ISSN 2308-3840 (online)

World Journal of *Meta-Analysis*

World J Meta-Anal 2019 July 31; 7(7): 339-372

Published by Baishideng Publishing Group Inc

WJMA

World Journal of Meta-Analysis

Contents

Irregular Volume 7 Number 7 July 31, 2019

Contents

World Journal of Meta-Analysis

Volume 7 Number 7 July 31, 2019

ABOUT COVER

Editorial Board of *World Journal of Meta-Analysis*, Edmundas Kadusevicius, PhD, Professor, Department of Clinical Pharmacology, Institute of Physiology and Pharmacology, Lithuanian University of Health Sciences, Kaunas LT-50009, Lithuania

AIMS AND SCOPE

The *World Journal of Meta-Analysis* (*WJMA*) is an online, open-access, single-blind peer-reviewed journal that is published by Baishideng Publishing Group. Accepting both solicited and unsolicited manuscripts, the *WJMA* mainly publishes high quality meta-analysis and systematic review articles to lead the development of various fields of clinical medicine. The type of articles published in the *WJMA* include the editorial, minireview, review, meta-analysis, meta-regression, systematic review, and evidence-based practice.

The primary aim of the *WJMA* is to provide scholars and readers from various fields of clinical medicine with a platform to publish high quality meta-analysis and systematic review articles and communicate their research findings online.

The *WJMA* mainly publishes research results and findings obtained through meta-analysis and systematic review in a wide range of areas, including medicine, pharmacy, *etc.*

INDEXING/ABSTRACTING

The *WJMA* is now abstracted and indexed in Emerging Sources Citation Index (Web of Science), China National Knowledge Infrastructure (CNKI), China Science and Technology Journal Database (CSTJ), and Superstar Journals Database

RESPONSIBLE EDITORS FOR THIS ISSUE

Responsible Electronic Editor: *Ji-Hong Liu*

Proofing Production Department Director: *Yun-Xiaojian Wu*

NAME OF JOURNAL
World Journal of Meta-Analysis

ISSN
ISSN 2308-3840 (online)

LAUNCH DATE
May 26, 2013

FREQUENCY
Irregular

EDITORS-IN-CHIEF
Giuseppe Biondi-Zoccai

EDITORIAL BOARD MEMBERS
https://www.wjgnet.com/2308-3840/editorialboard.htm

EDITORIAL OFFICE
Jin-Lei Wang, Director

PUBLICATION DATE
July 31, 2019

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 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 Meta-Analysis

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

World J Meta-Anal 2019 July 31; 7(7): 339-342

DOI: 10.13105/wjma.v7.i7.339

ISSN 2308-3840 (online)

EDITORIAL

Asymptomatic bacteriuria among hospitalized diabetic patients: Should they be treated?

Manuel J Ramos-Ramirez, Salim Surani

ORCID number: Manuel j Ramos-Ramirez (0000-0001-6693-8016); Salim Surani (0000-0001-7105-4266).

Author contributions: Ramos-Ramirez MJ and Surani S contributed to the writing of this manuscript; Final manuscript was approved by all authors.

Conflict-of-interest statement: The authors have declared no conflicts of interest.

Manuscript source: Invited Manuscript

Received: May 25, 2019
Peer-review started: June 10, 2019
First decision: July 26, 2019
Revised: July 26, 2019
Accepted: July 29, 2019
Article in press: July 29, 2019
Published online: July 31, 2019

P-Reviewer: Rakhshan V, Papazafeiropoulou A, Avtanski D
S-Editor: Tang JZ
L-Editor: A

Manuel J Ramos-Ramirez, Corpus Christi Medical Center, Corpus Christi, TX 78412, United States

Salim Surani, Medical Critical Care Services, Christus Spohn Hospitals-Corpus Christi, Corpus Christi, TX 78413, United States

Corresponding author: Salim Surani, BSc, FACC, FACP, FCCP, MD, Professor, Medical Critical Care Services, Christus Spohn Hospitals-Corpus Christi, 701 Ayers Street, Corpus Christi, TX 78404, United States. srsurani@hotmail.com
Telephone: +1-36-18857722
Fax: +1-36-18507563

Abstract

Diabetes Mellitus is a significant health care challenge in the United States. The Center for Disease Control and Prevention estimates approximately 9.4% of patients in the United States are afflicted by diabetes. The Infectious Disease Society of America asymptomatic bacteriuria in women as two consecutive clean-catch voided urine specimens with isolation of the same bacterial strain in counts ≥ 10^5 cfu/mL It is understood that diabetic patients tend to be at higher risk for infections than non-diabetics. Urinary tract infections (UTIs) tend to be the most common infection contracted by this population. UTIs are not only a significant cause of morbidity and mortality, they are also a significant financial burden. The data are conflicting, in regard to treating asymptomatic bacteriuria in diabetic patients to avoid hospital complications and ultimately decrease healthcare costs associated with these complications. However, clinicians continue to prescribe antibiotics empirically. Further randomized controlled study looking into the specific population as immunocompromised diabetic patients, patient with diabetic ketoacidosis and patient in intensive care unit needs to be undertaken.

Key words: Asymptomatic bacteriuria; Diabetes mellitus; Hospitalized diabetics; Urinary tract infection

Core tip: Urinary tract infections among diabetics can predispose patients to significant morbidity, mortality, and increased healthcare costs. Data remains controversial as it pertains to treatment of asymptomatic bacteriuria in hospitalized diabetics in reducing the risk of urinary tract infection, complications, and healthcare costs.

E-Editor: Liu JH

Citation: Ramos-Ramirez MJ, Surani S. Asymptomatic bacteriuria among hospitalized diabetic patients: Should they be treated? *World J Meta-Anal* 2019; 7(7): 339-342
URL: https://www.wjgnet.com/2308-3840/full/v7/i7/339.htm
DOI: https://dx.doi.org/10.13105/wjma.v7.i7.339

INTRODUCTION

It is known in the scientific community that diabetic patients are particularly susceptible to infections. Studies have suggested that diabetic patients are at four times higher risk of suffering from infections than non-diabetics[1]. Among infections in diabetics, urinary tract infections (UTIs) are the most common type of infection[2]. Prevalence of asymptomatic bacteriuria (ASB) is quite common among the diabetic population. The Infectious Disease Society of America (IDSA) ASB in women as two consecutive clean-catch voided urine specimens with isolation of the same bacterial strain in counts ≥ 10^5 cfu/mL in men IDSA recommends a single, clean-catch, voided urine specimen with one bacterial species isolated in a quantitative count of 10^5 cfu/mL to be defined as asymptomatic bacteriuria. For any asymptomatic patient, bacteriuria is defined as a single catheterized urine specimen with one bacterial species isolated in counts ≥ 10^5 cfu/mL[3].

In a cross-sectional study, the prevalence of urinary tract infection (UTI) in diabetic patients was 16%[2]. What's more important is that the prevalence of ASB in diabetic patients has been shown to be at four times higher than the general population, however, whether ASB is a common precursor to UTIs and should ASB be treated or not is still inconclusive[4-8].

STUDY ANALYSIS

The diabetic population as whole are at higher risk for suffering from complications of UTIs which include renal and peri-renal abscess, emphysematous pyelonephritis, emphysematous cystitis, fungal infections, xanthogronalumatous pyelonephritis, and renal papillary necrosis[2]. Among diabetics, women, were found to have a higher incidence of UTIs than their male counterparts[3]. Consequently, diabetic women are also at higher risk of suffering from increased morbidity and mortality from UTI[4]. A study suggested that diabetic women are as much as 6 times to 24 times more likely than non-diabetic women to be admitted for acute pyelonephritis[5]. Whereas, diabetic men are 3.4-17 times more likely than their nondiabetic counterparts to be admitted for the same condition[6-9].

