; C: Comparison of procollagen type I amino-terminal propeptide changes before and after treatment; D: Comparison of intact Paricalcitol hormone changes before and after treatment. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$, $^{d}P < 0.0001$. PINP: Procollagen type I amino-terminal propeptide; iPTH: intact Paricalcitol hormone.

DISCUSSION

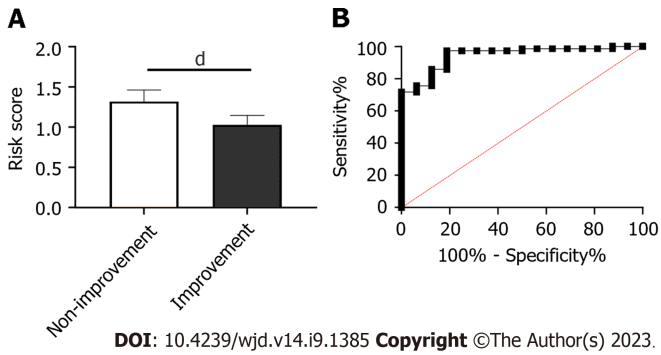
DM is characterized by high incidence, low control rate and various complications worldwide[15]. DN is a common complication of DM, especially in patients with a disease course > 10 years[16]. The long-term presence of hyperglycemia triggers activation of the polyol pathway and protein kinase C pathway, resulting in a series of pathophysiological changes, such as oxidative stress, disorder of renal glucose metabolism, inflammatory reaction, abnormal metabolism, and abnormal hemodynamics, and finally inducing CRF[17]. The kidneys of patients with CRF shrink and lose the ability to maintain normal renal function, which results in serious consequences such as imbalance of acid and potassium, retention of metabolites, and fluid and electrolyte imbalance, which increases the risk of death[18].

Hemodiafiltration is a type of renal replacement therapy, which is primarily used for blood purification in patients with renal failure[19]. This method removes metabolites, toxins, and excess liquids and electrolytes from the blood through a series of filtration and dialysis processes. In hemodiafiltration treatment, the patient's blood is filtered and dialyzed by special filters and dialyzers, and the removed waste and excess liquid are excreted through the urine[20]. Hemodiafiltration can ameliorate the symptoms and quality of life of patients with kidney disease, but it also needs close monitoring and adjustment of treatment regimens to avoid complications[21]. According to prior research[22], patients



DOI: 10.4239/wjd.v14.i9.1385 **Copyright** ©The Author(s) 2023.

Figure 3 Least absolute shrinkage and selection operator-based screening of predictors. A, B: The coefficient distribution of the least absolute shrinkage and selection operator repression analysis and the calculation of adjustment parameters (lambda) based on partial likelihood deviation of 10 times cross-validation.



DOI: 10.4239/wjd.v14.i9.1385 **Copyright** ©The Author(s) 2023.

Figure 4 Risk score in patients with different efficacy. A: Risk score in the non-improvement group and improvement group; B: Receiver operating characteristic curve of risk score in predicting efficacy. $^{d}P < 0.0001$.

often have calcium–phosphorus metabolism disorder during dialysis, which compromises its efficacy. Continuous hemodialysis can reduce urinary toxins, but it cannot replace normal renal metabolism and endocrine function. In this study, the clinical efficacy and renal function improvement of the joint group were significantly higher than those in the dialysis group after treatment. The results suggest that paricalcitol combined with hemodiafiltration can deliver significantly greater efficacy in patients with comorbid DN and CRF, and improve renal function.

Due to the loss of renal function, dialysis patients often have abnormal bone metabolism, including hyperphosphatemia, hypocalcemia, and hyperparicalcitolism. These abnormalities increase the risk of osteoporosis and fracture[23]. In hemodialysis therapy, the use of auxiliary drugs can improve this situation and reduce the occurrence of adverse reactions[24]. Therefore, in the bone metabolism management of dialysis patients, it is necessary to comprehensively consider factors including the clinical situation of patients and serum bone-metabolism-related indexes. In this study, the joint group showed significantly lower levels of phosphorus, PINP and iPTH than the dialysis group, and a significantly higher calcium level. The two groups showed no significant difference in the incidence of adverse reactions. The results indicate that paricalcitol combined with hemodiafiltration can control serum phosphorus and calcium levels, lower iPTH level, and improve bone-metabolism-related indexes, without increasing safety risks.

Predicting the clinical efficacy on patients is important in the treatment process[25]. For example, predictive models can help healthcare providers make more informed decisions and recommend specific treatments for individual patients, which can bring better results and more personalized care[26]. Predictive models can also help providers identify high-risk patients in certain situations, so that they can carry out early intervention to prevent or mitigate the development of these situations. In this study, we established a risk model to predict the efficacy based on laboratory indexes. In this study, UA, BUN, phosphorus and PINP were related to the efficacy in patients. The risk score of each patient was calculated. The non-improvement group had significantly higher risk scores than the improvement group. According to receiver operating characteristic curve-based analysis, the area under the curve of risk score for predicting efficacy was 0.945.

Our study confirmed that paricalcitol combined with hemodiafiltration improved the condition of patients with comorbid DN and CRF and corrected calcium–phosphorus metabolism disorder. We also successfully constructed a predictive model. However, the study still had some limitations. First, we did not collect data about long-term prognosis, and we only evaluated short-term efficacy, so whether there is any influence on long-term efficacy after treatment needs further study. Second, we need to verify whether the predictive model has high generality or not needs verification by more data. Finally, in such a single-center study, the lack of research samples may have led to bias in the analysis. We hope to carry out more studies in the future to improve the research conclusions.

CONCLUSION

In the treatment of comorbid DN and CRF, the combined use of paricalcitol and hemodiafiltration delivered greater clinical efficacy and improved the bone metabolism of patients, with good safety.

ARTICLE HIGHLIGHTS

Research background

Diabetic nephropathy (DN) is one of the common complications of diabetes, mainly manifested as glomerular damage. As it progresses, DN may lead to chronic renal failure (CRF), which seriously affects quality of life and life expectancy.

Research motivation

Hemodialysis filtration is an effective method for treating CRF, but patients receiving hemodialysis often experience abnormalities in blood calcium and phosphorus. Paroxycarbinol can promote intestinal calcium absorption and inhibit the secretion and differentiation of Paricalcitol cells by binding to vitamin D receptors. However, it is still unclear whether periostenol has an effect on calcium phosphate metabolism disorder in patients with CRF due to DN.

Research objectives

Supplement the blank of paracalcitol combined with hemodiafiltration in the treatment of CRF, and increases the clinical treatment plan for disorder of calcium and phosphate metabolism during hemodialysis and filtration.

Research methods

We retrospectively analyzed and observed the effect of paricalcitol combined with hemodiafiltration on calcium phosphate metabolism disorder in patients with CRF due to DN. For the first time, a risk model for predicting efficacy was established using a least absolute shrinkage and selection operator (LASSO) regression model.

Research results

We found that the combination of paricalcitol and hemodialysis filtration significantly improved the metabolic disorder of calcium phosphate metabolism disorder in patients, and improved efficacy. Using the LASSO model, we established a risk score to predict efficacy, which provides a new reference for clinical treatment and efficacy prediction.

Research conclusions

Paricalcitol can improve calcium phosphate metabolism disorder in hemodialysis patients, and the risk model established by LASSO regression model effectively predicts clinical efficacy.

Research perspectives

As a retrospective study, we cannot collect more samples and observe the prognosis of patients. We hope to conduct randomized controlled trials in future studies to observe the long-term prognosis.

FOOTNOTES

Author contributions: Ma XY designed and performed the research and wrote the paper; Sun FY designed the research and supervised the report; Sheng YP and Yang XM designed the research and contributed to the analysis; Zhang HR provided clinical advice.

Institutional review board statement: The study was reviewed and approved by the Cangzhou Central Hospital.

Informed consent statement: All participants have signed an informed consent form.

Conflict-of-interest statement: The authors declare no conflicts of interest for this article.

Data sharing statement: Clinical data for this study can be obtained from the corresponding author.

Country/Territory of origin: China

ORCID number: Xiao-Ying Ma 0000-0001-6942-0941; Yu-Ping Sheng 0000-0003-3335-9549; Xing-Meng Yang 0009-0001-3910-7732; Hao-Ran Zhang 0009-0009-7922-0711; Fu-Yun Sun 0000-0003-3947-9517.

S-Editor: Wang JL
L-Editor: A
P-Editor: Chen YX

REFERENCES

1 **Qi C**, Mao X, Zhang Z, Wu H. Classification and Differential Diagnosis of Diabetic Nephropathy. *J Diabetes Res* 2017; **2017**: 8637138 [PMID: 28316995 DOI: 10.1155/2017/8637138]
2 **Li X**, Lu L, Hou W, Huang T, Chen X, Qi J, Zhao Y, Zhu M. Epigenetics in the pathogenesis of diabetic nephropathy. *Acta Biochim Biophys Sin (Shanghai)* 2022; **54**: 163-172 [PMID: 35130617 DOI: 10.3724/abbs.2021016]
3 **Samsu N**. Diabetic Nephropathy: Challenges in Pathogenesis, Diagnosis, and Treatment. *Biomed Res Int* 2021; **2021**: 1497449 [PMID: 34307650 DOI: 10.1155/2021/1497449]
4 **Khan NU**, Lin J, Liu X, Li H, Lu W, Zhong Z, Zhang H, Waqas M, Shen L. Insights into predicting diabetic nephropathy using urinary biomarkers. *Biochim Biophys Acta Proteins Proteom* 2020; **1868**: 140475 [PMID: 32574766 DOI: 10.1016/j.bbapap.2020.140475]
5 **Iyengar SK**, Adler SG. The application of the HapMap to diabetic nephropathy and other causes of chronic renal failure. *Semin Nephrol* 2007; **27**: 223-236 [PMID: 17418690 DOI: 10.1016/j.semnephrol.2007.01.003]
6 **Pazmino PA**. Current Concepts on Diabetic Nephropathy and 2014 Data on Diabetic Renal Failure in Texas. *Tex Med* 2016; **112**: e1 [PMID: 27441426]
7 **Takada T**, Masaki T, Hoshiyama A, Toki T, Kamata Y, Shichiri M. Tolvaptan alleviates excessive fluid retention of nephrotic diabetic renal failure unresponsive to furosemide. *Nephrology (Carlton)* 2018; **23**: 883-886 [PMID: 29665203 DOI: 10.1111/nep.13390]
8 **Aniort J**, Heng AÉ, Deteix P, Souweine B, Lautrette A. [Epidemiology of acute renal failure]. *Nephrol Ther* 2019; **15**: 63-69 [PMID: 30639043 DOI: 10.1016/j.nephro.2018.11.002]
9 **Iyengar R**, Franzese J, Gianchandani R. Inpatient Glycemic Management in the Setting of Renal Insufficiency/Failure/Dialysis. *Curr Diab Rep* 2018; **18**: 75 [PMID: 30112652 DOI: 10.1007/s11892-018-1044-y]
10 **Kelly DM**, Ademi Z, Doehner W, Lip GYH, Mark P, Toyoda K, Wong CX, Sarnak M, Cheung M, Herzog CA, Johansen KL, Reinecke H, Sood MM. Chronic Kidney Disease and Cerebrovascular Disease: Consensus and Guidance From a KDIGO Controversies Conference. *Stroke* 2021; **52**: e328-e346 [PMID: 34078109 DOI: 10.1161/STROKEAHA.120.029680]
11 **Bae E**, Kim JH, Jung MH, Jang SJ, Lee TW, Jung S, Lee S, Jang HN, Chang SH, Park DJ. Paricalcitol Attenuates Contrast-Induced Acute Kidney Injury by Regulating Mitophagy and Senescence. *Oxid Med Cell Longev* 2020; **2020**: 7627934 [PMID: 33299530 DOI: 10.1155/2020/7627934]
12 **Martínez-Arias L**, Panizo S, Alonso-Montes C, Martín-Vírgala J, Martín-Carro B, Fernández-Villabrille S, García Gil-Albert C, Palomo-Antequera C, Fernández-Martín JL, Ruiz-Torres MP, Dusso AS, Carrillo-López N, Cannata-Andía JB, Naves-Díaz M. Effects of calcitriol and paricalcitol on renal fibrosis in CKD. *Nephrol Dial Transplant* 2021; **36**: 793-803 [PMID: 33416889 DOI: 10.1093/ndt/gfaa373]
13 **Lim JH**, Yook JM, Oh SH, Jeon SJ, Noh HW, Jung HY, Choi JY, Cho JH, Kim CD, Kim YL, Park SH. Paricalcitol Improves Hypoxia-Induced and TGF-β1-Induced Injury in Kidney Pericytes. *Int J Mol Sci* 2021; **22** [PMID: 34575914 DOI: 10.3390/ijms22189751]
14 **de Boer IH**, Khunti K, Sadusky T, Tuttle KR, Neumiller JJ, Rhee CM, Rosas SE, Rossing P, Bakris G. Diabetes Management in Chronic Kidney Disease: A Consensus Report by the American Diabetes Association (ADA) and Kidney Disease: Improving Global Outcomes (KDIGO). *Diabetes Care* 2022; **45**: 3075-3090 [PMID: 36189689 DOI: 10.2337/dci22-0027]
15 **Thipsawat S**. Early detection of diabetic nephropathy in patient with type 2 diabetes mellitus: A review of the literature. *Diab Vasc Dis Res* 2021; **18**: 14791641211058856 [PMID: 34791910 DOI: 10.1177/14791641211058856]
16 **Anders HJ**, Huber TB, Isermann B, Schiffer M. CKD in diabetes: diabetic kidney disease versus nondiabetic kidney disease. *Nat Rev Nephrol* 2018; **14**: 361-377 [PMID: 29654297 DOI: 10.1038/s41581-018-0001-y]
17 **Selby NM**, Taal MW. An updated overview of diabetic nephropathy: Diagnosis, prognosis, treatment goals and latest guidelines. *Diabetes Obes Metab* 2020; **22** Suppl 1: 3-15 [PMID: 32267079 DOI: 10.1111/dom.14007]
18 **Borisov VV**, Shilov EM. [Chronic renal failure]. *Urologiia* 2017; 11-18 [PMID: 28406591 DOI: 10.18565/urol.2017.1-supplement.11-18]
19 **Bouchard J**, Macedo E, Soroko S, Chertow GM, Himmelfarb J, Ikizler TA, Paganini EP, Mehta RL; Program to Improve Care in Acute Renal Disease. Comparison of methods for estimating glomerular filtration rate in critically ill patients with acute kidney injury. *Nephrol Dial Transplant* 2010; **25**: 102-107 [PMID: 19679558 DOI: 10.1093/ndt/gfp392]
20 **Filler G**, Clark WF, Huang SH. Tandem hemodialysis and plasma exchange. *Pediatr Nephrol* 2014; **29**: 2077-2082 [PMID: 24022368 DOI: 10.1007/s00467-013-2620-7]
21 **Navarrete JE**, Rajabalan A, Cobb J, Lea JP. Proportion of Hemodialysis Treatments with High Ultrafiltration Rate and the Association with Mortality. *Kidney360* 2022; **3**: 1359-1366 [PMID: 36176655 DOI: 10.34067/KID.0001322022]
22 **Akchurin OM**, Kaskel F. Update on inflammation in chronic kidney disease. *Blood Purif* 2015; **39**: 84-92 [PMID: 25662331 DOI: 10.1159/000368940]
23 **He J**, Xu S, Zhang B, Xiao C, Chen Z, Si F, Fu J, Lin X, Zheng G, Yu G, Chen J. Gut microbiota and metabolite alterations associated with reduced bone mineral density or bone metabolic indexes in postmenopausal osteoporosis. *Aging (Albany NY)* 2020; **12**: 8583-8604 [PMID: 32392181 DOI: 10.18632/aging.103168]
24 **Greenfield EM**. Anabolic effects of intermittent PTH on osteoblasts. *Curr Mol Pharmacol* 2012; **5**: 127-134 [PMID: 21787293]
25 **Kate RJ**, Pearce N, Mazumdar D, Nilakantan V. A continual prediction model for inpatient acute kidney injury. *Comput Biol Med* 2020; **116**: 103580 [PMID: 32001013 DOI: 10.1016/j.compbiomed.2019.103580]
26 **Archer L**, Snell KIE, Ensor J, Hudda MT, Collins GS, Riley RD. Minimum sample size for external validation of a clinical prediction model with a continuous outcome. *Stat Med* 2021; **40**: 133-146 [PMID: 33150684 DOI: 10.1002/sim.8766]

World Journal of Diabetes

Submit a Manuscript: https://www.f6publishing.com

World J Diabetes 2023 September 15; 14(9): 1393-1402

DOI: 10.4239/wjd.v14.i9.1393

ISSN 1948-9358 (online)

ORIGINAL ARTICLE

Retrospective Study

Early neonatal complications in pregnant women with gestational diabetes mellitus and the effects of glycemic control on neonatal infection

Bei-Bei Wang, Mei Xue

Specialty type: Endocrinology and metabolism

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C, C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Ibenthal A, Germany; Pagotto U, Italy; Horowitz M, Australia

Received: May 6, 2023
Peer-review started: May 6, 2023
First decision: May 19, 2023
Revised: May 24, 2023
Accepted: August 4, 2023
Article in press: August 4, 2023
Published online: September 15, 2023

Bei-Bei Wang, Mei Xue, Department of Neonatal Intensive Care Unit, Taizhou People's Hospital of Jiangsu Province, Taizhou 225300, Jiangsu Province, China

Corresponding author: Mei Xue, MM, Chief Physician, Department of Neonatal Intensive Care Unit, Taizhou People's Hospital of Jiangsu Province, No. 399 Hailing South Road, Hailing District, Taizhou 225300, Jiangsu Province, China. xuemei009260@163.com

Abstract

BACKGROUND

Gestational diabetes mellitus (GDM) has become increasingly prevalent globally. Glycemic control in pregnant women with GDM has a critical role in neonatal complications.

AIM

To analyze the early neonatal complications in GDM, and examine the effect of blood glucose control level on neonatal infection.

METHODS

The clinical data of 236 pregnant women with GDM and 240 healthy pregnant women and newborns during from March 2020 to December 2021 the same period were retrospectively analyzed, and the early complications in newborns in the two groups were compared. The patients were divided into the conforming glycemic control group (CGC group) and the non-conforming glycemic control group (NCGC group) based on whether glycemic control in the pregnant women with GDM conformed to standards. Baseline data, immune function, infection-related markers, and infection rates in neonates were compared between the two groups.

RESULTS

The incidence of neonatal complications in the 236 neonates in the GDM group was significantly higher than that in the control group ($P < 0.05$). Pregnant women with GDM in the NCGC group ($n = 178$) had significantly higher fasting plasma glucose, 2 h postprandial blood glucose and glycated hemoglobin $A1_c$ levels than those in the CGC group ($n = 58$) ($P < 0.05$). There were no differences in baseline data between the two groups ($P > 0.05$). Additionally, the NCGC group had significantly decreased peripheral blood $CD3^+$, $CD4^+$, $CD8^+$ T cell ratios, CD4/CD8

ratios and immunoglobulin G in neonates compared with the CGC group ($P < 0.05$), while white blood cells, serum procalcitonin and C-reactive protein levels increased significantly. The neonatal infection rate was also significantly increased in the NCGC group ($P < 0.05$).

CONCLUSION
The risk of neonatal complications increased in pregnant women with GDM. Poor glycemic control decreased neonatal immune function, and increased the incidence of neonatal infections.

Key Words: Gestational diabetes mellitus; Early neonatal complications; Glycemic control; Neonatal infection

Core Tip: Gestational diabetes mellitus (GDM) is an important complication that affects pregnancy outcome. Pregnant women with GDM and long-term abnormal glucose metabolism are closely associated with the risk of adverse maternal and neonatal outcomes. Some studies suggest that the immune function of newborns may be significantly affected by GDM, and that the effect of glycemic control is related to pregnancy outcomes and neonatal prognosis. In this study, we confirmed that the risk of neonatal complications increased in pregnant women with GDM, and poor glycemic control leads to impairment of fetal immune system and ultimately increases the risk of neonatal infections.

Citation: Wang BB, Xue M. Early neonatal complications in pregnant women with gestational diabetes mellitus and the effects of glycemic control on neonatal infection. *World J Diabetes* 2023; 14(9): 1393-1402
URL: https://www.wjgnet.com/1948-9358/full/v14/i9/1393.htm
DOI: https://dx.doi.org/10.4239/wjd.v14.i9.1393

INTRODUCTION

Gestational diabetes mellitus (GDM) is a glucose metabolism disorder during pregnancy. Studies have indicated that GDM tends to cause diabetes mellitus and cardiovascular disease in neonates after delivery by pregnant women with GDM[1]. Recently, studies reported that GDM incidence has shown a significant increasing trend worldwide[2] and has become a global health concern. Previous studies[3,4] have shown that GDM is considered to be one of the major risk factors for maternal and child complications in the perinatal phase and the incidence of cesarean section, polyhydramnios, premature birth, fetal deformities, neonatal infection, hyperbilirubinemia, fetal macrosomia, and neonatal hypoglycemia is higher in pregnant women with GDM who have abnormal glucose metabolism for a long period. Rational diet control, exercise, and glucose-lowering treatments have achieved good results in terms of glycemic control in pregnant women with GDM. However, 30% of pregnant women with GDM were reported to be affected by multiple factors, including irregular diet, lack of exercise, hormone secretion disorder, *etc*[5]. In addition, glycemic control was poor. This ultimately affected maternal and child health, and increased the risk of neonatal complications. A study found that the immune function of neonates may be significantly affected by GDM, and the effectiveness of glycemic control is strongly associated with adverse pregnancy outcomes and neonatal complications[6]. Early detection of abnormal glucose metabolism and achieving good glycemic control during pregnancy can effectively prevent adverse maternal and child outcomes in the perinatal stage of GDM patients[7]. However, there is still unclear whether glycemic control that does not conform to the standards in pregnant women with GDM decreases immune function in neonates and increases the incidence of neonatal infections.

In this study, we investigated the differences in neonatal complications between pregnant women with GDM and healthy controls to examine the effects of GDM on neonatal prognosis. We then divided the pregnant women with GDM into two groups based on whether glycemic control conformed to standards. Subsequently, we compared the blood glucose levels in pregnant women with GDM, immune function, infection-related marker levels, and the incidence of infection in neonates of the pregnant women with GDM in these two groups. The purpose of this study was to analyze the effects of glycemic control in GDM pregnant women on neonatal immune function and infection.

MATERIALS AND METHODS

Patient characteristics

The newborns delivered by 236 pregnant women with GDM in Taizhou People's Hospital of Jiangsu Province from March 2020 to December 2021 were retrospectively included in the GDM group. The neonates of 240 healthy pregnant women during the same period were selected as the control group. The patients were divided into the conforming glycemic control group (CGC group) and the non-conforming glycemic control group (NCGC group) based on whether glycemic control in pregnant women with GDM conformed to standards.

Inclusion criteria: (1) Natural singleton pregnancy; (2) age 20–40 years; and (3) for the GDM group, blood glucose measurement at week 24–28 of pregnancy conformed to the diagnostic criteria for GDM formulated by the American Diabetes Association in 2013[8]: 75 g oral glucose tolerance test result showed fasting plasma glucose (FPG) ≥ 5.1 mmol/L, blood glucose 1 h after test ≥ 10.0 mmol/L or blood glucose 2 h after test ≥ 8.5 mmol/L; the GDM group received diet and/or glucose-lowering treatment.

Exclusion criteria: (1) Comorbid hypertension, anemia, polyhydramnios, and other underlying diseases or pregnancy complications; (2) past history of adverse pregnancy outcomes; (3) gestational age at delivery < 28 wk; and (4) presence of heart, brain, lung, liver, and other organ diseases.

The screening process is shown in Figure 1. The study was reviewed and approved by the Taizhou People's Hospital of Jiangsu Province Institutional Review Board.

Treatment methods

Dietary control and/or insulin treatment was carried out in all pregnant women with GDM, which was specified as follows: total daily caloric intake was calculated based on 130 J/kg145 J/kg, and the proportions of carbohydrates, proteins, and fats were 55%–65%, 20%–25%, and 15%–25%, respectively. A routine diet complied with the principle of eating less in more meals and 4–6 meals were consumed each day. Insulin treatment of 0.6 U/kg–0.8 U/kg was administered every day, blood glucose level was closely monitored, and insulin dose was promptly adjusted in pregnant women with GDM with abnormal blood glucose after comprehensive dietary intervention. Follow-up was carried out every 2 wk in the form of hospital visits. Glycemic control criteria[9] were FPG ≤ 5.6 mmol/L, 2 h postprandial blood glucose (P2h-PG) ≤ 6.7 mmol/L, and glycated hemoglobin ($HbA1_C$) < 6%. All three criteria must be met to conform to glycemic control standards. Otherwise, the patient was considered not to conform to glycemic control standards.

Observation markers

Baseline data and blood glucose in pregnant women and immune function, infectionrelated markers, and infection rate in neonates were observed and compared. (1) Blood glucose: This was measured 24 h before delivery. Venous whole blood was collected from pregnant women, and a low-speed centrifuge was used to extract serum samples at 3000 r/min for 10 min. A Mindray glucose assay kit (glucose oxidase assay) was used to measure FPG and P2h-PG in serum samples. Heparin anticoagulant tubes were used to collect venous whole blood from pregnant women. Ion exchange chromatography and gradient elution were used to measure $HbA1_C$ after hemolysis of blood samples using hemolysin; (2) immune function: 5 mL of umbilical vein blood was collected from neonates after delivery. Blood samples were mixed with allophycocyanin (APC)/cyanine dye 7 (Cy7) fluorescently labeled mouse anti-human CD3 antibody, phycoerythrin/Cy7 mouse anti-human CD4 antibody, and APC/Cy7 mouse anti-human CD8, and incubated at room temperature for 15 min. BD FACS™ lysis solution (BD Inc., USA) was added and incubated in the dark for 15 min. A BD FACSCanto II flow cytometer (BD Inc., USA) was used to measure the proportions of CD3+ T cells, CD4+T cells, and CD8+ T cells with different fluorescent labels in peripheral blood. The CD4/CD8 ratio was then calculated; (3) the levels of immunoglobulin G (IgG), IgA, and IgM in the peripheral blood of newborns in both groups were measured by immunoturbidimetry; (4) a Roche Cobas 8000 fully automatic biochemical analyzer was used to measure the white blood cell (WBC) count in the umbilical vein blood in neonates; (5) enzyme-linked immunosorbent assay (ELISA) was used to measure procalcitonin (PCT) and C-reactive protein (CRP) levels. ELISA kits were purchased from Beyotime Biotechnology Co., Ltd; and (6) neonatal infections were observed and recorded, including upper respiratory tract infection, lower respiratory tract infection, skin infection, intestinal infection, and sepsis.

Statistical analysis

Statistical data were processed using SPSS 20.0 software, and quantitative data were tested for normal distribution. Quantitative data with normal distribution were expressed as mean ± SD. An independent sample *t*-test was used for inter-group comparisons. Quantitative data with abnormal distribution are represented by median (quartile), and a non-parametric test was used for inter-group comparisons. Qualitative data were expressed as % and the χ^2 test was used. A P value < 0.05 was considered statistically significant.

RESULTS

Comparison of baseline data

In the GDM group, the age of pregnant women ranged from 21–39 years and the mean age was 29.98 ± 4.65 years; body mass index (BMI) was 19.7–38.1 kg/m^2 and mean BMI was (29.75 ± 2.68) kg/m^2. There were 143 primipara and 93 multipara women. In the control group, the age ranged from 22–40 years and the mean age was 30.26 ± 4.74 years; BMI was 20.3–37.9 kg/m^2 and mean BMI was 30.01 ± 3.12 kg/m^2; there were 159 primipara and 181 multipara women. There were no differences in age, BMI, parity, and gravidity between the two groups and the groups were comparable (P > 0.05). as shown in Table 1.

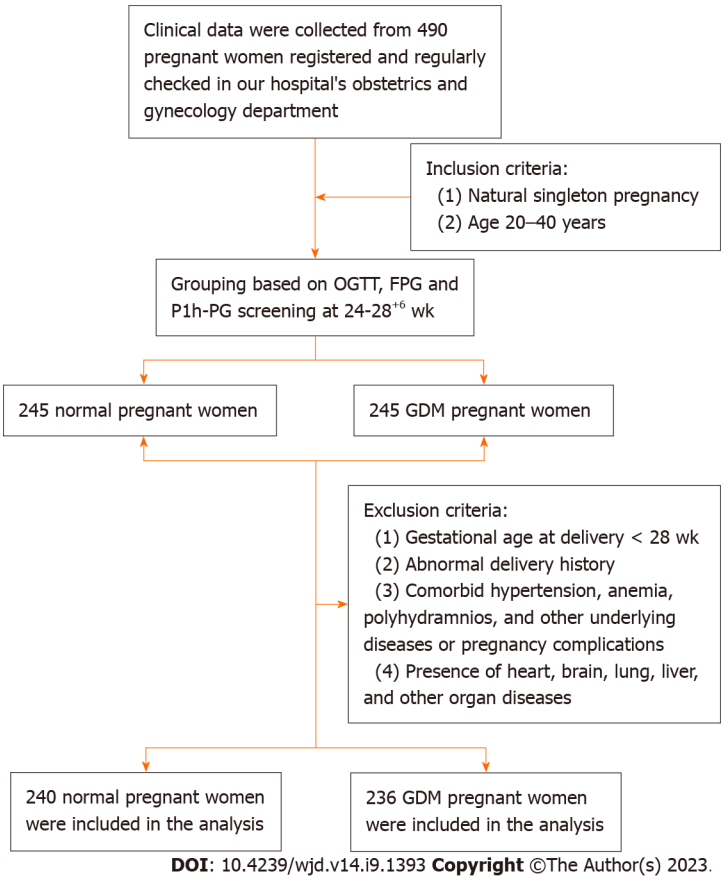
Comparison of early neonatal complications

The incidence of premature births, fetal macrosomia, hypoglycemia, hypocalcemia, and hyperbilirubinemia in neonates delivered by pregnant women with GDM was significantly higher than that in neonates delivered by women in the

Table 1 Comparison of baseline data in pregnant women with gestational diabetes mellitus between conforming glycemic control and non-conforming glycemic control groups

Groups	Cases	Age (yr)	BMI (kg/m²)	Type of pregnant woman, *n* (%)	
				Primipara	Multipara
CGC group	178	30.05 ± 4.46	29.55 ± 2.82	109 (61.24)	69 (38.76)
NCGC group	58	29.47 ± 3.75	30.08 ± 2.57	34 (56.90)	25 (43.10)
t/χ^2 value		0.893	1.270	0.344	
P value		0.373	0.206	0.558	

BMI: Body mass index; CGC: Conforming glycemic control; NCGC: Non-conforming glycemic control.



DOI: 10.4239/wjd.v14.i9.1393 **Copyright** ©The Author(s) 2023.

Figure 1 Screening, randomization and analysis of populations. OGTT: Oral glucose tolerance test; FPG: Fasting plasma glucose; P1h-PG: 1 h postprandial blood glucose; GDM: Gestational diabetes mellitus.

control group. These differences were statistically significant ($P < 0.05$) as shown in Table 2.

Comparison of blood glucose markers

Pregnant women with GDM were divided into the CGC group and the NCGC group based on whether their glycemic control conformed to standards. Blood glucose markers in the NCGC group, such as FPG, P2h-PG, and $HbA1_C$ levels, were significantly higher ($P < 0.05$) compared with pregnant women with GDM in the CGC group. These results are shown in Table 3.

Comparison of peripheral blood T cell subsets

Results of the flow cytometry analysis of peripheral blood T cell subsets were compared between the two groups of neonates. The ratio of peripheral blood $CD3^+$T cells, $CD4^+$T cells, and $CD8^+$T cells, and the CD4/CD8 ratio in neonates in the NCGC group were all significantly lower than those in the CGC group ($P < 0.05$). These results showed that immune function was significantly decreased in neonates from the NCGC group. These results are shown in Table 4.

Table 2 Comparison of early neonatal complications between the gestational diabetes mellitus and control groups, *n* (%)

Complications			χ^2 value	*P* value
Premature birth	45 (19.07)	12 (5.00)	22.341	< 0.001
Fetal macrosomia	57 (24.15)	23 (9.58)	18.064	< 0.001
Hypoglycemia	42 (17.80)	7 (2.92)	28.53	< 0.001
Hypocalcemia	22 (9.32)	5 (2.08)	11.653	< 0.001
Hyperbilirubinemia	29 (12.29)	13 (5.42)	6.984	0.008
Polycythemia	38 (16.10)	22 (9.17)	5.195	0.023
Hyaline membrane disease	13 (5.51)	2 (0.83)	8.522	0.004
Fetal distress	34 (14.41)	6 (2.50)	21.917	< 0.001
Congenital malformation	11 (4.66)	1 (0.42)	8.723	0.003
Neonatal asphyxia	21 (8.90)	6 (2.50)	9.104	0.003
Neonatal infection	35 (14.83)	11 (4.58)	14.312	< 0.001

GDM: Gestational diabetes mellitus.

Table 3 Comparison of blood glucose markers in pregnant women with gestational diabetes mellitus between conforming glycemic control and non-conforming glycemic control groups

Groups	Cases	FPG (mmol/L)	P2h-PG (mmol/L)	$HbA1_c$ (%)
CGC group	178	4.68 ± 0.60	5.51 ± 0.85	5.11 ± 0.45
NCGC group	58	5.96 ± 0.68	7.14 ± 1.04	6.38 ± 0.74
t/χ^2 value		13.645	11.979	15.691
P value		< 0.001	< 0.001	< 0.001

CGC: Conforming glycemic control; NCGC: Non-conforming glycemic control; FPG: Fasting plasma glucose; P2h-PG: 2 h postprandial blood glucose; $HbA1_C$: Hemoglobin.

Table 4 Comparison of $CD3^+$, $CD4^+$, $CD8^+$ and $CD4^+/CD8^+$ ratio between the two groups of neonates (mean ± SD, *n*)

Groups	Cases	$CD3^+$ (%)	$CD4^+$ (%)	$CD8^+$ (%)	CD4/CD8
CGC group	178	52.01 ± 10.78	39.21 ± 7.80	25.69 ± 5.47	1.61 ± 0.54
NCGC group	58	45.25 ± 7.33	22.46 ± 5.48	19.42 ± 2.95	1.17 ± 0.33
t value		4.449	15.170	8.335	5.854
P value		< 0.001	< 0.001	< 0.001	< 0.001

CGC: Conforming glycemic control; NCGC: Non-conforming glycemic control.

Comparison of immunoglobulin levels

The levels of IgG, IgM, and IgA, were compared between neonates in the two groups. The results showed that the level of IgG in the CGC group was significantly higher than that in the NCGC group, with a statistically significant difference ($P < 0.05$). There were no significant differences in the levels of IgM and IgA in peripheral blood between the two groups of newborns ($P > 0.05$), as shown in Table 5.

Comparison of infection-related inflammatory markers

Infection-related inflammatory markers, including WBC, serum PCT and CRP levels, were compared between neonates in the two groups. WBC, PCT, and CRP levels in neonates in the NCGC group were significantly higher than those in the CGC group, and these differences were statistically significant ($P < 0.05$), as shown in Table 6.

Table 5 Comparison of immunoglobulin G, immunoglobulin M and immunoglobulin A levels between the two groups of neonates (mean ± SD, *n*)

Groups	Cases	IgG (g/L)	IgM (g/L)	IgA (g/L)
CGC group	178	9.78 ± 1.38	0.181 ± 0.043	0.31 ± 0.08
NCGC group	58	7.21 ± 1.32	0.173 ± 0.040	0.29 ± 0.07
t value		12.447	1.251	1.688
P value		< 0.001	0.212	0.093

IG: Immunoglobulin; CGC: Conforming glycemic control; NCGC: Non-conforming glycemic control.

Table 6 Comparison of white blood cell, procalcitonin and C-reactive protein levels between the two groups of neonates (mean ± SD, *n*)

Groups	Cases	WBC (×10⁹/L)	PCT (μg/L)	CRP (mg/L)
CGC group	178	15.56 ± 5.47	0.43 ± 0.12	7.22 ± 2.07
NCGC group	58	25.80 ± 8.61	0.81 ± 0.24	12.38 ± 3.22
t value		10.618	15.920	14.212
P value		< 0.001	< 0.001	< 0.001

WBC: white blood cell; PCT: procalcitonin; CRP: C-reactive protein; CGC: Conforming glycemic control; NCGC: Non-conforming glycemic control.

Comparison of neonatal infections

The incidence of upper respiratory tract infection, lower respiratory tract infection, and skin infection in neonates in the NCGC group was 31.03%, which was significantly higher than neonates in the CGC group (9.35%). These differences were statistically significant ($P < 0.05$) and are shown in Table 7.

DISCUSSION

Glucose metabolism disorders and long-term blood glucose abnormalities in pregnant women with GDM are associated with decreased pancreatic islet function and insulin resistance[10], and GDM has a major impact on adverse maternal and child outcomes in the perinatal phase compared to the normal diabetic population. Currently, there are 210 million neonates affected by GDM globally, as shown in the 2017 International Diabetes Federation report[11]. Furthermore, GDM has become a major global public health concern. The results of this study show that the incidence of premature births, fetal macrosomia, hypoglycemia, hypocalcemia, hyperbilirubinemia, and infection in neonates delivered by pregnant women with GDM was significantly higher than in neonates delivered by healthy pregnant women. These results demonstrate that the health of neonates is severely affected by blood glucose abnormalities in the mother during pregnancy. The main harm caused by GDM is an increase in maternal and child adverse outcomes and mortality rate during the perinatal phase, resulting in fetal distress, developmental abnormalities, and increases the risk of hypoglycemia, deformities, and infection in neonates[12]. Therefore, it can be seen from these results that stringent glycemic control in pregnant women with GDM is an essential measure to prevent neonatal complications in the perinatal stage. Capobianco *et al*[13] also reported the GDM is an important complication that affects maternal and pregnancy outcomes, and good glycemic control can decrease the risk of pregnancy complications and the cesarean section rate, increase the rate of natural vaginal delivery, and has positive effects in decreasing premature births, fetal macrosomia, hypoglycemia, asphyxiation, and infection.

A recent study[14] found that interleukin (IL)-6 and tumor necrosis factor (TNF)-α levels were significantly increased in the umbilical vein blood from neonates delivered by women with GDM. The neonates also showed varying degrees of immune dysfunction, suggesting that the risk of infection is higher in neonates of GDM patients. Our study results showed that compared with healthy pregnant women, the incidence of neonatal infection was significantly higher in pregnant women with GDM. Blood glucose markers and neonatal infection rates were significantly increased in pregnant women with GDM in the NCGC group compared to those in the CGC group. This suggested that glycemic control has significant effects in decreasing neonatal infection caused by blood glucose abnormalities in GDM patients. Zarrin *et al*[15] found that the condition of GDM patients worsened as gestational age increased, and glucose metabolism abnormalities during pregnancy may directly affect maternal and fetal immune function. Maternal immune defects and fetal T lymphocyte developmental abnormalities will affect neonatal immune function. Neonatal immune dysfunction is an independent risk factor for infection[16]. T lymphocytes are the most important cell population in the immune system, of which the CD3+ subset represents mature T lymphocytes and immune function[17]. CD4+ T cells mainly regulate

Table 7 Comparison of neonatal infections between the two groups, *n* (%)

Groups	Cases	Upper respiratory tract infection	Lower respiratory tract infection	Skin infection	Intestinal infection	Sepsis	Total
CGC group	178	10 (5.62)	4 (2.25)	1 (0.56)	2 (1.12)	0 (0.00)	17 (9.55)
NCGC group	58	7 (12.07)	3 (5.17)	2 (3.45)	5 (8.62)	1 (1.72)	18 (31.03)
χ^2 value							15.985
P value							< 0.001

CGC: Conforming glycemic control; NCGC: Non-conforming glycemic control.

humoral immunity while CD8+ T cells are inhibitory/cytotoxic T lymphocytes that mainly regulate cellular immunity and play a critical role in regulating CD3+ and CD4+ functions[18,19]. The CD4+/CD8+ ratio can normally be used to reflect the equilibrium between humoral/cellular immunity. This cell ratio is an important marker for evaluating immune function and a low CD4+/CD8+ ratio usually means that the body is in an immunosuppressed state[20]. Kugler *et al*[21] confirmed that neonatal immune function defects were related to the inheritance of abnormal T lymphocyte development and maternal immune function defects, and were independent risk factors for neonatal infection. We compared the differences in peripheral blood T cell subsets in this study between neonates in the two groups. The results showed that peripheral blood $CD3^+$T cells, $CD4^+$T cells, and $CD8^+$T cells, and the CD4/CD8 ratio of neonates in the NCGC group were all significantly lower than those in the CGC group. These results demonstrated that blood glucose abnormalities in pregnant women with GDM may affect peripheral blood T cell subsets in neonates, resulting in decreased immune function and immune regulation disorders, thus reducing infection resistance.

Immunoglobulins are an important class of immune effector molecules, including IgG, IgA, IgM, and so on. IgG in the peripheral blood of newborns is mainly from the mother, accounting for about 75% of the total serum immunoglobulin content, and plays an important role in preventing infection[22]. This study found that IgG in neonates from women with GDM in the NCGC group significantly decreased compared to that in the CGC group, suggesting that poor blood glycemic control in GDM pregnant women can lead to a decline in neonatal immune function. It is speculated that for patients with GDM, abnormal glucose metabolism itself is an inflammatory reaction, which hinders the production of IgG, thereby reducing the amount of IgG entering the fetus *via* the placenta[23]. However, IgA and IgM cannot pass through the placental barrier, resulting in extremely low levels in the peripheral blood of newborns, leading to insignificant changes in levels.

WBC are the most commonly used marker for early diagnosis and treatment of neonatal infections, and PCT and CRP are important serum markers for diagnosing neonatal infection[24]. This study compared these markers in pregnant women with GDM and neonates from the two groups. The results revealed that WBC, serum PCT and CRP levels in neonates in the NCGC group were significantly greater than those in the CGC group, suggesting that GDM patients who did not meet glycemic control standards have neonates with elevated inflammatory markers and an increased risk of infection. This may be because blood glucose abnormalities during pregnancy can promote the transcription of placental CRP, IL-6, and PCT, which are important mechanisms that directly affect the fetal immune system[25]. Li *et al*[26] found that a hyperglycemic environment activated placental HIF-1a and TLR4/MyD88/NF-kB pathways in pregnant women with GDM, induced IL-6 and IL-8 secretions, promoted placental inflammation and autophagy to disrupt placental homeostasis and cell renewal, and increased the risk of infection in neonates delivered by pregnant women with GDM. From these findings combined with the results from the present study, we believe that poor glycemic control in pregnant women with GDM can result in long-term blood glucose abnormalities, which may stimulate inflammatory responses in the fetal placenta, thereby affecting the fetal immune system and ultimately increase the risk of neonatal infections.

The innovation of this study is that the relationship between the blood glucose control level in pregnant women with GDM and neonatal immune function was analyzed, which opens up a new direction for predicting neonatal infectious pathology. However, a larger multicenter clinical study is needed in the future to validate the results of this study as this was a single center study with a limited sample size due to the strict screening conditions. In addition, immunoglobulin, as an antibody related to the immune response *in vivo*, can be used as an early diagnostic indicator of infection, and IgG antibodies can also enhance the anti-infection ability of newborns and prevent related infectious diseases. However, this study lacks the assessment of neonatal peripheral blood immunoglobulin level to verify neonatal immune function. Finally, the neonates included in this study were only followed up for a short time. The influence of blood glucose control level in GDM patients on the long-term immune function of neonates still requires further research.

CONCLUSION

In summary, we speculate that glucose metabolism disorders and long-term blood glucose abnormalities in GDM patients with poor glycemic control may be considered a type of inflammatory response which affects the T lymphocyte subsets in neonates, resulting in immune dysfunction, and ultimately decreasing immune function and increasing the risk of infection.

ARTICLE HIGHLIGHTS

Research background

Gestational diabetes mellitus (GDM) is related to obesity in pregnant women, older age in pregnant women, excessive nutrition during pregnancy, lack of exercise, genetic history of familial type 2 diabetes, excessive sugar consumption and other factors. GDM often causes obstetric complications, which seriously threaten the life and health of pregnant women and newborns. Blood sugar control measures have a considerable impact on pregnancy outcome and newborn status in patients with GDM.

Research motivation

The long-term abnormal glucose metabolism in GDM pregnant women affects the immune function of newborns, and it is unclear whether poor glucose control in GDM pregnant women increases the risk of neonatal infectious diseases.

Research objectives

The purpose of this study was to determine the correlation between GDM pregnant women and neonatal complications, and to analyze the impact of blood glucose control on the risk of neonatal infectious diseases.

Research methods

The clinical data of 236 pregnant women with GDM and 240 healthy pregnant women and newborns were retrospectively analyzed to compare early neonatal complications in the two groups of pregnant women. The 236 pregnant women with GDM were divided into two groups based on whether their blood sugar control reached the standard. The baseline data, neonatal immune function, infection related indicators, and neonatal infection rate in the two groups of pregnant women with GDM were compared.

Research results

The incidence of neonatal complications in GDM pregnant women was significantly higher than that in normal pregnant women. Compared with GDM pregnant women who achieved glycemic control, the proportion of CD3+, CD4+, and CD8+T cells in peripheral blood and the ratio of CD4/CD8 cells in newborns from mothers who did not achieve glycemic control significantly decreased, while the white blood cell count, serum procalcitonin, and C-reactive protein levels significantly increased, and the neonatal infection rate significantly increased.

Research conclusions

The risk of neonatal complications is increased in pregnant women with GDM, and poor glycemic control leads to impairment of the fetal immune system and ultimately increases the risk of neonatal infections.

Research perspectives

The effect of blood glucose control is related to pregnancy outcome and neonatal prognosis.

FOOTNOTES

Author contributions: Wang BB conceived the study; Wang BB and Xue M performed the data collection and extraction and analyzed the data; Wang BB and Xue M interpreted and reviewed the data and drafts; Xue M reviewed the final draft.

Institutional review board statement: The study was reviewed and approved by the Taizhou People's Hospital of Jiangsu Province Institutional Review Board.

Informed consent statement: The analysis used anonymous clinical data that were obtained after each patient agreed to treatment by written consent. According to institutional policy, this study was exempt from the informed consent process.

Conflict-of-interest statement: The authors declare no conflicts of interest for this article.

Data sharing statement: statement: The dataset is available from the corresponding author at xuemei009260@163.com.

Country/Territory of origin: China

ORCID number: Bei-Bei Wang 0009-0004-2122-1088; Mei Xue 0009-0006-5025-5783.

S-Editor: Wang JL

L-Editor: A
P-Editor: Yu HG

REFERENCES

1 **Schaefer-Graf UM**, Pawliczak J, Passow D, Hartmann R, Rossi R, Bührer C, Harder T, Plagemann A, Vetter K, Kordonouri O. Birth weight and parental BMI predict overweight in children from mothers with gestational diabetes. *Diabetes Care* 2005; **28**: 1745-1750 [PMID: 15983329 DOI: 10.2337/diacare.28.7.1745]
2 **Zhu Y**, Zhang C. Prevalence of Gestational Diabetes and Risk of Progression to Type 2 Diabetes: a Global Perspective. *Curr Diab Rep* 2016; **16**: 7 [PMID: 26742932 DOI: 10.1007/s11892-015-0699-x]
3 **Crowther CA**, Hiller JE, Moss JR, McPhee AJ, Jeffries WS, Robinson JS; Australian Carbohydrate Intolerance Study in Pregnant Women (ACHOIS) Trial Group. Effect of treatment of gestational diabetes mellitus on pregnancy outcomes. *N Engl J Med* 2005; **352**: 2477-2486 [PMID: 15951574 DOI: 10.1056/NEJMoa042973]
4 **Wendland EM**, Torloni MR, Falavigna M, Trujillo J, Dode MA, Campos MA, Duncan BB, Schmidt MI. Gestational diabetes and pregnancy outcomes--a systematic review of the World Health Organization (WHO) and the International Association of Diabetes in Pregnancy Study Groups (IADPSG) diagnostic criteria. *BMC Pregnancy Childbirth* 2012; **12**: 23 [PMID: 22462760 DOI: 10.1186/1471-2393-12-23]
5 **Miremberg H**, Ben-Ari T, Betzer T, Raphaeli H, Gasnier R, Barda G, Bar J, Weiner E. The impact of a daily smartphone-based feedback system among women with gestational diabetes on compliance, glycemic control, satisfaction, and pregnancy outcome: a randomized controlled trial. *Am J Obstet Gynecol* 2018; **218**: 453.e1-453.e7 [PMID: 29425836 DOI: 10.1016/j.ajog.2018.01.044]
6 **Buhary BM**, Almohareb O, Aljohani N, Alzahrani SH, Elkaissi S, Sherbeeni S, Almaghamsi A, Almalki M. Glycemic control and pregnancy outcomes in patients with diabetes in pregnancy: A retrospective study. *Indian J Endocrinol Metab* 2016; **20**: 481-490 [PMID: 27366714 DOI: 10.4103/2230-8210.183478]
7 **Hashimoto K**, Koga M. Indicators of glycemic control in patients with gestational diabetes mellitus and pregnant women with diabetes mellitus. *World J Diabetes* 2015; **6**: 1045-1056 [PMID: 26240701 DOI: 10.4239/wjd.v6.i8.1045]
8 **American Diabetes Association**. Standards of medical care in diabetes--2013. *Diabetes Care* 2013; **36** Suppl 1: S11-S66 [PMID: 23264422 DOI: 10.2337/dc13-S011]
9 **Miazgowski T**, Bikowska M, Ogonowski J, Taszarek A. The Impact of Health Locus of Control and Anxiety on Self-Monitored Blood Glucose Concentration in Women with Gestational Diabetes Mellitus. *J Womens Health (Larchmt)* 2018; **27**: 209-215 [PMID: 28829663 DOI: 10.1089/jwh.2017.6366]
10 **McIntyre HD**, Catalano P, Zhang C, Desoye G, Mathiesen ER, Damm P. Gestational diabetes mellitus. *Nat Rev Dis Primers* 2019; **5**: 47 [PMID: 31296866 DOI: 10.1038/s41572-019-0098-8]
11 **Carracher AM**, Marathe PH, Close KL. International Diabetes Federation 2017. *J Diabetes* 2018; **10**: 353-356 [PMID: 29345068 DOI: 10.1111/1753-0407.12644]
12 **Egan AM**, Bogdanet D, Griffin TP, Kgosidialwa O, Cervar-Zivkovic M, Dempsey E, Allotey J, Alvarado F, Clarson C, Cooray SD, de Valk HW, Galjaard S, Loeken MR, Maresh MJA, Napoli A, O'Shea PM, Wender-Ozegowska E, van Poppel MNM, Thangaratinam S, Crowther C, Biesty LM, Devane D, Dunne FP; INSPIRED research group. A core outcome set for studies of gestational diabetes mellitus prevention and treatment. *Diabetologia* 2020; **63**: 1120-1127 [PMID: 32193573 DOI: 10.1007/s00125-020-05123-6]
13 **Capobianco G**, Gulotta A, Tupponi G, Dessole F, Pola M, Virdis G, Petrillo M, Mais V, Olzai G, Antonucci R, Saderi L, Cherchi PL, Dessole S, Sotgiu G. Materno-Fetal and Neonatal Complications of Diabetes in Pregnancy: A Retrospective Study. *J Clin Med* 2020; **9** [PMID: 32825775 DOI: 10.3390/jcm9092707]
14 **Teng Y**, Xuan S, Jiang M, Tian L, Tian J, Chang Q. Expression of H(2)S in Gestational Diabetes Mellitus and Correlation Analysis with Inflammatory Markers IL-6 and TNF-α. *J Diabetes Res* 2020; **2020**: 3085840 [PMID: 32280713 DOI: 10.1155/2020/3085840]
15 **Zarrin M**, Grossen-Rösti L, Bruckmaier RM, Gross JJ. Elevation of blood β-hydroxybutyrate concentration affects glucose metabolism in dairy cows before and after parturition. *J Dairy Sci* 2017; **100**: 2323-2333 [PMID: 28109586 DOI: 10.3168/jds.2016-11714]
16 **Allam G**, Alsulaimani AA, Alzaharani AK, Nasr A. Neonatal infections in Saudi Arabia: Association with cytokine gene polymorphisms. *Cent Eur J Immunol* 2015; **40**: 68-77 [PMID: 26155186 DOI: 10.5114/ceji.2015.50836]
17 **Dong D**, Zheng L, Lin J, Zhang B, Zhu Y, Li N, Xie S, Wang Y, Gao N, Huang Z. Structural basis of assembly of the human T cell receptor-CD3 complex. *Nature* 2019; **573**: 546-552 [PMID: 31461748 DOI: 10.1038/s41586-019-1537-0]
18 **Ruterbusch M**, Pruner KB, Shehata L, Pepper M. In Vivo CD4(+) T Cell Differentiation and Function: Revisiting the Th1/Th2 Paradigm. *Annu Rev Immunol* 2020; **38**: 705-725 [PMID: 32340571 DOI: 10.1146/annurev-immunol-103019-085803]
19 **Martin MD**, Badovinac VP. Defining Memory CD8 T Cell. *Front Immunol* 2018; **9**: 2692 [PMID: 30515169 DOI: 10.3389/fimmu.2018.02692]
20 **Behairy BE**, Ehsan N, Anwer M, Allam A, El-Henawy I, Hameed NA, Zakaria HM. Expression of intrahepatic CD3, CD4, and CD8 T cells in biliary atresia. *Clin Exp Hepatol* 2018; **4**: 7-12 [PMID: 29594193 DOI: 10.5114/ceh.2017.71394]
21 **Kugler DG**, Flomerfelt FA, Costa DL, Laky K, Kamenyeva O, Mittelstadt PR, Gress RE, Rosshart SP, Rehermann B, Ashwell JD, Sher A, Jankovic D. Systemic toxoplasma infection triggers a long-term defect in the generation and function of naive T lymphocytes. *J Exp Med* 2016; **213**: 3041-3056 [PMID: 27849554 DOI: 10.1084/jem.20151636]
22 **Gudelj I**, Lauc G, Pezer M. Immunoglobulin G glycosylation in aging and diseases. *Cell Immunol* 2018; **333**: 65-79 [PMID: 30107893 DOI: 10.1016/j.cellimm.2018.07.009]
23 **Dall'Olio F**, Malagolini N. Immunoglobulin G Glycosylation Changes in Aging and Other Inflammatory Conditions. *Exp Suppl* 2021; **112**: 303-340 [PMID: 34687015 DOI: 10.1007/978-3-030-76912-3_10]
24 **Yang AP**, Liu J, Yue LH, Wang HQ, Yang WJ, Yang GH. Neutrophil CD64 combined with PCT, CRP and WBC improves the sensitivity for the early diagnosis of neonatal sepsis. *Clin Chem Lab Med* 2016; **54**: 345-351 [PMID: 26351925 DOI: 10.1515/cclm-2015-0277]
25 **Wojcik M**, Zieleniak A, Mac-Marcjanek K, Wozniak LA, Cypryk K. The elevated gene expression level of the A(2B) adenosine receptor is associated with hyperglycemia in women with gestational diabetes mellitus. *Diabetes Metab Res Rev* 2014; **30**: 42-53 [PMID: 23956030 DOI: 10.1002/dmrr.2446]

26 **Li YX**, Long DL, Liu J, Qiu D, Wang J, Cheng X, Yang X, Li RM, Wang G. Gestational diabetes mellitus in women increased the risk of neonatal infection *via* inflammation and autophagy in the placenta. *Medicine (Baltimore)* 2020; **99**: e22152 [PMID: 33019392 DOI: 10.1097/MD.0000000000022152]

World Journal of Diabetes

Submit a Manuscript: https://www.f6publishing.com

World J Diabetes 2023 September 15; 14(9): 1403-1411

DOI: 10.4239/wjd.v14.i9.1403

ISSN 1948-9358 (online)

ORIGINAL ARTICLE

Retrospective Study

Risk factors of concurrent urinary sepsis in patients with diabetes mellitus comorbid with upper urinary tract calculi

Jian-Jie Gou, Chao Zhang, Hai-Song Han, Hong-Wei Wu

Specialty type: Endocrinology and metabolism

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B, B, B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Sachmechi I, United States; Selvin E, United States; Cai L, United States

Received: June 6, 2023
Peer-review started: June 6, 2023
First decision: June 21, 2023
Revised: June 30, 2023
Accepted: July 18, 2023
Article in press: July 18, 2023
Published online: September 15, 2023

Jian-Jie Gou, Chao Zhang, Hai-Song Han, Department of Urology Surgery, People's Hospital of Jincheng, Jincheng 048000, Shanxi Province, China

Hong-Wei Wu, Emergency Department, People's Hospital of Jincheng, Jincheng 048000, Shanxi Province, China

Corresponding author: Hong-Wei Wu, MM, Associate Chief Physician, Emergency Department, People's Hospital of Jincheng, No. 456 Wenchang East Street, Zhongjiazhuang, Jincheng 048000, Shanxi Province, China. 13935660657@163.com

Abstract

BACKGROUND

Urinary sepsis is frequently seen in patients with diabetes mellitus (DM) complicated with upper urinary tract calculi (UUTCs). Currently, the known risk factors of urinary sepsis are not uniform.

AIM

To analyze the risk factors of concurrent urinary sepsis in patients with DM complicated with UUTCs by logistic regression.

METHODS

We retrospectively analyzed 384 patients with DM complicated with UUTCs treated in People's Hospital of Jincheng between February 2018 and May 2022. The patients were screened according to the inclusion and exclusion criteria, and 204 patients were enrolled. The patients were assigned to an occurrence group (n = 78) and a nonoccurrence group (n = 126). Logistic regression was adopted to analyze the risk factors for urinary sepsis, and a risk prediction model was established.

RESULTS

Gender, age, history of lumbago and abdominal pain, operation time, urine leukocytes (U-LEU) and urine glucose (U-GLU) were independent risk factors for patients with concurrent urinary sepsis ($P < 0.05$). Risk score = 0.794 × gender + 0.941 × age + 0.901 × history of lumbago and abdominal pain - 1.071 × operation time + 1.972 × U-LEU + 1.541 × U-GLU. The occurrence group had notably higher risk scores than the nonoccurrence group ($P < 0.0001$). The area under the curve of risk score for forecasting concurrent urinary sepsis in patients was 0.801, with specificity of 73.07%, sensitivity of 79.36% and Youden index of 52.44%.

CONCLUSION
Sex, age, history of lumbar and abdominal pain, operation time, ULEU and UGLU are independent risk factors for urogenic sepsis in diabetic patients with UUTC.

Key Words: Diabetes mellitus; Upper urinary tract calculi; Urinary sepsis; Risk factors; Risk prediction model; Logistic regression; Concurrent urinary sepsis

Core Tip: This study was to determine risk factors of concurrent urinary sepsis in patients with diabetes mellitus comorbid with upper urinary tract calculi and construct a risk prediction model. Gender, age, history of lumbago and abdominal pain, operation time, urinary leukocytes and urinary glucose were independent risk factors for concurrent urinary sepsis. It is helpful to identify high-risk patients at an early stage and implement active and effective intervention measures to reduce complications and improve the prognosis of patients.

Citation: Gou JJ, Zhang C, Han HS, Wu HW. Risk factors of concurrent urinary sepsis in patients with diabetes mellitus comorbid with upper urinary tract calculi. *World J Diabetes* 2023; 14(9): 1403-1411
URL: https://www.wjgnet.com/1948-9358/full/v14/i9/1403.htm
DOI: https://dx.doi.org/10.4239/wjd.v14.i9.1403

INTRODUCTION

With the improvement of living standards, the incidence of urinary calculi worldwide is increasing gradually. Its incidence in adults in China is 1%-5%, and upper urinary tract calculi (UUTCs) are a more frequently seen problem[1]. Calculi are caused by several factors, such as socioeconomic status, environmental factors and eating habits[2]. The incidence of calculi in northern and southern areas of China differs, with a high incidence in southern areas (5%-10%)[3]. Other factors such as gender and genetic susceptibility can also affect the occurrence of calculi, and the incidence rate among males is three times that among females[4]. In China, the annual incidence of urinary calculi is 150-200 cases per 100000 people, and approximately 25% of patients need hospitalization and surgical treatment. After treatment, the recurrence rate is high at approximately 50% within 10 years[5].

The urethra connects the urinary system with the outside world, providing a way for bacteria and other pathogens to invade the urinary system[6]. Usually, the flushing action during urination and urethral mucosa form a natural protective barrier to prevent bacteria from remaining, growing and reproducing[7]. However, various factors hinder the defensive function of the urinary system. For example, obstruction can result in stagnant water above the obstruction, making it easier for pathogenic bacteria to invade and colonize the urinary system, giving rise to infection[8]. Without timely intervention, the infection may develop into urinary sepsis or even septic shock, endangering the life of patients.

Sepsis is a serious disease and an acute physical reaction caused by infection, with associated physiological, pathological and biochemical abnormalities[9]. Sepsis is defined as organ dysfunction due to the host's uncontrolled immune response to infection. Unfortunately, sepsis is a global health threat with high mortality[10]. Without timely treatment, sepsis may develop into septic shock and multiple organ dysfunctions and even cause death[11].

The incidence of diabetes mellitus (DM) is increasing. According to the latest statistics of the International Diabetes Federation, the global prevalence of DM has reached 9.3%. DM can give rise to systemic damage, leading to immune dysfunction and proneness to serious infection[12]. However, currently, the risk factors for concurrent urinary sepsis in patients with UUTCs and DM are still under investigation.

Accordingly, this study aimed to determine the risk factors for concurrent urinary sepsis in patients with DM comorbid with UUTCs to provide a reference for clinical therapy and prevention.

MATERIALS AND METHODS

Patients

We retrospectively analyzed 384 patients with DM complicated with UUTCs treated in People's Hospital of Jincheng between February 2018 and May 2022. Inclusion criteria were: (1) Imaging results, such as urinary ultrasound, intravenous urography or abdominal computed tomography, suggested the presence of UUTCs; (2) a clear history of DM that met the guidelines for the diagnosis and treatment of senile DM in China[13]; and (3) a complete medical history and laboratory and imaging data. Exclusion criteria were: (1) Age < 18 years; (2) pregnant women; (3) bilateral UUTCs; (4) hematological disease, immune system disease, or malignant tumors; (5) treated with immunomodulatory drugs; and (6) other primary infection, such as lung or abdominal infection. The 384 patients were screened according to the inclusion and exclusion criteria, and 204 patients were enrolled. According to Guidelines for Emergency Treatment of Sepsis/Septic

Shock in China (2018)[14] and the diagnostic criteria for urinary sepsis[14], patients were assigned to an occurrence group (n = 78) and nonoccurrence group (n = 126).

Collection of clinical data

The clinical data were collected through the medical record system of our hospital, including: (1) General information: Gender, age and body mass index; (2) medical history: Lumbago and abdominal pain, hematuria, symptoms of urinary tract irritation, hypertension, and DM; (3) urine examination data: Urinary leukocytes (U-LEU), urinary nitrite (U-NIT), urinary glucose (U-GLU), and urinary occult blood; and (4) imaging examination data: Lateral classification, location, maximum diameter and hydronephrosis of calculi.

Statistical analysis

We used R language 4.1.1 software (R Foundation for Statistical Computing, Vienna, Austria) for data cleaning and analysis, and constructed a model. Logistic regression was adopted for screening the risk factors, and receiver operating characteristic (ROC) curve was adopted for value verification. This study used Graph Pad Prism 8.0 for data visualization. $P < 0.05$ indicated a significant difference.

RESULTS

Analysis of clinical data

The occurrence and nonoccurrence groups did not differ significantly for hematuria and hypertension ($P > 0.05$) (Table 1), but they did differ significantly for gender, age, history of lumbago and abdominal pain, symptoms of urinary tract irritation and operation time ($P < 0.05$) (Table 1).

Comparison of urinary examination indexes

The occurrence and nonoccurrence groups did not differ significantly for urinary occult blood ($P > 0.05$) (Table 2), but they did differ significantly for U-LEU, U-NIT, and U-GLU ($P < 0.01$) (Table 2).

Imaging index detection

The occurrence and nonoccurrence groups did not differ significantly for lateral classification of calculi, obstruction position, maximum calculi diameter and severity of hydronephrosis ($P > 0.05$) (Table 3).

Logistic regression analysis

According to the above results, meaningful indicators were assigned (Table 4). The backward logistic regression method was used. Gender, age, history of lumbago and abdominal pain, operation time, U-LEU and U-GLU were independent risk factors for concurrent urinary sepsis ($P < 0.05$) (Table 5).

Construction of risk model

Based on the β coefficient of logistic regression, a risk score for predicting concurrent urinary sepsis was constructed. Risk score = 0.794 × gender + 0.941 × age + 0.901 × history of lumbago and abdominal pain - 1.071 × operation time + 1.972 × U-LEU + 1.541 × U-GLU. According to the comparison results, the occurrence group had notably higher risk scores than the nonoccurrence group (Figure 1A) ($P < 0.0001$). According to ROC curve analysis, the area under the curve (AUC) of risk score for forecasting concurrent urinary sepsis was 0.801, with specificity of 73.07%, sensitivity of 79.36% and Youden index of 52.44% (Figure 1B).

DISCUSSION

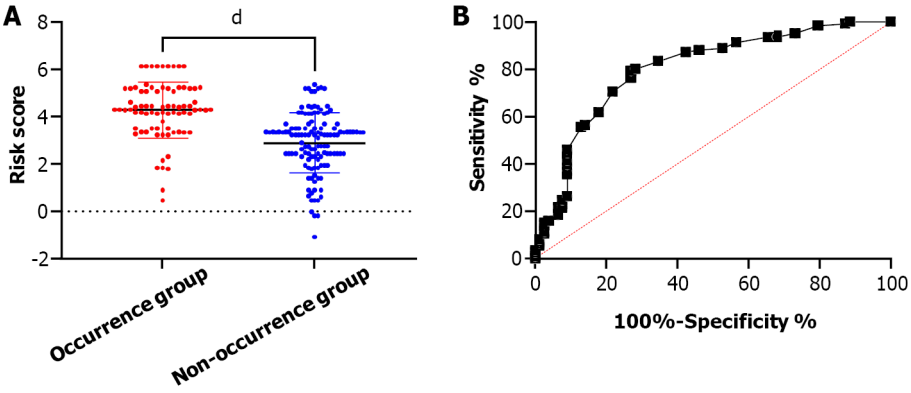
Urinary sepsis is a dangerous disease. Without timely diagnosis and treatment, its prognosis is unfavorable[15]. There are approximately 2.8 to 9.8 million new cases of urinary sepsis every year, with 1.6 million deaths[16]. Thus, it is important to quickly identify urinary sepsis and provide effective timely treatment. In the guidelines of the European Association of Urology (2017 edition), the definition of urinary sepsis has been updated, which emphasizes that the disease is more serious than uncomplicated infection and may cause organ dysfunction and become life-threatening[17]. Patients with UUTCs and DM are more likely to have urinary sepsis[18]. This is because calculi may trigger infection, and DM makes patients susceptible to various diseases[19]. Therefore, early identification of high-risk factors in these patients and effective intervention have become the focus of many hospitals, which has also been recognized by the World Health Organization.

Our study retrospectively analyzed the risk factors for concurrent urinary sepsis in patients with DM complicated with UUTCs. Gender, age, history of lumbago and abdominal pain, operation time, U-LEU and U-GLU were independent risk factors for concurrent urinary sepsis. The risk of urinary sepsis was about 2.212 times higher in women than in men. Prior research has revealed that women with ureteral calculi or who undergo endoscopic lithotripsy face an independently increased risk of urinary sepsis[20]. However, one other study has revealed no independent correlation between gender and incidence of urinary sepsis[21]. Kumar *et al*[22] have revealed that the reasons why older women are prone to urinary

Table 1 Analysis of clinical data

Factors		Occurrence group (n = 78)	Nonoccurrence group (n = 126)	χ^2 value	P value
Gender				9.219	0.002
	Male	30	76		
	Female	48	50		
Age				5.732	0.016
	≥ 60 yr	45	51		
	< 60 yr	33	75		
BMI				0.292	0.588
	≥ 25 kg/m^2	28	50		
	< 25 kg/m^2	50	76		
History of lumbago and abdominal pain				8.459	0.003
	Yes	38	36		
	No	40	90		
Hematuria				0.569	0.450
	Yes	36	65		
	No	42	61		
Symptoms of urinary tract irritation				4.036	0.044
	Yes	60	80		
	No	18	46		
Hypertension				0.533	0.465
	Yes	30	55		
	No	48	71		
Operation time		64.32 ± 15.35	59.94 ± 7.04	4.662	0.001

BMI: Body mass index.



DOI: 10.4239/wjd.v14.i9.1403 **Copyright** ©The Author(s) 2023.

Figure 1 Value of risk score in predicting urinary sepsis in patients with diabetes mellitus and upper urinary tract calculi. A: Risk score in predicting urinary sepsis; B: Area under the curve of risk score for prediction of urinary sepsis. $^dP < 0.0001$.

tract infection and progression to urinary sepsis may include poor perineal hygiene, postmenopausal estrogen deficiency, atrophic vaginitis, uterine and bladder prolapse and the use of vaginal supports.

With the increase of age, the functions of various organs or systems tend to decline, including liver and kidney dysfunction, cardiovascular system defects, and immune system defects[23]. Weakened compensatory ability of organs and systems in patients gives rise to a decline in overall physical function, and patients with DM are more susceptible to

Table 2 Detection of urinary indexes

Factors		Occurrence group (*n* = 78)	Nonoccurrence group (*n* = 126)	χ^2	*P* value
Urine occult blood				1.210	0.750
	-	18	35		
	1+	22	28		
	2+	24	38		
	3+	14	25		
U-LEU				15.330	0.002
	-	4	30		
	1+	26	38		
	2+	20	37		
	3+	28	25		
U-NIT				12.499	0.001
	-	33	85		
	+	45	41		
U-GLU				11.596	0.008
	-	7	33		
	1+	17	31		
	2+	40	50		
	3+	14	12		

U-LEU: Urinary leukocytes; U-NIT: Urinary nitrite; U-GLU: Urinary glucose.

Table 3 Comparison of imaging indexes

Factors		Occurrence group (*n* = 78)	Nonoccurrence group (*n* = 126)	χ^2	*P* value
Lateral classification of calculi				1.494	0.221
	Left	48	88		
	Right	30	38		
Obstruction position				2.938	0.086
	Ureter	64	90		
	Kidney	14	36		
Maximum diameter of calculi				0.048	0.825
	≥ 20 mm	39	65		
	< 20 mm	39	61		
Degree of hydronephrosis				0.400	0.526
	Yes	11	22		
	No	67	104		

infection in such cases[24]. Urinary tract obstruction can easily give rise to secondary infection, systemic inflammatory reaction and even sepsis[25]. In this study, the risk of urinary sepsis in patients aged > 60 years was 2.563 times that in patients < 60 years old, which is in agreement with previous studies.

Similar to prior research, U-LEU was an independent risk factor for UUTCs complicated with urinary sepsis in our study[26]. Some researchers believe that positive urine bacterial culture can more accurately predict the occurrence of urinary sepsis[27]. However, our study did not include the results of urine bacterial culture as a predictor because of the lag time of urine bacterial culture. Usually, it takes 2-3 d or even longer to achieve the results of urine bacterial culture, which leads to a lag in forecasting ability. Therefore, we mainly adopted U-LEU as an indicator, which suggests purulent

Table 4 meaningful indicators were assigned

Factors	Assignment
Gender	Male = 0, female = 1
Age	≥ 60 yr = 1, < 60 yr = 0
History of lumbago and abdominal pain	Yes = 1, no = 0
Symptoms of urinary tract irritation	Yes = 1, no = 0
Operation time	≥ 60 yr = 1, < 60 yr = 0
U-LEU	- = 0, 1 + -3 + = 1
U-NIT	- = 0, + = 1
U-GLU	- = 0, 1 + -3 + = 1
Occurrence	Occurrence group = 1, nonoccurrence group = 0

U-LEU: Urinary leukocytes; U-NIT: Urinary nitrite; U-GLU: Urinary glucose.