UTIs are not only a significant cause of morbidity and mortality by elevating the risk of pyelonephritis, premature delivery, impaired renal function, and end-stage renal disease in patients, but also is a significant financial burden. The estimated annual cost of community-acquired UTI is significant, at approximately 1.6 billion USD/year and treatment of the same incurs in significant cost in the United States to about 1.6 billion dollars in 1995 with and about 25.5 billion USD over the course of 20 years[10].

Given the clinical burden and economic cost of UTI it raises the question if the data that we currently have can be directly translated from a community over to a hospital setting and if ASB, a potentially treatable cause of UTI should be taken into consideration among the hospitalized diabetic patient?

In a study performed on hospitalized patients from 1996-2003 the rate of ASB was 12.76% (117 out of 917) and 11.4% (296 out of 2596) respectively in diabetic and nondiabetic males. The rate of ASB was 14.97% (229 out of 1529) and 13.1% (679 out of 5175) in diabetic and nondiabetic females, respectively[17]. Furthermore, prospective study done in two tertiary care university affiliated teaching hospitals demonstrated an overall prevalence of ASB to be 7.9% (85 cases per 1072 women) and a higher likelihood to have occult upper UTIs in certain aboriginal diabetic populations with lower level of education and socioeconomic status (53% of aboriginals *vs* 20% of non-aboriginals, $P = 0.016$)[6,9]. Studies among diabetic populations have found that the length of time with diabetes rather than diabetic control, as interpreted by hemoglobin A1c was shown to have an increased risk for both ASB and UTIs, however studies failed to mention how many, if any at all, of these patients were diagnosed in inpatient *vs* outpatient setting[1,11]. In another study, conducted in the year 2000, risk factors for ASB for Type II Diabetic women included: Age,

macroalbuminuria, a lower BMI, and a UTI during the previous year. ASB in Type II Diabetic women was noted to be an independent risk factor for UTIs[12]. The same study failed to demonstrate ASB as a risk factor for UTI in Type I Diabetics[12]. Sexual activity has also consequently been associated with ASB[13]. However, none of these studies manage to conclude any significance within the hospitalized population nor do they answer the question should treatment of ASB be beneficial to diabetic patients, particularly women. Some contest that rather than being condition or findings, we should consider this to be a complication of longstanding diabetes, along with albuminuria, and peripheral neuropathy[12,14]. In a large multicenter prospective study with an 18-month follow up did not demonstrate any significant association with ASB and renal function decline[15]. A long-term prospective study confirmed no significant association with ASB and renal function impairment in diabetic women at 6 years[16].

Although, ASB does have some data that supports increased risk for symptomatic UTI, does this mean should we treat all ASB? Well, the question is more complicated than that. Should we treat depending on the pathogen? Several studies have demonstrated *E. Coli* to be the most commonly isolated bacteria in diabetic patients with ASB, this however is in keeping community acquired UTIs in non-diabetic patients as well[1]. Other studies in hospitalized patients demonstrate the contrary with more pathogenic organisms being isolated (*Klebsiella, Pseudomonas aeruginosa*) however no changes in antibiotic resistance were noted in comparison with non-diabetic patients[17,18]. Another emergency room based study demonstrated a correlation between diabetes and bacterial antibiotic resistance[19].

CONCLUSION

The question still stands; should ASB in diabetic patients be treated? Prospective randomized control trial done in 2002 comparing antimicrobial *vs* non antimicrobial therapy approach in diabetic women with ASB and followed for 36 mo, the study found no decrease in number of symptomatic episodes of hospitalizations during long term follow up and a high rate of recurrent bacteriuria after antibiotic treatment was given. The study went further to conclude no benefit to continued screening for and treatment of asymptomatic bacteriuria[20]. Current recommendations from the Infectious Disease Society of American Guidelines and United States Preventive Services Task Force recommend against routine screening of diabetic patients for asymptomatic bacteriuria[4,21].

In conclusion, However, to our knowledge quality data and multiple high-quality studies are lacking. The conclusions are limited due to most of these studies focusing on the female population thus educated decisions for management of male diabetic patients is largely unclear. Further studies are required to determine the certain subpopulations of diabetic patients that would benefit, if any, especially for patients in ICU, patients with DKA or patients with significant immunosuppression from routine treatment of ASB. Some studies however have suggested the use of prophylactic measures such as probiotics may be beneficial to avoiding UTIs and its possible complications[22]. However, significant gaps in knowledge exists among the hospitalized patients. The jury is still out!

REFERENCES

1 **Shah BR**, Hux JE. Quantifying the risk of infectious diseases for people with diabetes. *Diabetes Care* 2003; **26**: 510-513 [PMID: 12547890 DOI: 10.2337/diacare.26.2.510]

2 **Patterson JE**, Andriole VT. Bacterial Urinary Tract Infections in Diabetes. *Infect Dis Clin* 1997; **11**: 735-750 [DOI: 10.1016/S0891-5520(05)70383-4]

3 **Turan H**, Serefhanoglu K, Torun AN, Kulaksizoglu S, Kulaksizoglu M, Pamuk B, Arslan H. Frequency, risk factors, and responsible pathogenic microorganisms of asymptomatic bacteriuria in patients with type 2 diabetes mellitus. *Jpn J Infect Dis* 2008; **61**: 236-238 [PMID: 18503181]

4 **Nicolle LE**, Bradley S, Colgan R, Rice JC, Schaeffer A, Hooton TM; Infectious Diseases Society of America; American Society of Nephrology; American Geriatric Society. Infectious Diseases Society of America guidelines for the diagnosis and treatment of asymptomatic bacteriuria in adults. *Clin Infect Dis* 2005; **40**: 643-654 [PMID: 15714408 DOI: 10.1086/427507]

5 **Nicolle LE**. Asymptomatic bacteriuria in diabetic women. *Diabetes Care* 2000; **23**: 722-723 [PMID: 10840984 DOI: 10.2337/diacare.23.6.722]

6 **Nicolle LE**, Friesen D, Harding GK, Roos LL. Hospitalization for acute pyelonephritis in Manitoba, Canada, during the period from 1989 to 1992; impact of diabetes, pregnancy, and aboriginal origin. *Clin Infect Dis* 1996; **22**: 1051-1056 [PMID: 8783709 DOI: 10.1093/clinids/22.6.1051]

7 **Boroumand MA**, Sam L, Abbasi SH, Salarifar M, Kassaian E, Forghani S. Asymptomatic bacteriuria in type 2 Iranian diabetic women: a cross sectional study. *BMC Womens Health* 2006; **6**: 4 [PMID: 16504076 DOI: 10.1186/1472-6874-6-4]

8 **Zhanel GG**, Harding GK, Nicolle LE. Asymptomatic bacteriuria in patients with diabetes mellitus. *Rev Infect Dis* 1991; **13**: 150-154 [PMID: 2017615 DOI: 10.1093/clinids/12.5.150]

9 **Zhanel GG**, Nicolle LE, Harding GK. Prevalence of asymptomatic bacteriuria and associated host factors in women with diabetes mellitus. The Manitoba Diabetic Urinary Infection Study Group. *Clin Infect Dis* 1995; **21**: 316-322 [PMID: 8562738 DOI: 10.1093/clinids/21.2.316]

10 **Foxman B**. Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. *Am J Med* 2002; **113**: 5S-13S [PMID: 12113866 DOI: 10.1016/s0002-9343(02)01054-9]