Table 5 Logistic multivariate regression

Factors	β	Standard error	χ^2	*P* value	OR	95%CI	
						Lower limit	Upper limit
Gender	0.794	0.335	5.603	0.018	2.212	1.146	4.268
Age	0.941	0.346	7.408	0.006	2.563	1.301	5.047
History of lumbago and abdominal pain	0.901	0.348	6.700	0.010	2.462	1.245	4.871
Symptoms of urinary tract irritation	0.628	0.379	2.753	0.097	1.875	0.892	3.939
Operation time	-1.071	0.345	9.619	0.002	0.342	0.174	0.674
U-LEU	1.972	0.602	10.725	0.001	7.182	2.207	23.373
U-NIT	0.491	0.339	2.101	0.147	1.634	0.841	3.172
U-GLU	1.541	0.509	9.171	0.002	4.668	1.722	12.652

U-LEU: Urinary leukocytes; U-NIT: Urinary nitrite; U-GLU: Urinary glucose.

inflammation in the urinary tract, so it can be used to predict the risk of urinary sepsis.

In patients with DM, the reasons for positive U-GLU may include an increase in blood glucose and decrease in renal glucose threshold. Hyperglycemia causes failure of glucose absorption in the renal tubules, so that glucose is excreted in the urine[18]. Additionally, DM can decrease the ability of renal tubules to absorb glucose, and the glucose in urine cannot be completely reabsorbed[28]. Both of these conditions may lead to positive U-GLU. Positive U-GLU may indicate poor control of DM or diabetic nephropathy. Diabetic nephropathy can easily damage the genitourinary system, causing difficulty in controlling urinary tract infection or recurrence. High concentration of U-GLU provides heat for the growth and metabolism of pathogenic bacteria, which in turn leads to disorder of the body's defense mechanisms.

In this study, a history of lumbago and abdominal pain had a strong correlation with concurrent urinary sepsis in patients with DM and UUTCs. Lumbago and abdominal pain are frequent symptoms of UUTCs, and one of the manifestations of many patients with urinary sepsis[29]. UUTCs can give rise to urinary retention, bacterial reproduction and infection, increasing the risk of urinary sepsis. DM complicated with UUTCs is a risk factor for urinary sepsis[30]. Patients with DM are often accompanied by various pathophysiological changes such as decreased immune function and metabolic disorder, which can lead to urinary tract infection. Therefore, patients with DM and UUTCs are at higher risk of urinary sepsis.

Long operation time increases the risk of infection. Long-term exposure of wound tissue increases the probability of infection by surrounding flora, and long operation time also increases bleeding and absorption of perfusion fluid, which increase the risk of infection. Urethral obstruction during surgery may also lead to urinary retention, creating favorable conditions for bacterial reproduction[24,31].

We constructed a risk prediction model based on the regression coefficient. A prediction model is a mathematical model that estimates the probability of a specific event or disease according to the combination of multiple risk factors. Through the analysis and assessment of risk factors, a data-based model can be established to help doctors and researchers better understand the risk factors of a disease and develop better prevention and treatment plans. In this

study, the occurrence group had notably higher risk scores than the nonoccurrence group, and the AUC of risk score in forecasting urinary sepsis was > 0.8, indicating a high value of the risk model in predicting urinary sepsis.

We analyzed the risk factors for concurrent urinary sepsis in patients with DM complicated with UUTCs by logistic regression model, and successfully constructed a prediction model. However, our study had some limitations. First, there was no external verification of our results. This was because in such a single-center study, it was impossible to establish an effective verification set because of the small number of samples collected. Second, as a single-center research model, its universality needs further verification. Therefore, we hope to carry out prospective research and collect more samples in the future to improve the conclusions.

CONCLUSION

Gender, age, history of lumbago and abdominal pain, operation time, U-LEU and U-GLU were independent risk factors for concurrent urinary sepsis in patients with DM and UUTCs. It is helpful to identify high-risk patients at an early stage and implement effective intervention measures to reduce complications and improve prognosis.

ARTICLE HIGHLIGHTS

Research background

In patients with diabetes mellitus (DM), long-term hyperglycemia can trigger increases in sugar, protein and other substances in urine, promoting formation of calculi. Urinary calculi can lead to urinary tract infection, renal insufficiency and other complications, and even become life-threatening in severe cases, causing a serious impact on the health of patients with DM. However, at present, the risk factors for urinary sepsis are not uniform. The purpose of this study was to analyze the risk factors for urinary sepsis in patients with DM complicated with upper urinary tract calculi (UUTCs) to provide potential indicators for clinical observation.

Research motivation

It is helpful to identify high-risk patients at an early stage and implement and effective intervention measures by constructing a prediction model, thus reducing complications and improving prognosis.

Research objectives

We successfully predicted high-risk patients by establishing a risk model, which was beneficial to clinical and targeted treatment and prevention.

Research methods

We constructed a risk model of urinary sepsis by logistic regression model, which provided an observation model for the prediction of high-risk patients.

Research results

Although we successfully established a risk model, due to the small number of patients, it was impossible to carry out external verification, so more data are needed to verify whether the model is universal.

Research conclusions

Gender, age, history of lumbago and abdominal pain, operation time, and urinary leukocytes and urinary glucose were independent risk factors for concurrent urinary sepsis in patients with DM and UUTCs, and we predicted high-risk patients using a risk model.

Research perspectives

The universality of the model could be verified based on multicenter data, and then extended to clinical practice.

FOOTNOTES

Author contributions: Gou JJ designed and performed the research and wrote the paper; Zhang C designed the research and supervised the report; Han HS designed the research and contributed to the analysis; Wu HW provided clinical advice; Zhang C, Han HS, and Wu HW supervised the report.

Institutional review board statement: The study was reviewed and approved by the Institutional review board of People's Hospital of Jincheng (Approval No. JCPH.No20230401001).

Informed consent statement: All study participants or their legal guardian provided informed written consent about personal and medical data collection prior to study enrolment.

Conflict-of-interest statement: The authors declare no conflicts of interest for this article.

Data sharing statement: Dataset available from the corresponding author.

Country/Territory of origin: China

ORCID number: Jian-Jie Gou 0009-0003-1174-6094; Chao Zhang 0009-0002-2945-3241; Hai-Song Han 0009-0007-4986-0895; Hong-Wei Wu 0009-0006-5516-8769.

S-Editor: Chen YL
L-Editor: A
P-Editor: Chen YL

REFERENCES

1 **Abedi G**, Monga M. Flexible Ureteroscopy for Treatment of Upper Urinary Tract Calculus. *J Endourol* 2021; **35**: S56-S61 [PMID: 34499545 DOI: 10.1089/end.2020.1018]
2 **Wang X**, Zhang Y, Zhao F, Li J, Yian Y. Symptomatic recurrence rate of upper urinary tract calculi in children after endourological procedures. *J Pediatr Urol* 2022; **18**: 141.e1-141.e7 [PMID: 35300915 DOI: 10.1016/j.jpurol.2021.09.025]
3 **Lou B**, Sun Y, Lin J, Yuan Z, He L, Long C, Lin X. Clinical Features of Endogenous Endophthalmitis Secondary to Minimally Invasive Upper Urinary Tract Calculus Removal. *Ocul Immunol Inflamm* 2022; **30**: 104-110 [PMID: 32809901 DOI: 10.1080/09273948.2020.1778732]
4 **Prasongwatana V**, Bovornpadungkitti S, Chotikawanich E, Pachitrat K, Suwanatrai S, Sriboonlue P. Chemical components of urinary stones according to age and sex of adult patients. *J Med Assoc Thai* 2008; **91**: 1589-1594 [PMID: 18972904]
5 **Lai L**, Zhang W, Zheng F, Wang T, Bai P, Liu Z, Zheng J, Shao Z, Duan B, Wang H, Xing J, Chen H, Huang Y, Chen B. Comparison of the Efficacy of ShuoTong Ureteroscopy and Simple Flexible Ureteroscopy in the Treatment of Unilateral Upper Ureteral Calculi. *Front Surg* 2021; **8**: 707022 [PMID: 34646858 DOI: 10.3389/fsurg.2021.707022]
6 **Nicolle LE**. Urinary tract infection. *Crit Care Clin* 2013; **29**: 699-715 [PMID: 23830659 DOI: 10.1016/j.ccc.2013.03.014]
7 **Petrosillo N**, Granata G, Boyle B, Doyle MM, Pinchera B, Taglietti F. Preventing sepsis development in complicated urinary tract infections. *Expert Rev Anti Infect Ther* 2020; **18**: 47-61 [PMID: 31795788 DOI: 10.1080/14787210.2020.1700794]
8 **Tan N**, Xu L, Wu J. Analysis of the Causes and Preventive Strategies of Urogenic Sepsis after Flexible Ureteroscopic Lithotripsy. *Evid Based Complement Alternat Med* 2022; **2022**: 5332101 [PMID: 36118082 DOI: 10.1155/2022/5332101]
9 **Purcarea A**, Sovaila S. Sepsis, a 2020 review for the internist. *Rom J Intern Med* 2020; **58**: 129-137 [PMID: 32396142 DOI: 10.2478/rjim-2020-0012]
10 **Salomão R**, Ferreira BL, Salomão MC, Santos SS, Azevedo LCP, Brunialti MKC. Sepsis: evolving concepts and challenges. *Braz J Med Biol Res* 2019; **52**: e8595 [PMID: 30994733 DOI: 10.1590/1414-431X20198595]
11 **Rello J**, Valenzuela-Sánchez F, Ruiz-Rodriguez M, Moyano S. Sepsis: A Review of Advances in Management. *Adv Ther* 2017; **34**: 2393-2411 [PMID: 29022217 DOI: 10.1007/s12325-017-0622-8]
12 The Prevention of Diabetes Mellitus. *JAMA* 2021; **325**: 190 [PMID: 33433568 DOI: 10.1001/jama.2020.17738]
13 **Deng MQ**, Pan Q, Xiao XH, Guo LX. [Interpretations of guideline for the management of diabetes mellitus in the elderly in China (2021 edition)]. *Zhonghua Nei Ke Za Zhi* 2021; **60**: 954-959 [PMID: 34689515 DOI: 10.3760/cma.j.cn112138-20210305-00183]
14 **Napolitano LM**. Sepsis 2018: Definitions and Guideline Changes. *Surg Infect (Larchmt)* 2018; **19**: 117-125 [PMID: 29447109 DOI: 10.1089/sur.2017.278]
15 **Porat A**, Bhutta BS, Kesler S. Urosepsis. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing, 2023
16 **Guliciuc M**, Maier AC, Maier IM, Kraft A, Cucuruzac RR, Marinescu M, Şerban C, Rebegea L, Constantin GB, Firescu D. The Urosepsis-A Literature Review. *Medicina (Kaunas)* 2021; **57** [PMID: 34577795 DOI: 10.3390/medicina57090872]
17 **Cotterill N**, Madersbacher H, Wyndaele JJ, Apostolidis A, Drake MJ, Gajewski J, Heesakkers J, Panicker J, Radziszewski P, Sakakibara R, Sievert KD, Hamid R, Kessler TM, Emmanuel A. Neurogenic bowel dysfunction: Clinical management recommendations of the Neurologic Incontinence Committee of the Fifth International Consultation on Incontinence 2013. *Neurourol Urodyn* 2018; **37**: 46-53 [PMID: 28640977 DOI: 10.1002/nau.23289]
18 **Hong X**, Liu G, Chi Z, Yang T, Zhang Y. Predictive model for urosepsis in patients with Upper Urinary Tract Calculi based on ultrasonography and urinalysis using artificial intelligence learning. *Int Braz J Urol* 2023; **49**: 221-232 [PMID: 36638148 DOI: 10.1590/S1677-5538.IBJU.2022.0450]
19 **Sabih A**, Leslie SW. Complicated Urinary Tract Infections. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing, 2023
20 **Liu J**, Yang Q, Lan J, Hong Y, Huang X, Yang B. Risk factors and prediction model of urosepsis in patients with diabetes after percutaneous nephrolithotomy. *BMC Urol* 2021; **21**: 74 [PMID: 33910537 DOI: 10.1186/s12894-021-00799-3]
21 **Kino M**, Hayashi T, Hino D, Nakada T, Kitoh H, Akakura K. Patients' poor performance status is an independent risk factor for urosepsis induced by kidney and ureteral stones. *Urolithiasis* 2021; **49**: 477-484 [PMID: 33755744 DOI: 10.1007/s00240-021-01256-4]
22 **Kumar S**, Bag S, Ganesamoni R, Mandal AK, Taneja N, Singh SK. Risk factors for urosepsis following percutaneous nephrolithotomy: role of 1 week of nitrofurantoin in reducing the risk of urosepsis. *Urol Res* 2012; **40**: 79-86 [PMID: 21567157 DOI: 10.1007/s00240-011-0386-6]
23 **Cao JD**, Wang ZC, Wang YL, Li HC, Gu CM, Bai ZG, Chen ZQ, Wang SS, Xiang ST. Risk factors for progression of Urolith Associated with

Obstructive Urosepsis to severe sepsis or septic shock. *BMC Urol* 2022; **22**: 46 [PMID: 35346141 DOI: 10.1186/s12894-022-00988-8]

24 **Bhojani N**, Miller LE, Bhattacharyya S, Cutone B, Chew BH. Risk Factors for Urosepsis After Ureteroscopy for Stone Disease: A Systematic Review with Meta-Analysis. *J Endourol* 2021; **35**: 991-1000 [PMID: 33544019 DOI: 10.1089/end.2020.1133]

25 **Chugh S**, Pietropaolo A, Montanari E, Sarica K, Somani BK. Predictors of Urinary Infections and Urosepsis After Ureteroscopy for Stone Disease: a Systematic Review from EAU Section of Urolithiasis (EULIS). *Curr Urol Rep* 2020; **21**: 16 [PMID: 32211969 DOI: 10.1007/s11934-020-0969-2]

26 **Amier Y**, Zhang Y, Zhang J, Yao W, Wang S, Wei C, Yu X. Analysis of Preoperative Risk Factors for Postoperative Urosepsis After Mini-Percutaneous Nephrolithotomy in Patients with Large Kidney Stones. *J Endourol* 2022; **36**: 292-297 [PMID: 34569289 DOI: 10.1089/end.2021.0406]

27 **Zhu Z**, Cui Y, Zeng H, Li Y, Zeng F, Chen Z, Hequn C. The evaluation of early predictive factors for urosepsis in patients with negative preoperative urine culture following mini-percutaneous nephrolithotomy. *World J Urol* 2020; **38**: 2629-2636 [PMID: 31828354 DOI: 10.1007/s00345-019-03050-9]

28 **Sun L**, Diao X, Gang X, Lv Y, Zhao X, Yang S, Gao Y, Wang G. Risk Factors for Cognitive Impairment in Patients with Type 2 Diabetes. *J Diabetes Res* 2020; **2020**: 4591938 [PMID: 32377520 DOI: 10.1155/2020/4591938]

29 **Wang S**, Yuan P, Peng E, Xia D, Xu H, Wang S, Ye Z, Chen Z. Risk Factors for Urosepsis after Minimally Invasive Percutaneous Nephrolithotomy in Patients with Preoperative Urinary Tract Infection. *Biomed Res Int* 2020; **2020**: 1354672 [PMID: 31998778 DOI: 10.1155/2020/1354672]

30 **Liang X**, Huang J, Xing M, He L, Zhu X, Weng Y, Guo Q, Zou W. Risk factors and outcomes of urosepsis in patients with calculous pyonephrosis receiving surgical intervention: a single-center retrospective study. *BMC Anesthesiol* 2019; **19**: 61 [PMID: 31039739 DOI: 10.1186/s12871-019-0729-3]

31 **Zhu H**, Liu B, Karagöz MA, Yue G, Lei Y, Dou S, Xu Z, Liu Y. Reasons and risk factors for delayed discharge after day-surgery percutaneous nephrolithotomy. *BMC Urol* 2022; **22**: 209 [PMID: 36544109 DOI: 10.1186/s12894-022-01159-5]

World Journal of Diabetes

Submit a Manuscript: https://www.f6publishing.com

World J Diabetes 2023 September 15; 14(9): 1412-1421

DOI: 10.4239/wjd.v14.i9.1412

ISSN 1948-9358 (online)

ORIGINAL ARTICLE

Retrospective Study

Effect of sitagliptin combined with Yiqi yangyin huoxue decoction on clinical efficacy and hemorheology in early diabetic nephropathy

Jun Ling, Yan-Hua Yang

Specialty type: Endocrinology and metabolism

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C, C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Ceriello A, Italy; Olvera-Barrios A, United Kingdom

Received: July 4, 2023
Peer-review started: July 4, 2023
First decision: July 19, 2023
Revised: July 27, 2023
Accepted: August 7, 2023
Article in press: August 7, 2023
Published online: September 15, 2023

Jun Ling, Yan-Hua Yang, Department of Pharmacy, Changzhou Geriatric Hospital Affiliated to Soochow University, Changzhou NO. 7 People's Hospital, Changzhou 213000, Jiangsu Province, China

Corresponding author: Yan-Hua Yang, MM, Associate Chief Physician, Department of Pharmacy, Changzhou Geriatric Hospital Affiliated to Soochow University, Changzhou NO. 7 People's Hospital, No. 288 Yanling East Road, Economic Development Zone, Changzhou 213000, Jiangsu Province, China. yyh0728@163.com

Abstract

BACKGROUND

Early diabetic nephropathy (DN) is a complication of diabetes mellitus. It mainly affects kidney microvessels and glomerular function, and its timely and effective treatment is critical for early DN. However, the effects of treatments comprising simple Western medicine are not optimal. With the promotion and implementation of integrated Chinese and western medicine treatments, remarkable results have been achieved for many diseases. To this end, we explored the clinical efficacy of integrated traditional Chinese and western medicines for the treatment of early DN.

AIM

To investigate the effect of sitagliptin tablets combined with Yiqi yangyin huoxue decoction on clinical efficacy and hemorheology in patients with early DN.

METHODS

Through a retrospective analysis, 123 patients with early DN were admitted to the endocrinology clinic of the Changzhou NO. 7 People's Hospital from January 2021 to October 2022 and were selected as study subjects. After rigorous screening, 100 patients with early DN were enrolled. The control group (CG, n = 50) and the observation group (OG, n = 50) were divided according to the treatment method. The CG were treated with sitagliptin, and the OG were treated with sitagliptin plus the Yiqi yangyin huoxue decoction. Both groups were treated for 3 mo. For both groups, the baseline data and clinical efficacy were compared, and changes in blood glucose levels, lipid levels, renal function, and hematological indicators before (T0) and after (T1) treatment were assessed.

RESULTS

The total effective rate for the OG was 94.00% and that of the CG was 80.00% (P <

0.05). After treatment (T1), the levels of fasting blood glucose, 2 h postprandial glucose, total cholesterol, triacylglycerol, and low-density lipoprotein cholesterol in OG patients were obviously lower than those in the CG ($P < 0.05$), and cystatin C, homocysteine, urinary microalbumin, and blood creatinine values in OG patients were also obviously lower than those in the CG ($P < 0.05$); erythrocyte deposition, plasma viscosity, whole blood high shear viscosity, and whole blood low shear viscosity were markedly lower in OG patients than in the CG ($P < 0.05$).

CONCLUSION

Sitagliptin combined with Yiqi yangyin huoxue decoction has a remarkable effect when used to treat patients with early DN. Further, it is helpful in improving hemorheological indices and controlling disease progression.

Key Words: Sitagliptin; Yiqi yangyin huoxue decoction; Early diabetic nephropathy; Renal function; Clinical effects; Hemorheology

Core Tip: Early diabetic nephropathy (DN) mainly involves kidney microvessels and glomerular functions, and its timely and effective treatment is critical. In clinical practice, the overall treatment effect of medication alone is not ideal. The development of traditional Chinese medicine has demonstrated its superiority for the treatment of many diseases. In the present study, sitagliptin tablets combined with a blood decoction were used to treat patients with early DN. The results showed that sitagliptin combined with Yiqi yangyin huoxue decoction had a significant effect on the treatment of early DN, which was helpful in improving hemorheological indices.

Citation: Ling J, Yang YH. Effect of sitagliptin combined with Yiqi yangyin huoxue decoction on clinical efficacy and hemorheology in early diabetic nephropathy. *World J Diabetes* 2023; 14(9): 1412-1421
URL: https://www.wjgnet.com/1948-9358/full/v14/i9/1412.htm
DOI: https://dx.doi.org/10.4239/wjd.v14.i9.1412

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease characterized by defective insulin secretion and activity. The prevalence of diabetes in China has been increasing, and that of DM in people over 18 years of age is as high as 11.2%[1]. Approximately 20%–40% of patients with diabetes in China have diabetic nephropathy (DN), and 21.8% of type 2 diabetes patients have this condition[2]. Early DN renal injury involves the glomeruli, renal tubules, renal interstitium, and renal blood vessels, with an increase in persistent urinary albumin excretion and a progressive decrease in the glomerular filtration rate, Early DN with glucose and lipid metabolism disorders, insulin resistance, hemodynamic changes, oxidative stress, inflammation, autophagy disorders, and other mechanisms[2-3]. Some patients with Type 2 DM (T2DM) have elevated urinary albumin levels upon diagnosis. If the microalbuminuria period is not treated promptly, it will rapidly progress to a lengthy albuminuria period until end-stage renal disease develops[4]. End-stage treatment of DN is more difficult than that of other renal diseases, with high disability rates. Accordingly, delaying the progression of DN is the focus of current research.

Presently, the key to clinical and early stage DN management is timely and effective treatment, such that disease development can be effectively controlled, which is beneficial for improving prognosis. Western medicine mainly involves lifestyle adjustments, a low-protein diet, lowering glucose and blood pressure, adjusting fat, reducing proteinuria, and improving renal function, as fundamental treatment principles[5]. Sitagliptin is a dipeptidyl peptidase-4 inhibitor that effectively improves fasting and postprandial blood glucose[6] in patients with T2DM[6]. Clinically, sitagliptin attenuates the oxidative stress response in kidney tissues, delays the progression of DN, and prevents the deterioration of renal function[7]. However, owing to the poor prognosis of DN, the overall treatment impact of individual medications is not ideal; therefore, other therapeutic drugs must be used in combination to improve the curative effect on early DN. With the development of traditional Chinese medicine (TCM), targeted protection of the potentially involved viscera, the predictable control of disease progress, and intervention-based treatment, significant results have been achieved for many diseases[8]. Therefore, this study was based on the basic pathogenesis of early DN with Qi and Yin insufficiency, dampness and heat, the application of sitagliptin tablets with Yiqi yangyin huoxue decoction for the treatment of early DN patients, an observation of its clinical impact on early DN patients, and its influence on blood rheology for early clinical intervention and to delay the progression of DN.

MATERIALS AND METHODS

Patient characteristics

Through a retrospective analysis, 123 patients with early DN admitted to the endocrinology clinic of the Changzhou NO. 7 People's Hospital from January 2021 to October 2022 were selected as the study participants. After rigorous screening, 100 patients with early DN were enrolled. The control group (CG, n = 50) and the observation group (OG, n = 50) were divided according to the treatment method.

The inclusion criteria were as follows: (1) All patients were between 18 and 75 years of age, independent of sex; (2) All patients met the diagnostic criteria for T2DM; and (3) Baseline data, clinical characteristics, laboratory indicators, and other data of all patients were complete. The exclusion criteria were as follows: (1) Patients with type 1 DM or other special types of DM; (2) Patients with infectious diseases, liver disease, cardiovascular disease, urinary system diseases, and kidney diseases; (3) Patients with malignant tumors; (4) Those unable to cooperate in clinical treatment or with mental illness; (5) Pregnant or lactating women; (6) Recent cases of diabetic ketoacidosis; and (7) Patients whose renal lesions were not induced by DM.

Diagnostic criteria for T2DM were as follows, based on the 1999 World Health Organization expert consultation report [9,10]: (1) Typical diabetes symptoms (polydipsia, polyuria, polyphagia, unexplained weight loss) plus random blood glucose ≥ 11.1 mmol/L; (2) Fasting blood glucose (FBG) ≥ 7.0 mmol/L; and (3) Oral glucose tolerance test 2 h blood glucose test ≥ 11.1 mmol/L, for patients without diabetes symptoms, which needed to be repeated on another day. Diagnostic criteria for early DN[11] were as follows: Referring to the 2012 updated Kidney Disease Outcomes Quality Initiative Clinical Practice Guidelines for Diabetes and Chronic Kidney Disease, excluding other causes (24 h infection, fever, congestive heart failure) of chronic kidney disease, the urine microalbumin (mg/L)/urine creatinine (g/L) (UACR) was significantly elevated in two of three consecutive examinations (30 mg/g ≤ UACR ≤ 300 mg/g). DN staging using the international universal Mogensen staging[12] was performed as follows: Mogensen I, II, and III stages represent early DN; the urinary albumin excretion rate in early DN was examined three consecutive times within 3 mo and was approximately 20–200 μg/min or 30–300 mg/d. Diagnostic criteria for TCM symptoms[13] were as follows: Consistent with Qi and Yin insufficiency and blood stasis syndrome, the main symptoms were turbid urine, dull complexion, hand-foot-heart heat, fatigue, limb edema, and low back pain; secondary symptoms were frequent urination, palpitations, dry throat, dry mouth, skin nails, and limb numbness; and the tongue pulse was as follows: Tongue thin, red or dark purple, sublingual vein, ecchymosis, and a weak or string pulse.

Therapeutic method

All enrolled participants received basic treatment as follows: (1) Diabetes health knowledge education, focusing on explaining the relevant knowledge of early DN to the participants and persuading patients to correctly understand and treat such diseases so that they can maintain a good mood throughout the treatment process; (2) Diet control, developing a high-quality, low-protein diet for each patient, a personalized diet plan, and a low-salt diet for patients with hypertension; (3) Exercise guidance, according to the specific circumstances of the patient, personalized exercise should be arranged for the patient to ensure that aerobic exercise and anaerobic exercises are performed alternately, with the exercise time for each patient being ≥ 150 min per week, thus gradually improving the exercise level of the patient; (4) Blood glucose-lowering therapy, based on the actual blood glucose status of the patient, oral medicine or insulin was selected and the blood glucose index was monitored in real time to ensure that it was stable and met the inclusion criteria; FBG was controlled between 5 and 8 mmol/L, and 2 h postprandial glucose (2 h PG) was controlled between 7 and 10 mmol/L; and (5) Symptomatic treatment, other treatments that did not interfere with the purpose of the study and non-angiotensin receptor blockers drugs were used to regulate blood pressure to ensure that this met the inclusion criteria.

The CG patients were treated with sitagliptin (Merck Sharp & Dohme Ltd., Chinese medicine approval word: J20140095, specification: 100 mg, seven tablets and four boards) at 100 mg/time, po, qd, and treatment was performed for 3 mo. OG patients were orally administered sitagliptin combined with Yiqi yangyin huoxue decoction daily in the morning and evening once each, after meals, and treatment was performed for 3 mo. The Yiqi yangyin huoxue decoction proposed by the investigator and the prescription composition are listed in Table 1.

Observational indicators

(1) General observation measures included sex, age, disease duration, systolic blood pressure (SBP), diastolic blood pressure (DBP), and body mass index (BMI); (2) Based on the Guiding Principles for Clinical Research on the Treatment of Diabetes with New TCM and the Guiding Principles for Clinical Research of New TCM in the Treatment of Chronic Nephritis, complete cure: 24 h urine albumin excretion rate (UAER) normal, renal function is normal; excellent: 24 h UAER reduced by 40%, normal or basically normal renal function; effective, 24 h UAER reduced by < 40%, renal function is normal or improved; invalid: Clinical manifestations and laboratory examination are not improved or aggravated; (3) Improvements in pre-treatment (T0) and posttreatment (T1) blood glucose levels, namely FBG and 2 h PG; (4) The blood lipid contents before treatment (T0) and after treatment (T1), namely total cholesterol, triacylglycerol, and low-density lipoprotein cholesterol (LDL-C), were compared between the two groups; (5) Pre-and post-treatment (T0) renal functions, including improvements in cystatin C (Cys-C), homocysteine (HCY), urinary microalbumin (U-mAlb), and blood creatinine (Cr) levels, were also compared; and (6) Blood rheological indices before (T0) and after treatment (T1), including changes in red blood cell deposition, plasma viscosity, high resection viscosity of whole blood, and low resection viscosity of whole blood, were assessed.

Table 1 Composition of decoction used for nourishing Qi, nourishing Yin, and promoting blood circulation

Type	Dosage, g
Astragalus	20
Chinese yam	15
Poria cocos	10
Dwarf lilyturf root	10
Danpi	10
Common peony root	10
Prepared *Rehmannia* root	10
Dogberry	15
Sealwort	10
Root of Zhejiang figwort	10
Ligusticum wallichii	10

Statistical analysis

Data were analyzed using SPSS statistical software version 25.0. The measurement data were expressed as the mean ± SD and analyzed based on a *t*-test. Count data are expressed as the frequency percentage (n%) and were compared by performing a χ^2 test. Statistical significance was set at $P < 0.05$.

RESULTS

Basic data analysis

The number of patients included and the flow chart of the analysis method are shown in Figure 1. There was no statistically significant difference in gender, age, course of disease, SBP, DBP, and BMI clinical data between the two groups of patients ($P > 0.05$) are presented in Table 2.

Evaluation of therapeutic efficiency

After 3 mo of treatment, the total effective rate was 94.00% for OG patients; the total effective rate was 80.00% for CG patients, the difference between the two groups is statistically significant ($P < 0.05$; Figure 2).

Improvement in blood glucose levels

After treatment (T1), the FBG and 2 h PG levels in the OG patients were markedly lower than those in the CG patients ($P < 0.05$; Figure 3).

Improvement in blood lipid levels

At T0, a comparison of TC, TG, and LDL-C contents was not significantly differ between the two groups ($P > 0.05$), and at T1, the contents of TC, TG, and LDL-C in the OG were markedly lower than those in the CG ($P < 0.05$; Table 3).

Improvement in renal function

At T0, serum Cys-C, HCY, mAlb, and Cr contents was not significantly differ between the two groups ($P > 0.05$); however, At T1, serum Cys-C, HCY, U-mAlb, and Cr values of patients in the OG were markedly lower than those of patients in the CG ($P < 0.05$; Table 4).

Improvement in hemorheology

At T1, erythrocyte deposition, plasma viscosity, whole blood high shear viscosity, and whole blood low shear viscosity in the OG were markedly lower than those in the CG ($P < 0.05$; Table 5).

DISCUSSION

The basic pathogenesis of diabetes is dryness-heat due to insufficiency. Qi Yin deficiency and meridian obstruction are the pathological causes of diabetes. The early stage of diabetes is dominated by Yin deficiency with heat excess, but when it progresses to the stage of DN, the disease is mostly at the stage of Qi and Yin insufficiency, and blood stasis becomes an important aspect of its pathogenesis[14]. Clinically, early DN is commonly characterized by the insufficiency of both Qi

Table 2 Analysis of basic data between the two groups (mean ± SD)

Index	OG (*n* = 50)	CG (*n* = 50)	χ^2/t value	*P* value
Sex (male/female)	28/22	26/24	0.161	0.688
Age (yr)	51.24 ± 4.43	51.28 ± 4.28	−0.046	0.963
Disease course (yr)	1.48 ± 0.47	1.49 ± 0.57	−0.188	0.851
SBP (mmHg)	123.47 ± 10.28	124.16 ± 11.33	−0.319	0.750
DBP (mmHg)	78.26 ± 4.15	79.51 ± 4.36	−1.480	0.142
BMI (kg/m^2)	25.21 ± 1.27	25.19 ± 1.44	0.038	0.970

The course of the disease was diabetic nephropathy. OG: Observation group; CG: Control group; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; BMI: Body mass index.

Table 3 Improvement in blood lipid levels in both groups (mean ± SD)

Group	Time	OG (*n* = 50)	CG (*n* = 50)	*t*-value	*P* value
TC (mmol/L)	T0	7.05 ± 1.21	7.08 ± 1.19	−0.166	0.868
	T1	4.97 ± 0.73	6.13 ± 0.84	−7.366	< 0.001
TC (mmol/L)	T0	1.65 ± 0.17	1.63 ± 0.14	0.637	0.525
	T1	1.32 ± 0.28	1.49 ± 0.21	−3.475	< 0.001
LDL-C (mmol/L)	T0	4.13 ± 0.68	4.09 ± 0.71	0.219	0.827
	T1	1.87 ± 0.42	2.45 ± 0.67	−5.196	< 0.001

OG: Observation group; CG: Control group; TC: Total cholesterol; TG: Triacylglycerol; LDL-C: Low-density lipoprotein cholesterol; T0: Pre-treatment; T1: Post-treatment.

Table 4 Improvement in renal function in the two groups (mean ± SD)

Group	Time	OG (*n* = 50)	CG (*n* = 50)	*t*-value	*P* value
Cys-C (mg/L)	T0	1.59 ± 0.43	1.57 ± 0.45	0.333	0.74
	T1	1.57 ± 0.45	1.48 ± 0.39	−2.566	0.012
HCY (μmol/L)	T0	30.41 ± 5.73	30.44 ± 5.69	−0.018	0.986
	T1	19.28 ± 3.15	22.36 ± 4.28	−4.097	< 0.001
U-mAlb (mg/L)	T0	195.67 ± 15.28	195.73 ± 15.14	−0.017	0.987
	T1	162.38 ± 11.54	174.63 ± 13.45	−4.887	< 0.001
Cr (μmol/L)	T0	102.54 ± 9.47	102.71 ± 9.24	−0.096	0.924
	T1	89.24 ± 7.36	96.53 ± 8.47	-4.591	< 0.001

OG: Observation group; CG: Control group; Cys-C: Cystatin C; HCY: Homocysteine; U-mAlb: Urinary microalbumin; Cr: Blood creatinine.

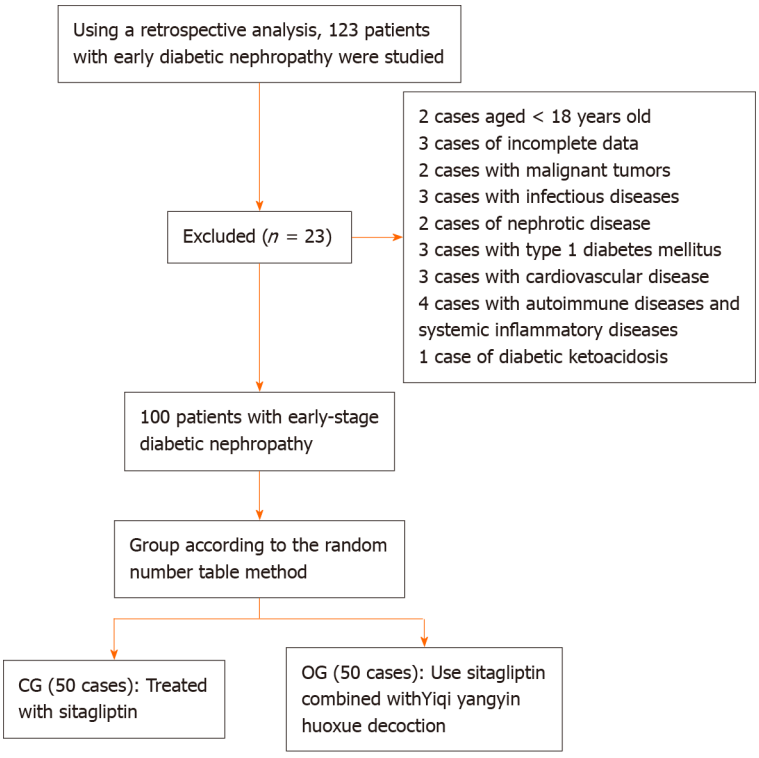
and Yin, as well as blood stasis of the venation. Its pathogenesis includes meridian infarction, blocked veins, and deficiency of both Qi and Yin. For DN (see TCM), the following are noted: Urinary turbidity, fatigue, hot hands and feet, dull complexion, swollen limbs, low back pain or frequent urination, palpitations and restlessness, dry throat and mouth, skin and nail lesions, numbness of limbs, tongue thinning and exhibiting a dark red or purple texture, tortuous sublingual veins, ecchymosis, and weak or astringent veins[15]. Therefore, the early detection of DN is beneficial for controlling disease progression. Owing to the poor efficacy of drug treatment alone, we used western medicine and TCM for targeted treatment.