11 **Boyko EJ**, Fihn SD, Scholes D, Abraham L, Monsey B. Risk of urinary tract infection and asymptomatic bacteriuria among diabetic and nondiabetic postmenopausal women. *Am J Epidemiol* 2005; **161**: 557-564 [PMID: 15746472 DOI: 10.1093/aje/kwi078]

12 **Geerlings SE**, Stolk RP, Camps MJ, Netten PM, Collet TJ, Hoepelman AI; Diabetes Women Asymptomatic Bacteriuria Utrecht Study Group. Risk factors for symptomatic urinary tract infection in women with diabetes. *Diabetes Care* 2000; **23**: 1737-1741 [PMID: 11128343 DOI: 10.2337/diacare.23.12.1737]

13 **Strom BL**, Collins M, West SL, Kreisberg J, Weller S. Sexual activity, contraceptive use, and other risk factors for symptomatic and asymptomatic bacteriuria. A case-control study. *Ann Intern Med* 1987; **107**: 816-823 [PMID: 3688674 DOI: 10.7326/0003-4819-107-6-816]

14 **Geerlings SE**, Stolk RP, Camps MJ, Netten PM, Hoekstra JB, Bouter KP, Bravenboer B, Collet JT, Jansz AR, Hoepelman AI. Asymptomatic bacteriuria may be considered a complication in women with diabetes. Diabetes Mellitus Women Asymptomatic Bacteriuria Utrecht Study Group. *Diabetes Care* 2000; **23**: 744-749 [PMID: 10840989 DOI: 10.2337/diacare.23.6.744]

15 **Geerlings SE**, Stolk RP, Camps MJ, Netten PM, Collet JT, Schneeberger PM, Hoepelman AI. Consequences of asymptomatic bacteriuria in women with diabetes mellitus. *Arch Intern Med* 2001; **161**: 1421-1427 [PMID: 11386891 DOI: 10.1001/archinte.161.11.1421]

16 **Meiland R**, Geerlings SE, Stolk RP, Netten PM, Schneeberger PM, Hoepelman AI. Asymptomatic bacteriuria in women with diabetes mellitus: effect on renal function after 6 years of follow-up. *Arch Intern Med* 2006; **166**: 2222-2227 [PMID: 17101940 DOI: 10.1001/archinte.166.20.2222]

17 **Bonadio M**, Costarelli S, Morelli G, Tartaglia T. The influence of diabetes mellitus on the spectrum of uropathogens and the antimicrobial resistance in elderly adult patients with urinary tract infection. *BMC Infect Dis* 2006; **6**: 54 [PMID: 16545130 DOI: 10.1186/1471-2334-6-54]

18 **Meiland R**, Geerlings SE, De Neeling AJ, Hoepelman AI. Diabetes mellitus in itself is not a risk factor for antibiotic resistance in Escherichia coli isolated from patients with bacteriuria. *Diabet Med* 2004; **21**: 1032-1034 [PMID: 15317610 DOI: 10.1111/j.1464-5491.2004.01169.x]

19 **Wright SW**, Wrenn KD, Haynes ML. Trimethoprim-sulfamethoxazole resistance among urinary coliform isolates. *J Gen Intern Med* 1999; **14**: 606-609 [PMID: 10571705 DOI: 10.1046/j.1525-1497.1999.10128.x]

20 **Harding GK**, Zhanel GG, Nicolle LE, Cheang M; Manitoba Diabetes Urinary Tract Infection Study Group. Antimicrobial treatment in diabetic women with asymptomatic bacteriuria. *N Engl J Med* 2002; **347**: 1576-1583 [PMID: 12432044 DOI: 10.1056/NEJMoa021042]

21 **Lin K**, Fajardo K; U. S. Preventive Services Task Force. Screening for asymptomatic bacteriuria in adults: evidence for the U.S. Preventive Services Task Force reaffirmation recommendation statement. *Ann Intern Med* 2008; **149**: W20-W24 [PMID: 18591632 DOI: 10.7326/0003-4819-149-1-200807010-00009-w1]

22 **Ng QX**, Peters C, Venkatanarayanan N, Goh YY, Ho CYX, Yeo WS. Use of Lactobacillus spp. to prevent recurrent urinary tract infections in females. *Med Hypotheses* 2018; **114**: 49-54 [PMID: 29602464 DOI: 10.1016/j.mehy.2018.03.001]

World Journal of Meta-Analysis

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

World J Meta-Anal 2019 July 31; 7(7): 343-345

DOI: 10.13105/wjma.v7.i7.343

ISSN 2308-3840 (online)

EDITORIAL

Artificial intelligence for endoscopy

Hiroyuki Imaeda, Yoshikazu Tsuzuki, Kazuya Miyaguchi, Keigo Ashitani, Hideki Ohgo, Hidetomo Nakamoto

ORCID number: Hiroyuki Imaeda (0000-0003-0898-6540); Yoshikazu Tsuzuki (0000-0003-3029-7469); Kazuya Miyaguchi (0000-0003-0701-3608); Keigo Ashitani (0000-0002-5043-892X); Hideki Ohgo (0000-0002-3916-2918); Hidetomo Nakamoto (0000-0003-3975-5067).

Author contributions: Imaeda H made the conception, designed the study and drafted of the article; Miyaguchi K, Ashitani K, Tsuzuki Y, Nakamoto H critical revised the article for important intellectual content; Imaeda H, Tsuzuki Y, Miyaguchi K, Ashitani K, Nakamoto H made the final approval of the article.

Conflict-of-interest statement: The authors have no conflict of interest to declare.

Manuscript source: Invited manuscript

Received: June 25, 2019
Peer-review started: June 25, 2019
First decision: July 20, 2019
Revised: July 27, 2019

Hiroyuki Imaeda, Yoshikazu Tsuzuki, Hideki Ohgo, Department of Gastroenterology, Saitama Medical University, Iruma-gun, Saitama 3500495, Japan

Kazuya Miyaguchi, Keigo Ashitani, Hidetomo Nakamoto, Department of General Internal Medicine, Saitama Medical University, Iruma-gun, Saitama 3500495, Japan

Corresponding author: Hiroyuki Imaeda, MD, Professor, Department of Gastroenterology, Saitama Medical University, Morohongo 38, Moroyama-machi, Iruma-gun, Saitama 3500495, Japan. imaedahi@yahoo.co.jp
Telephone: +81-49-2761667
Fax: +81-49-2761667

Abstract

In recent times, there has been progressive development in artificial intelligence (AI) following the introduction of deep learning in the medical field including gastroenterology and endoscopy. Most of the reported studies were based on retrospective data. Several prospective studies of real-time diagnosis of moving images using the AI system are expected to match the real clinical situation and to aid the endoscopists in the detection and diagnosis of neoplasms without missing any lesion. AI can read a large number of endoscopic images in a few minutes and make a diagnosis; therefore, it is expected to cover the lack of support for the screening esophagogastroduodenoscopy in the health check-up and a large number of capsule images, thereby freeing the endoscopists from this burden. AI can help make the diagnosis during the endoscopic procedure and thereby prevent an unnecessary biopsy for patients taking antithrombotic drugs. AI can also be useful for education and training in endoscopy. Trainees can learn to perform endoscopy and the detection and diagnosis of lesions by the support of AI. In the near future, real-time endoscopic diagnosis using AI is expected to lessen the burden of endoscopists, to enhance the quality level of endoscopists, to overcome the miss of lesions and to make optimal diagnosis.

Key words: Artificial Intelligence; Endoscopy; Gastric cancer; Colonic neoplasm

Core tip: Artificial intelligence (AI) has an increasing role in medical imaging in recent times. It has numerous benefits in the field of endoscopy. It aids in the accurate identification and diagnosis of lesions. AI also helps in reading and accurately interpreting large volumes of endoscopic images. It can play a role in the training of endoscopists as well.

Accepted: July 29, 2019
Article in press: July 29, 2019
Published online: July 31, 2019

P-Reviewer: De Quadros LG, Jun C, Skok P, Barreto SG
S-Editor: Dou Y
L-Editor: A
E-Editor: Liu JH

Citation: Imaeda H, Tsuzuki Y, Miyaguchi K, Ashitani K, Ohgo H, Nakamoto H. Artificial intelligence for endoscopy. *World J Meta-Anal* 2019; 7(7): 343-345
URL: https://www.wjgnet.com/2308-3840/full/v7/i7/343.htm
DOI: https://dx.doi.org/10.13105/wjma.v7.i7.343

ARTIFICIAL INTELLIGENCE FOR ENDOSCOPY

In recent times, there has been progressive development in artificial intelligence (AI) following the introduction of deep learning in various fields. As expected, AI has been introduced into the medical field, and many papers have reported on its use in different specialties in the medical field, including gastroenterology and endoscopy[1].

In the field of endoscopy, conventional endoscopy, magnifying endoscopy and endocytoscopy using white light images and optical digital images for gastrointestinal neoplasia, gastritis related to *Helicobacter pylori* infection, and ulcerative colitis have been reported. Most of the reported studies were based on retrospective data. In addition, the results from these retrospective studies have been based on good-quality still images and not on either low-quality images or moving images[2,3]. Real-time imaging involves moving images from different levels in the endoscopic fields and not still images. Therefore, the results from retrospective studies might not match the real images. Several prospective studies of real-time diagnosis using the AI system have also been reported[4]. The data from these studies are expected to match the real clinical situation and to be more beneficial. For more robust clinical verification, well-designed multicenter prospective studies with adequate inclusion/ exclusion criteria that represent the target population are needed[1]. Moreover, the efficiency and accuracy of AI increases as the amount of data increases: For example, the use of moving images. Based on the prospective studies, real-time detection and diagnosis of lesions during endoscopic procedure are expected to become feasible. At present there is high sensitivity for the detection of gastric cancers; however, the specificity is not so good[5], because most gastric cancers involve gastric inflammation as well, which makes the gastric mucosa red or white, irregular, and granular. Gastric inflammation causes erosion, ulcer, or polyp, and is relatively similar in appearance to the mucosa in gastric cancer. On the other hand, the diagnosis of colonic neoplasms using narrow band imaging (NBI), magnifying endoscopy with NBI, or endocytoscopy shows high sensitivity as well as specificity. Recently, EndoBRAIN software (Olympus Medical Systems, Japan) which is an AI system used in endocytoscopy for colonic neoplasms has become available in Japan. This allows the differential diagnosis of a colonic lesion and the confirmation of a colonic neoplasm to be made in a very short time. The endocytoscopy involves a special scope which magnifies the target lesion 520 times and enables observation at the cell level as with a microscope, whereas, EndoBRAIN software has not detected colonic polyps automatically yet.

Until now, it usually took huge time and effort for endoscopists to learn about the many gastrointestinal diseases and train in the endoscopic detection and diagnosis of gastric cancer or colonic neoplasm. Even when the endoscopists are experts, they might sometimes miss the detection and diagnosis of the neoplasms due to similar color of the lesions to the surrounding area, small size of the lesion, difficult location such as behind the folds or on the bending site, and lesions endoscopically observed in a moment among a lot of visual images. AI is expected to aid the endoscopists in the detection and diagnosis of neoplasms without missing any lesion.

AI also helps in the quick interpretation of endoscopic images taken in screening esophagogastroduodenoscopy (EGD) without waste of time. In Japan, screening EGD is conducted as part of the health check-up, and a double check of the screening EGD images is necessary. However, the number of endoscopists is not enough, especially in the local suburbs. Extensive time is required to interpret numerous endoscopic images, and the interpretation of endoscopic images is a burden for Japanese endoscopists. AI can read a large number of endoscopic images in a few minutes and make a diagnosis; therefore, it is expected to cover the lack of support for the screening EGD in the health check-up, thereby freeing the endoscopists from this burden. Moreover, there are numerous software that can easily detect lesions in capsule endoscopy. The interpretation of a large number of capsule images by the endoscopists takes a lot of time, and AI can free the endoscopists from the burden of capsule endoscopy image interpretation.

Lately, there has been a gradual increase in the number of patients taking antithrombotic drugs, and there is a hesitancy in performing a biopsy in these

patients. Although the guidelines for patients taking antithrombotic drugs is available not only in Western countries[6], but also in Eastern countries[7,8], and endoscopic biopsies are allowed in these patients, bleeding following a biopsy is sometimes observed. In addition, colonic polyps were sometimes resected and discard during colonoscopic procedure, therefore, it is important to decide the indication of resection and discard for colonic polyps. AI can help make the diagnosis in vivo during the endoscopic procedure and thereby prevent an unnecessary biopsy and resection, and careless discard.

AI can also be useful for education and training in endoscopy. Generally, instructors teach trainees on the various aspects of endoscopy. Even in the absence of an instructor in some hospitals, trainees can learn to perform endoscopy and the detection and diagnosis of lesions by the support of AI. However, if they always rely on AI for the diagnosis, the diagnostic acumen of the trainees does not improve. The trainees need to learn detection and diagnosis of lesions without the use of AI as well.

On the other hand, as deep learning algorithm is black-box, the machine-generated decision is sometimes hard to understand for endoscopists and it does not match the diagnosis by endoscopists. Therefore, the level of detection and diagnosis sometimes has to be checked periodically.

In the near future, real-time endoscopic diagnosis during endoscopic procedures with real moving images using AI is expected to become widespread in all endoscopic fields, to lessen the burden of endoscopists and to enhance the quality level of endoscopists. AI is desired to overcome the miss of lesions by endoscopy and to make endoscopic diagnosis comparable with optimal diagnostic accuracy by histopathological findings, and to reduce medical costs by avoiding pathological examination and unnecessary resection.

REFERENCES

1 **Yang YJ**, Bang CS. Application of artificial intelligence in gastroenterology. *World J Gastroenterol* 2019; **25**: 1666-1683 [PMID: 31011253 DOI: 10.3748/wjg.v25.i14.1666]

2 **Mori Y**, Kudo SE, Mohmed HEN, Misawa M, Ogata N, Itoh H, Oda M, Mori K. Artificial intelligence and upper gastrointestinal endoscopy: Current status and future perspective. *Dig Endosc* 2019; **31**: 378-388 [PMID: 30549317 DOI: 10.1111/den.13317]

3 **Kudo SE**, Mori Y, Misawa M, Takeda K, Kudo T, Itoh H, Oda M, Mori K. Artificial intelligence and colonoscopy: Current status and future perspectives. *Dig Endosc* 2019; **31**: 363-371 [PMID: 30624835 DOI: 10.1111/den.13340]

4 **Mori Y**, Kudo SE, Chiu PW, Singh R, Misawa M, Wakamura K, Kudo T, Hayashi T, Katagiri A, Miyachi H, Ishida F, Maeda Y, Inoue H, Nimura Y, Oda M, Mori K. Impact of an automated system for endocytoscopic diagnosis of small colorectal lesions: an international web-based study. *Endoscopy* 2016; **48**: 1110-1118 [PMID: 27494455 DOI: 10.1055/s-0042-113609]