This study shows that the clinical effectiveness of Yiqi yangyin huoxue decoction for the treatment of early DN, as well as that compared with conventional western medicine treatment. We found that the total response rate of patients in the OG was markedly better than that in the CG, indicating that sitagliptin and Yiqi yangyin huoxue decoction, used to treat early diabetic kidney disease, have a good synergistic effect, which can effectively relieve the clinical symptoms of early

Table 5 Improvement in hemorheology in the two groups (mean ± SD)

Group	Time	OG (*n* = 50)	CG (*n* = 50)	*t*-value	*P* value
Red blood cell deposition (%)	T0	46.88 ± 5.23	46.91 ± 5.19	−0.029	0.977
	T1	37.15 ± 4.13	43.96 ± 4.87	−7.542	< 0.001
Plasma viscosity (mPa·s)	T0	3.26 ± 0.38	3.29 ± 0.43	−0.490	0.625
	T1	1.52 ± 0.25	2.27 ± 0.31	−13.465	< 0.001
Whole blood high shear viscosity (mPa·s)	T0	5.94 ± 1.13	5.59 ± 1.07	1.598	0.113
	T1	4.06 ± 0.75	5.13 ± 0.81	−6.854	< 0.001
Whole blood low shear viscosity (mPa·s)	T0	10.29 ± 2.34	10.33 ± 2.29	−0.108	0.914
	T1	7.70 ± 1.25	8.72 ± 1.64	−3.527	0.001

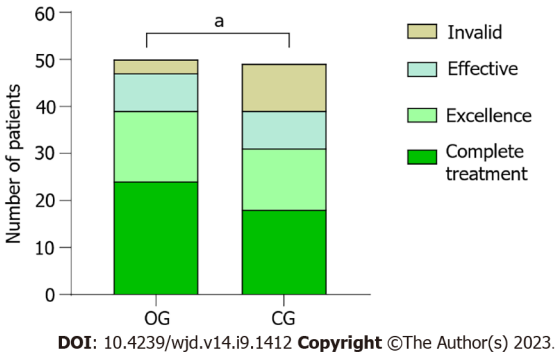
OG: Observation group; CG: Control group.



DOI: 10.4239/wjd.v14.i9.1412 **Copyright** ©The Author(s) 2023.

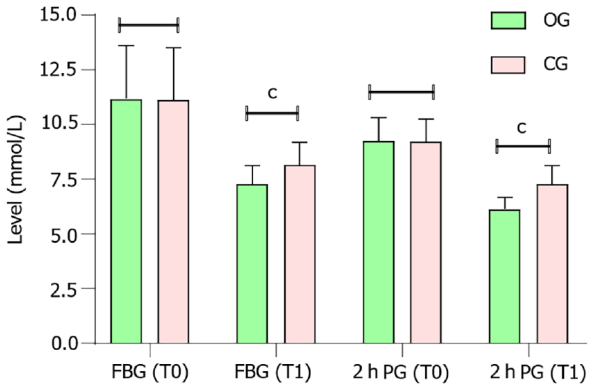
Figure 1 Number of patients included, and flow chart of analysis method. OG: Observation group; CG: Control group.

diabetic kidney disease, improve renal function, and reverse or delay early kidney injury. Our study also found that after treatment, compared with those before treatment, the levels of serum FBG, 2 h PG, TC, TG, and LDL-C were markedly lower in the OG compared to those in the CG. This shows that sitagliptin combined with Yiqi yangyin huoxue decoction can effectively reduce blood glucose and lipid levels, improve the state of hyperglycemia and hyperlipidemia, inhibit thrombosis formation, and slowed disease progression in patients with early DN. This could be because sitagliptin is a dipeptidyl peptidase-4 inhibitor. It is also a type of hypoglycemic drug that has an inhibitory effect on pancreatic islet α-cell hyperplasia and ultimately increases insulin secretion, such that blood sugar can be effectively regulated[16,17]. There are many drugs in the Yiqi yangyin huoxue decoction that have related effects. For example, when used in combination with astragalus and *Rehmannia*, blood sugar levels can be effectively reduced. By combining the tonifying effect of astragalus with the nourishing and kidney-strengthening effect of *Rehmannia*, the Qi and blood of the patient can also be replenished. *Astragalus membranaceus* can enhance immunity, help to resist oxidation, and have a certain regulatory effect on blood lipid and blood glucose levels in the body[18]. Further, *Rehmannia glutinosa* can improve hormone levels, especially the level of adrenaline, and has a certain improving effect on blood sugar and blood lipids in the body.



DOI: 10.4239/wjd.v14.i9.1412 **Copyright** ©The Author(s) 2023.

Figure 2 Efficacy evaluation based on the two groups. OG: Observation group; CG: Control group. $^{a}P < 0.05$.



DOI: 10.4239/wjd.v14.i9.1412 **Copyright** ©The Author(s) 2023.

Figure 3 Comparison of blood glucose levels between the two groups. OG: Observation group; CG: Control group; FBG: Fasting blood glucose; 2 h PG: 2 h postprandial blood glucose; T0: Pre-treatment; T1: Post-treatment. $^{c}P < 0.001$.

Oxidative stress reactions comprise one component of the pathogenesis of DN, and the reactive oxygen species produced by this reaction have an important impact on renal function. CysC is a small-molecule protein that is relatively stable in blood and is mostly used clinically to evaluate renal function. Moreover, it is an independent risk factor for diabetic complications. When renal function is impaired, serum levels increase significantly[19]. HCY is a non-essential amino acid with a sulfur-containing group, demethylated by methionine, and is closely related to renal function and metabolism. Research has shown that HCY can promote the excessive generation of oxygen free radicals and hydrogen peroxide, leading to the aggravation of renal tissue damage and acceleration of disease progression in DN[20]. U-mALB is a negatively charged protein secreted by the liver, and the vast majority of it fails to cross the glomerular filtration membrane charge barrier. An increase in urinary U-mALB content indicates altered glomerular permeability, and glomerular filtration membrane charge barrier is impaired[21]. Serum Cr is also a biochemical index used to evaluate renal function; its increase is related to a decrease in the glomerular filtration rate, and with this, renal function damage or renal failure is considered[22]. The results of our study showed that the levels of CysC, HCY, mALB, and Cr in the OG were markedly lower than those in the CG, indicating that sitagliptin combined with Yiqi yangyin huoxue decoction could reduce albuminuria, improve tubular function, and exert protective effects on renal function. *Astragalus* in Yiqi yangyin huoxue decoction can improve the balance of water and sodium in the body, change the permeability of blood vessels to reduce proteinuria, and effectively regulate renal function in patients with early DN[23]. *Ligusticum wallichi*i can effectively prevent the production of advanced glycosylation end products in the renal cortex and inhibit the apoptosis of renal cells, thus improving renal function.

Microcirculation disorders are an important pathological basis of DN. Microangiopathy is the pathological basis of typical clinical manifestations, such as proteinuria, in patients with early DN[24]. Hemodynamic abnormalities are also important causes of proteinuria and glomerulosclerosis in patients with DN[25]. The results of this study showed that after treatment, the erythrocyte deposition, plasma viscosity, whole blood high shear viscosity, and whole blood low shear viscosity in the OG were markedly lower than those in the CG. This indicated that sitagliptin combined with Yiqi yangyin huoxue decoction could significantly improve blood rheological indices in patients with early DN. The Yiqi yangyin huoxue decoction uses *A. membranaceus* and prepared *Rehmannia* root as monarch drugs. *Astragalus membranaceus* has a slightly warm and sweet taste, which is beneficial for reducing water-swelling, supplementing Qi, consolidating the surface, strengthening the spleen, and tonifying the middle. The *Rehmannia* root is a processed product of *R. glutinosa*. It has sweet and bitter tastes and is beneficial for the heart, liver, and lung meridians. It nourishes the blood, promotes fluid production, clears heat, and cools the blood. When used in combination, it has the effect of strengthening Qi and Yin. The use of Chinese yam, Dogberry, *Poria cocos*, Sealwort, and Dwarf lilyturf root as medicinal herbs has a role in nourishing

Qi in patients, and it is the main treatment for patients with spleen deficiency caused by fatigue and lumbar debility. The roots of Zhejiang figwort, Danpi, *L. wallichii*, and common peony root act as adjuvants, prevent thrombosis, improve hypercoagulability, promote blood circulation, and resolve blood stasis; after entering the body, they can improve blood viscosity, speed up blood flow, and reduce glomerular filtration, nourish the kidney, repair the kidney, and work together to complement each other, and thus, they have a marked influence on the treatment of diseases. Limitations of this study are as follows. Owing to time and sample size limitations, the selected subjects were all admitted to our hospital. Next, we will expand the sample size in clinical practice or conduct multicenter research and further explore the mechanism underlying the effects of Yiqi yangyin huoxue decoction for the treatment of early DN.

CONCLUSION

In conclusion, treatment with sitagliptin combined with Yiqi yangyin huoxue decoction in patients with Qi insufficiency and early DN has a significant effect, which is helpful in improving blood glucose, blood lipids, renal function, and hematological indicators and helping control disease progression, which is worthy of further clinical research.

ARTICLE HIGHLIGHTS

Research background

Early diabetic nephropathy (DN) is a major complication of diabetes, a disease induced by glomerular sclerosis caused by a long-term glucose metabolism disorder. Progressive renal damage, edema, polyuria, and proteinuria are the main clinical symptoms of this disease. The main treatment plan comprising Western medicine for early DN is lipid-lowering and blood pressure regulation; however, the treatment effect is not good. Therefore, we used traditional Chinese medicine (TCM) to control disease progression from the root cause.

Research motivation

With the development of TCM, we already know that the main pathogenic mechanism of early DN is "Kidney deficiency and blood stasis, Qi and Yin insufficiency." Therefore, we used western medicine combined with a TCM program for targeted treatment.

Research objectives

This study aimed to use sitagliptin tablets combined with Yiqi yangyin huoxue decoction to treat patients with early diabetic nephropathy, to observe the clinical efficacy and blood rheology in these patients, and to provide a basis for early clinical intervention to delay the progression of this disease.

Research methods

Using a retrospective approach, patients with early DN were randomized into control group (CG) (n = 50) and observation group (OG) (n = 50). CG patients were treated with sitagliptin, and OG patients were treated with sitagliptin plus the Yiqi yangyin huoxue decoction. Both groups were treated for 3 mo.

Research results

The total response rate of the patients in the OG was 94.00%, and the total response rate of those in the CG was 80.00%. After treatment (T1), fasting blood glucose, 2 h postprandial glucose, total cholesterol, triacylglycerol, low-density lipoprotein cholesterol, cystatin C, homocysteine, urinary microalbumin, and blood creatinine levels in OG patients were markedly lower than those in the CG; further, red blood cell deposition, plasma viscosity, and hyperviscosity of whole blood in OG patients were markedly lower than those in the CG (all $P < 0.05$).

Research conclusions

Sitagliptin combined with Yiqi yangyin huoxue decoction has achieved remarkable results in patients with early DN, helping to improve blood glucose, blood lipid, renal function, and hematological indicators.

Research perspectives

In this retrospective study, patients with early DN were randomized into the CG and OG; the CG patients were treated with sitagliptin, and The OG patients were treated with sitagliptin plus Yiqi yangyin huoxue decoction; both groups were treated for 3 mo. Comparing the baseline data and clinical efficacy between the two groups, changes in blood glucose, blood lipid, renal function, and the hematology index were observed before (T0) and after treatment (T1). Thus, we explored the efficacy of sitagliptin in combination with Yiqi yangyin huoxue decoction for early DN treatment and its effect on blood rheology.

FOOTNOTES

Author contributions: This article was written by Ling J, who independently completed the research design and data analysis; and Yang YH provided important guidance for solving difficult and complex problems.

Institutional review board statement: The study was reviewed and approved by the Biomedical Research Ethics Committee, Changzhou NO. 7 People's Hospital.

Informed consent statement: This is a retrospective study and has applied for exemption from informed consent.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Data sharing statement: Data used in this study were obtained from the corresponding author at yyh0728@163.com.

Country/Territory of origin: China

ORCID number: Jun Ling 0009-0003-7957-6722; Yan-Hua Yang 0000-0003-0382-1894.

S-Editor: Li L
L-Editor: A
P-Editor: Yu HG

REFERENCES

1 **Li Y**, Teng D, Shi X, Qin G, Qin Y, Quan H, Shi B, Sun H, Ba J, Chen B, Du J, He L, Lai X, Li Y, Chi H, Liao E, Liu C, Liu L, Tang X, Tong N, Wang G, Zhang JA, Wang Y, Xue Y, Yan L, Yang J, Yang L, Yao Y, Ye Z, Zhang Q, Zhang L, Zhu J, Zhu M, Ning G, Mu Y, Zhao J, Teng W, Shan Z. Prevalence of diabetes recorded in mainland China using 2018 diagnostic criteria from the American Diabetes Association: national cross sectional study. *BMJ* 2020; **369**: m997 [PMID: 32345662 DOI: 10.1136/bmj.m997]

2 **Lu WN**, Li H. [The interpretation of national guidelines for the prevention and control of diabetes in primary care (2018), early diagnosis, standard evaluation and treatment of diabetic kidney disease]. *Zhonghua Nei Ke Za Zhi* 2019; **58**: 789-792 [PMID: 31594180 DOI: 10.3760/cma.j.issn.0578-1426.2019.10.013]

3 **Barrera-Chimal J**, Jaisser F. Pathophysiologic mechanisms in diabetic kidney disease: A focus on current and future therapeutic targets. *Diabetes Obes Metab* 2020; **22** Suppl 1: 16-31 [PMID: 32267077 DOI: 10.1111/dom.13969]

4 **Aggarwal N**, Kare PK, Varshney P, Kalra OP, Madhu SV, Banerjee BD, Yadav A, Raizada A, Tripathi AK. Role of angiotensin converting enzyme and angiotensinogen gene polymorphisms in angiotensin converting enzyme inhibitor-mediated antiproteinuric action in type 2 diabetic nephropathy patients. *World J Diabetes* 2017; **8**: 112-119 [PMID: 28344754 DOI: 10.4239/wjd.v8.i3.112]

5 **Rando MM**, Guthoff M, Tiwari V, Biscetti F. Editorial: Diagnosis, prevention and treatment in diabetic nephropathy. *Front Endocrinol (Lausanne)* 2022; **13**: 1011665 [PMID: 36120444 DOI: 10.3389/fendo.2022.1011665]

6 **Kushiyama A**, Kikuchi T, Tanaka K, Tahara T, Takao T, Onishi Y, Yoshida Y, Kawazu S, Iwamoto Y. Prediction of the effect on antihyperglycaemic action of sitagliptin by plasma active form glucagon-like peptide-1. *World J Diabetes* 2016; **7**: 230-238 [PMID: 27326345 DOI: 10.4239/wjd.v7.i11.230]

7 **Scott R**, Morgan J, Zimmer Z, Lam RLH, O'Neill EA, Kaufman KD, Engel SS, Raji A. A randomized clinical trial of the efficacy and safety of sitagliptin compared with dapagliflozin in patients with type 2 diabetes mellitus and mild renal insufficiency: The CompoSIT-R study. *Diabetes Obes Metab* 2018; **20**: 2876-2884 [PMID: 30019498 DOI: 10.1111/dom.13473]

8 **Guo MF**, Dai YJ, Gao JR, Chen PJ. Uncovering the Mechanism of Astragalus membranaceus in the Treatment of Diabetic Nephropathy Based on Network Pharmacology. *J Diabetes Res* 2020; **2020**: 5947304 [PMID: 32215271 DOI: 10.1155/2020/5947304]

9 **Grimaldi A**, Heurtier A. [Diagnostic criteria for type 2 diabetes]. *Rev Prat* 1999; **49**: 16-21 [PMID: 9926711]

10 **Roden M**. [Diabetes mellitus: definition, classification and diagnosis]. *Wien Klin Wochenschr* 2016; **128** Suppl 2: S37-S40 [PMID: 27052219 DOI: 10.1007/s00508-015-0931-3]

11 **National Kidney Foundation**. KDOQI Clinical Practice Guideline for Diabetes and CKD: 2012 Update. *Am J Kidney Dis* 2012; **60**: 850-886 [PMID: 23067652 DOI: 10.1053/j.ajkd.2012.07.005]

12 **Mogensen CE**, Schmitz A, Christensen CK. Comparative renal pathophysiology relevant to IDDM and NIDDM patients. *Diabetes Metab Rev* 1988; **4**: 453-483 [PMID: 3061756 DOI: 10.1002/dmr.5610040504]

13 **Dou Z**, Xia Y, Zhang J, Li Y, Zhang Y, Zhao L, Huang Z, Sun H, Wu L, Han D, Liu Y. Syndrome Differentiation and Treatment Regularity in Traditional Chinese Medicine for Type 2 Diabetes: A Text Mining Analysis. *Front Endocrinol (Lausanne)* 2021; **12**: 728032 [PMID: 35002950 DOI: 10.3389/fendo.2021.728032]

14 **Liu XJ**, Hu XK, Yang H, Gui LM, Cai ZX, Qi MS, Dai CM. A Review of Traditional Chinese Medicine on Treatment of Diabetic Nephropathy and the Involved Mechanisms. *Am J Chin Med* 2022; **50**: 1739-1779 [PMID: 36222120 DOI: 10.1142/S0192415X22500744]

15 **Sun GD**, Li CY, Cui WP, Guo QY, Dong CQ, Zou HB, Liu SJ, Dong WP, Miao LN. Review of Herbal Traditional Chinese Medicine for the Treatment of Diabetic Nephropathy. *J Diabetes Res* 2016; **2016**: 5749857 [PMID: 26649322 DOI: 10.1155/2016/5749857]

16 **Scott LJ**. Sitagliptin: A Review in Type 2 Diabetes. *Drugs* 2017; **77**: 209-224 [PMID: 28078647 DOI: 10.1007/s40265-016-0686-9]

17 **Wang D**, Zhang G, Chen X, Wei T, Liu C, Chen C, Gong Y, Wei Q. Sitagliptin ameliorates diabetic nephropathy by blocking TGF-β1/Smad signaling pathway. *Int J Mol Med* 2018; **41**: 2784-2792 [PMID: 29484381 DOI: 10.3892/ijmm.2018.3504]

18 **Tian H**, Lu J, He H, Zhang L, Dong Y, Yao H, Feng W, Wang S. The effect of Astragalus as an adjuvant treatment in type 2 diabetes mellitus: A (preliminary) meta-analysis. *J Ethnopharmacol* 2016; **191**: 206-215 [PMID: 27269392 DOI: 10.1016/j.jep.2016.05.062]

19 **Liu L**, Wang H, Ning J, Han J, Yu C, Guan Q. The Predictability of Cystatin C for Peripheral Arterial Disease in Chinese Population with Type 2 Diabetes Mellitus. *J Diabetes Res* 2022; **2022**: 5064264 [PMID: 35392484 DOI: 10.1155/2022/5064264]

20 **Ding S**, Yang Y, Zheng Y, Xu J, Cheng Y, Wei W, Yu F, Li L, Li M, Wang M, Wang Z, Xiang G. Diagnostic Value of the Combined Measurement of Serum HCY and NRG4 in Type 2 Diabetes Mellitus with Early Complicating Diabetic Nephropathy. *J Pers Med* 2023; **13** [PMID: 36983737 DOI: 10.3390/jpm13030556]

21 **Song LL**, Wang N, Zhang JP, Yu LP, Chen XP, Zhang B, Yang WY. Postprandial glucagon-like peptide 1 secretion is associated with urinary albumin excretion in newly diagnosed type 2 diabetes patients. *World J Diabetes* 2023; **14**: 279-289 [PMID: 37035218 DOI: 10.4239/wjd.v14.i3.279]

22 **Qi MY**, He YH, Cheng Y, Fang Q, Ma RY, Zhou SJ, Hao JQ. Icariin ameliorates streptozocin-induced diabetic nephropathy through suppressing the TLR4/NF-κB signal pathway. *Food Funct* 2021; **12**: 1241-1251 [PMID: 33433547 DOI: 10.1039/d0fo02335c]

23 **Lu Z**, Zhong Y, Liu W, Xiang L, Deng Y. The Efficacy and Mechanism of Chinese Herbal Medicine on Diabetic Kidney Disease. *J Diabetes Res* 2019; **2019**: 2697672 [PMID: 31534972 DOI: 10.1155/2019/2697672]

24 **Verma N**, Liu M, Ly H, Loria A, Campbell KS, Bush H, Kern PA, Jose PA, Taegtmeyer H, Bers DM, Despa S, Goldstein LB, Murray AJ, Despa F. Diabetic microcirculatory disturbances and pathologic erythropoiesis are provoked by deposition of amyloid-forming amylin in red blood cells and capillaries. *Kidney Int* 2020; **97**: 143-155 [PMID: 31739987 DOI: 10.1016/j.kint.2019.07.028]

25 **Lee S**, Lee MY, Nam JS, Kang S, Park JS, Shin S, Ahn CW, Kim KR. Hemorheological Approach for Early Detection of Chronic Kidney Disease and Diabetic Nephropathy in Type 2 Diabetes. *Diabetes Technol Ther* 2015; **17**: 808-815 [PMID: 26214546 DOI: 10.1089/dia.2014.0295]

World Journal of Diabetes

Submit a Manuscript: https://www.f6publishing.com

World J Diabetes 2023 September 15; 14(9): 1422-1449

DOI: 10.4239/wjd.v14.i9.1422

ISSN 1948-9358 (online)

META-ANALYSIS

Effectiveness and safety of traditional Chinese medicine for diabetic retinopathy: A systematic review and network meta-analysis of randomized clinical trials

Hong-Dian Li, Ming-Xuan Li, Wen-Hua Zhang, Shu-Wen Zhang, Yan-Bing Gong

Specialty type: Endocrinology and metabolism

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): A
Grade B (Very good): B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Rahmati M, Iran; Salceda R, Mexico; Horowitz M, Australia

Received: April 17, 2023
Peer-review started: April 17, 2023
First decision: June 1, 2023
Revised: June 14, 2023
Accepted: July 29, 2023
Article in press: July 29, 2023
Published online: September 15, 2023

Hong-Dian Li, Dongfang Hospital, Beijing University of Chinese Medicine, Beijing 100700, China

Ming-Xuan Li, Capital Medical University, Beijing Hospital of Traditional Chinese Medicine, Beijing 100010, China

Wen-Hua Zhang, Shu-Wen Zhang, Yan-Bing Gong, Dongzhimen Hospital, Beijing University of Chinese Medicine, Beijing 100700, China

Corresponding author: Yan-Bing Gong, PhD, Dean, Dongzhimen Hospital, Beijing University of Chinese Medicine, No. 5 Haiyuncang Hutong, Dongcheng District, Beijing 100700, China. gyb_1226@163.com

Abstract

BACKGROUND

Diabetic retinopathy (DR) is currently recognized as one of the most serious diabetic microangiopathies and a major cause of adult blindness. Commonly used clinical approaches include etiological control, microvascular improvement, and surgical intervention, but they are ineffective and have many side effects. Oral Chinese medicine (OCM) has been used for thousands of years to treat DR and is still widely used today, but it is unclear which OCM is more effective for DR.

AIM

To estimate relative effectiveness and safety profiles for different classes of OCMs for DR, and provide rankings of the available OCMs.

METHODS

The search time frame was from the creation of the database to January 2023. RevMan 5.3 and Stata 14.0 software were used to perform the systematic review and Network meta-analyses (NMA).

RESULTS

A total of 107 studies and 9710 patients were included, including 4767 cases in the test group and 4973 cases in the control group. Based on previous studies and clinical reports, and combined with the recommendations of Chinese guidelines for the prevention and treatment of DR, 9 OCMs were finally included in this study, namely Compound Xueshuantong Capsules, Qiming Granules, Compound

Danshen Dripping Pills, Hexue Mingmu Tablets (HXMM), Qiju Dihuang Pills (QJDH), Shuangdan Mingmu Capsules (SDMM), Danggui Buxue Decoction (DGBX), Xuefu Zhuyu Decoction and Buyang Huanwu Decoction. When these nine OCMs were analyzed in combination with conventional western medicine treatment (CT) compared with CT alone, the NMA results showed that HXMM + CT has better intervention effect on the overall efficacy of DR patients, HXMM + CT has better effect on improving patients' visual acuity, SDMM + CT has better effect on inhibiting vascular endothelial growth factor, DGBX + CT has better effect on reducing fundus hemorrhage area, HXMM + CT has better effect on reducing fasting blood glucose, and QJDH + CT has better effect on reducing glycated hemoglobin. When there are not enough clinical indicators for reference, SDMM + CT or HXMM + CT treatments can be chosen because they are effective for more indicators and demonstrate multidimensional efficacy.

CONCLUSION

This study provides evidence that combining OCMs with CT leads to better outcomes in all aspects of DR compared to using CT alone. Based on the findings, we highly recommend the use of SDMM or HXMM for the treatment of DR. These two OCMs have demonstrated outstanding efficacy across multiple indicators.

Key Words: Diabetic retinopathy; Network meta-analysis; Traditional Chinese medicine; Therapeutic effect; Systematic review

Core Tip: To our knowledge, this study represents the first network meta-analysis (NMA) examining the effectiveness of traditional Chinese medicine in treating diabetic retinopathy (DR). Notably, this NMA includes the largest number of original studies, subjects, and variety of Chinese medicines to date. While the efficacy of Chinese medicine for DR has been widely recognized in China, no previous studies have systematically evaluated which Chinese medicine treatment is the most effective. Therefore, this study fills an important gap in the field.

Citation: Li HD, Li MX, Zhang WH, Zhang SW, Gong YB. Effectiveness and safety of traditional Chinese medicine for diabetic retinopathy: A systematic review and network meta-analysis of randomized clinical trials. *World J Diabetes* 2023; 14(9): 1422-1449
URL: https://www.wjgnet.com/1948-9358/full/v14/i9/1422.htm
DOI: https://dx.doi.org/10.4239/wjd.v14.i9.1422

INTRODUCTION

Diabetic retinopathy (DR) is currently recognized as one of the serious diabetic microangiopathies and is the leading cause of blindness in adults. According to the International Diabetes Federation, it is estimated that the number of people with diabetes will reach 642 million worldwide in 2040, and 34.6% of these patients will have DR[1]. DR causes irreversible visual impairment, including abnormal vision, blurred vision, and even blindness. In addition, the presence of DR implies an increased risk of life-threatening systemic vascular complications[2]. Currently, the main treatments for DR include retinal laser photocoagulation, pharmacotherapy, hormonal therapy, and surgery. However, these treatments may lead to adverse effects such as increased angiogenesis, increased intraocular pressure, and retinal hemorrhage[3,4]. Studies have shown that age is a key factor affecting DR, and the number of DR patients in the elderly population will reach new highs as the world ages[5]. In view of the current situation, the pathogenesis of DR is being actively explored around the world and effective therapeutic drugs are being explored.

In fact, traditional Chinese medicine (TCM) has long been considered a promising complementary therapy that dates back more than 1000 years. In China, many facts have proven that herbal medicine can effectively improve the fundus condition of patients, relieve the pain of the disease, and obtain a better quality of life for patients through multi-target and multi-path interventions in DR[6]. Many Oral Chinese medicine (OCM), including proprietary Chinese medicine preparations and herbal granules are widely used in the treatment of DR. For example, Qiming Granules (QM), the first OCM approved by the State Food and Drug Administration for the treatment of DR, whose main ingredients are *Hedysarum Multijugum Maxim.* (Huangqi in chinese), *Radix Puerariae* (Gegen in chinese), *Lycii Fructus* (Gouqizi in chinese), and *Cassiae Semen* (Juemingzi in chinese), *etc.*, were shown to alleviate retinal hypoxia and ischemia by increasing retinal blood flow and improving blood circulation in a multicenter, randomized, parallel controlled clinical trial[7]. Then for example, Compound Xueshuantong Capsules (XST), an OCM commonly used for DR, was shown to effectively improve the disorder of retinal structure and edema in streptozotocin-induced type 2 diabetic rats by activating the PPAR signaling pathway, reversing the reduction in retinal thickness and retinal ganglion cell number, and reducing the apoptotic index of retinal cells[8]. Compound Danshen dripping pills, an oral proprietary Chinese medicine containing Danshen, was found to improve vision and clinical symptoms and reduce the incidence of macular edema compared to captopril in a retrospective study[9].

In addition to these well known OCMs, there are many lesser known OCMs that are widely used in clinical practice. However, the selection of these OCMs remains a challenge for patients with different disease states. Network meta-analyses (NMA) allow for the comparison of multiple treatments (*i.e.* three or more) using both direct comparisons of interventions within randomized controlled trials (RCT) and indirect comparisons across trials based on a common comparator[10]. To date, there are no studies comparing different types of OCMs used for the treatment of DR. Therefore, we searched all RCTs of OCMs used for the treatment of DR and initiated this NMA to compare the efficacy between them, hoping to provide some suggestions for clinical practice.

MATERIALS AND METHODS

The study protocol was registered on PROSPERO (International Prospective Register of Systematic Reviews). Registration No. CRD42022352250 (https://www.crd.york.ac.uk/PROSPERO/#myprosperoID=CRD42022352250). This program was developed in accordance with the Preferred Reporting Items For Systematic Review And Meta-analysis Protocols (PRISMA-P)[11]. The PRISMA Extension Statement is used to ensure that all aspects of the methods and results are reported[12].

Eligibility criteria

The Population-Intervention-Comparators-Outcomes-Studydesign framework was adopted as the eligibility criteria for the review as following.

Study type

The RCT is the original study that we agreed to include. We did not place any restrictions on the language, country, publication date, or phase of the RCT. Duplicate publications, summaries of personal experience, purely theoretical studies, reviews, animal or cellular experiments, and original studies with incorrect or incomplete data in the literature should also be excluded.

Population

Patients with DR who meet the standard diagnosis rely on fundus fluorescence angiography and fundus signs to detect microangiomas, exudates, hemorrhages, neovascularization, and other fundus changes. No restriction of age, gender, occupation and region. No concomitant ocular diseases caused by non-hyperglycemic factors, such as primary glaucoma and senile cataract; no acute metabolic diseases, such as diabetic ketoacidosis, within a short period of time before enrollment.

Intervention

Regarding the RCT of OCM for DR, the blinding and language are not limited. The control group received only oral western medicine, conventional western medicine treatment (CT) mainly included hypoglycemic drugs, lipid-lowering drugs and antihypertensive drugs, among which hypoglycemic drugs could include subcutaneous injection of insulin, in addition, other injectable drugs were not acceptable; antioxidant and microcirculation improvement drugs, such as calcium dobesilate, pancreatic kininogenase; drugs to promote retinal metabolism and nerve nutrition of the eye, such as lecithin complex iodine, *etc.* The test group was added to the control group with OCM. By combining previous studies and actual clinical observations, and also referring to the latest published Guideline for the prevention and treatment of type 2 diabetes mellitus in China (2020 edition)[13], we decided to study the 9 most commonly used OCMs which are XST (composed of *Panax Notoginseng (Burk.) F. H. Chen Ex C. Chow/Figwort Root/Hedysarum Multijugum Maxim./Radix Salviae*), Compound Danshen Dripping Pills (DS, composed of *Radix Salviae/Borneolum Syntheticum/Panax Notoginseng (Burk.) F. H. Chen Ex C. Chow*), Qiming Granules (QM, composed of *Hedysarum Multijugum Maxim./Radix Puerariae/Rehmanniae Radix Praeparata/Lycii Fructus/Leonuri Fructus/Cassiae Semen/Pollen Typhae/Whitm.ania Pigra Whitman*), Hexue Mingmu Tablets (HXMM, *Pollen Typhae/Rehmanniae Radix Praeparata/Radix Salviae/Ecliptae Herba/Chrysanthemi Flos/Scutellariae Radix/Cassiae Semen/Plantaginis Semen/Leonuri Fructus/Fructus Ligustri Lucidi/Prunellae Spica/Gentianae Radix Et Rhozima/Curcumae Radix/Equiseti Hiemalis Herba/Radix Paeoniae Rubra/Cortex Moutan/Angelicae Sinensis Radix/Chuanxiong Rhizoma*), Qiju Dihuang Pills (QJDH, *Lycii Fructus/Rehmanniae Radix Praeparata/Chrysanthemi Flos/Cornus Officinalis Sieb. Et Zucc./Rhizoma Dioscoreae/Poria Cocos(Schw.) Wolf./Alisma Orientale (Sam.) Juz./Cortex Moutan*), Shuangdan Mingmu Capsules (SDMM, *Ecliptae Herba/Fructus Ligustri Lucidi*), Danggui Buxue Decoction (DGBX, *Hedysarum Multijugum Maxim./Angelicae Sinensis Radix*), Xuefu Zhuyu Decoction (XFZY, *Persicae Semen/Carthami Flos/Radix Paeoniae Rubra/Chuanxiong Rhizoma/Radix Bupleuri/Platycodon Grandiforus/Licorice/Angelicae Sinensis Radix/Rehmanniae Radix Praeparata/Achyranthis Bidentatae Radix/Aurantii Fructus*) and Buyang Huanwu Decoction (BYHW, *Hedysarum Multijugum Maxim./Angelicae Sinensis Radix/Radix Paeoniae Rubra/Chuanxiong Rhizoma/Persicae Semen/Carthami Flos/Pheretima*). To obtain the most accurate efficacy results for OCM, all studies performing non-oral treatments such as laser photocoagulation, surgery, injectable fluids, acupuncture, tui-na, and traditional Chinese gongfu were excluded from this study.

Outcome measures

The main efficacy indicators in this study encompass total clinical effectiveness and visual acuity. Following the international DR efficacy determination standard, the efficacy after treatment was categorized into three groups: Significantly

effective, effective, and ineffective. Significantly effective cases were identified based on two criteria: (1) Improvement in visual acuity by ≥ 2 lines; and (2) improvement in two or more of the three fundus indices (microvessel count, hemorrhage, and exudation), or significant improvement in one or more of the three indices without deterioration in the remaining indices. Effective cases were determined by improvement in visual acuity and improvement in at least one of the three fundus indices without deterioration in the remaining indices. Ineffective cases were those that did not meet the criteria for effectiveness based on the indices. For assessing visual acuity, the international standard visual acuity table was utilized, where the counting included 2 rows for no light perception to light perception, and 1 row for each interval including light perception, manual, index, 0.02, 0.04, 0.06, 0.08, and 0.1, while refractive error measurements accounted for corrected visual acuity. The efficacy index considered the total clinical efficiency (comprising significantly effective and effective populations), reflecting the actual determination of clinical effects. Secondary indicators include fundus hemorrhagic area (FHA), fasting blood glucose (FBG), glycated hemoglobin (HbA1c) and vascular endothelial growth factor (VEGF).

Data sources and search strategy

A total of seven databases were searched by computer: China national knowledge infrastructure, Wanfang Database, Weipu Journal Database, Chinese Biomedical Literature Database, PubMed, Cochrane Library, and Web of Science database, and the search time of each database was built until January 2023. In addition, references to the literature were incorporated retrospectively to supplement access to relevant literature. The search takes a combination of subject terms and free words. English search terms include: "Diabetic Retinopathy", "Diabetic Retinopathies", "Traditional Chinese medicine", "Compound Xueshuantong Capsules", "Compound Danshen Dripping Pills", "Qiju Dihuang Pills", "Qiming Granules", "Hexue Mingmu Tablets", "Randomized Controlled Trial", *etc.* The detailed search strategy is described in Supplementary Table 1.

Data screening and quality evaluation

Two investigators (LMX and LHD) performed literature screening and data extraction, excluding duplicates and then first read the title and abstract to exclude literature that clearly did not meet the requirements, and then read the remaining literature in full to clarify whether it met the inclusion criteria, and if there was disagreement, a third investigator (WZ) had to be consulted for a decision after discussion. Data extraction included: (1) Basic information: Article title, first author, publication time, country/region, *etc.*; (2) study characteristics: Interventions in the trial and control groups, number of cases, age, duration of intervention, and adverse effects in the study subjects; (3) key information required for risk of bias evaluation in the literature; and (4) outcome indicators included in the test and control groups.