5 **Hirasawa T**, Aoyama K, Tanimoto T, Ishihara S, Shichijo S, Ozawa T, Ohnishi T, Fujishiro M, Matsuo K, Fujisaki J, Tada T. Application of artificial intelligence using a convolutional neural network for detecting gastric cancer in endoscopic images. *Gastric Cancer* 2018; **21**: 653-660 [PMID: 29335825 DOI: 10.1007/s10120-018-0793-2]

6 **ASGE Standards of Practice Committee**. Acosta RD, Abraham NS, Chandrasekhara V, Chathadi KV, Early DS, Eloubeidi MA, Evans JA, Faulx AL, Fisher DA, Fonkalsrud L, Hwang JH, Khashab MA, Lightdale JR, Muthusamy VR, Pasha SF, Saltzman JR, Shaukat A, Shergill AK, Wang A, Cash BD, DeWitt JM. The management of antithrombotic agents for patients undergoing GI endoscopy. *Gastrointest Endosc* 2016; **83**: 3-16 [PMID: 26621548 DOI: 10.1016/j.gie.2015.09.035]

7 **Fujimoto K**, Fujishiro M, Kato M, Higuchi K, Iwakiri R, Sakamoto C, Uchiyama S, Kashiwagi A, Ogawa H, Murakami K, Mine T, Yoshino J, Kinoshita Y, Ichinose M, Matsui T; Japan Gastroenterological Endoscopy Society. Guidelines for gastroenterological endoscopy in patients undergoing antithrombotic treatment. *Dig Endosc* 2014; **26**: 1-14 [PMID: 24215155 DOI: 10.1111/den.12183]

8 **Kato M**, Uedo N, Hokimoto S, Ieko M, Higuchi K, Murakami K, Fujimoto K. Guidelines for Gastroenterological Endoscopy in Patients Undergoing Antithrombotic Treatment: 2017 Appendix on Anticoagulants Including Direct Oral Anticoagulants. *Dig Endosc* 2018; **30**: 433-440 [PMID: 29733468 DOI: 10.1111/den.13184]

World Journal of Meta-Analysis

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

World J Meta-Anal 2019 July 31; 7(7): 346-349

DOI: 10.13105/wjma.v7.i7.346

ISSN 2308-3840 (online)

OPINION REVIEW

Phantom of the inflammasome in the gut: Cytomegalovirus

Ferhat Arslan, Haluk Vahaboglu

ORCID number: Ferhat Arslan (0000-0004-6020-1488); Haluk Vahaboglu (0000-0001-8217-1767).

Author contributions: All authors equally contributed to this paper with conception and design of the study, literature review and analysis, drafting and critical revision and editing, and final approval of the final version.

Conflict-of-interest statement: No potential conflicts of interest. No financial support.

Manuscript source: Invited manuscript

Received: July 19, 2019
Peer-review started: July 21, 2019
First decision: July 23, 2019
Revised: July 25, 2019
Accepted: July 29, 2019
Article in press: July 29, 2019
Published online: July 31, 2019

P-Reviewer: Pellicano R, Perse M, Soldera J
S-Editor: Yan JP
L-Editor: A

Ferhat Arslan, Haluk Vahaboglu, Department of Infectious Diseases and Clinical Microbiology, Istanbul Medeniyet University, Istanbul 34722, Turkey

Corresponding author: Ferhat Arslan, MD, Associate Professor, Department of Infectious Diseases and Clinical Microbiology, Istanbul Medeniyet University, Istanbul 34722, Turkey.
ferhat.arslan@medeniyet.edu.tr
Telephone: +90-5055802245

Abstract

Cytomegalovirus (CMV) is frequently detected in inflammatory bowel tissue, especially in corticosteroid-refractory patients, and it has been blamed for adverse outcomes. However, the first acquisition of CMV does not involve the colon. In particular in the colonic mucosa, which evolved due to the gut microbial relationship, CMV promotes inflammation *via* recruited monocytes and not through replication in resident macrophages. Whether CMV is the last straw in the process of mucosal inflammation, a doomed agent, or an innocent bystander is a difficult question that remains elusive. With this work, we will try to review the relationship between intestinal mucosa and CMV in the framework of basic virological principles.

Key words: Cytomegalovirus; Ulcerative colitis; Gancyclovir; Inflammatory bowel disease

Core tip: We will here draw an analogy between the cytomegalovirus and the hero of the Gaston Leroux's "The Phantom of the Opera" novel, with the intestinal mucosa as the opera building. We aimed to emphasize the viral pathogenesis process to understand the elusive character of cytomegalovirus in the inflammatory bowel diseases.

Citation: Arslan F, Vahaboglu H. Phantom of the inflammasome in the gut: Cytomegalovirus. *World J Meta-Anal* 2019; 7(7): 346-349
URL: https://www.wjgnet.com/2308-3840/full/v7/i7/346.htm
DOI: https://dx.doi.org/10.13105/wjma.v7.i7.346

INTRODUCTION

E-Editor: Liu JH

Gaston Leroux's "The Phantom of the Opera" novel features a Paris opera building haunted by a phantom, an ugly genius of a man who had devoted his life to music and desperate love. The dungeons and tunnels that he created led to dynamic movements, enabling him to appear and disappear like a phantom all over the opera building. The protagonist may be interpreted as creative or destructive throughout the novel. He is a great composer and he makes clever devices, while strangling people with a Punjab lasso at the same time. What circumstances push the protagonist to lash out with violence and destruction? While that question is of interest to literary critics, we will here draw an analogy between the cytomegalovirus (CMV) and the hero of the aforementioned novel, with the intestinal mucosa as the opera building. For that, however, we must look at the basic viral pathogenesis before any deeper analysis.

VIRUSES

Viruses are nonliving particles, and their replication is entirely dependent on the ability to infect the cells of their hosts[1]. This obligate dependence should suggest that every conditional change must be of some interest to the host as well as the virus. The first step in viral infection is entry into the target cell *via* specific receptors that provide tropism and fusion to the cells. Glycoprotein complexes act as an entry and fusion activator[2]. The second step in viral infection is replication and the production of new virions to spread[3]. After this step, there is another important component to complete infection: The immune response. A nonspecific role is played by the host cell's intrinsic defenses: Apoptosis, autophagy, RNA silencing, and antiviral proteins, while pathogen-specific responses harness the innate and adaptive immunity process[4]. Intracellular detection of viral infection occurs *via* receptors (Toll-like receptors, RIG, MDA) on cellular compartments (cytoplasm, plasma, and endosomal membranes)[5-7]. Following recognition, virus-infected cells and uninfected sentinel cells (dendritic cells, macrophages, natural killer cells) produce interferons in response to cellular products. Cytokines, both proinflammatory and anti-inflammatory, and chemokines are complementary elements in the ongoing inflammation[8]. Encoding cytokine homologs to block receptors and soluble cytokine receptors to neutralize cytokines, or altering the cytokine signaling pathway, are the preferred targets in herpesvirus survival strategies[9]. This diversity in the virus-host interaction causes different inflammatory stimulations that differ in clinical presentation. In particular, non-cytopathic viruses do not stimulate inflammation and may persist over a long duration. However, encoding at least one regulator of intrinsic/innate defenses is an essential component of viral pathogenesis.

CYTOMEGALOVIRUS

CMV is a member of the β-herpesvirus subfamily. The virion consists of a 235-kb double-stranded linear DNA core in an icosahedral nucleocapsid, enveloped by a proteinaceous matrix. Nearly 200 of its genes encode proteins, but some express only noncoding RNAs, including approximately 14 microRNAs[10]. The genes with functions beyond transcription and proliferation necessitate a look at the human-CMV interaction from a co-evolutionary point of view. For instance, some virally encoded proteins show homology with the human chemokine receptor family[9]. Thus, this lifelong interaction may have a positive effect on our immunity, which can be revealed through animal studies[11].

Worldwide CMV seroprevalence has increased from 40% to 99%, and population-based studies have shown that young children are an important source of CMV for childbearing women[12,13]. A survey has also demonstrated that primary school-age children continue to shed CMV in urine and live viremia at higher rates when compared with older children[14]. Moreover, 18-30 year-old college students shed CMV in saliva and urine without antibody response[15]. Notably, neither children nor college students experienced any clinical conditions. In contrast, mother-to-child transmission can occur even in the womb or during birth, as well as through breastfeeding. The association between fetal infection or frailty and human-CMV interactions from the beginning to the end of life is being investigated by researchers[16].

In light of the latest data, platelet-derived growth factor receptor alpha (PDGFR-α) has been identified as an entry receptor that forms a heterotrimeric complex with gH/gL/gO in fibroblasts[17]. Additionally, while cell line-based in vitro studies show some proteins, such as neuropilin 2, act as epithelial/endothelial receptors, in real life most of these receptors are found inside the immune cells[18,19]. Fibroblasts, a type of

stromal cells, endogenously express PDGFR. In a previous report, perivascular stromal cells were found to be susceptible to CMV infection in an ulcerative colitis murine model *via* PDGFR-β and CXC chemokine ligand 12[20]. Additionally, in another investigation, more PDGFRα$^+$ cells (smooth muscle cells) were found in the distal than in the proximal colon, which may be related to the frequency of CMV colitis rather than cell involvement[21]. If the inflammasome affects stromal and not epithelial cells, it may be inferred that CMV participates in the ongoing process at least *via* its immunomodulatory effect. In contrast, it is interesting to think that the lifelong persistence of the virus and the protective and dormant structure of the epithelial/endothelial cells may interact in terms of the infectious process.

CMV persists (latency) over the host's lifetime in specific progenitor cells that undergo reprogramming from hemopoietic stem cells[22,23]. This latency is broken intermittently through viral reactivation that is controlled by the adaptive immunity[24]. Moreover, monocyte recruitment to the relevant locations is the main mechanism in clinical manifestations of CMV[25]. In particular in the colonic mucosa, which evolved due to the gut microbial relationship, CMV promotes inflammation *via* recruited monocytes and not through replication in resident macrophages[26]. Although monocyte recruitment is essential in the effective control and elimination of viral, bacterial, fungal, and protozoal infections, it is worth questioning whether the intruder, here CMV, can alter the infection dynamics on its own. As mentioned before, we postulate that CMV plays a role akin to "The Phantom of the Opera" in the mucosa, with a balance between creative and destructive behaviors. Like the Phantom, CMV has gained a bad reputation, especially where inflammatory diseases are concerned, whereas a viral genome study revealed a higher ebstein barr virus (EBV) load in mucosal samples[27]. Both the CMV and EBV encode a viral ortholog of cellular interleukin-10 that impedes inflammatory responses and modulates host immunity[28].

The rumors about the tortures inflicted by the Phantom show similarities with the existence of CMV and inflammations flaring in the mucosa. Be they a sign of direct or indirect pathogenicity, the first acquisition of CMV does not involve the colon. Whether CMV is the last straw in the process of mucosal inflammation, a doomed agent, or an innocent bystander is a difficult question that remains elusive. Thus, another important question follows on: To treat or not to treat?

CMV is frequently detected in inflammatory bowel tissue, especially in corticosteroid-refractory patients, and it has been blamed for adverse outcomes. Ganciclovir treatment is preferred by some clinicians, with or without other immunomodulatory drugs. Since clinical relevance and treatment efficacy have not been determined precisely, an accepted approach is not available. Many observational studies and a few metanalyses have been carried out on the effect that ganciclovir treatment has on CMV reactivation in inflammatory bowel diseases[29]. Unfortunately, these uncontrolled and selection bias studies have not delivered adequate conclusions. Colectomy rates show high variability in both ganciclovir-treated and untreated groups.

CONCLUSION

Researchers should focus the novel basic scientific data about the host and CMV interaction and re-review the clinical definitions, and treatment effectiveness of antivirals in the light of the evolutionary perspective.

REFERENCES

1 **Sun X**, Ilca SL, Huiskonen JT, Poranen MM. Dual Role of a Viral Polymerase in Viral Genome Replication and Particle Self-Assembly. *MBio* 2018; **9**: pii: e01242-18 [PMID: 30279282 DOI: 10.1128/mBio.01242-18]

2 **Sathiyamoorthy K**, Chen J, Longnecker R, Jardetzky TS. The COMPLEXity in herpesvirus entry. *Curr Opin Virol* 2017; **24**: 97-104 [PMID: 28538165 DOI: 10.1016/j.coviro.2017.04.006]

3 **Morrison KM**, Beucler MJ, Campbell EO, White MA, Boody RE, Wilson KC, Miller WE. Development of a Primary Human Cell Model for the Study of Human Cytomegalovirus Replication and Spread within Salivary Epithelium. *J Virol* 2019; **93**: pii: e01608-18 [PMID: 30404806 DOI: 10.1128/JVI.01608-18]

4 **Landolfo S**, De Andrea M, Dell'Oste V, Gugliesi F. Intrinsic host restriction factors of human cytomegalovirus replication and mechanisms of viral escape. *World J Virol* 2016; **5**: 87-96 [PMID: 27563536 DOI: 10.5501/wjv.v5.i3.87]

5 **Lee N**, Wong CK, Hui DS, Lee SK, Wong RY, Ngai KL, Chan MC, Chu YJ, Ho AW, Lui GC, Wong BC, Wong SH, Yip SP, Chan PK. Role of human Toll-like receptors in naturally occurring influenza A infections. *Influenza Other Respir Viruses* 2013; **7**: 666-675 [PMID: 23552014 DOI: 10.1111/irv.12109]

6 **Luangsay S**, Ait-Goughoulte M, Michelet M, Floriot O, Bonnin M, Gruffaz M, Rivoire M, Fletcher S, Javanbakht H, Lucifora J, Zoulim F, Durantel D. Expression and functionality of Toll- and RIG-like