Quality evaluation of RCTs was performed usingRevMan 5.3 (Cochrane Collaboration, Copenhagen, The Nordic Cochrane Centre). Assessed by 2 investigators (HL and ML) using the tool for assessing risk of bias recommended in the Cochrane systematic reviewers' handbook 5.1[14], including the following 7 aspects: Random-sequence generation (selection bias); allocation concealment (selection bias); blinding of participants and personnel (performance bias); blinding of outcome assessment (detection bias); incomplete outcome data (attrition bias); selective reporting (reporting bias); others bias. Each aspect can be further categorized as “low risk of bias”, “unclear risk of bias”, and “high risk of bias”. When there is a disagreement, it can be decided by mutual discussion or by consulting 3 investigators (SZ).

Statistical analysis

Stata 14.0 (MRC Biostatistics Unit, Cambridge, United Kingdom) software was used to implement the statistical process. The odds ratio (OR) and its 95% confidence interval (CI) were used for statistical data, and the mean difference and its 95%CI were used for measurement data. Data were preprocessed using the "Network" command, and two comparisons were made between different interventions, according to this case there is no closed loop, so the consistency model is used. The efficacy was ranked according to the surface under the cumulativeranking curve (SUCRA), and the results of the NMA analysis were finally presented in tabular form to obtain the relatively best interventions. Considering the inclusion of multiple observations, we combined the individual observations two by two and used a multivariate approach to determine the dependencies between the results. Use the "Clustering" command to obtain a clustering ranking chart[15]. Sensitivity analysis is performed for factors that may affect the stability of the results. Finally, "correction-comparison" funnel plots were drawn for publication bias assessment.

RESULTS

Literature search results and basic characteristics

A total of 1667 relevant papers were identified and screened for inclusion in 107 randomized controlled trials, involving a total of 9710 patients (4767 in the trial group and 4973 in the control group). Baseline characteristics were carefully matched between the groups. The individual sample sizes of the trials ranged from 30 to 256 individuals, and the observation periods varied from 4 wk to 24 wk. The trials encompassed 9 different OCMs, namely XST (26 studies[16-41]), QM (23 studies[42-64]), DS (21 studies, references[65-85]), HXMM (9 studies[86-94]), QJDH (6 studies[95-100]), SDMM (5 studies[101-105]), DGBX (6 studies[106-111]), XFZY (7 studies[112-118]), and BYHW (4 studies[119-122]). All studies evaluated at least one of the two primary efficacy measures, namely total clinical effectiveness and visual acuity. It should be noted that all included studies were conducted exclusively in China. The literature screening process is shown in Figure 1, the characteristics of the literature are shown in Table 1, and the OCMs involved in the study and their details

Table 1 Basic characteristics of included randomized controlled trials

Ref.	Sample size	Random method	Interventions		Period of treatment	Age	Outcomes	Adverse reactions
	C/E		C	E		C/E		C/E
Men[16], 2020	40/40	TRD	CT + Calcium Dobesilate	C + XST	8 wk	67.46 ± 2.52/63.12 ± 2.21	1, 2, 3, 4	-
Bai[17], 2016	38/38	-	CT + Calcium Dobesilate	C + XST	24 wk	51.08 ± 4.73/50.63 ± 5.51	1, 2, 7	3/2
An[18], 2021	30/30	-	Calcium Dobesilate	C + XST	20 wk	69.51 ± 5.19/70.32 ± 5.39	1, 2	-
Yan and Song[19], 2020	46/46	TRD	Calcium Dobesilate	C + XST	12 wk	47.4 ± 4.6/48.5 ± 4.9	1, 7	22/8
Li[20], 2021	20/20	-	Calcium Dobesilate	C + XST	20 wk	59.67 ± 1.78/58.51 ± 1.31	1, 3	-
Jin[21], 2020	40/40	-	Calcium Dobesilate	C + XST	20 wk	66.12 ± 3.45/66.58 ± 3.16	2, 3	-
Wu[22], 2015	50/50	TRD	CT	C + XST	12 wk	54.1 ± 6.6/54.6 ± 6.2	1	-
Qu[23], 2018	35/35	-	CT + Calcium Dobesilate	C + XST	20 wk	59.7 ± 6.3/60.4 ± 7.2	1	-
Sui[24], 2017	47/49	-	CT + Calcium Dobesilate	C + XST	4 wk	-	1	-
Jiang *et al* [25], 2019	46/46	-	Calcium Dobesilate	C + XST	20 wk	58.72 ± 2.20/58.69 ± 2.15	1, 2, 4	-
Zhu *et al* [26], 2016	48/48	-	CT	C + XST	12 wk	56.38 ± 12.19/56.15 ± 12.21	2	-
Sun[27], 2021	18/18	-	CT	C + XST	12 wk	61.38 ± 3.69/62.47 ± 3.01	1	-
Liu *et al* [28], 2018	45/45	TRD	CT + Calcium Dobesilate	C + XST	12 wk	48.56 ± 7.64/48.34 ± 6.49	1	-
Li[29], 2019	49/49	TRD	CT + Calcium Dobesilate	C + XST	12 wk	66.41 ± 4.11/66.82 ± 4.03	1, 2, 4	-
Xiao[30], 2016	110/110	-	CT + Calcium Dobesilate	C + XST	12 wk	50.2 ± 6.4/49.5 ± 5.9	1, 7	0/0
Chen[31], 2019	39/39	-	Calcium Dobesilate	C + XST	12 wk	58.17 ± 3.82/59.34 ± 3.27	1, 3, 4	-
Zhao[32], 2020	43/44	-	Calcium Dobesilate	C + XST	24 wk	53.71 ± 5.52/53.66 ± 5.49	3	-
An[33], 2020	35/35	-	Calcium Dobesilate	C + XST	12 wk	52.12 ± 15.76/51.17 ± 17.83	1, 4, 7	1/2
Zhang[34], 2019	19/19	-	Calcium Dobesilate	C + XST	8 wk	51.86 ± 1.92/53.28 ± 2.64	1, 7	0/0
Wei *et al* [35], 2017	34/34	TRD	CT	C + XST	35 wk	62.94 ± 3.48/61.31 ± 3.54	1, 3, 4, 7	0/0
Meng *et al* [36], 2012	38/40	-	CT + Calcium Dobesilate	C + XST	24 wk	49.2 ± 7.8/48.8 ± 6.7	1	-
Zhu and Sui [37], 2022	76/76	TRD	CT + Calcium Dobesilate	C + XST	12 wk	55.89 ± 4.17/55.94 ± 4.13	1, 2, 3, 7	12/13
Li[38], 2017	33/33	-	Calcium Dobesilate	C + XST	12 wk	-	2	-
Wang *et al* [39], 2020	42/44	TRD	CT + Calcium Dobesilate	C + XST	20 wk	68.35 ± 6.82/69.52 ± 7.11	1, 3, 7	4/3
Hu[40],	30/30	-	Calcium	C + XST	20 wk	55.30 ±	1, 3, 7	0/0

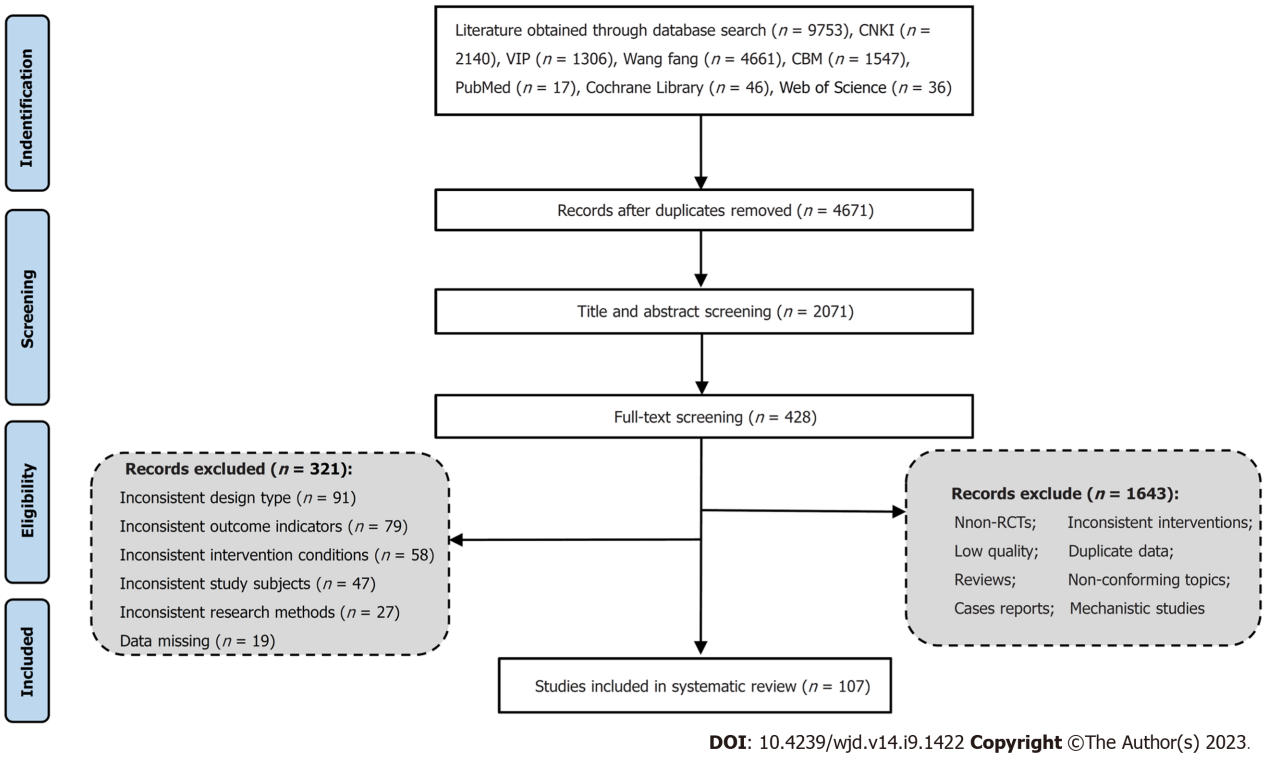
2017			Dobesilate			2.15/55.67 ± 2.08		
Xu and Ru [41], 2020	46/46	-	Calcium Dobesilate	C + XST	12 wk	52.3 ± 3.2/52.1 ± 3.6	1, 3	-
Wang[42], 2016	38/38	-	Iodized Lecithin	C + QM	8 wk	43 ± 6/42 ± 5	1	-
Wang[43], 2018	44/44	TRD	Calcium Dobesilate	C + QM	12 wk	58.4 ± 7.5/57.8 ± 6.2	1, 7	12/5
Wang *et al* [44], 2020	32/32	-	Amlodipine besylate	C + QM	12 wk	38.94 ± 4.89/39.87 ± 5.13	1, 2	-
Chen[45], 2016	45/45	TRD	CT	C + QM	12 wk	62.05 ± 5.47/63.11 ± 5.64	1	-
Wang *et al* [46], 2015	38/41	-	Calcium Dobesilate	C + QM	24 wk	52.1 ± 5.6/52.5 ± 5.3	1, 2, 7	0/0
Sui *et al*[47], 2014	43/43	-	Calcium Dobesilate	C + QM	12 wk	50.53 ± 11.28/50.22 ± 14.82	1, 2, 5, 6, 7	0/0
Huang[48], 2017	63/63	-	CT + Calcium Dobesilate	C + QM	12 wk	55.9 ± 4.1/55.6 ± 4.2	1	-
Feng *et al* [49], 2016	41/42	Lottery -	Calcium Dobesilate	C + QM	12 wk	55.89 ± 6.13/55.26 ± 6.29	1, 2, 5, 6	-
Ge[50], 2018	53/53	-	CT + Calcium Dobesilate	C + QM	24 wk	50.87 ± 3.71/51.25 ± 3.64	1	-
Yan[51], 2020	38/46	Lottery	CT + Calcium Dobesilate	C + QM	8 wk	56.96 ± 4.59/56.65 ± 4.02	1, 2, 5, 6, 7	8/2
Meng *et al* [52], 2016	21/21	-	Calcium Dobesilate	C + QM	24 wk	-	1	-
Zhang *et al* [53], 2016	46/45	-	CT	C + QM	24 wk	-	1	-
Zhang[54], 2013	34/34	-	CT	C + QM	36 wk	-	1	-
Wu *et al* [55], 2022	50/50	Lottery	Pancreatic Kinino-genase	C + QM	24 wk	53.82 ± 5.42/54.06 ± 4.93	1, 2, 4, 7	3/5
Zhou and Femng[56], 2018	60/60	-	CT + Calcium Dobesilate	C + QM	12 wk	58.5 ± 6.7/57.9 ± 6.2	1, 2	-
Wang *et al* [57], 2019	48/52	-	CT + Calcium Dobesilate	C + QM	24 wk	66.8 ± 6.3/66.7 ± 6.2	1, 7	0/0
Wang[58], 2017	47/47	-	CT + Calcium Dobesilate	C + QM	12 wk	54.3 ± 4.9/54.5 ± 4.8	1, 4, 7	0/0
Yin[59], 2018	46/50	-	Calcium Dobesilate	C + QM	12 wk	55.27 ± 5.42/54.63 ± 5.28	1, 2, 7	0/0
Xin *et al* [60], 2019	38/38	-	Epalrestat	C + QM	12 wk	55.1 ± 3.3/55.5 ± 3.2	1, 3, 7	0/0
Zhang[61], 2017	39/39	-	Calcium Dobesilate	C + QM	12 wk	56.8 ± 2.5/56.9 ± 2.1	1, 2, 5, 6, 7	0/0
Yue[62], 2016	38/57	-	CT	C + QM	24 wk	49.82 ± 6.17/50.67 ± 5.23	1	-
Zheng *et al* [63], 2014	15/15	-	CT	C + QM	24 wk	50.4 ± 3.1/55.2 ± 4.7	1	-
Yang *et al* [64], 2013	36/35	-	CT	C + QM	24 wk	50.94 ± 8.01/50.23 ± 7.15	1	-
Huang[65], 2020	20/20	-	Calcium Dobesilate	C + DS	16 wk	52.16 ± 2.45/53.16 ± 2.26	1, 2, 3, 5, 6	-
Zheng and Ji[66], 2021	43/44	-	CT	C + DS	8 wk	57.52 ± 6.41/58.21 ± 6.35	1, 4	-
Meng *et al* [67], 2011	28/30	-	CT + Calcium Dobesilate	C + DS	24 wk	51.20 ± 7.90/50.60 ± 8.70	1, 7	0/0

Wang[68], 2004	16/28	-	CT + Calcium Dobesilate	C + DS	16 wk	50.5 ± 9.36/50.4 ± 8.70	1	-
Zhao[69], 2019	53/53	TRD	CT	C + DS	8 wk	57.5 ± 14.8/56.8 ± 13.4	1, 3, 4, 7	0/0
Ma *et al*[70], 2016	34/48	-	CT	C + DS	24 wk	59.16 ± 9.73/59.01 ± 10.58	5, 6, 7	0/0
Chen *et al*[71], 2007	25/25	-	CT	C + DS	8 wk	60.56/62.42	2, 3	-
Xu[72], 2019	43/43	TRD	Calcium Dobesilate	C + DS	16 wk	53.06 ± 4.39/53.11 ± 4.41	1, 3, 7	0/0
Huang *et al*[73], 2021	45/45	TRD	CT + Calcium Dobesilate	C + DS	24 wk	67.3 ± 5.1/67.5 ± 5.3	2, 4, 7	3/4
Jiao[74], 2018	75/75	-	CT + Calcium Dobesilate	C + DS	8 wk	56.31 ± 2.19/56.24 ± 3.86	1	-
Li[75], 2017	89/89	-	CT + Calcium Dobesilate	C + DS	8 wk	55.8 ± 6.8/56.5 ± 7.2	1, 5, 6	-
Yan and Yuan[76], 2014	20/60	-	CT	C + DS	24 wk	68.8/65.6	2, 3	-
Zhou[77], 2008	18/28	-	Calcium Dobesilate	C + DS	24 wk	50.50 ± 9.36/50.40 ± 8.70	1	-
Miao[78], 2020	24/34	TRD	Calcium Dobesilate	C + DS	16 wk	57.46 ± 4.41/57.33 ± 4.26	2, 3	-
Ruan *et al*[79], 2017	35/35	-	Calcium Dobesilate	C + DS	16 wk	52.8 ± 1.7/52.5 ± 1.1	1, 3, 5, 6, 7	0/0
Yin *et al*[80], 2013	50/50	-	CT + Calcium Dobesilate	C + DS	8 wk	59.7/57.9	1, 3	-
Zhu[81], 2018	57/57	-	CT	C + DS	12 wk	64.12 ± 1.36/64.17 ± 1.38	1, 2, 3	-
Yang *et al*[82], 2013	32/33	-	CT	C + DS	8 wk	54.2 ± 10.8/55.4 ± 12.1	5, 6	-
Bai[83], 2017	38/38	TRD	CT + Calcium Dobesilate	C + DS	16 wk	-	1, 2, 3, 4, 7	0/0
Guo[84], 2015	35/100	-	CT	C + DS	24 wk	59.6 ± 9.7/59.0 ± 10.6	5, 6	-
Liu[85], 2018	89/89	TRD	CT	C + DS	4 wk	54.97 ± 4.88/55.02 ± 5.01	5, 6	-
Liu[86], 2019	41/42	-	Pancreatic Kininogenase	C + HXMM	12 wk	-	3, 7	2/2
Zhao and Liu[87], 2021	30/30	-	CT + Calcium Dobesilate	C + HXMM	12 wk	64.84 ± 4.26/65.09 ± 4.37	1, 7	4/2
Gao *et al*[88], 2020	128/128	TRD	CT + Calcium Dobesilate	C + HXMM	12 wk	57.65 ± 7.82/58.14 ± 7.63	1, 3, 7	4/3
Li[89], 2021	34/34	Lottery	CT + Calcium Dobesilate	C + HXMM	12 wk	68.49 ± 4.62/67.84 ± 4.57	1, 3	-
Wang *et al*[90], 2018	100/100	-	Pancreatic Kininogenase	C + HXMM	12 wk	50.62 ± 6.91/50.96 ± 6.71	1, 3, 7	4/2
Zhang and Wang[91], 2018	39/39	-	CT	C + HXMM	12 wk	-	1, 2	-
Ye *et al*[92], 2019	88/88	TRD	Calcium Dobesilate	C + HXMM	12 wk	60.9 ± 13.4/60.5 ± 13.4	1, 5, 6	-
Yu[93], 2019	30/30	-	Epalrestat	C + HXMM	12 wk	-	1, 7	2/3
Du *et al*[94], 2015	26/25	TRD	CT	C + HXMM	12 wk	53.39 ± 4.96/52.13 ± 5.01	1, 7	0/0

Song[95], 2013	40/40	-	CT	C + QJDH	12 wk	-	1, 7	0/0
Li[96], 2019	40/40	-	Mecobalamin	C + QJDH	24 wk	61.25 ± 6.75/60.85 ± 6.57	1, 3, 4, 7	0/0
Li and Wei [97], 2019	54/54	-	CT + Calcium Dobesilate	C + QJDH	20 wk	56.1 ± 3.7/55.4 ± 3.1	1, 3, 4, 5, 6, 7	0/0
Wu[98], 2018	29/29	TRD	Pancreatic Kinino-genase	C + QJDH	12 wk	52.4 ± 10.8/52.3 ± 9.2	1, 5, 7	0/0
Guan[99], 2017	40/40	-	CT + Calcium Dobesilate	C + QJDH	24 wk	40.0 ± 3.1/41.1 ± 2.0	1, 5, 6, 7	6/5
Ainu *et al* [100], 2019	50/50	-	CT + Calcium Dobesilate	C + QJDH	4 wk	53.02 ± 5.39/52.61 ± 5.39	2, 3, 4	-
Fu[101], 2019	40/40	-	Calcium Dobesilate	C + SDMM	24 wk	-	5, 7	7/2
Ji and Liu [102], 2022	52/52	Lottery	Calcium Dobesilate	C + SDMM	12 wk	56.53 ± 4.09/56.63 ± 4.02	1, 3, 4, 7	7/5
Jin and Zhang[103], 2019	71/71	-	CT + Calcium Dobesilate	C + SDMM	16 wk	62.39 ± 8.34/63.07 ± 8.08	1, 7	9/2
Liu *et al* [104], 2019	60/60	TRD	CT + Calcium Dobesilate	C + SDMM	16 wk	57.10 ± 9.26/57.54 ± 8.11	1, 3, 4, 7	0/0
Pang[105], 2015	40/40	-	CT	C + SDMM	16 wk	49.6 ± 5.3/49.4 ± 5.7	1	-
Deng *et al* [106], 2018	40/40	TRD	Calcium Dobesilate	C + DGBX	12 wk	59.48 ± 8.22/59.62 ± 8.30	1, 3, 7	0/0
Wang and Chen[107], 2020	75/75	-	Calcium Dobesilate	C + DGBX	12 wk	62.38 ± 2.00/62.40 ± 2.02	1, 4	-
Wu[108], 2013	33/34	-	CT	C + DGBX	12 wk	-	1, 2, 5, 6, 7	0/0
Sun *et al* [109], 2019	90/92	-	CT	C + DGBX	12 wk	55.3 ± 3.7/55.9 ± 3.5	1, 3, 7	10/6
Yu[110], 2020	28/28	-	CT + Calcium Dobesilate	C + DGBX	12 wk	60.01 ± 8.26/60.48 ± 8.11	1, 2	-
Xu[111], 2018	43/43	TRD	CT	C + DGBX	12 wk	56.2 ± 7.3/56.6 ± 7.1	1, 7	4/0
Huang [112], 2017	54/54	TRD	Iodized Lecithin	C + XFZY	8 wk	62.15 ± 11.80/61.84 ± 12.11	2	-
Zhu[113], 2010	36/36	TRD	CT	C + XFZY	8 wk	55.42 ± 5.35/55.73 ± 5.10	1, 5, 6, 7	0/0
Xiong and Chen[114], 2019	43/43	TRD	Calcium Dobesilate	C + XFZY	16 wk	53.14 ± 7.25/54.28 ± 7.13	2, 5, 6	-
Liu[115], 2020	57/57	Lottery	CT	C + XFZY	16 wk	50.38 ± 3.67/50.47 ± 3.22	2	-
Gong *et al* [116], 2014	40/40	-	CT	C + XFZY	12 wk	57.24 ± 10.60/55.36 ± 9.28	1, 5	-
Hao[117], 2012	84/66	-	CT	C + XFZY	4 wk	-	1	-
Huang [118], 2004	50/70	-	CT	C + XFZY	12 wk	64.5/65	1	-
Zhang[119], 2019	46/46	-	CT	C + BYHW	8 wk	64.37 ± 9.13/66.46 ± 9.90	1	-
Yang[120], 2019	40/40	TRD	CT + Calcium Dobesilate	C + BYHW	8 wk	48.66 ± 8.82/50.35 ± 9.06	1, 2, 5	-
Tian[121],	27/27	-	Metformin	C + BYHW	12 wk	53.05 ±	1	-

2019						1.18/53.12 ± 1.12		
Qu and Yao [122], 2009	30/32	-	CT + Iodized Lecithin	C + BYHW	12 wk	-	1	-

A total of 6 studies[95-100] used Qiju Dihuang Pills (QJDH) (consisting of *Lycii Fructus*/Gouqizi in chinese; *Rehmanniae Radix Praeparata*/Dihuang in chinese; *Chrysanthemi Flos*/Juhua in chinese; *Cornus Officinalis Sieb. Et Zucc.*/Shanzhuyu in chinese; *Rhizoma Dioscoreae*/Shanyao in chinese; *Poria Cocos(Schw.) Wolf.*/Fuling in chinese; *Alisma Orientale (Sam.) Juz.*/Zexie in chinese; *Cortex Moutan*/Mudanpi in chinese). Four[95-97,100] of these six studies added *Paeoniae Radix Alba* (Baishao in Chinese), *Tribulifructus* (Jili in Chinese), *Cassiae Semen* (Juemingzi in Chinese), and *Angelicae Sinensis Radix* (Danggui in Chinese). Although there are some changes in components, the whole is still dominated by QJDH, which has the same efficacy as QJDH, so in this study, we classified these 6 studies as using QJDH. The composition of all Oral Chinese medicine (OCM) in this study is shown in Supplementary Table 2, and the source of OCMs, quality control report and chemical analysis report are shown in Supplementary Table 3. TRD: Table of random digit; CT: Conventional treatment, including blood glucose control, blood pressure lowering, lipid regulation and other conventional treatments; E: Experimental group; C: Control group; QM: Qiming Granules; DS: Compound Danshen Dripping Pills; XFZY: Xuefu Zhuyu Decoction; DGBX: Danggui Buxue Decoction; HXMM: Hexue Mingmu Tablets; SDMM: Shuangdan Mingmu Capsules; BYHW: Buyang Huanwu Decoction; QJDH: Qiju Dihuang Pills; XST: Compound Xueshuantong Capsule; -: Not mentioned; 1: Total effective rate; 2: Visual acuity; 3: Fundus hemorrhage area; 4: Vascular endothelial growth factor; 5: Fasting blood glucose; 6: Glycated hemoglobin; 7: Adverse reactions.



DOI: 10.4239/wjd.v14.i9.1422

Figure 1 The PRISMA flow diagram for search and selection processes of the meta- analysis. PRISMA: Preferred Reporting Items for Systematic Reviews and Meta- Analyses; RCT: Randomized controlled trials.

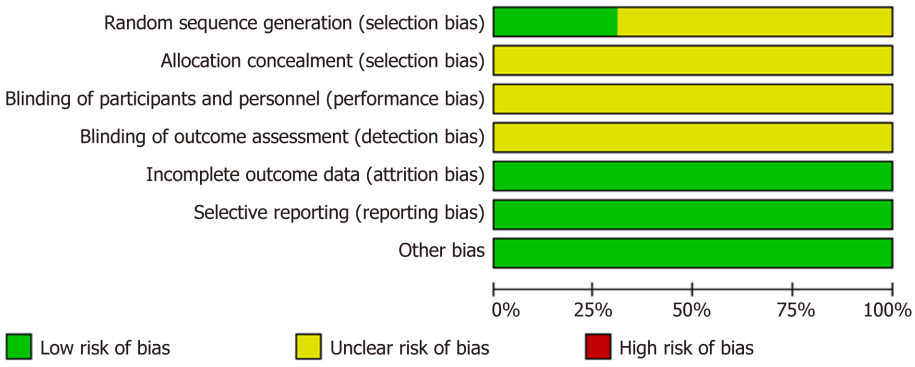
are shown in Supplementary Tables 2 and 3, respectively.

Risk of bias evaluation results

The quality of the included literature was evaluated using the "Risk Assessment Tool" recommended by the Cochrane Collaboration: 33 studies[16,19,22,28,29,35,37,39,43,45,49,51,55,69,72,73,78,83,85,88,89,92,94,98,102,104,106,111-115,120] mentioned the specific randomization method used and therefore assessed as "Low risk". The other 74 studies only mentioned the randomized grouping without mentioning the specific method used for allocation and were therefore evaluated as "Unclear risk". None of the included studies mentioned allocation concealment and blinding, and were evaluated as "Unclear risk". All studies had clear outcome indicators and were evaluated as "Low risk"; no duplicate publications or published biases were found in any of the studies and were evaluated as "Low risk"; other biases were unknown and were evaluated as "Unclear risk". All data were reported completely and were comparable between groups (Figure 2 and Supplementary Figure 1).

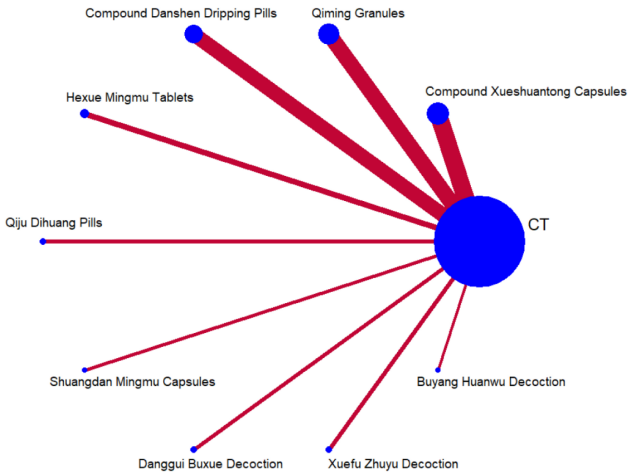
Results of reticulated meta-analysis

Mesh relationship diagram and consistency testing: The reticulation between the nine included OCM is shown in Figure 3. The total number of arms in the 107 papers totals 214. Lines between nodes indicate direct comparative evidence



DOI: 10.4239/wjd.v14.i9.1422 **Copyright** ©The Author(s) 2023.

Figure 2 Results of risk of bias graphs.



DOI: 10.4239/wjd.v14.i9.1422 **Copyright** ©The Author(s) 2023.

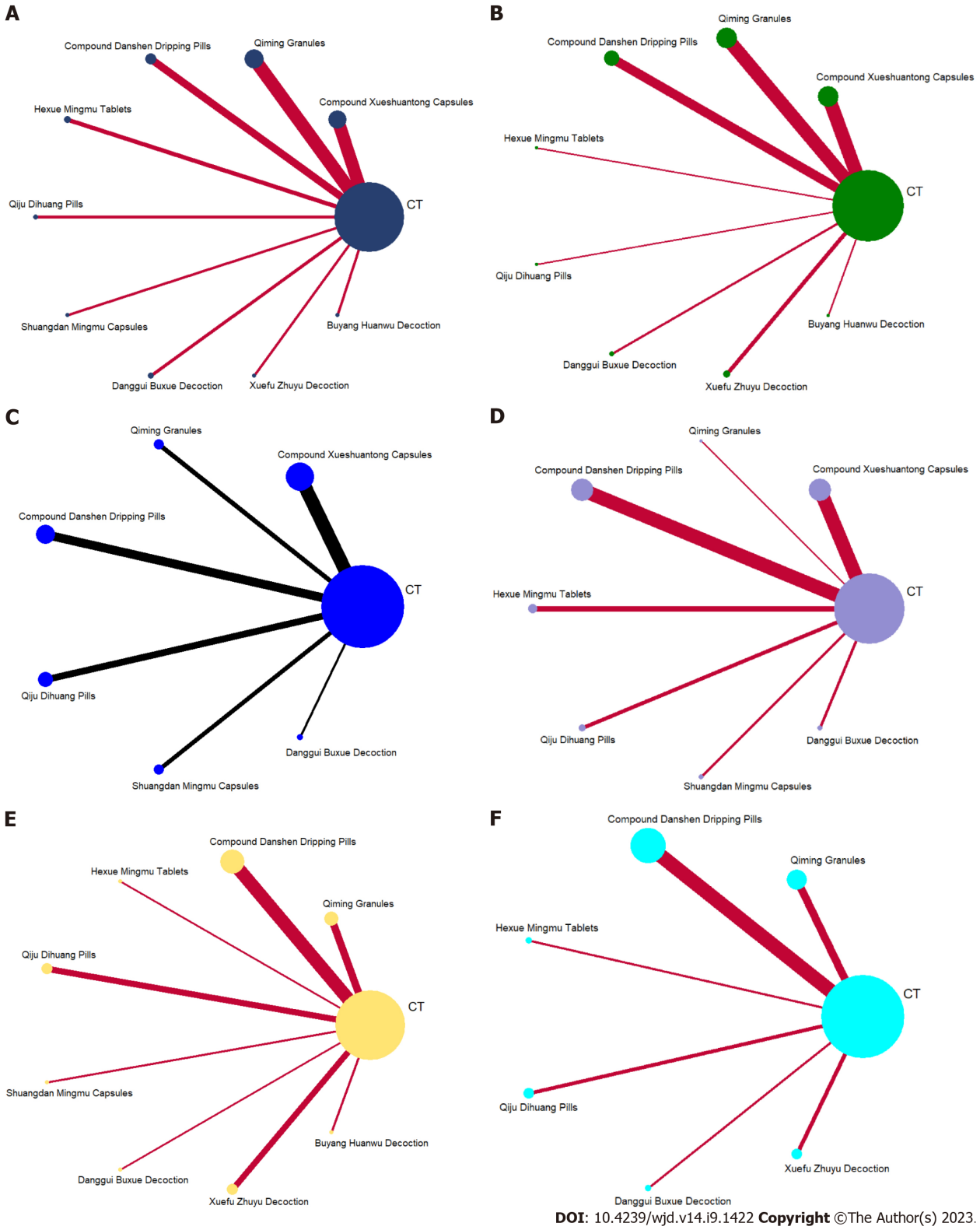
Figure 3 Reticulation of diabetic retinopathy treated with socio-comportemental and medical indicator. CT: Conventional western medicine treatment.

between the two interventions, no lines indicate no direct comparison, indirect comparisons can be made through reticulated Meta-analysis. The thickness of the line represents the number of included studies comparing each treatment, and the circular area represents the sample size of the population using the measure. All interventions involved in this study did not form a closed loop and did not require consistency testing.

Total effective rate: A total of 89 studies[16-20,22-25,27-31,33-37,39-69,72,74,75,77,79-81,83,87-99,102-111,113,116-122] were included to compare the total effective rate after OCM + CT treatment. The evidence diagram is shown in Figure 4A. The difference was statistically significant ($P < 0.05$) for the total effective rate for these nine OCM + CT treatments compared with CT (Figure 5A). The nine OCMs + CT total effective rate in descending order are: DS (SUCRA = 75.0%) > DGBX (SUCRA = 72.8%) > SDMM (SUCRA = 71.4%) > QM (SUCRA = 66.4%) > QJDH (SUCRA = 62.4%) > XST = HXMM (SUCRA = 52.5%) > BYHW (SUCRA = 26.8%) > XFZY (SUCRA = 20.1%) > CT (SUCRA = 0.0%). The probability ranking is shown in Figure 6A.

Visual acuity: A total of 34 studies[16-18,21,23,25,26,29,37,38,44,46,47,49,51,55,56,59,60,65,71,73,76,78,81,83,91,100,108,110,112,114,115,120] with 8 OCMs were included to compare visual acuity after OCM + CT treatment. The evidence diagram is shown in Figure 4B. In terms of visual acuity, supplemental XST, QM, DS, HXMM, QJDH, DGBX and XFZY treatments were statistically significant compared with CT treatment alone ($P < 0.05$) (Figure 5B). The eight OCMs + CT in order of highest to lowest improvement in visual acuity are: HXMM (SUCRA = 76.3%) > XST (SUCRA = 67.4%) > DS (SUCRA = 67.2%) > DGBX (SUCRA = 64.8%) > BYHW (SUCRA = 50.3%) > XFZY (SUCRA = 44.4%) > QM (SUCRA = 40.1%) > QJDH (SUCRA = 36.2%) > CT (SUCRA = 3.4%). The probability ranking is shown in Figure 6B.

VEGF: A total of 18 studies[16,25,29,31,33,35,55,58,66,69,73,83,96,97,100,102,104,107] with 6 OCMs were included to compare VEGF after OCM + CT treatment. The evidence diagram is shown in Figure 4C. In terms of VEGF, supplemental XST, DS, QJDH and SDMM were statistically significant compared with CT treatment alone ($P < 0.05$) (Figure 5C). The seven OCMs + CT in order of VEGF reduction from highest to lowest are: SDMM (SUCRA = 97.7%) > DS (SUCRA = 74.4%) > QJDH (SUCRA = 57.9%) > DGBX (SUCRA = 45.9%) > XST (SUCRA = 44.8%) > QM (SUCRA = 24.7%) > CT



DOI: 10.4239/wjd.v14.i9.1422

Figure 4 Network diagrams of comparisons on different outcomes of treatments in different groups (Oral Chinese medicines + conventional western medicine treatment) of patients with diabetic retinopathy. A: Total effective rate; B: Visual acuity; C: Vascular endothelial growth factor; D: Fundus hemorrhage area; E: Fasting blood glucose; F: Glycated hemoglobin. CT: Conventional western medicine treatment.

(SUCRA = 4.7%). The probability ranking is shown in Figure 6C.