receptors in HepaRG cells. *J Hepatol* 2015; **63**: 1077-1085 [PMID: 26144659 DOI: 10.1016/j.jhep.2015.06.022]
7 **Tengroth L**, Millrud CR, Kvarnhammar AM, Kumlien Georén S, Latif L, Cardell LO. Functional effects of Toll-like receptor (TLR)3, 7, 9, RIG-I and MDA-5 stimulation in nasal epithelial cells. *PLoS One* 2014; **9**: e98239 [PMID: 24886842 DOI: 10.1371/journal.pone.0098239]
8 **Chaaitanya IK**, Muruganandam N, Sundaram SG, Kawalekar O, Sugunan AP, Manimunda SP, Ghosal SR, Muthumani K, Vijayachari P. Role of proinflammatory cytokines and chemokines in chronic arthropathy in CHIKV infection. *Viral Immunol* 2011; **24**: 265-271 [PMID: 21830898 DOI: 10.1089/vim.2010.0123]
9 **Scarborough JA**, Paul JR, Spencer JV. Evolution of the ability to modulate host chemokine networks via gene duplication in human cytomegalovirus (HCMV). *Infect Genet Evol* 2017; **51**: 46-53 [PMID: 28315475 DOI: 10.1016/j.meegid.2017.03.013]
10 **Crough T**, Khanna R. Immunobiology of human cytomegalovirus: From bench to bedside. *Clin Microbiol Rev* 2009; **22**: 76-98, Table of Contents [PMID: 19136435 DOI: 10.1128/CMR.00034-08]
11 **Smithey MJ**, Venturi V, Davenport MP, Buntzman AS, Vincent BG, Frelinger JA, Nikolich-Žugich J. Lifelong CMV infection improves immune defense in old mice by broadening the mobilized TCR repertoire against third-party infection. *Proc Natl Acad Sci U S A* 2018; **115**: E6817-E6825 [PMID: 29967140 DOI: 10.1073/pnas.1719451115]
12 **Staras SA**, Flanders WD, Dollard SC, Pass RF, McGowan JE, Cannon MJ. Cytomegalovirus seroprevalence and childhood sources of infection: A population-based study among pre-adolescents in the United States. *J Clin Virol* 2008; **43**: 266-271 [PMID: 18778968 DOI: 10.1016/j.jcv.2008.07.012]
13 **Mussi-Pinhata MM**, Yamamoto AY, Aragon DC, Duarte G, Fowler KB, Boppana S, Britt WJ. Seroconversion for Cytomegalovirus Infection During Pregnancy and Fetal Infection in a Highly Seropositive Population: "The BraCHS Study". *J Infect Dis* 2018; **218**: 1200-1204 [PMID: 29868783 DOI: 10.1093/infdis/jiy321]
14 **Amin MM**, Bialek SR, Dollard SC, Wang C. Urinary Cytomegalovirus Shedding in the United States: The National Health and Nutrition Examination Surveys, 1999-2004. *Clin Infect Dis* 2018; **67**: 587-592 [PMID: 29471326 DOI: 10.1093/cid/ciy143]
15 **Huang Y**, Guo X, Song Q, Wang H, Yu H, Zhang Y, Qiao E, Xue W, Li X, Zhuang S, Wei F, Li T, Ge S, Wu T, Xia N, Zhang J. Cytomegalovirus Shedding in Healthy Seropositive Female College Students: A 6-Month Longitudinal Study. *J Infect Dis* 2018; **217**: 1069-1073 [PMID: 29294037 DOI: 10.1093/infdis/jix679]
16 **Araújo Carvalho AC**, Tavares Mendes ML, Santos VS, Tanajura DM, Prado Nunes MA, Martins-Filho PRS. Association between human herpes virus seropositivity and frailty in the elderly: A systematic review and meta-analysis. *Ageing Res Rev* 2018; **48**: 145-152 [PMID: 30391341 DOI: 10.1016/j.arr.2018.10.009]
17 **Wu K**, Oberstein A, Wang W, Shenk T. Role of PDGF receptor-α during human cytomegalovirus entry into fibroblasts. *Proc Natl Acad Sci U S A* 2018; **115**: E9889-E9898 [PMID: 30275317 DOI: 10.1073/pnas.1806305115]
18 **Martinez-Martin N**, Marcandalli J, Huang CS, Arthur CP, Perotti M, Foglierini M, Ho H, Dosey AM, Shriver S, Payandeh J, Leitner A, Lanzavecchia A, Perez L, Ciferri C. An Unbiased Screen for Human Cytomegalovirus Identifies Neuropilin-2 as a Central Viral Receptor. *Cell* 2018; **174**: 1158-1171.e19 [PMID: 30057110 DOI: 10.1016/j.cell.2018.06.028]
19 **Schellenburg S**, Schulz A, Poitz DM, Muders MH. Role of neuropilin-2 in the immune system. *Mol Immunol* 2017; **90**: 239-244 [PMID: 28843905 DOI: 10.1016/j.molimm.2017.08.010]
20 **Matsumura K**, Nakase H, Kosugi I, Honzawa Y, Yoshino T, Matsuura M, Kawasaki H, Arai Y, Iwashita T, Nagasawa T, Chiba T. Establishment of a novel mouse model of ulcerative colitis with concomitant cytomegalovirus infection: in vivo identification of cytomegalovirus persistent infected cells. *Inflamm Bowel Dis* 2013; **19**: 1951-1963 [PMID: 23839229 DOI: 10.1097/MIB.0b013e318293c5bf]
21 **Lu C**, Huang X, Lu HL, Liu SH, Zang JY, Li YJ, Chen J, Xu WX. Different distributions of interstitial cells of Cajal and platelet-derived growth factor receptor-α positive cells in colonic smooth muscle cell/interstitial cell of Cajal/platelet-derived growth factor receptor-α positive cell syncytium in mice. *World J Gastroenterol* 2018; **24**: 4989-5004 [PMID: 30510374 DOI: 10.3748/wjg.v24.i44.4989]
22 **Zhu D**, Pan C, Sheng J, Liang H, Bian Z, Liu Y, Trang P, Wu J, Liu F, Zhang CY, Zen K. Human cytomegalovirus reprogrammes haematopoietic progenitor cells into immunosuppressive monocytes to achieve latency. *Nat Microbiol* 2018; **3**: 503-513 [PMID: 29588542 DOI: 10.1038/s41564-018-0131-9]
23 **Krishna BA**, Poole EL, Jackson SE, Smit MJ, Wills MR, Sinclair JH. Latency-Associated Expression of Human Cytomegalovirus US28 Attenuates Cell Signaling Pathways To Maintain Latent Infection. *MBio* 2017; **8**: pii: e01754-17 [PMID: 29208743 DOI: 10.1128/mBio.01754-17]
24 **Goodrum F**. Human Cytomegalovirus Latency: Approaching the Gordian Knot. *Annu Rev Virol* 2016; **3**: 333-357 [PMID: 27501258 DOI: 10.1146/annurev-virology-110615-042422]
25 **Shi C**, Pamer EG. Monocyte recruitment during infection and inflammation. *Nat Rev Immunol* 2011; **11**: 762-774 [PMID: 21984070 DOI: 10.1038/nri3070]
26 **Dennis EA**, Smythies LE, Grabski R, Li M, Ballestas ME, Shimamura M, Sun JJ, Grams J, Stahl R, Niederweis ME, Britt WJ, Smith PD. Cytomegalovirus promotes intestinal macrophage-mediated mucosal inflammation through induction of Smad7. *Mucosal Immunol* 2018; **11**: 1694-1704 [PMID: 30076393 DOI: 10.1038/s41385-018-0041-4]
27 **Lopes S**, Andrade P, Conde S, Liberal R, Dias CC, Fernandes S, Pinheiro J, Simões JS, Carneiro F, Magro F, Macedo G. Looking into Enteric Virome in Patients with IBD: Defining Guilty or Innocence? *Inflamm Bowel Dis* 2017; **23**: 1278-1284 [PMID: 28617757 DOI: 10.1097/MIB.0000000000001167]
28 **Young VP**, Mariano MC, Tu CC, Allaire KM, Avdic S, Slobedman B, Spencer JV. Modulation of the Host Environment by Human Cytomegalovirus with Viral Interleukin 10 in Peripheral Blood. *J Infect Dis* 2017; **215**: 874-882 [PMID: 28453840 DOI: 10.1093/infdis/jix043]
29 **Pillet S**, Pozzetto B, Roblin X. Cytomegalovirus and ulcerative colitis: Place of antiviral therapy. *World J Gastroenterol* 2016; **22**: 2030-2045 [PMID: 26877608 DOI: 10.3748/wjg.v22.i6.2030]

World Journal of Meta-Analysis

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

World J Meta-Anal 2019 July 31; 7(7): 350-357

DOI: 10.13105/wjma.v7.i7.350

ISSN 2308-3840 (online)

SYSTEMATIC REVIEWS

Pediatric recurrent *Clostridium difficile* infections in immunocompetent children: Lessons learned from case reports of the first twelve consecutive patients

Angela Chu, Sonia Michail

ORCID number: Angela Chu (0000-0003-3873-0565); Sonia Michail (0000-0002-0528-7202).