FHA: A total of 32 studies[16,20,21,31,32,35,37,39-41,60,65,69,71,72,76,78-81,83,86,88-90,96,97,100,102,104,106,109] with 7 OCMs were included to compare FHA after OCM + CT treatment. The evidence diagram is shown in Figure 4D. These seven OCM + CT were statistically significant ($P < 0.05$) compared with CT for FHA, respectively (Figure 5D). The seven OCMs + CT reduce FHA in the following order from highest to lowest: DGBX (SUCRA = 87.5%) > SDMM (SUCRA =

A

Compound Xueshuantong Capsules									
0.90 (0.62,1.29)	Qiming Granules								
0.82 (0.52,1.30)	0.92 (0.59,1.44)	Compound Danshen Dripping Pills							
1.01 (0.63,1.64)	1.13 (0.71,1.81)	1.23 (0.71,2.12)	Hexue Mingmu Tablets						
0.91 (0.44,1.90)	1.02 (0.49,2.11)	1.11 (0.51,2.42)	0.90 (0.41,1.98)	Qiju Dihuang Pills					
0.82 (0.38,1.77)	0.91 (0.43,1.97)	0.99 (0.44,2.24)	0.81 (0.35,1.84)	0.90 (0.33,2.42)	Shuangdan Mingmu Capsules				
0.84 (0.52,1.36)	0.94 (0.58,1.51)	1.02 (0.59,1.77)	0.83 (0.47,1.46)	0.92 (0.41,2.03)	1.02 (0.45,2.34)	Danggui Buxue Decoction			
1.57 (0.89,2.78)	1.75 (1.00,3.08)	1.91 (1.02,3.57)	1.55 (0.81,2.94)	1.72 (0.74,4.01)	1.92 (0.79,4.63)	1.87 (0.98,3.58)	Xuefu Zhuyu Decoction		
1.43 (0.74,2.78)	1.60 (0.83,3.09)	1.74 (0.85,3.55)	1.41 (0.68,2.92)	1.57 (0.63,3.91)	1.75 (0.68,4.50)	1.71 (0.82,3.55)	0.91 (0.41,2.01)	Buyang Huanwu Decoction	
3.69 (2.83,4.80)	4.11 (3.20,5.30)	4.48 (3.08,6.50)	3.63 (2.44,5.40)	4.03 (2.04,7.98)	4.50 (2.18,9.27)	4.40 (2.93,6.60)	2.35 (1.42,3.89)	2.57 (1.40,4.72)	CT

B

Compound Xueshuantong Capsules								
1.05 (0.97,1.14)	Qiming Granules							
1.00 (0.91,1.10)	0.95 (0.87,1.04)	Compound Danshen Dripping Pills						
0.96 (0.78,1.17)	0.91 (0.74,1.12)	0.96 (0.77,1.18)	Hexue Mingmu Tablets					
1.08 (0.90,1.29)	1.02 (0.85,1.23)	1.08 (0.89,1.30)	1.13 (0.87,1.47)	Qiju Dihuang Pills				
1.00 (0.86,1.16)	0.95 (0.82,1.11)	1.00 (0.85,1.17)	1.05 (0.82,1.33)	0.93 (0.74,1.16)	Danggui Buxue Decoction			
1.05 (0.93,1.18)	1.00 (0.89,1.12)	1.05 (0.92,1.19)	1.10 (0.88,1.37)	0.97 (0.80,1.19)	1.05 (0.88,1.25)	Xuefu Zhuyu Decoction		
1.03 (0.85,1.25)	0.98 (0.81,1.19)	1.04 (0.85,1.26)	1.08 (0.83,1.42)	0.96 (0.75,1.24)	1.04 (0.82,1.30)	0.99 (0.80,1.22)	Buyang Huanwu Decoction	
1.18 (1.11,1.25)	1.12 (1.06,1.19)	1.18 (1.10,1.27)	1.23 (1.01,1.50)	1.09 (0.92,1.30)	1.18 (1.02,1.36)	1.13 (1.02,1.25)	1.14 (0.95,1.37)	CT

C

Compound Xueshuantong Capsules						
0.51 (0.10,2.72)	Qiming Granules					
2.04 (0.74,5.61)	3.97 (0.70,22.41)	Compound Danshen DrippingPills				
1.36 (0.45,4.13)	2.66 (0.44,15.90)	0.67 (0.20,2.23)	Qiju Dihuang Pills			
5.69 (1.57,20.65)	11.09 (1.65,74.54)	2.80 (0.71,11.00)	4.17 (0.99,17.62)	Shuangdan Mingmu Capsule		
0.98 (0.19,5.10)	1.90 (0.22,16.65)	0.48 (0.09,2.67)	0.72 (0.12,4.22)	0.17 (0.03,1.14)	Danggui Buxue Decoction	
0.28 (0.15,0.54)	0.55 (0.12,2.58)	0.14 (0.06,0.31)	0.21 (0.08,0.51)	0.05 (0.02,0.15)	0.29 (0.06,1.33)	CT

D

Compound Xueshuantong Capsules							
0.84 (0.52,1.38)	Qiming Granules						
0.88 (0.71,1.10)	1.05 (0.64,1.71)	Compound Danshen Dripping Pills					
0.64 (0.49,0.85)	0.76 (0.45,1.29)	0.730 (0.55,0.97)	Hexue Mingmu Tablets				
0.95 (0.69,1.30)	1.12 (0.66,1.93)	1.07 (0.78,1.47)	1.47 (1.03,2.11)	Qiju Dihuang Pills			
1.09 (0.75,1.57)	1.29 (0.73,2.29)	1.23 (0.85,1.78)	1.69 (1.12,2.55)	1.15 (0.75,1.77)	Shuangdan Mingmu Capsules		
1.18 (0.82,1.70)	1.40 (0.80,2.48)	1.34 (0.93,1.93)	1.84 (1.23,2.75)	1.25 (0.82,1.91)	1.09 (0.68,1.74)	Danggui Buxue Decoction	
0.44 (0.38,0.51)	0.52 (0.33,0.83)	0.50 (0.42,0.58)	0.68 (0.54,0.87)	0.46 (0.35,0.61)	0.40 (0.29,0.57)	0.37 (0.27,0.52)	CT

E

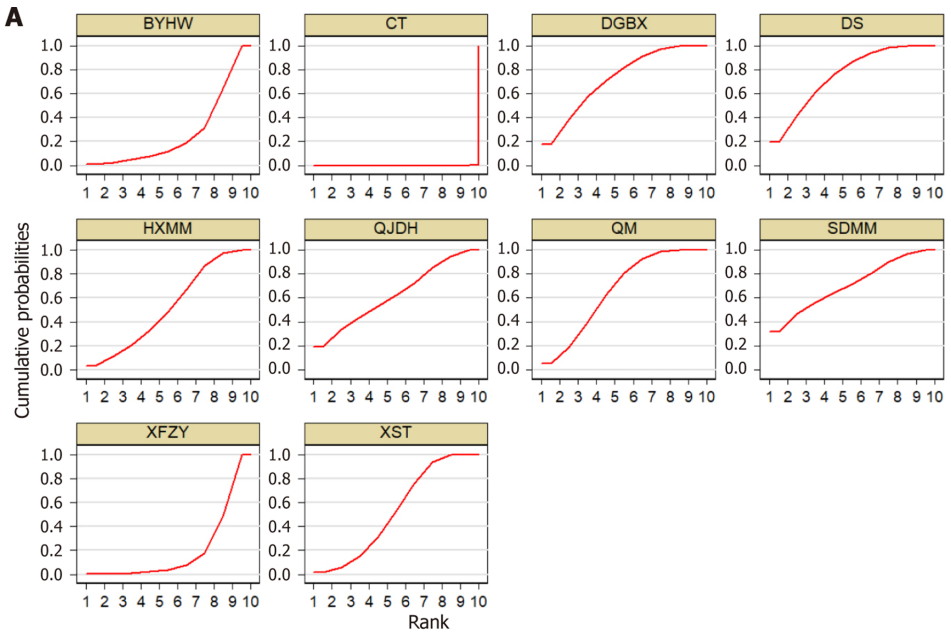
Qiming Granules							
1.39 (0.77,2.48)	Compound Danshen Dripping Pills						
3.48 (1.17,10.33)	2.51 (0.90,6.98)	Hexue Mingmu Tablets					
1.93 (0.95,3.95)	1.39 (0.75,2.59)	0.56 (0.18,1.68)	Qiju Dihuang Pills				
2.99 (0.97,9.19)	2.16 (0.75,6.23)	0.86 (0.21,3.50)	1.55 (0.50,4.84)	Shuangdan Mingmu Capsules			
1.06 (0.41,2.75)	0.76 (0.32,1.84)	0.30 (0.09,1.09)	0.55 (0.21,1.45)	0.35 (0.10,1.30)	Danggui Buxue Decoction		
1.10 (0.56,2.19)	0.80 (0.45,1.42)	0.32 (0.11,0.94)	0.57 (0.28,1.16)	0.37 (0.12,1.13)	1.04 (0.40,2.70)	Xuefu Zhuyu Decoction	
1.06 (0.37,3.02)	0.76 (0.29,2.04)	0.30 (0.08,1.17)	0.55 (0.19,1.59)	0.35 (0.09,1.40)	1.00 (0.29,3.46)	0.96 (0.34,2.73)	Buyang Huanwu Decoction
0.86 (0.53,1.40)	0.62 (0.45,0.85)	0.25 (0.09,0.65)	0.44 (0.26,0.75)	0.29 (0.10,0.79)	0.81 (0.36,1.84)	0.78 (0.48,1.26)	0.81 (0.32,2.05)	CT

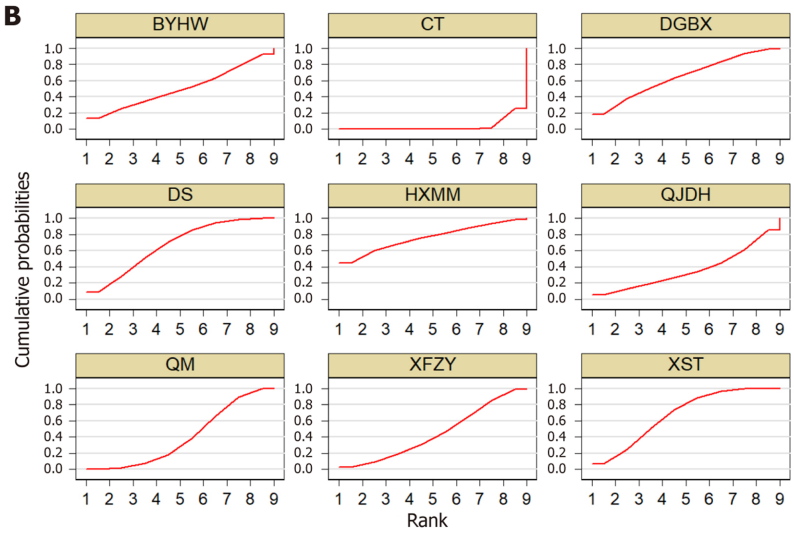
F

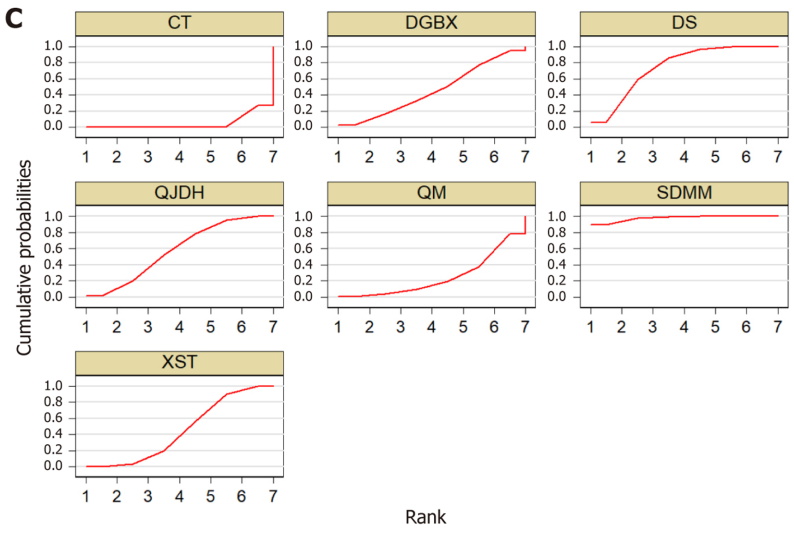
Qiming Granules						
0.97 (0.48,1.96)	Compound Danshen Dripping Pills					
1.43 (0.45,4.56)	1.47 (0.50,4.35)	Hexue Mingmu Tablets				
2.77 (1.07,7.15)	2.85 (1.21,6.69	1.94 (0.55,6.81)	Qiju Dihuang Pills			
0.96 (0.29,3.11)	0.99 (0.33,2.97)	0.67 (0.16,2.83)	0.35 (0.10,1.24)	Danggui Buxue Decoction		
1.08 (0.42,2.78)	1.11 (0.48,2.60)	0.76 (0.22,2.65)	0.39 (0.14,1.12)	1.13 (0.32,4.02)	Xuefu Zhuyu Decoction	
0.64 (0.36,1.14)	0.66 (0.44,0.99)	0.45 (0.16,1.23)	0.23 (0.11,0.49)	0.67 (0.24,1.87)	0.59 (0.28,1.25)	CT

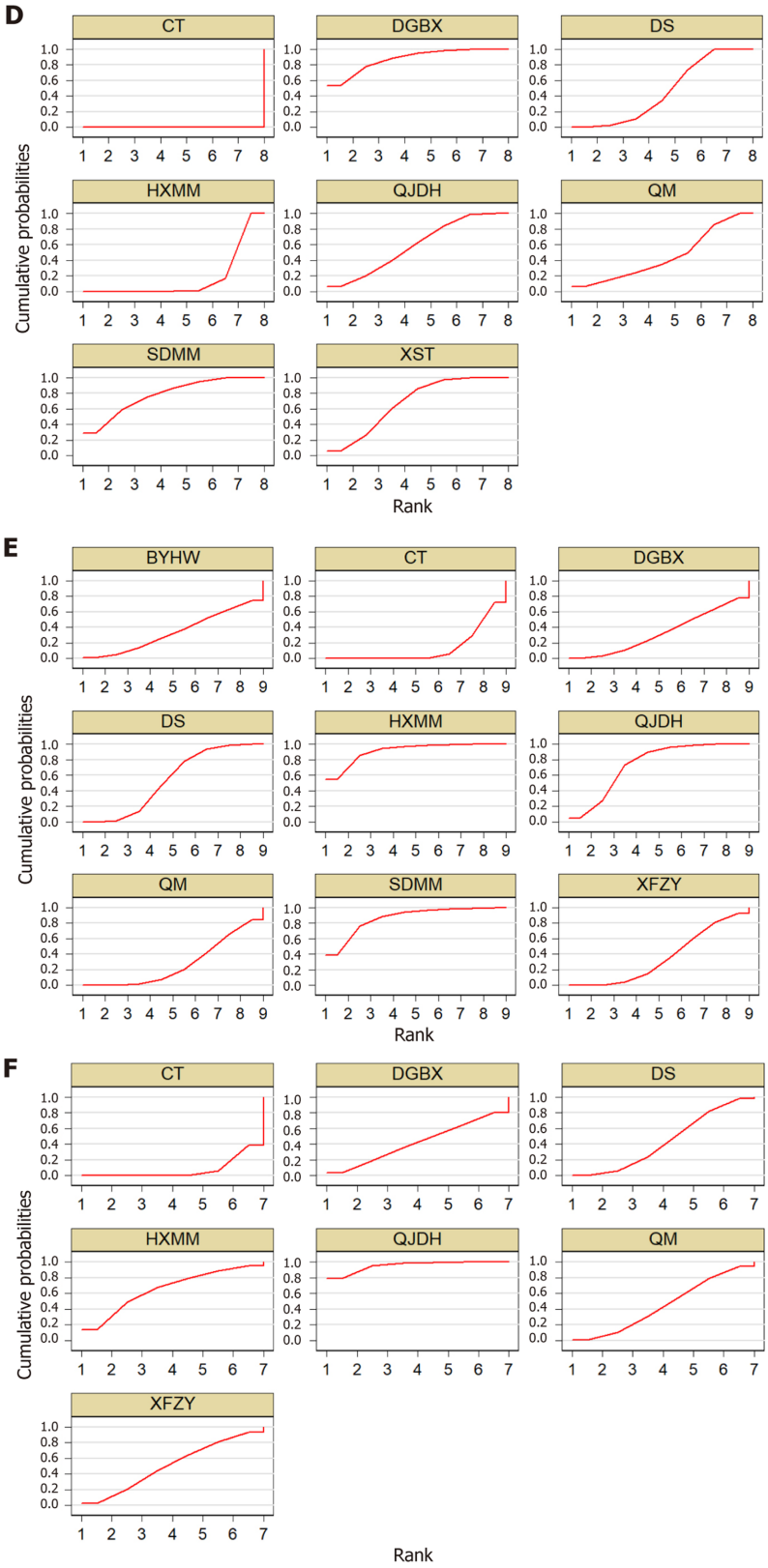
DOI: 10.4239/wjd.v14.i9.1422

Figure 5 Pooled estimates of the network meta-analysis. A: Pooled risk d ratios (95% credible intervals) for the total effective rate; B: Pooled risk d ratios (95% credible intervals) for visual acuity; C: Pooled risk d ratios (95% credible intervals) for vascular endothelial growth factor; D: Pooled risk d ratios (95% credible intervals) for fundus hemorrhage area; E: Pooled risk d ratios (95% credible intervals) for fasting blood glucose; F: Pooled risk d ratios (95% credible intervals) for glycated hemoglobin. CT: Conventional western medicine treatment.


A
BYHW
CT
DGBX
DS
HXMM
QJDH
QM
SDMM
XFZY
XST
Cumulative probabilities
Rank


B
BYHW
CT
DGBX
DS
HXMM
QJDH
QM
XFZY
XST
Cumulative probabilities
Rank


C
CT
DGBX
DS
QJDH
QM
SDMM
XST
Cumulative probabilities
Rank



DOI: 10.4239/wjd.v14.i9.1422

Figure 6 Surface under the cumulativeranking curve for outcomes. A: Total effective rate; B: Visual acuity; C: Vascular endothelial growth factor; D: Fundus hemorrhage area; E: Fasting blood glucose; F: Glycated hemoglobin. CT: Conventional western medicine treatment; XST: Xueshuantong Capsules; QM: Qiming Granules; DS: Compound Danshen Dripping Pills; HXMM: Hexue Mingmu Tablets; QJDH: Qiju Dihuang Pills; SDMM: Shuangdan Mingmu Capsules; DGBX: Danggui Buxue Decoction; XFZY: Xuefu Zhuyu Decoction; BYHW: Buyang Huanwu Decoction.

77.7%) > XST (SUCRA = 67.9%) > QJDH (SUCRA = 58.8%) > DS (SUCRA = 45.9%) > QM (SUCRA = 45.1%) > HXMM (SUCRA = 17.0%) > CT (SUCRA = 0.0%). The probability ranking is shown in Figure 6D.

FBG: A total of 21 studies[47,49,51,61,65,70,75,79,82,84,85,92,97-99,101,108,113,114,116,120] with 8 OCMs were included to compare FBG after OCM + CT treatment. The evidence diagram is shown in Figure 4E. In terms of FBG, supplemental DS, HXMM, QJDH and SDMM treatments were statistically significant compared with CT treatment alone ($P < 0.05$) (Figure 5E). The eight OCMs + CT lower FBG in the following order from highest to lowest: HXMM (SUCRA = 91.1%) > SDMM (SUCRA = 86.4%) > QJDH (SUCRA= 73.8%) > DS (SUCRA = 54.2%) > XFZY (SUCRA = 36.1%) > BYHW (SUCRA = 34.0%) > DGBX (SUCRA = 33.2%)> QM (SUCRA =27.8%)> CT (SUCRA =13.5%). The probability ranking is shown in Figure 6E.

HbA1c: A total of 17 studies[47,49,51,61,65,70,75,79,82,84,85,92,97,99,108,113,114] with 6 OCMs were included to compare HbA1c after OCM + CT treatment. The evidence diagram is shown in Figure 4F. In terms of HbA1c, supplemental DS and QJDH treatments were statistically significant compared with CT treatment alone ($P < 0.05$) (Figure 5F). The six OCMs + CT lower HbA1c in the following order from highest to lowest: QJDH (SUCRA = 95.5%) > HXMM (SUCRA = 65.3%) > XFZY (SUCRA = 50.8%) > QM (SUCRA = 45.0%) > DS (SUCRA = 43.6%) > DGBX (SUCRA = 42.2%) > CT (SUCRA = 7.6%). The probability ranking is shown in Figure 6F.

Cluster analysis and meta-analysis two-by-two comparison results: The key outcome indicators included in this study were cluster analyzed to derive the intervention of different OCMs + CT for two outcome indicators at the same time. In terms of total effective rate and visual acuity, HXMM, XST, DS and DGBX in the upper right corner of Figure 7A performed better; in terms of total effective rate and VEGF, SDMM and DS performed better (Figure 7B); in terms of total effective rate and FHA, DGBX and SDMM in the upper right corner of Figure 7C performed better; in terms of FBG and HbA1c, QJDH and HXMM in the upper right corner of Figure 7D performed better.

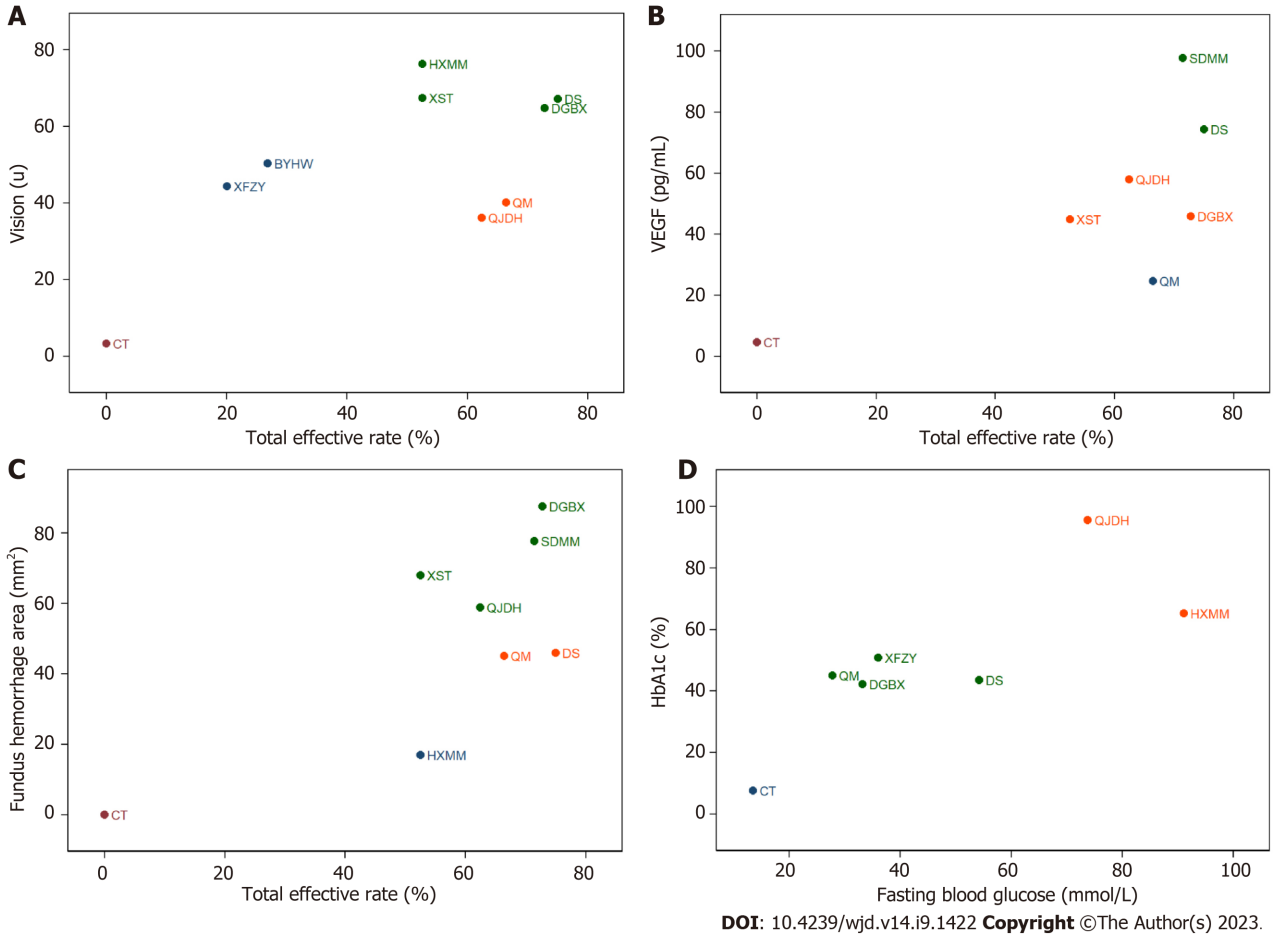
A two-by-two comparison of the nine OCMs + CT and CT was performed at each index. The total clinical efficiency of DR treatment with all 9 OCMs + CT was found to be higher than that of CT alone; CT in combination with XST, QM, DS, HXMM, DGBX and XFZY were superior to CT alone in improving visual acuity, respectively; in anti-VEGF, CT in combination with XST, DS, QJDH and SDMM were better than CT alone, respectively; CT in combination with XST, QM, DS, HXMM, QJDH, SDMM and DGBX were superior to CT alone in reducing FHA, respectively; CT in combination with DS, HXMM, QJDH and SDMM were superior to CT alone in lowering FBG, respectively; CT in combination with DS and QJDH were superior to CT alone in lowering HbA1c, respectively. All results are plotted as forest plots shown in Supplementary Figure 2.

Sensitivity analysis

To verify the stability of the above results, we performed NMA with sample size and duration of treatment as sensitivity factors that may affect the results. Of the total 107 studies, 54 studies[16-18,20,21,23,27,31-36,38,40,42,44,46,52,54,60,61,63-68,71,76-79,82,83,87,89,91,93-96,98,99,101,105,106,108,110,113,116,120-122] with a case load of no more than 80 were included in the sensitivity analysis. The results revealed that there was no significant difference in total effective rate for CT + SDMM compared with CT (OR: 0.94, 95%CI: 0.94-23.98, $P > 0.05$), unlike the original NMA; for HbA1c, DGBX + CT was effective in reducing HbA1c compared with CT (OR: 0.67, 95%CI: 0.48-0.94, $P < 0.05$), unlike the original NMA. There was no significant change in the remaining indicators, so the sample size was considered as a possible factor influencing the results (Supplementary Figure 3). When using duration of treatment as a sensitivity factor, we divided all studies according to 12 wk of treatment, and a total of 65 studies[16,19,22,24,26-31,33,34,37,38,41-45,47-49,51,56,58-61,66,69,71,72,75,80-82,85-95,98,100,102,106-113,116-122] with no more than 12 wk of treatment were included in the sensitivity analysis. Results found that DGBX + CT was more effective than CT in terms of VEGF (OR: 0.29, 95%CI: 0.16-0.53, $P < 0.05$), unlike the original NMA; for FBG, CT + QJDH was not significantly different from CT (OR: 0.67, 95%CI: 0.39-1.16, $P > 0.05$), unlike the original NMA; for HbA1c, the effect of DS + CT was not significantly different from CT (OR: 0.77, 95%CI: 0.38-1.57, $P > 0.05$), unlike the original NMA. Consideration of sample size may have influenced this result (Supplementary Figure 4).

The small sample effect and publication bias

The total effective rate (significantly effective + effective), visual acuity, VEGF, FHA, FBG and HbA1c were used as evaluation indicators to produce a comparative corrected funnel plot of the study to assess the small sample effect, see Figure 8. The results showed that the total effective rate and FBG comparison corrected funnel plots showed basic symmetry, with studies roughly symmetrically distributed on both sides of the midline, suggesting that a small sample effect is less likely. The poor symmetry of the corrected funnel plot for visual acuity, VEGF, FHA, and HbA1c comparisons suggests the possibility of a small sample effect. The reasons may be related to the mixed quality of included studies, small sample size, inconsistent treatment regimens, and different pathological stages of study subjects. Further analysis of publication bias using Begg's and Egger's tests revealed the presence of publication bias for total clinical effectiveness ($P_{Begg} < 0.001$, $P_{Egger} < 0.001$). Additionally, there was a potential publication bias for FBG ($P_{Begg} = 0.01$; $P_{Egger} = 0.151$) and FHA ($P_{Begg} = 0.224$, $P_{Egger} = 0.041$). However, no publication bias was observed for visual acuity, VEGF, and HbA1c ($P_{Begg} > 0.05$; $P_{Egger} > 0.05$). The presence of bias in these particular outcomes may be attributed to various factors, including the mixed quality of the included studies, small sample sizes, variations in treatment regimens, and differences in the pathological stages of the study subjects. These factors could contribute to heterogeneity and potential reporting biases within the literature.



DOI: 10.4239/wjd.v14.i9.1422

Figure 7 Cluster analysis for outcome indicators. A: Total effective rate and vision; B: Total effective rate and VEGF; C: Total effective rate and fundus hemorrhage area ; D: Fasting blood glucose and HbA1c. CT: Conventional western medicine treatment; XST: Xueshuantong Capsules; QM: Qiming Granules; DS: Compound Danshen Dripping Pills; HXMM: Hexue Mingmu Tablets; QJDH: Qiju Dihuang Pills; SDMM: Shuangdan Mingmu Capsules; DGBX: Danggui Buxue Decoction; XFZY: Xuefu Zhuyu Decoction; BYHW: Buyang Huanwu Decoction.

Adverse reactions

Of the total 107 RCTs, 47 mentioned adverse reactions, but only 20 of these studies[17,19,33,37,39,43,51,55,73,86-88,90,93,99,101-103,109,111] had patients with adverse reactions, and the other 26 studies[30,34,35,40,46,47,57-61,67-70,72,79,83,94-98,104,106,108,113] in which all patients had no adverse reactions. A total of 76 patients in the experimental group had adverse reactions during the treatment, and 127 patients in the control group had adverse reactions. A total of 7 OCMs were involved, and the results are shown in Table 2.

DISCUSSION

To the best of our knowledge, this study is the first NMA focused on the combination of OCM with conventional Western medicine for the treatment of DR, and is the NMA with the largest number of original studies included, the largest number of subjects, and the largest variety of OCM included to date. Only five NMAs[123-127] have reported the therapeutic effect on DR, and only one[125] of them is about the efficacy of herbal injections for DR. This study comprehensively collected RCTs involving 9 commonly used OCMs in China and included 6 clinical indicators commonly used to evaluate the efficacy of DR, the largest number of included indicators we are aware of to date in a similar report.

Although surgical treatment and intravitreal drug injection for DR are becoming popular, there are still some problems that are difficult to solve in a short period of time. First, invasive therapies have a limited audience. Total retinal photocoagulation is the primary treatment for proliferative DR and is not advocated for the treatment of non-proliferative DR; intraocular injections of VEGF inhibitors are more effective primarily in the treatment of DR with macular edema. In some forms of non-clearing vitreous hemorrhage, vitrectomy has been shown to remain the only method for removing fibrous proliferation and relieving traction detachment, with mixed results[128]. Second, safety issues need to be kept in mind, for example, one study found that although intraocular steroid injections led to rapid regression of dimethyl ether, however, this improvement did not persist and was associated with a significant increase in the incidence of elevated intraocular pressure and cataracts[129]. Furthermore, we must take into account the additional financial burden incurred by this type of treatment. A study from Canada reported that Grid laser therapy adds an additional cost benefit per

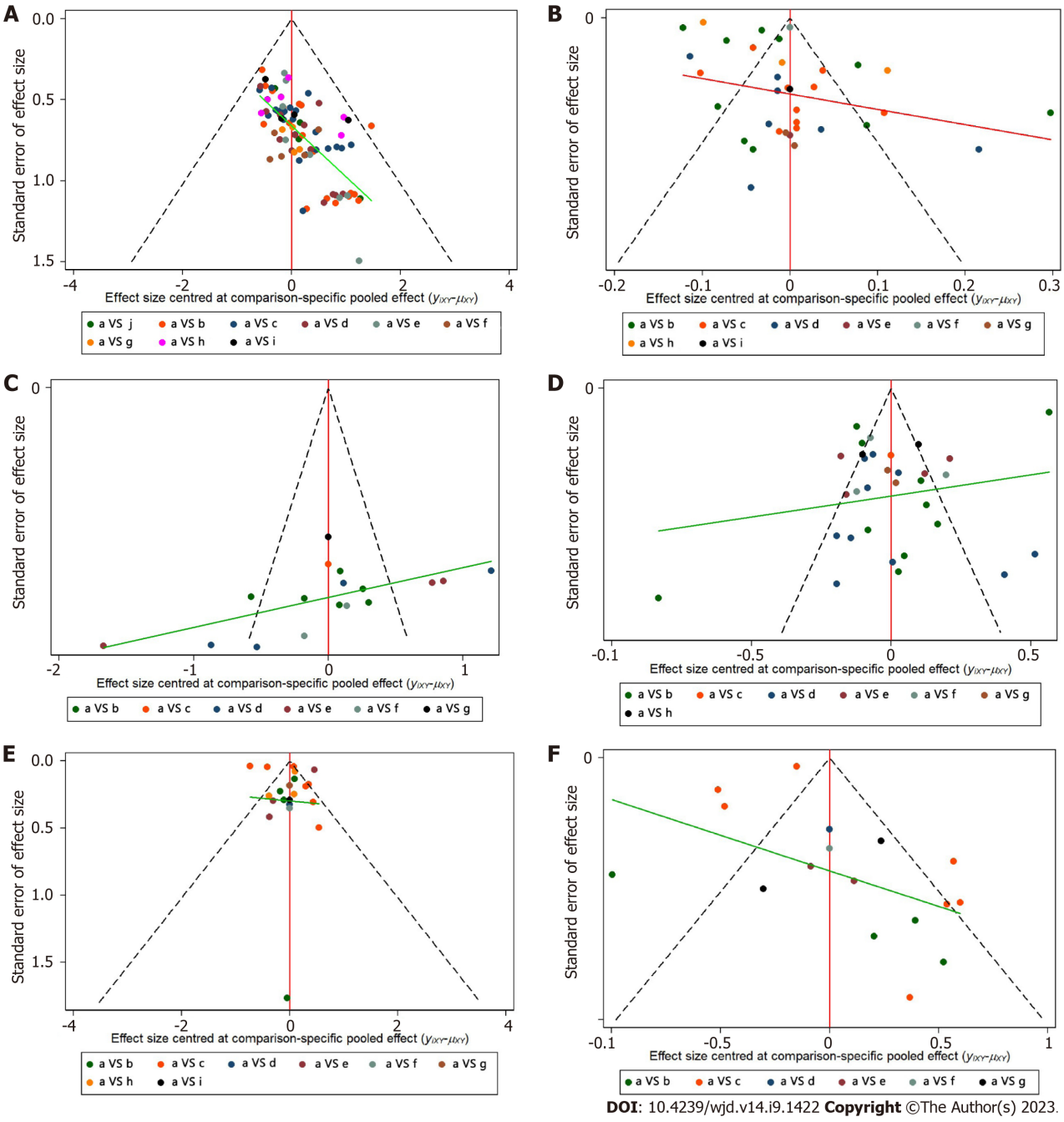
Table 2 Occurrence of adverse reactions of Oral Chinese medicine

OCMs compound	Group	Sample	Hypoglycemia	Stomach upset (*n*)	Loss of appetite	Insomnia	Fever	Dizzy	Nausea	Liver damage	Kidney damage	Macular edema	Corneal damage	Itchy skin	Fatigue
Xueshuantong Capsules	C	42	0	14	5	0	0	0	6	8	9	0	0	0	0
	E	28	0	8	8	0	0	0	8	2	2	0	0	0	0
Qiming Granules	C	23	0	1	0	0	0	0	0	3	2	8	7	1	1
	E	12	0	2	0	0	0	0	0	1	0	3	3	1	2
Compound Danshen Dripping Pills	C	3	0	3	0	0	0	0	0	0	0	0	0	0	0
	E	4	0	4	0	0	0	0	0	0	0	0	0	0	0
Hexue Mingmu Tablets	C	16	2	4	3	0	0	0	3	0	0	0	0	3	1
	E	12	0	6	2	0	0	0	1	0	0	0	0	2	1
Qiju Dihuang Pills	C	6	0	0	0	0	0	0	6	0	0	0	0	0	0
	E	5	0	0	0	0	0	0	5	0	0	0	0	0	0
Shuangdan Mingmu Capsules	C	23	0	5	0	0	5	2	8	0	0	0	0	0	3
	E	9	0	2	0	0	2	2	3	0	0	0	0	0	0
Danggui Buxue Decoction	C	14	2	4	0	2	0	4	0	0	0	0	2	0	0
	E	6	2	2	0	0	0	0	1	0	0	0	0	0	1

OCM: Oral Chinese medicine; E: Experimental group; C: Control group; The numbers in the table represent the number of patient cases.

quality of life adjusted year[130]. In contrast, OCM is not only increasingly proving to have surprising clinical efficacy [131], is affordable[132], and is indicated for patients in almost all stages of DR with a broad universal indication. Therefore, a systematic and comprehensive evaluation of the therapeutic efficacy of OCM for DR is essential.

This study found that DS showed excellent efficacy in improving visual acuity levels and total clinical effectiveness. DS is composed of three herbs *Radix Salviae* (Danshen in Chinese), *Panax notoginseng (Burkill) F. H. Chen ex C. H.* (Sanqi in Chinese) and *borneol* (bingpian in Chinese). According to Chinese medicine, Danshen and Sanqi have the effect of activating blood circulation and dispelling blood stasis, and are commonly used herbs for treating diseases of blood stasis and obstruction; Bingpian is obtained from the stem of *Blumea balsamifera (L.) DC.* or the leaves of *Cinnamomum camphora* by water steam distillation and recrystallization, and is documented in the famous Chinese medical work *Annotation of Materia Medica* from the Tang Dynasty (about 659 AD) as a treatment for eye diseases. Each of the three herbs has its own characteristics and at the same time exerts the effect of activating blood circulation and removing blood stasis, which is in line with modern pharmacological research. It was found[133] that Tanshinone IIa (the main active component of Danshen) promoted phosphorylation of AMP-activated protein kinase AMPK at T172 in retinal pigment epithelial cells and inhibited monolayer permeability of human retinal epithelial cells under high glucose conditions, similar to that



DOI: 10.4239/wjd.v14.i9.1422

Figure 8 Risk of bias funnel chart. A: Total effective rate; B: Visual acuity; C: Vascular endothelial growth factor; D: Fundus hemorrhage area; E: Fasting blood glucose; F: Glycated hemoglobin. a: CT: Conventional treatment, including blood glucose control, blood pressure lowering, lipid regulation and other conventional treatments; b: Compound Xueshuantong Capsules; c: Qiming Granules; d: Compound Danshen Dripping Pills; e: Hexue Mingmu Tablets; f: Qiju Dihuang Pills; g: Shuangdan Mingmu Capsules; h: Danggui Buxue Decoction; i: Xuefu Zhuyu Decoction; j: Buyang Huanwu Decoction.

under normal glucose, while apparently preventing co-localization of NF-κB and p300 and inhibiting their binding, thereby reducing ARPE-19 cell monolayer permeability. A study by Fan *et al*[134] found that Tanshinone IIa significantly downregulated the expression levels of VEGF and intercellular adhesion molecule-1 (ICAM-1) in a dose-dependent manner under HG conditions, probably by mediating proliferation, migration and inhibition of angiogenesis in human retinal endothelial cells. Sanqi and its extracts have anti-inflammatory[135-138], antioxidant, inhibit platelet aggregation, regulate blood glucose[139,140], regulate blood pressure[141-143], improve insulin resistance[144,145], inhibit neuronal apoptosis[146-148] and neuronal protection[149-152]. In particular, ginsenoside Rb1 (the main active compound extracted from the rhizome of Sanqi) has been widely demonstrated to be promising as an antidiabetic and its complications, improving diabetes-related complications by regulating oxidative stress, apoptosis, inflammatory response, enhancing insulin sensitivity, improving leptin resistance, activating the activation of lipocalin signaling pathway, and inhibiting fibronectin expression[153-161]. In addition to showing good clinical treatment rates, DGBX has shown excellent performance in improving the fundus hemorrhage area. According to TCM theory, DGBX is mainly used for treating diseases caused by fatigue and internal injury, blood deficiency and qi weakness. Although there are only two herbs in the formula, Radix Astragali Mongolici (Huangqi in Chinese) and Angelicae Sinensis Radix (Danggui in Chinese), the

formula is short but powerful and can promote the production of tangible blood from invisible qi, which is a classic OCM with the effect of tonifying qi and promoting blood production. It is a classic OCM with the effect of nourishing Qi and promoting blood circulation. DGBX was found to affect lipid metabolism in the early stages of atherosclerosis in diabetic Goto-Kakizaki rats, and the mechanism may be related to the regulation of intravascular lipid metabolism genes[162]. Astragalus polysaccharides are the main active components of Huangqi, can reduce the levels of tumor necrosis factor-α, ICAM-1, vascular VEGF and p-Akt in the retina of diabetic rats, and affect the Akt-VEGF signaling pathway by anti-inflammatory and reducing the adhesion of leukocytes to the diabetic retina[163], while inhibiting peripapillary cell apoptosis and basement membrane thickening. Dangui and its active ingredients were able to inhibit the VEGF-α pathway to improve the inflammatory response and apoptosis in the retina of diabetic rats[164], and inhibit α-glucosidase, protein tyrosine phosphatase 1B, rat lens aldose reductase, acetylcholinesterase, butyrylcholinesterase andβ-site amyloid precursor protein cleaving enzyme 1 to exert antidiabetic effects[165].

An interesting finding is that SDMM, QJDH and HXMM are all OCMs with the main effect of nourishing the Yin of the liver and kidney, and they all showed good results in lowering glucose. Chinese medicine theory believes that diabetes is a disease with Yin deficiency as the fundamental pathogenesis, and DR develops from diabetes, so the treatment should focus on replenishing Yin. Ligustrum lucidum (Nvzhenzi in Chinese) and Ecliptae Herba (Mohanlian in Chinese) are common components of HXMM and SDMM, which are widely used in China for the treatment of liver-kidney yin deficiency syndrome[166] (A TCM pathological diagnostic pattern caused by the imbalance of yin and yang[167,168], which is closely related to the development of diabetes). They were found to increase insulin sensitivity, enhance the function of islet β-cells INS-1 and β-tc-6[169], reduce retinal oxidative stress levels, repair diabetes-induced abnormal transcriptome[170], improve retinal cell apoptosis[171], inhibit NLRP3 inflammasome and autophagy signaling pathways [172], regulate homocysteine pathway, reduce lipid peroxidation and scavenge free radicals[173], thereby reducing fundus microangiopathy and protecting normal retinal barrier function in diabetic mice. Meanwhile, SDMM was confirmed to be the most effective complementary and alternative drug for inhibiting VEGF in this study, exerting anti-VEGF effects by inhibiting VEGF-induced RF/6A cell tube formation[174], inhibiting NF-κB activity to regulate advanced glycosylation end-product accumulation, oxidative stress and mitochondrial function, and thus improving retinal cell apoptosis by downregulating PKCδ, P47phox and ERK1/2[175,176]. QJDH is a very well-known Chinese OCM for the treatment of eye diseases, which is composed of Liuwei Dihuang Pills (an ancient remedy with very good treatment of diabetes and its complications) plus *Chrysanthemi Flos* and *Lycii Fructus*, and has been shown to possibly inhibit the development of DR by interfering with multiple biological pathways such as regulation of response to insulin, glucose homeostasis, and angiogenesis[177]. It was found that *Lycii Fructus* extract and the active ligand taurine dose-dependently enhanced cell viability, reduced apoptosis, downregulated caspase-3 protein expression and caspase-3 enzymatic activity, and downregulated mRNA encoding pro-inflammatory mediators of MMP-9 and fibronectin as well as COX-2 and iNOS protein expression in human retinal epithelial cell lines after HG treatment in order to achieve a protective effect on human retinal epithelial cell lines under HG exposure, thereby delaying the progression of DR[178, 179]. Several network pharmacological and experimental validations found that chemical components such as luteolin, kaempferol, beta-sitosterol, and thymol were able to improve apoptosis-related protein expression by regulating the NLRP/NOX4 signaling pathway, downregulate network hub genes of tumor necrosis factor, and other multibiological pathways, inhibit VEGF-induced RF/6A cell tube formation, and slow down the DR process[171,174,180,181], and these chemicals were also found in *Chrysanthemi Flos*.

It is noteworthy that metabolic diseases, such as hyperglycemia, hyperlipidemia, and hypertension, serve as crucial risk factors contributing to the development of DR[2]. Effective management of these risk factors is imperative in the prevention and treatment of DR. In this regard, incorporating exercise training and neuromuscular electrical stimulation [182,183] can prove to be a valuable strategy. The guidelines established by the American College of Sports Medicine and the American Diabetes Association emphasize the significance of initial guidance from a qualified exercise trainer for individuals with type 2 diabetes[184]. These guidelines advocate for the implementation of appropriate exercise training to optimize outcomes related to glycemic control, blood pressure, lipid levels, and cardiovascular risk management.

Despite the clear strengths of this study, there are some limitations. First, the quality of the included studies in this study warrants improvement, as most of them were short-term, single-center, and had small sample sizes. Second, clinical effectiveness, being a commonly used measure in clinical practice, may vary slightly in its definition across different randomized controlled trials, leading to a certain degree of heterogeneity in the results. And then, due to the relatively strict inclusion criteria applied in this study, some high-quality individualized TCM treatment studies were excluded, which may limit the comprehensive representation of the characteristics of TCM. Finally, the included literature lacked direct comparisons between different TCMs, and a closed loop was not formed in the evidence network, thereby allowing only indirect comparisons to assess the efficacy advantages and disadvantages of different interventions. Despite these limitations, the present study remains one of the most comprehensive studies available and holds significant clinical reference value.

This study aims to optimize patient outcomes by tailoring treatment strategies to each patient's unique condition. We recommend the utilization of SDMM + CT or HXMM + CT for treatment due to their demonstrated efficacy across multiple indicators. Specifically, HXMM + CT has shown greater effectiveness in improving patients' visual acuity, while SDMM + CT exhibits stronger inhibitory effects on VEGF. Furthermore, DGBX + CT has proven to be more effective in reducing FHA, HXMM + CT excels in reducing FBG, and QJDH + CT demonstrates superior efficacy in reducing HbA1c. Additionally, we suggest combining OCMs with western drugs for the treatment of DR, as this combination has been shown to yield superior outcomes compared to interventions with western drugs alone. Hence, it is crucial to select appropriate treatment methods in clinical practice based on the individual circumstances of patients with DR to attain maximum benefits from combined Chinese and Western medicine interventions.

CONCLUSION

This study provides evidence that combining OCMs with western drugs leads to better outcomes in all aspects of DR compared to using western drugs alone. Based on the findings, we highly recommend the use of SDMM or HXMM for the treatment of DR. These two OCMs have demonstrated outstanding efficacy across multiple indicators.

ARTICLE HIGHLIGHTS

Research background

Diabetic retinopathy (DR) is one of the most important factors in adult blindness, yet rationalized DR treatment protocols are currently not systematically updated.

Research motivation

Current traditional Chinese medicine treatment options for DR need to be re-evaluated.

Research objectives

To investigate which complementary alternative treatment with herbs is the most effective for the different clinical characteristics of DR patients.

Research methods

Alternative treatment options to traditional Chinese medicine were incorporated and assessed by employing a mesh meta-analysis to prioritize the therapeutic effects of these options based on various clinical observations.

Research results

When these nine Oral Chinese medicines were analyzed in combination with conventional western medicine treatment (CT) compared with CT alone, the results showed that Hexue Mingmu Tablets has better intervention effect on the overall efficacy, visual acuity and reducing fasting blood glucose, Shuangdan Mingmu Capsules has better effect on inhibiting vascular endothelial growth factor, Danggui Buxue Decoction has better effect on reducing fundus hemorrhage area, and Qiju Dihuang Pills has better effect on reducing glycated hemoglobin.

Research conclusions

Shuangdan Mingmu Capsules or Hexue Mingmu Tablets in combination with western drugs for DR may be the ideal treatment option.

Research perspectives

Bringing guidance to the clinical use of DR, as well as providing direction to basic experiments.

FOOTNOTES

Author contributions: Li HD and Li MX conceived and designed the study, performed the initial search and screening of the literature; Zhang SW was involved in the decision of literature inclusion; Zhang WH assessed the quality of the literature; Li HD and Li MX entered the literature data, including author information, year of publication, outcome indicators, and the profile of the study population; Li MX performed the network meta-analysis and produced the figures; Li HD performed the first draft of the manuscript; Zhang WH and Zhang SW performed the preliminary check and revision of the first draft; Gong YB performed the final revision of the manuscript and critically reviewed the results of the data; In addition, all personnel were familiar with the content of the manuscript; Li HD and Li MX contributed equally to this study.

Supported by The National Administration of Traditional Chinese Medicine for the Youth Qihuang Scholar Support Project.

Conflict-of-interest statement: All authors have no conflict of interest of any kind.

PRISMA 2009 Checklist statement: The authors have read the PRISMA 2009 Checklist, and the manuscript was prepared and revised according to the PRISMA 2009 Checklist.

Country/Territory of origin: China

ORCID number: Hong-Dian Li 0000-0002-4888-3469; Ming-Xuan Li 0000-0003-2250-5213; Yan-Bing Gong 0009-0002-7108-446X.

S-Editor: Fan JR
L-Editor: A
P-Editor: Zhang YL

REFERENCES

1 **Bourne RRA**, Flaxman SR, Braithwaite T, Cicinelli MV, Das A, Jonas JB, Keeffe J, Kempen JH, Leasher J, Limburg H, Naidoo K, Pesudovs K, Resnikoff S, Silvester A, Stevens GA, Tahhan N, Wong TY, Taylor HR; Vision Loss Expert Group. Magnitude, temporal trends, and projections of the global prevalence of blindness and distance and near vision impairment: a systematic review and meta-analysis. *Lancet Glob Health* 2017; **5**: e888-e897 [PMID: 28779882 DOI: 10.1016/S2214-109X(17)30293-0]

2 **Cheung N**, Mitchell P, Wong TY. Diabetic retinopathy. *Lancet* 2010; **376**: 124-136 [PMID: 20580421 DOI: 10.1016/S0140-6736(09)62124-3]

3 **Whitcup SM**, Cidlowski JA, Csaky KG, Ambati J. Pharmacology of Corticosteroids for Diabetic Macular Edema. *Invest Ophthalmol Vis Sci* 2018; **59**: 1-12 [PMID: 29297055 DOI: 10.1167/iovs.17-22259]

4 **Stitt AW**, Curtis TM, Chen M, Medina RJ, McKay GJ, Jenkins A, Gardiner TA, Lyons TJ, Hammes HP, Simó R, Lois N. The progress in understanding and treatment of diabetic retinopathy. *Prog Retin Eye Res* 2016; **51**: 156-186 [PMID: 26297071 DOI: 10.1016/j.preteyeres.2015.08.001]

5 **Youngblood H**, Robinson R, Sharma A, Sharma S. Proteomic Biomarkers of Retinal Inflammation in Diabetic Retinopathy. *Int J Mol Sci* 2019; **20** [PMID: 31557880 DOI: 10.3390/ijms20194755]

6 **Zhao ZH**, Xu M, Fu C, Huang Y, Wang TH, Zuo ZF, Liu XZ. A Mechanistic Exploratory Study on the Therapeutic Efficacy of Astragaloside IV Against Diabetic Retinopathy Revealed by Network Pharmacology. *Front Pharmacol* 2022; **13**: 903485 [PMID: 35814228 DOI: 10.3389/fphar.2022.903485]

7 **Luo XX**, Duan JG, Liao PZ, Wu L, Yu YG, Qiu B, Wang YL, Li YM, Yin ZQ, Liu XL, Yao K. Effect of qiming granule on retinal blood circulation of diabetic retinopathy: a multicenter clinical trial. *Chin J Integr Med* 2009; **15**: 384-388 [PMID: 19802544 DOI: 10.1007/s11655-009-0384-5]

8 **Sun HH**, Chai XL, Li HL, Tian JY, Jiang KX, Song XZ, Wang XR, Fang YS, Ji Q, Liu H, Hao GM, Wang W, Han J. Fufang Xueshuantong alleviates diabetic retinopathy by activating the PPAR signalling pathway and complement and coagulation cascades. *J Ethnopharmacol* 2021; **265**: 113324 [PMID: 32890714 DOI: 10.1016/j.jep.2020.113324]

9 **Wang F**, Li L, Xiao H. Analysis of the efficacy and safety of Danshen Dripping pills on the eyes of diabetic retinopathy patients with Qi stagnation and blood stasis. *Am J Transl Res* 2021; **13**: 13059-13066 [PMID: 34956524]

10 **Li T**, Puhan MA, Vedula SS, Singh S, Dickersin K; Ad Hoc Network Meta-analysis Methods Meeting Working Group. Network meta-analysis-highly attractive but more methodological research is needed. *BMC Med* 2011; **9**: 79 [PMID: 21707969 DOI: 10.1186/1741-7015-9-79]

11 **Shamseer L**, Moher D, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart LA; PRISMA-P Group. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. *BMJ* 2015; **350**: g7647 [PMID: 25555855 DOI: 10.1136/bmj.g7647]

12 **Hutton B**, Salanti G, Caldwell DM, Chaimani A, Schmid CH, Cameron C, Ioannidis JP, Straus S, Thorlund K, Jansen JP, Mulrow C, Catalá-López F, Gøtzsche PC, Dickersin K, Boutron I, Altman DG, Moher D. The PRISMA extension statement for reporting of systematic reviews incorporating network meta-analyses of health care interventions: checklist and explanations. *Ann Intern Med* 2015; **162**: 777-784 [PMID: 26030634 DOI: 10.7326/M14-2385]

13 **Chinese Elderly Type 2 Diabetes Prevention and Treatment of Clinical Guidelines Writing Group**; Geriatric Endocrinology and Metabolism Branch of Chinese Geriatric Society; Geriatric Endocrinology and Metabolism Branch of Chinese Geriatric Health Care Society; Geriatric Professional Committee of Beijing Medical Award Foundation; National Clinical Medical Research Center for Geriatric Diseases (PLA General Hospital). [Clinical guidelines for prevention and treatment of type 2 diabetes mellitus in the elderly in China (2022 edition)]. *Zhonghua Nei Ke Za Zhi* 2022; **61**: 12-50 [PMID: 34979769 DOI: 10.3760/cma.j.cn112138-20211027-00751]

14 **Cumpston M**, Li T, Page MJ, Chandler J, Welch VA, Higgins JP, Thomas J. Updated guidance for trusted systematic reviews: a new edition of the Cochrane Handbook for Systematic Reviews of Interventions. *Cochrane Database Syst Rev* 2019; **10**: ED000142 [PMID: 31643080 DOI: 10.1002/14651858.ED000142]

15 **Chaimani A**, Higgins JP, Mavridis D, Spyridonos P, Salanti G. Graphical tools for network meta-analysis in STATA. *PLoS One* 2013; **8**: e76654 [PMID: 24098547 DOI: 10.1371/journal.pone.0076654]

16 **Men LB**. [Effect of Compound Xueshuantong Capsules combined with Calcium Dobesilate on visual acuity and inflammatory factor levels in patients with diabetic retinopathy]. *Linchuang Yixue* 2020; **40**: 124-125 [DOI: 10.19528/j.issn.1003-3548.2020.01.051]

17 **Bai YX**. [Observation on treating diabetic retinopathy in integrative medicine and the safety evaluation]. *Zhongyi Linchang Yanjiu* 2016; **8**: 17-19 [DOI: 10.3969/j.issn.1674-7860.2016.31.006]

18 **An LN**. [Efficacy of compound thromboxane capsules supplemented with calcium hydroxybenzenesulfonate tablets in the treatment of non-proliferative diabetic retinopathy and its effect on retinal hemodynamics]. *Shiyong Tangniaobing Zazhi* 2021; **17**: 127-128

19 **Yan H**, Song JH. [Effectiveness of compound thromboxane capsules in the adjuvant treatment of non-proliferative diabetic retinopathy and the effect on OA hemodynamics in patients]. *Haixia Yaoxue* 2020; **32**: 141-142 [DOI: 10.3969/j.issn.1006-3765.2020.05.060]

20 **Li D**. [Effect of compound thromboxane capsule combined with calcium hydroxybenzenesulfonate in the treatment of diabetic retinopathy with macular edema]. *Tangniaobing Tiandi* 2021; **18**: 88

21 **Jin SJ**. [Observation on the effect of treatment with compound thromboxane capsules combined with calcium hydroxybenzenesulfonate in patients with diabetic retinopathy]. *Kang Yi* 2020; **17**: 249 [DOI: 10.12332/j.issn.2095-6525.2020.17.243]

22 **Wu ZX**. [Clinical effects and blood rheology of thromboxane in the treatment of type 2 diabetic retinopathy]. *Haixia Yaoxue* 2015; **27**: 3 [DOI: 10.3969/j.issn.1006-3765.2015.12.076]

23 **Qu ZX**. [Therapeutic effect of compound Xueshuantong capsule combined with calcium dobesilate in the treatment of diabetic retinopathy]. *ZhongguoXiandai Yisheng* 2018; **56**: 30-32

24 **Sui YN**. [Efficacy of compound Xueshuantong Capsules combined with Calcium Dobesilatein the treatment of diabetic retinopathy].

Neimenggu Zhongyiyao 2017; **36**: 93 [DOI: 10.16040/j.cnki.cn15-1101.2017.08.09]
25 **Jiang YN**, Jiang XD, Li S, Wang HM. [Effect of Compound Thrombosis on Early Diabetic Retinopathy and Its Influence on Serum Levels of Vascular Endothelial Growth Factor and NSE]. *Xiandai Zhenduan Zhiliao* 2019; **30**: 1782-1784
26 **Zhu YX**, Li J, Ying J. [Efficacy of compound Xueshuantong capsules in the treatment of non-proliferative diabetic retinopathy]. *Zhongguo Xiandai Yisheng* 2016; **54**: 60-62+66
27 **Sun H**. [Observation on the effect of compound thromboxane capsule in the treatment of diabetic retinopathy]. *Tangniaobing Tiandi* 2021; **18**: 57
28 **Liu X**, Zhou Q, Zhang Y. [Effects of compound Xueshuantong combined with calcium dobesilate on the levels of HIF-1 and SDF-1 in patients with non-proliferative diabetic retinopathy]. *Hebei Yiyao* 2018; **40**: 1838-1841 [DOI: 10.3969/j.issn.1002-7386.2018.12.019]
29 **Li Y**. [Effect of compound Xueshuantong combined with calcium dobesilate on vascular growth factor levels and visual acuity in patients with diabetic retinopathy]. *Tangniaobing Xintiandi* 2019; **22**: 180-181 [DOI: 10.16658/j.cnki.1672-4062.2019.07.180]
30 **Xiao LP**. [Clinical efficacy of compound Xueshuantong combined with calcium dobesilate in the treatment of non-proliferative diabetic retinopathy]. *Jilin Yixue* 2016; **37**: 2254-2255 [DOI: 10.3969/j.issn.1004-0412.2016.09.085]
31 **Chen KF**. [Clinical effect observation of compound Xueshuantong combined with calcium dobesilate in the treatment of early diabetic retinopathy]. *Huaihai Yiyao* 2019; **37**: 72-74 [DOI: 10.14126/j.cnki.1008-7044.2019.01.026]
32 **Zhao Y**. [Clinical effects of compound Xueshuantong combined with calcium dobesilate in the treatment of early diabetic retinopathy]. *Shoudu Shipin Yiyao* 2020; **27**: 95
33 **An LN**. [Clinical study of compound Xueshuantong combined with calcium dobesilate in the treatment of diabetic retinopathy]. *Shiyong Zhongxiyi Jiehe Linchuang* 2020; **20**: 69-71 [DOI: 10.13638/j.issn.1671-4040.2020.14.034]
34 **Zhang L**. [Clinical efficacy of compound Xueshuantong combined with calcium dobesilate in the treatment of early diabetic retinopathy]. *Tangniaobing Tiandi* 2019; **16**: 91
35 **Wei M**, Chen C, Liao T, Peng J, Liu J. [Clinical Effect of Compound Xueshuantong on Early Diabetic Retinopathy and Serum Levels of VEGF, IGF-1 and NSE]. *Xiandai Shengwu Yixue Jinzhan* 2017; **17**: 3901-3904 [DOI: 10.13241/j.cnki.pmb.2017.20.023]
36 **Meng X**, Zhang S, Duan Y. [Clinical observation on the treatment of diabetic retinopathy with Compound Xueshuantong]. *Henan Daxue Xuebao* 2012; **31**: 151-152 [DOI: 10.15991/j.cnki.41-1361/r.2012.02.019]
37 **Zhu Y**, Sui A. [Effects of two different treatment regimens on fundus microvasculature and blood rheology in patients with early diabetic retinopathy]. *Zhongguo Yixue Chuangxin* 2022; **19**: 35-38 [DOI: 10.3969/j.issn.1674-4985.2022.10.009]
38 **Li BJ**. Clinical efficacy of compound Xueshuantong combined with calcium dobesilate in the treatment of proliferative diabetic retinopathy. 2017 Fifth World Congress of Integrative Medicine Abstracts Collection (Previous); 2017 Dec 6; Guangzhou, GuangDong, China: Guoji Huiyi, 2017: 13. Available from: https://kns.cnki.net/kcms2/article/abstract?v=zrtWY6fLGG6aXSpR1SYn5cOFoTKcnoLRVUAXqH0FI1QCAceY09lAbBhMYYljvCp5i5xrdbocLTN1kW6aMDBZd6Xc0kqT6nyytP6hkxdUyiWvGTu0upJMqfkNJ8vE-khRHBn_yfbae5-aiQpCcLN7og==&uniplatform=NZKPT
39 **Wang J**, Du W, Li Y. [Efficacy of compound Xueshuantong combined with calcium dobesilate capsules in the treatment of diabetic retinopathy in the elderly and its effect on blood rheology]. *Zhongguo Laonianxue Zazhi* 2020; **40**: 1603-1606 [DOI: 10.3969/j.issn.1005-9202.2020.08.010]
40 **Hu D**. [Clinical efficacy of Compound Xueshuantong combined with calcium dobesilate in patients with visual field defects due to diabetic retinopathy]. *Yixue Lilun Shijian* 2017; **30**: 670-671 [DOI: 10.19381/j.issn.1001-7585.2017.05.022]
41 **Xu J**, Ru J. [Effect of Compound Xueshuantong combined with calcium dobesilate on ocular blood flow in patients with early diabetic retinopathy]. *Xiandai Zhongxiyi Jiehe Zazhi* 2020; **29**: 4064-4067 [DOI: 10.3969/j.issn.1008-8849.202036.018]
42 **Wang X**. [Clinical observation of Iodized Lecithin combined with Qiming Granules in the treatment of macular cystoid edema in diabetic retinopathy]. *Zhongyiyao Xinxi* 2016; **33**: 111-113
43 **Wang Q**. [Efficacy of Qiming Granules in non-proliferative diabetic retinopathy. *Shenzhen Zhongxiyi Jiehe Zazhi* 2018; **28**: 48-49 [DOI: 10.16458/j.cnki.1007-0893.2018.10.022]
44 **Wang M**, Yan ZJ, Zhang LM, Zhang YH, Xu WH. [Clinical efficacy of Qiming Granules combined with Levamlodipine Besylate in the treatment of diabetic retinopathy combined with hypertension]. *Hubei Zhongxiyi Jiehe Zazhi* 2020; **22**: 58-60 [DOI: 10.3969/j.issn.1008-987x.2020.06.15]
45 **Chen JQ**. [Clinical observation of 45 cases of diabetic retinopathy treated with Qiming Granules combined with conventional drugs]. *Xinzhongyi* 2016; **48**: 50-51 [DOI: 10.13457/j.cnki.jncm.2016.09.023]
46 **Wang B**, Li XY, Liu MC, Gao ZN. [Efficacy of Qiming Granules combined with calcium dobesilate in diabetic retinopathy]. *Zhongwai Yiliao* 2015; **34**: 114-115 [DOI: 10.16662/j.cnki.1674-0742.2015.03.048]
47 **Sui HL**, Yu CY, Xue HM, Wang RN. [Clinical efficacy of Qiming Granules combined with calcium dobesilate Capsules in the treatment of patients with non-proliferative diabetic retinopathy]. *Zhongwai Yixue Chuangxin* 2014; **11**: 99-102 [DOI: 10.3969/j.issn.1674-4985.2014.20.036]
48 **Huang Q**. [Efficacy of Qiming Granules combined with calcium dobesilate in the treatment of type 2 diabetic retinopathy]. *Zhongxiyi Jiehe Xinxueguan Dianzi Zazhi* 2017; **5**: 34-35 [DOI: 10.3969/j.issn.2095-6681.2017.30.023]
49 **Feng JL**, Zhou P, Wu QL, Chen YN, Tian J. [Observation on the effect of Qiming Granules combined with calcium dobesilate in the treatment of diabetic retinopathy in phase III]. *Hebei Yiyao* 2016; **38**: 3430-3433 [DOI: 10.3969/j.issn.1002-7386.2016.22.018]
50 **Ge AL**. [Clinical observation on the treatment of diabetic retinopathy with Qiming Granules combined with western medicine]. *Zhongguo Minjian Liaofa* 2018; **26**: 76-77 [DOI: 10.19621/j.cnki.11-3555/r.2018.1147]
51 **Yan JH**. [Effectiveness of Qiming Granules combined with calcium dobesilate in the treatment of non-proliferative diabetic retinopathy]. *Henan Yixue Yanjiu* 2020; **29**: 6477-6479 [DOI: 10.3969/j.issn.1004-437X.2020.34.055]
52 **Meng FC**, Yu GJ, Hou J. [Clinical efficacy of Qiming Granules for diabetic retinopathy]. *Zhongguo Shequ Yishi* 2016; **32**: 111,113 [DOI: 10.3969/j.issn.1007-614x.2016.13.67]
53 **Zhang Y**, Ma JY, Wang C. [Analysis of the effect of Qiming Granules in the treatment of non-proliferative diabetic retinopathy]. *Zhongguo Shiyong Yiyao* 2016; **11**: 151-152 [DOI: 10.14163/j.cnki.11-5547/r.2016.12.110]
54 **Zhang M**. [Analysis of the efficacy of Qiming Granules in the treatment of simple diabetic retinopathy]. *Zhongguo Yaowu Jingjixue* 2013; **S3**: 93-94
55 **Wu Z**, Dong N, Shang LX. [Clinical study on Qiming Granules combined with pancreatic kininogenase in treatment of type 2 diabetic

retinopathy]. *Xiandai Yaowu Linchuang* 2022; **37**: 795-799 [DOI: 10.7501/j.issn.1674-5515.2022.04.021]
56 **Zhou P**, Feng JL. [Analysis of the effect of Qiming Granules combined with calcium dobesilate in the treatment of non-proliferative diabetic retinopathy]. *Dongfang Shiliao Baojian* 2018; **1**: 108 [DOI: 10.3969/j.issn.1672-5018.2018.01.096]
57 **Wang SQ**, Lei Y, Zhang R. [Improvement of Qiming granule combined with calcium dobesilate on fundus microcirculation in the treatment of patients with non-proliferative diabetic retinopathy]. *Linchuang Yixue Yanjiu Shijian* 2019; **4**: 117-118 [DOI: 10.19347/j.cnki.2096-1413.201931049]
58 **Wang Z**. [Qiming Granules combined with calcium dobesilate in treatment of non-proliferative diabetic retinopathy]. *Guoji Yanke Zazhi* 2017; **17**: 4 [DOI: 10.3980/j.issn.1672-5123.2017.4.28]
59 **Yin XD**. [Efficacy and safety evaluation of the combination regimen of Qiming Granules and calcium dobesilate in the treatment of non-proliferative diabetic retinopathy]. *Yixue Lilun Shijian* 2018; **31**: 83-84 [DOI: 10.19381/j.issn.1001-7585.2018.01.045]
60 **Xin W**, Yang L, Cui S, Wang D, Guo YH, Yang ZX. [Effects of Qiming Granules combined with Epalrestat on fundus angiography, blood rheology and oxidative stress in patients with diabetic retinopathy]. *Xiandai Zhongxiyi Jiehe Zazhi* 2019; **28**: 2677-2680 [DOI: 10.3969/j.issn.1008-8849.2019.24.013]
61 **Zhang JT**. [Curative effect and safety analysis of calcium dobesilate combined with Qiming granules to treated with 78 cases diabetic retinopathy]. *Dangdai Yixue* 2017; **23**: 88-90 [DOI: 10.3969/j.issn.1009-4393.2017.15.037]
62 **Yue YJ**. [Clinical effect of Qiming Granules on treating non-proliferative diabetic retinopathy]. *Zhongguo Yiyao Zhinan* 2016; **14**: 8-9 [DOI: 10.15912/j.cnki.gocm.2016.09.006]
63 **Zheng JQ**, Huang JS, Liu ZF. [Efficacy of Qiming Granules in the treatment of diabetic retinopathy]. *Manxing Bingxue Zazhi* 2014; **15**: 657-658 [DOI: 10.16440/j.cnki.1674-8166.2014.08.007]
64 **Yang L**, Zhang GY, Quan XL, Jia N. [Efficacy of Qiming granules in 71 cases of non-proliferative diabetic retinopathy]. *Hangkong Hangtian Zazhi* 2013; **24**: 686-687 [DOI: 10.3969/j.issn.2095-1434.2013.06.027]
65 **Huang H**. [Analysis of the effect of Compound Danshen Dripping Pills in the adjuvant treatment of patients with diabetic retinopathy]. *Beifang Yaoxue* 2020; **17**: 100-101 [DOI: 10.3969/j.issn.1672-8351.2020.04.072]
66 **Zheng XH**, Ji YJ. [Effect of Compound Danshen Dripping Pills on the efficacy of diabetic retinopathy patients]. *Beifang Yaoxue* 2021; **18**: 41-42 [DOI: 10.3969/j.issn.1672-8351.2021.06.020]
67 **Meng XM**, Zhang SS, Duan YC. [Clinical observation on the treatment of diabetic retinopathy with Compound Danshen Dripping Pills combined with Calcium Dobesilate]. *Yixue Luntan Zazhi* 2011; **32**: 137-138
68 **Wang B**. [Clinical efficacy of Compound Danshen Dripping Pills in the treatment of diabetic retinopathy]. *Zhonghua Yixue Quanke Zazhi* 2004; **3**: 58-59
69 **Zhao CQ**. [Clinical study on the treatment of diabetic retinopathy with Compound Danshen Dripping Pills]. *Yixue Linchuang Yanjiu* 2019; **36**: 4-7 [DOI: 10.3969/j.issn.1671-7171.2019.01.002]
70 **Ma MJ**, Tian CG, Zhao ZG, Qin GJ, Zheng LL, Jiang HW. [The Curative Effect of Compound Danshen Dripping Pills Treatment Early Diabetic Retinopathy]. *Shijie Zhongyiyao* 2016; **11**: 450-453 [DOI: 10.3969/j.issn.1673-7202.2016.03.021]
71 **Chen Y**, Guo TS, Chen XW. [Clinical observation on the treatment of early diabetic retinopathy with Compound Danshen Dripping Pills]. *Shizhen Guoyi Guoyao* 2007; **18**: 2539-2540 [DOI: 10.3969/j.issn.1008-0805.2007.10.130]
72 **Xu HT**. [Observation on the effect of Compound Danshen Dripping Pills combined with Calcium Dobesilate in the treatment of diabetic retinopathy]. *Zhongguo Liaoyang Yixue* 2019; **28**: 884-886 [DOI: 10.13517/j.cnki.ccm.2019.08.041]
73 **Huang YX**, Sun HP, Tang YQ, Chen X. [Effects of Compound Danshen Dripping Pills on inflammatory mediators, cytokines and visual function in patients with diabetic retinopathy]. *Zhongxiyi Jiehe Xinnao Xueguanbing Zazhi* 2021; **19**: 1364-1366, 1408 [DOI: 10.12102/j.issn.1672-1349.2021.08.030]
74 **Jiao SF**. [Application of Calcium Dobesilate combined with Compound Danshen Dripping Pills in the treatment of diabetic retinopathy]. *Zhongwai Nvxing Jiankang Zazhi* 2018; **24**: 82-83 [DOI: 10.3969/j.issn.2096-0417.2018.24.056]
75 **Li Y**. [Clinical efficacy evaluation of the combination regimen of Compound Danshen Dripping Pills and Calcium Dobesilate in the treatment of diabetic retinopathy]. *Hangkong Hangtian Yixue Zazhi* 2017; **28**: 1229-1231 [DOI: 10.3969/j.issn.2095-1434.2017.10.037]
76 **Yan XQ**, Yuan MR. [Observation on the efficacy of applying Compound Danshen Dripping Pills in the treatment of 60 cases of senile simple diabetic retinopathy]. *Shizhen Guoyi Guoyao* 2014; **25**: 2187-2188 [DOI: 10.3969/j.issn.1008-0805.2014.09.058]
77 **Zhou LJ**. [Clinical efficacy of Compound Danshen Dripping Pills in the treatment of diabetic retinopathy]. *Zhongguo Yiyao Daobao* 2008; **5**: 77 [DOI: 10.3969/j.issn.1673-7210.2008.01.056]
78 **Miao CX**. [Clinical efficacy of Compound Danshen Dripping Pills with Calcium Dobesilate in the treatment of diabetic retinopathy]. *Dongfang Yaoshan* 2020; **3**: 87
79 **Ruan YX**, Chen M, Liu ZQ, Wang YL. , Sun N, Huang X, Gan H. [Clinical study on the treatment of retinopathy in diabetic patients with Compound Danshen Dripping Pills combined with Calcium Dobesilate]. *Zhongnan Yixue Kexue Zazhi* 2017; **45**: 18-20,23 [DOI: 10.15972/j.cnki.43-1509/r.2017.01.004]
80 **Yin L**, Zhang DL, Ren Q, Li L, Sun P, Sun RX, Yu H, Sun CH. [Observation on the effect of Compound Danshen Dripping Pills with Calcium Dobesilate in the treatment of diabetic retinopathy]. *Xiandai Zhongxiyi Jiehe Zazhi* 2013; **22**: 2449-2450 [DOI: 10.3969/j.issn.1008-8849.2013.22.023]
81 **Zhu J**. [Efficacy of Compound Danshen Dripping Pills in the treatment of early diabetic retinopathy]. *Jiankang Shiye* 2018; **10**: 99-100 [DOI: 10.3969/j.issn.1005-0019.2018.10.124]
82 **Yang PJ**, Li SM, Lv YP, Huang ZY, Huang H. [Effects of Compound Danshen Dripping Pills on vascular endothelial function in patients with early diabetic retinopathy]. *Zhongguo Shiyan Fangjixue Zazhi* 2013; **19**: 340-343 [DOI: 10.11653/syfj2013080340]
83 **Bai YX**. [Efficacy of Calcium Dobesilate combined with Compound Danshen Dripping Pills in the treatment of diabetic retinopathy and the effect on serum inflammatory factors]. *Qiqihar Yixueyuan Xuebao* 2017; **38**: 2641-2643 [DOI: 10.3969/j.issn.1002-1256.2017.22.014]
84 **Guo BD**. [Efficacy of Compound Danshen Dripping Pills in the treatment of early diabetic retinopathy]. *Linchuang Yixue Wenxian Dianzi Zazhi* 2015; **8**: 1418-1418, 1419 [DOI: 10.16281/j.cnki.jocml.2015.08.021]
85 **Liu J**. [Effect of Compound Danshen Dripping Pills on vascular endothelial function in patients with early diabetic retinopathy]. *Xinli Yisheng* 2018; **24**: 118-119
86 **Liu YZ**. [Clinical analysis of Hexue Mingmu Tablets + Pancreatic Kininogenase Enteric-coated Tablets in the treatment of diabetic retinopathy]. *Yixue Lilun Shijian* 2019; **32**: 3 [DOI: 10.19381/j.issn.1001-7585.2019.18.048]
87 **Zhao ZH**, Liu JX. [Clinical effects of Hexue Mingmu Tablets with Calcium Dobesilate in the treatment of early diabetic retinopathy].

Jiankang Nvxing 2021; **13**: 136

88 **Gao L**, Qi T, Xu WB, Wan L. [Clinical study on the treatment of early diabetic retinopathy with Hexue Mingmu Tablets combined with Calcium Dobesilate]. *Zhongguo Yaoye* 2020; **29**: 133-135 [DOI: 10.3969/j.issn.1006-4931.2020.10.040]

89 **Li M**. [Clinical observation on the treatment of early diabetic retinopathy with Hexue Mingmu Tablets combined with Calcium Dobesilate. *Jiankang Nvxing* 2021; **35**: 99

90 **Wang YA**, Wang WM, Kong FN. [Efficacy observation of Hexue Mingmu Tablets combined with Pancreatic Kininogenase Enteric-coated Tablets in the treatment of diabetic retinopathy]. *Xiandai Yaowu Linchuang* 2018; **33**: 2978-2981 [DOI: 10.7501/j.issn.1674-5515.2018.11.048]

91 **Zhang YR**, Wang WX. [Effect of Hexuemingmu Tablet on Diabetic Retinopathy]. *Zhongguo Yiyao Zhinan* 2018; **16**: 42-43 [DOI: 10.15912/j.cnki.gocm.2018.11.030]

92 **Ye XL**, Guo XN, Xiong F. [Clinical observation of oral calcium dobesilate combined with HeXue MingMu tablet in the treatment of nonproliferative diabetic retinopathy]. *Chuanbei Yixueyuan Xuebao* 2019; **34**: 223-225 [DOI: 10.3969/j.issn.1005-3697.2019.02.16]

93 **Yu MX**. [Clinical study on the treatment of diabetic retinopathy with Epalrestat Tablets in combination with Hexue Mingmu Tablets]. *Linchuang Yiyao Shijian* 2019; **28**: 573-576 [DOI: 10.16047/j.cnki.cn14-1300/r.2019.08.004]

94 **Du J**, Li SG, Lv YY. [Clinical observation on the treatment of diabetic retinopathy with Hexue Mingmu Tablets]. *Zhejiang Zhongxiyi Jiehe Zazhi* 2015; **25**: 1140-1141 [DOI: 10.3969/j.issn.1005-4561.2015.12.022]

95 **Song R**. [Efficacy of Mingmu Dihuang Pills in diabetic retinopathy]. *Zhongyi Linchuang Yanjiu* 2013; **11**: 36-37 [DOI: 10.3969/j.issn.1674-7860.2013.11.016]

96 **Li YP**. [Clinical study on the treatment of diabetic retinopathy with Mingmu Dihuang Pills combined with Mecobalamin Tablets]. *Xiandai Yaowu Linchuang* 2019; **34**: 3044-3049 [DOI: 10.7501/j.issn.1674-5515.2019.10.034]

97 **Li JB**, Wei XY. [Clinical study on Mingmu Dihuang Pills combined with calcium dobesilate in treatment of early diabetic retinopathy]. *Xiandai Yaowu Linchuang* 2019; **34**: 1197-1201 [DOI: 10.7501/j.issn.1674-5515.2019.04.068]

98 **Wu YF**. [Observation on the effect of Qiju Dihuang Pills in the adjuvant treatment of diabetic retinopathy]. *Zhongguo Xiangcun Yiyao* 2018; **25**: 22-23 [DOI: 10.19542/j.cnki.1006-5180.002078]

99 **Guan W**. [Analysis of the efficacy of Qiju Dihuang Pills combined with calcium hydroxybenzene sulfonate in the treatment of diabetic retinopathy]. *Zhongxiyi Jiehe Yanjiu* 2017; **9**: 90-92 [DOI: 10.3969/j.issn.1674-4616.2017.02.013]

100 **Ainu N**, Wang Y, Bu Q, Zhao Y. [Clinical efficacy of calcium dobesilate dispersible tablets combined with Mingmu Dihuang pills in treatment of NPDR]. *Guoji Yanke Zazhi* 2019; **19**: 992-996 [DOI: 10.3980/j.issn.1672-5123.2019.6.23]

101 **Fu ZJ**. [Safety analysis of Shuangdan Mingmu Capsules combined with Calcium Dobesilate in the treatment of diabetic retinopathy]. *Shijie Zuixin Yixue Xinxi Wenzhai* 2019; **19**: 205-206 [DOI: 10.19613/j.cnki.1671-3141.2019.102.129]

102 **Ji XD**, Liu WT. [Safety and efficacy of Shuangdan Mingmu capsule combined with calcium dobesilate dispersible tablets in the treatment of diabetic retinopathy]. *Linchuang Yixue Yanjiu Shijian* 2022; **7**: 85-87 [DOI: 10.19347/j.cnki.2096-1413.202211024]

103 **Jin L**, Zhang LJ. [Clinical study on Shuangdan Mingmu Capsules combined with calcium dobesilate in treatment of diabetic retinopathy]. *Xiandai Yaowu Linchuang* 2019; **34**: 159-163 [DOI: 10.7501/j.issn.1674-5515.2019.01.035]

104 **Liu JP**, Kong YH, Wang L. [Clinical Efficacy of Shuangdan Mingmu Capsules Combined with Calcium Dobesilate in the Treatment of Diabetic Retinopathy and its Effects on Serum Levels of Vascular Endothelial Growth Factor, Platelet-Derived Growth Factor and Interleukin-1]. *Zhongguo Yiyuan Yongyao Pingjia Fenxi* 2019; **19**: 1332-1334,1338 [DOI: 10.14009/j.issn.1672-2124.2019.11.015]

105 **Pang YH**. [Clinical observation on the treatment of diabetic retinopathy with Shuangdan Mingmu Capsules]. *Tangniaobing Xinshijie* 2015; **3**: 39-39 [DOI: 10.16658/j.cnki.1672-4062.2015.03.013]

106 **Deng C**, Li J, Tang XZ. [Effect of Danggui Buxue Decoction on the efficacy of diabetic retinopathy and the effect of serum ICAM-1 and ET-1 levels]. *Zhongyiyao Xinxi* 2018; **35**: 90-93 [DOI: 10.19656/j.cnki.1002-2406.180026]

107 **Wang D**, Chen SF. [Clinical Effect of Danggui Buxue Decoction Combined with Calcium Hydroxybenzenesulfonate on Diabetic Retinopathy]. *Zhongwai Yiliao* 2020; **39**: 162-164 [DOI: 10.16662/j.cnki.1674-0742.2020.02.162]

108 **Wu YL**. [Clinical Survey of Xi-shiyin in Treatment of Non-proliferative Diabetic Retinopathy]. *Fujian Zhongyiyao Daxue* 2013; **(401)**: 12-18 [DOI: 10.7666/d.Y2299119]

109 **Sun XH**, Qian KW, Zhou Y, Gu HY. [Study on the efficacy and mechanism of Danggui Buxue Decoction in the treatment of diabetic retinopathy]. *Xiandai Zhongxiyi Jiehe Zazhi* 2019; **28**: 3152-3155,3170 [DOI: 10.3969/j.issn.1008-8849.2019.28.019]

110 **Yu RC**. [Effects of the Danggui Buxue decoction on non-proliferative diabetic retinopathy and ICAM-1 and ET-1]. *Zhongwai Linchuang Yanjiu* 2020; **12**: 62-64 [DOI: 10.3969/j.issn.1674-7860.2020.17.021]

111 **Xu W**. [Study on the therapeutic effect of Chinese traditional Chinese medicine on the treatment of diabetic retinopathy]. *Yiyao Qianyan* 2018; **8**: 362-363

112 **Huang SC**. [Observation on the efficacy of Xuefu Zhuyu Decoction in the treatment of simple diabetic retinopathy]. *Shenzhen Zhongxiyi Jiehe Zazhi* 2017; **27**: 27-28 [DOI: 10.16458/j.cnki.1007-0893.2017.21.013]

113 **Zhu JX**. [Observation of Xuefu Zhuyu Decoction on Clinical Efficacy of Qi and Yin Deficiency and Blood Stasis type Diabetic Retinopathy Bleeding Period]. *Shandong Zhongyiyao Daxue* 2015; **401**: 10-12

114 **Xiong P**, Chen R. [Effects of Xuefu Zhuyu Decoction treatment on glucolipid metabolism, inflammatory factors and visual acuity levels in patients with non-proliferative diabetic retinopathy]. *Sichuan Zhongyi* 2019; **37**: 137-139

115 **Liu T**. [The effect of Xuefu Zhuyu Decoction treatment on the visual acuity level of patients with non-proliferative diabetic retinopathy]. *Jiankang Zhiyou* 2020; **3**: 262-263

116 **Gong MF**, Xiao QF, Huang XY. [Treatment of 40 cases of simple diabetic retinopathy with Xuefu Zhuyu Decoction]. *Liaoning Zhongyi Zazhi* 2014; **41**: 500-501 [DOI: 10.13192/j.issn.1000-1719.2014.03.049]

117 **Hao XF**. [Treatment of 66 cases of simple diabetic retinopathy with Xuefu Zhuyu Decoction]. *Henan Zhongyi* 2012; **32**: 916 [DOI: 10.16367/j.issn.1003-5028.2012.07.007]

118 **Huang YF**. [Combination of Chinese and Western medicine in the treatment of diabetic retinopathy in 70 cases. *Sichuan Zhongyi* 2004; **08**: 94 [DOI: 10.3969/j.issn.1000-3649.2004.08.064]

119 **Zhang FF**. [Synergistic Effect of Buyang Huanwu Decoction on Non-proliferative Diabetic Retinopathy]. *Fujian Zhongyiyao Daxue* 2019; **(401)**: 12-16

120 **Yang L**. [Clinical observation on the treatment of diabetic retinopathy by combining Buyang Huanwu Decoction with Calcium Dobesilate Tablets]. *Zhongguo Minjian Liaofa* 2019; **27**: 60-61 [DOI: 10.19621/j.cnki.11-3555/r.2019.1732]

121 **Tian L**. [Efficacy of the Yiqi Huoxue Therapy in the Adjunctive Treatment of Diabetic Retinopathy]. *Shenzhen Zhongxiyi Jiehe Zazhi* 2019; **29**: 30-31 [DOI: 10.16458/j.cnki.1007-0893.2019.13.014]

122 **Qu L**, Yao XY. [Efficacy of combined Chinese and Western medicine in the treatment of simple diabetic retinopathy]. *Henan Zhongyi* 2009; **29**: 1019-1020 [DOI: 10.16367/j.issn.1003-5028.2009.10.061]

123 **Li S**, Liu H, Zhu X. The effect of psychotherapy on anxiety, depression, and quality of life in patients with diabetic retinopathy: A protocol for systematic review and network meta-analysis. *Medicine (Baltimore)* 2021; **100**: e28386 [PMID: 34941170 DOI: 10.1097/MD.0000000000028386]

124 **Zhang B**, Zhou Z, Zhang B, Wang D. Efficacy and Safety of Various Treatments for Proliferative Diabetic Retinopathy: A Systematic Review and Network Meta-Analysis. *Front Pharmacol* 2021; **12**: 709501 [PMID: 34858166 DOI: 10.3389/fphar.2021.709501]

125 **Sun W**, Li J, Yan X, Liao L, Li S, Wang X, Xiao C, Shang M, Chao G, Zhou J. Traditional Chinese Medicine Injections for Diabetic Retinopathy: A Systematic Review and Network Meta-Analysis of Randomized Controlled Trials. *J Integr Complement Med* 2022; **28**: 927-939 [PMID: 35861710 DOI: 10.1089/jicm.2021.0392]

126 **Fallico M**, Maugeri A, Lotery A, Longo A, Bonfiglio V, Russo A, Avitabile T, Pulvirenti A, Furino C, Cennamo G, Barchitta M, Agodi A, Reibaldi M. Intravitreal anti-vascular endothelial growth factors, panretinal photocoagulation and combined treatment for proliferative diabetic retinopathy: a systematic review and network meta-analysis. *Acta Ophthalmol* 2021; **99**: e795-e805 [PMID: 33326183 DOI: 10.1111/aos.14681]

127 **Wang DY**, Zhao XY, Zhang WF, Meng LH, Chen YX. Perioperative anti-vascular endothelial growth factor agents treatment in patients undergoing vitrectomy for complicated proliferative diabetic retinopathy: a network meta-analysis. *Sci Rep* 2020; **10**: 18880 [PMID: 33144606 DOI: 10.1038/s41598-020-75896-8]

128 **Hooper P**, Boucher MC, Cruess A, Dawson KG, Delpero W, Greve M, Kozousek V, Lam WC, Maberley DAL. Excerpt from the Canadian Ophthalmological Society evidence-based clinical practice guidelines for the management of diabetic retinopathy. *Can J Ophthalmol* 2017; **52** Suppl 1: S45-S74 [PMID: 29074014 DOI: 10.1016/j.jcjo.2017.09.027]

129 **Diabetic Retinopathy Clinical Research Network**. A randomized trial comparing intravitreal triamcinolone acetonide and focal/grid photocoagulation for diabetic macular edema. *Ophthalmology* 2008; **115**: 1447-1449, 1449.e1 [PMID: 18662829 DOI: 10.1016/j.ophtha.2008.06.015]

130 **Sharma S**, Brown GC, Brown MM, Hollands H, Shah GK. The cost-effectiveness of grid laser photocoagulation for the treatment of diabetic macular edema: results of a patient-based cost-utility analysis. *Curr Opin Ophthalmol* 2000; **11**: 175-179 [PMID: 10977223 DOI: 10.1097/00055735-200006000-00004]

131 **An X**, Jin D, Duan L, Zhao S, Zhou R, Lian F, Tong X. Direct and indirect therapeutic effect of traditional Chinese medicine as an add-on for non-proliferative diabetic retinopathy: a systematic review and meta-analysis. *Chin Med* 2020; **15**: 99 [PMID: 32963587 DOI: 10.1186/s13020-020-00380-4]

132 **Wang H**, Jin C, Jiang Q. Price adjustment for traditional Chinese medicine procedures: Based on a standardized value parity model. *Biosci Trends* 2017; **11**: 524-532 [PMID: 29070761 DOI: 10.5582/bst.2017.01227]

133 **Guo ZL**, Li Y, Liu XW, Wu MY, Guo Q, Yao XC, Wang YD, Wu WY. Sodium Tanshinone IIA Silate Alleviates High Glucose Induced Barrier Impairment of Human Retinal Pigment Epithelium through the Reduction of NF-κB Activation *via* the AMPK/p300 Pathway. *Curr Eye Res* 2020; **45**: 177-183 [PMID: 31576759 DOI: 10.1080/02713683.2019.1668419]

134 **Fan K**, Li S, Liu G, Yuan H, Ma L, Lu P. Tanshinone IIA inhibits high glucoseinduced proliferation, migration and vascularization of human retinal endothelial cells. *Mol Med Rep* 2017; **16**: 9023-9028 [PMID: 29039498 DOI: 10.3892/mmr.2017.7743]

135 **Allison DJ**, Ditor DS. The common inflammatory etiology of depression and cognitive impairment: a therapeutic target. *J Neuroinflammation* 2014; **11**: 151 [PMID: 25178630 DOI: 10.1186/s12974-014-0151-1]

136 **Jeon SW**, Kim YK. Neuroinflammation and cytokine abnormality in major depression: Cause or consequence in that illness? *World J Psychiatry* 2016; **6**: 283-293 [PMID: 27679767 DOI: 10.5498/wjp.v6.i3.283]

137 **Nagata H**, Sai B, Tada F, Tonomura T, Ise C. [Anemia and milk secretion - with special reference to anemia in the postpartum period]. *Josanpu Zasshi* 1979; **33**: 98-101 [PMID: 254729 DOI: 10.1124/jpet.114.218750]

138 **Zheng X**, Liang Y, Kang A, Ma SJ, Xing L, Zhou YY, Dai C, Xie H, Xie L, Wang GJ, Hao HP. Peripheral immunomodulation with ginsenoside Rg1 ameliorates neuroinflammation-induced behavioral deficits in rats. *Neuroscience* 2014; **256**: 210-222 [PMID: 24161284 DOI: 10.1016/j.neuroscience.2013.10.023]

139 **Xie T**, Zhou XP, Lin LL, Xu JY, Shen CS, Feng Z, Zhou LL, Shan JJ. [Metabolomics analysis of Tripterygium wilfordii formulation based on theory of detoxicity compatibility]. *Zhongguo Zhong Yao Za Zhi* 2016; **41**: 1124-1129 [PMID: 28875681 DOI: 10.4268/cjcmm20160625]

140 **Zhang H**, Li Z, Zhou Z, Yang H, Zhong Z, Lou C. Antidepressant-like effects of ginsenosides: A comparison of ginsenoside Rb3 and its four deglycosylated derivatives, Rg3, Rh2, compound K, and 20(S)-protopanaxadiol in mice models of despair. *Pharmacol Biochem Behav* 2016; **140**: 17-26 [PMID: 26528894 DOI: 10.1016/j.pbb.2015.10.018]

141 **Pang HH**, Li MY, Wang Y, Tang MK, Ma CH, Huang JM. Effect of compatible herbs on the pharmacokinetics of effective components of Panax notoginseng in Fufang Xueshuantong Capsule. *J Zhejiang Univ Sci B* 2017; **18**: 343-352 [PMID: 28378572 DOI: 10.1631/jzus.B1600235]

142 **Tian Z**, Pang H, Du S, Lu Y, Zhang L, Wu H, Guo S, Wang M, Zhang Q. Effect of Panax notoginseng saponins on the pharmacokinetics of aspirin in rats. *J Chromatogr B Analyt Technol Biomed Life Sci* 2017; **1040**: 136-143 [PMID: 27978468 DOI: 10.1016/j.jchromb.2016.12.007]

143 **Zhao S**, Zheng MX, Chen HE, Wu CY, Wang WT. Effect of panax notoginseng saponins injection on the p38MAPK pathway in lung tissue in a rat model of hypoxic pulmonary hypertension. *Chin J Integr Med* 2015; **21**: 147-151 [PMID: 25523598 DOI: 10.1007/s11655-014-1790-2]

144 **Zhai Y**, Meng X, Luo Y, Wu Y, Ye T, Zhou P, Ding S, Wang M, Lu S, Zhu L, Sun G, Sun X. Notoginsenoside R1 ameliorates diabetic encephalopathy by activating the Nrf2 pathway and inhibiting NLRP3 inflammasome activation. *Oncotarget* 2018; **9**: 9344-9363 [PMID: 29507694 DOI: 10.18632/oncotarget.24295]

145 **Zhang TT**, Jiang JG. Active ingredients of traditional Chinese medicine in the treatment of diabetes and diabetic complications. *Expert Opin Investig Drugs* 2012; **21**: 1625-1642 [PMID: 22862558 DOI: 10.1517/13543784.2012.713937]

146 **Hou QL**, Wang Y, Li YB, Hu XL, Wang SL. [Protective effect of notoginsenoside R1 on neuron injury induced by OGD/R through ATF6/Akt signaling pathway]. *Zhongguo Zhong Yao Za Zhi* 2017; **42**: 1167-1174 [PMID: 29027434 DOI: 10.19540/j.cnki.cjcmm.20170121.014]

147 **Li W**, Ling S, Yang Y, Hu Z, Davies H, Fang M. Systematic hypothesis for post-stroke depression caused inflammation and neurotransmission and resultant on possible treatments. *Neuro Endocrinol Lett* 2014; **35**: 104-109 [PMID: 24878979]

148 **Yang X**, Yang S, Hong C, Yu W, Guonian W. Panax Notoginseng Saponins attenuates sevofluraneinduced nerve cell injury by modulating

AKT signaling pathway. *Mol Med Rep* 2017; **16**: 7829-7834 [PMID: 28944861 DOI: 10.3892/mmr.2017.7519]

149 **Wang T**, Guo R, Zhou G, Zhou X, Kou Z, Sui F, Li C, Tang L, Wang Z. Traditional uses, botany, phytochemistry, pharmacology and toxicology of Panax notoginseng (Burk.) F.H. Chen: A review. *J Ethnopharmacol* 2016; **188**: 234-258 [PMID: 27154405 DOI: 10.1016/j.jep.2016.05.005]

150 **Berge LI**, Riise T. Comorbidity between Type 2 Diabetes and Depression in the Adult Population: Directions of the Association and Its Possible Pathophysiological Mechanisms. *Int J Endocrinol* 2015; **2015**: 164760 [PMID: 26457080 DOI: 10.1155/2015/164760]

151 **Xie W**, Meng X, Zhai Y, Zhou P, Ye T, Wang Z, Sun G, Sun X. Panax Notoginseng Saponins: A Review of Its Mechanisms of Antidepressant or Anxiolytic Effects and Network Analysis on Phytochemistry and Pharmacology. *Molecules* 2018; **23** [PMID: 29673237 DOI: 10.3390/molecules23040940]

152 **Xie W**, Zhou P, Sun Y, Meng X, Dai Z, Sun G, Sun X. Protective Effects and Target Network Analysis of Ginsenoside Rg1 in Cerebral Ischemia and Reperfusion Injury: A Comprehensive Overview of Experimental Studies. *Cells* 2018; **7** [PMID: 30545139 DOI: 10.3390/cells7120270]

153 **Chen CF**, Chiou WF, Zhang JT. Comparison of the pharmacological effects of Panax ginseng and Panax quinquefolium. *Acta Pharmacol Sin* 2008; **29**: 1103-1108 [PMID: 18718179 DOI: 10.1111/j.1745-7254.2008.00868.x]

154 **Chen F**, Wen Q, Jiang J, Li HL, Tan YF, Li YH, Zeng NK. Could the gut microbiota reconcile the oral bioavailability conundrum of traditional herbs? *J Ethnopharmacol* 2016; **179**: 253-264 [PMID: 26723469 DOI: 10.1016/j.jep.2015.12.031]

155 **Li S**, Tang Y, Liu C, Zhang Y. Development of a method to screen and isolate potential α-glucosidase inhibitors from Panax japonicus C.A. Meyer by ultrafiltration, liquid chromatography, and counter-current chromatography. *J Sep Sci* 2015; **38**: 2014-2023 [PMID: 25847676 DOI: 10.1002/jssc.201500064]

156 **Li X**, Wu L, Fan X, Zhang B, Gao X, Wang Y, Cheng Y. [Network pharmacology study on major active compounds of Fufang Danshen formula]. *Zhongguo Zhong Yao Za Zhi* 2011; **36**: 2911-2915 [PMID: 22308672]

157 **Park MJ**, Bae CS, Lim SK, Kim DI, Lim JC, Kim JC, Han HJ, Moon JH, Kim KY, Yoon KC, Park SH. Effect of protopanaxadiol derivatives in high glucose-induced fibronectin expression in primary cultured rat mesangial cells: role of mitogen-activated protein kinases and Akt. *Arch Pharm Res* 2010; **33**: 151-157 [PMID: 20191356 DOI: 10.1007/s12272-010-2237-3]

158 **Tabandeh MR**, Jafari H, Hosseini SA, Hashemitabar M. Ginsenoside Rb1 stimulates adiponectin signaling in C2C12 muscle cells through up-regulation of AdipoR1 and AdipoR2 proteins. *Pharm Biol* 2015; **53**: 125-132 [PMID: 25311947 DOI: 10.3109/13880209.2014.912237]

159 **Wu Y**, Yu Y, Szabo A, Han M, Huang XF. Central inflammation and leptin resistance are attenuated by ginsenoside Rb1 treatment in obese mice fed a high-fat diet. *PLoS One* 2014; **9**: e92618 [PMID: 24675731 DOI: 10.1371/journal.pone.0092618]

160 **Xue B**, Sun L, Li X, Wang X, Zhang Y, Mu Y, Liang L. Ginsenoside Rb1 relieves glucose fluctuation-induced oxidative stress and apoptosis in Schwann cells. *Neural Regen Res* 2012; **7**: 2340-2346 [PMID: 25538758 DOI: 10.3969/j.issn.1673-5374.2012.30.003]

161 **Yu X**, Ye L, Zhang H, Zhao J, Wang G, Guo C, Shang W. Ginsenoside Rb1 ameliorates liver fat accumulation by upregulating perilipin expression in adipose tissue of db/db obese mice. *J Ginseng Res* 2015; **39**: 199-205 [PMID: 26199550 DOI: 10.1016/j.jgr.2014.11.004]

162 **Xue M**, Bian Y, Liu Y, Zhou J, Xu J, Zhang L, Huang X. Danggui Buxue decoction ameliorates lipid metabolic defects involved in the initiation of diabetic atherosclerosis; identification of active compounds. *J Tradit Chin Med* 2020; **40**: 414-421 [PMID: 32506855 DOI: 10.19852/j.cnki.jtcm.2020.03.009]

163 **Liu JH**, Li CJ, Li YT. The protective effects of Astragalus polysaccharide on diabetic rats with retinopathy. *Hebei Yike Daxue Xuebao* 2017; **38**: 797-800 [DOI: 10.3969/ji.ssn.1007-3205.2017.07.013]

164 **Yang B**, Ma G, Liu Y. Z-Ligustilide Ameliorates Diabetic Rat Retinal Dysfunction Through Anti-Apoptosis and an Antioxidation Pathway. *Med Sci Monit* 2020; **26**: e925087 [PMID: 33011733 DOI: 10.12659/MSM.925087]

165 **Yousof Ali M**, Jung HA, Choi JS. Anti-diabetic and anti-Alzheimer's disease activities of Angelica decursiva. *Arch Pharm Res* 2015; **38**: 2216-2227 [PMID: 26152875 DOI: 10.1007/s12272-015-0629-0]

166 **Zhai Y**, Xu J, Feng L, Liu Q, Yao W, Li H, Cao Y, Cheng F, Bao B, Zhang L. Broad range metabolomics coupled with network analysis for explaining possible mechanisms of Er-Zhi-Wan in treating liver-kidney Yin deficiency syndrome of Traditional Chinese medicine. *J Ethnopharmacol* 2019; **234**: 57-66 [PMID: 30690072 DOI: 10.1016/j.jep.2019.01.019]

167 **Jiang M**, Lu C, Zhang C, Yang J, Tan Y, Lu A, Chan K. Syndrome differentiation in modern research of traditional Chinese medicine. *J Ethnopharmacol* 2012; **140**: 634-642 [PMID: 22322251 DOI: 10.1016/j.jep.2012.01.033]

168 **Lee S**, Park J, Lee H, Kim K. Development and validation of Yin-Deficiency Questionnaire. *Am J Chin Med* 2007; **35**: 11-20 [PMID: 17265546 DOI: 10.1142/S0192415X07004576]

169 **Xu H**, Su ZR, Huang W, Choi RC, Zheng YZ, Lau DT, Dong TT, Wang ZT, Tsim KW. Er Zhi Wan, an ancient herbal decoction for woman menopausal syndrome, activates the estrogenic response in cultured MCF-7 cells: an evaluation of compatibility in defining the optimized preparation method. *J Ethnopharmacol* 2012; **143**: 109-115 [PMID: 22710293 DOI: 10.1016/j.jep.2012.06.009]

170 **Yao F**, Jiang X, Qiu L, Peng Z, Zheng W, Ding L, Xia X. Long-Term Oral Administration of Salidroside Alleviates Diabetic Retinopathy in db/db Mice. *Front Endocrinol (Lausanne)* 2022; **13**: 861452 [PMID: 35370972 DOI: 10.3389/fendo.2022.861452]

171 **Yang Y**, Zhou M, Liu H. Luteolin, an aryl hydrocarbon receptor antagonist, alleviates diabetic retinopathy by regulating the NLRP/NOX4 signalling pathway: Experimental and molecular docking study. *Physiol Int* 2021 [PMID: 34143751 DOI: 10.1556/2060.2021.00148]

172 **Zhou L**, Zhang T, Lu B, Yu Z, Mei X, Abulizi P, Ji L. Lonicerae Japonicae Flos attenuates diabetic retinopathy by inhibiting retinal angiogenesis. *J Ethnopharmacol* 2016; **189**: 117-125 [PMID: 27196298 DOI: 10.1016/j.jep.2016.05.039]

173 **Li R**, Chen L, Yao GM, Yan HL, Wang L. Effects of quercetin on diabetic retinopathy and its association with NLRP3 inflammasome and autophagy. *Int J Ophthalmol* 2021; **14**: 42-49 [PMID: 33469482 DOI: 10.18240/ijo.2021.01.06]

174 **Wang S**, Du S, Wang W, Zhang F. Therapeutic investigation of quercetin nanomedicine in a zebrafish model of diabetic retinopathy. *Biomed Pharmacother* 2020; **130**: 110573 [PMID: 32745912 DOI: 10.1016/j.biopha.2020.110573]

175 **Kim CS**, Kim J, Kim YS, Jo K, Lee YM, Jung DH, Lee IS, Kim JH, Kim JS. Improvement in Diabetic Retinopathy through Protection against Retinal Apoptosis in Spontaneously Diabetic Torii Rats Mediated by Ethanol Extract of Osteomeles schwerinae C.K. Schneid. *Nutrients* 2019; **11** [PMID: 30836664 DOI: 10.3390/nu11030546]

176 **Chen B**, He T, Xing Y, Cao T. Effects of quercetin on the expression of MCP-1, MMP-9 and VEGF in rats with diabetic retinopathy. *Exp Ther Med* 2017; **14**: 6022-6026 [PMID: 29285153 DOI: 10.3892/etm.2017.5275]

177 **Yuan M**, He Q, Long Z, Zhu X, Xiang W, Wu Y, Lin S. Exploring the Pharmacological Mechanism of Liuwei Dihuang Decoction for Diabetic Retinopathy: A Systematic Biological Strategy-Based Research. *Evid Based Complement Alternat Med* 2021; **2021**: 5544518 [PMID:

34394383 DOI: 10.1155/2021/5544518]

178 **Song MK**, Salam NK, Roufogalis BD, Huang TH. Lycium barbarum (Goji Berry) extracts and its taurine component inhibit PPAR-γ-dependent gene transcription in human retinal pigment epithelial cells: Possible implications for diabetic retinopathy treatment. *Biochem Pharmacol* 2011; **82**: 1209-1218 [PMID: 21820420 DOI: 10.1016/j.bcp.2011.07.089]

179 **Song MK**, Roufogalis BD, Huang TH. Reversal of the Caspase-Dependent Apoptotic Cytotoxicity Pathway by Taurine from Lycium barbarum (Goji Berry) in Human Retinal Pigment Epithelial Cells: Potential Benefit in Diabetic Retinopathy. *Evid Based Complement Alternat Med* 2012; **2012**: 323784 [PMID: 22567031 DOI: 10.1155/2012/323784]

180 **Li H**, Li B, Zheng Y. Exploring the Mechanism of Action Compound-Xueshuantong Capsule in Diabetic Retinopathy Treatment Based on Network Pharmacology. *Evid Based Complement Alternat Med* 2020; **2020**: 8467046 [PMID: 32963574 DOI: 10.1155/2020/8467046]

181 **Li Y**, Huang Y, Tu C. Systems-Pharmacology-Based Identification of Antitumor Necrosis Factor Effect in Mimeng Flower Decoction for the Treatment of Diabetic Retinopathy. *Evid Based Complement Alternat Med* 2019; **2019**: 5107103 [PMID: 31827551 DOI: 10.1155/2019/5107103]

182 **Rahmati M**, Gondin J, Malakoutinia F. Effects of Neuromuscular Electrical Stimulation on Quadriceps Muscle Strength and Mass in Healthy Young and Older Adults: A Scoping Review. *Phys Ther* 2021; **101** [PMID: 34106246 DOI: 10.1093/ptj/pzab144]

183 **Rahmati M**, Malakoutinia F. Aerobic, resistance and combined exercise training for patients with amyotrophic lateral sclerosis: a systematic review and meta-analysis. *Physiotherapy* 2021; **113**: 12-28 [PMID: 34555670 DOI: 10.1016/j.physio.2021.04.005]

184 **Colberg SR**, Sigal RJ, Fernhall B, Regensteiner JG, Blissmer BJ, Rubin RR, Chasan-Taber L, Albright AL, Braun B; American College of Sports Medicine; American Diabetes Association. Exercise and type 2 diabetes: the American College of Sports Medicine and the American Diabetes Association: joint position statement executive summary. *Diabetes Care* 2010; **33**: 2692-2696 [PMID: 21115771 DOI: 10.2337/dc10-1548]

Published by **Baishideng Publishing Group Inc**
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-3991568
E-mail: bpgoffice@wjgnet.com
Help Desk: https://www.f6publishing.com/helpdesk
https://www.wjgnet.com