Author contributions: Chu A and Michail S contributed equally to this work. Chu A wrote most of the manuscript, Michail S conceptualized idea and supervised work.

Conflict-of-interest statement: All the authors declare that they have no competing interests.

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.

Manuscript source: Unsolicited Manuscript

Received: April 22, 2019
Peer-review started: April 22, 2019

Angela Chu, Sonia Michail, Department of Pediatrics, Miller Children's and Women's Hospital, Long Beach, CA 90826, United States

Angela Chu, Sonia Michail, Department of Pediatrics, UC Irvine School of Medicine, Irvine, CA 92612, United States

Sonia Michail, Pediatric Gastroenterology, Hepatology and Nutrition Center, Miller Children's and Women's Hospital, Long Beach, CA 90826, United States

Sonia Michail, University of Southern California, Los Angeles, CA 92708, United States

Corresponding author: Sonia Michail, MD, Department of Pediatrics, 2801 Atlantic Ave, Long Beach CA 90806 United States. sonia.michail@hotmail.com
Telephone: +1-7143787230

Abstract

BACKGROUND

Recurrent *Clostridium difficile* infection (CDI) in children can be difficult to manage and may represent an unidentified underlying pathology. Recurrence can be frequently encountered in immunodeficiency disorders and inflammatory bowel disease (IBD).

AIM

To report cases of a select population of children with recurrent CDI who are immunocompetent and do not have an identified IBD and examine the potential for any underlying risk factors, disease course and disease outcome.

METHODS

Review of charts for children aged 1-21 years with recurrent CDI referred to see pediatric gastroenterology service was performed. All subjects with known immunosuppression or IBD were excluded. Subjects were followed for at least 24 mo.

RESULTS

Twelve children seen consecutively were identified. All patients were treated with antibiotic courses for CDI prior to their referral. Five out of 12 patients had an underlying pathology that was not previously identified, including eosinophilic colitis and IBD. CDI symptoms resolved after treatment of underlying colitis without the need to target therapy for CDI. There were 9

First decision: June 10, 2019
Revised: June 13, 2019
Accepted: July 26, 2019
Article in press: July 26, 2019
Published online: July 31, 2019

P-Reviewer: Matowicka-Karna J, Trifan A
S-Editor: Dou Y
L-Editor: A
E-Editor: Liu JH

patients that failed antibiotic treatment of CDI and required fecal microbiome transplant, which was safe and highly effective in preventing recurrence (100% efficacy). The gut microbial changes after fecal transplant were characterized by a remarkable and durable increase in diversity and in abundance of *Bacteroides*.

CONCLUSION
Pediatric patients with frequent recurrence of CDI may have an unidentified underlying gastrointestinal pathology that may warrant further investigation by a specialist who can identify these diseases and help optimize management. Many of these children may benefit from fecal microbial transplant which appears to be a safe, highly effective therapy that results in long term changes in the gut microbiome.

Key words: Recurrent *Clostridium difficile* infection; Eosinophilic colitis; Inflammatory bowel disease; Fecal microbiome transplant

Core tip: Children with recurrent *Clostridium difficile* infection who do not have known immunodeficiency or inflammatory bowel disease deserve a thorough workup as many may have an underlying gastrointestinal disease.

Citation: Chu A, Michail S. Pediatric recurrent *Clostridium difficile* infections in immunocompetent children: Lessons learned from case reports of the first twelve consecutive patients. *World J Meta-Anal* 2019; 7(7): 350-357
URL: https://www.wjgnet.com/2308-3840/full/v7/i7/350.htm
DOI: https://dx.doi.org/10.13105/wjma.v7.i7.350

INTRODUCTION

The incidence of *Clostridium difficile* infections (CDI) in both adults and pediatrics is increasing[1-5]. CDI can result in a spectrum of disorders that ranges from carrier and asymptomatic state to causing significant morbidity and even mortality[6]. Infants frequently test positive but are asymptomatic[7]. Part of the rise in CDI could be from increasing testing among infants, which needs to be done with caution given the high prevalence of asymptomatic colonization in young infants[7]. There is also a higher incidence of colonization and colitis with *C. difficile* in pediatric inflammatory bowel disease (IBD) compared to adult IBD as well as patients with celiac disease[8].

There have been multiple studies showing correlation between certain risk factors predisposing to the development of CDI. Risk factors such as acid suppressing agents, especially H2 receptor antagonists, exposure to antibiotics and immunosuppressants, comorbidities such as cancer, cystic fibrosis and IBD, and hospitalization have been known to increase the incidence of CDI for some time[1,9]. These studies are charged with the task of understanding the risk for developing the infection in general, however, there is a paucity of studies that describe a select population of children that have *recurrence* of this infection. While community acquired CDI is more common in pediatrics than adults, recurrent CDI is not common in children[10]. A study by Kociolek in 2015[11] showed an association between recurrent CDI and malignancy and IBD. The study identified thirty children with recurrent infection and demonstrated that the majority of these subjects (19 subjects or 63%) have malignancy, underwent solid organ transplant or have IBD.

In this study, we aimed to understand CDI in a very unique population of children who are not immunocompromised and do not have any identified IBD. This study describes important discoveries of unidentified underlying gastrointestinal conditions which may not be recognized unless the child is adequately evaluated by a specialist in the field. The study also describes the success, and the durable gut microbial changes after fecal microbial transplant in this population. These discoveries contribute to the successful outcome in management of these subjects by identifying and addressing the underlying disease.

MATERIALS AND METHODS

Institutional Review Board (IRB) approval was obtained to study pediatric patients with recurrent CDI, defined as two or more distinct episodes of CDI associated with diarrhea or bloody diarrhea who were referred for evaluation to pediatric gastroenterology service. Subjects younger than one year and older than twenty-one years of age were excluded. All subjects with known immunosuppression or IBD prior to referral were excluded. Subjects had been followed up for at least one year.

Stool microbiome methods

The 16S bacterial DNA region from stool DNA and negative controls were amplified by PCR using a shared forward primer 806rB (CAAGCAGAAGACGGCATACGAGATAGTCAGCCAGCCGGACTACNVGGGTWTCTAAT) for all samples, while each sample had its own unique identifying reverse primer, which were modified from the original 515F-806R primer pairs. All samples were pooled and sequenced using custom sequencing primers; R1 (TATGGTAATTGTGTGYCAGCMGCCGCGGTAA), R2 (AGTCAGCCAGCCGGACTACNVGGGTWTCTAAT) and Index (AATGATACGGCGACCACCGAGATCTACACGCT). Paired-end sequencing (2 × 150bp) using Illumina MiSeq Reagent Kit v2 flowcell was performed on an Illumina MiSeq System.

Reads were de-multiplexed using QIIME v1.9.1. Statistical analyses were performed using the "phyloseq" (v1.20.0) package in the R statistical environment.

RESULTS

Twelve consecutive children were identified that fit the criteria described above. Children averaged 7.5 years of age (range 1-17 years). All children were treated with at least one course of metronidazole and one course of enteral vancomycin prior to referral. Nine children were exposed to antibiotic therapy prior to their first CDI. Three children had multiple antibiotic courses including amoxicillin. The most common single antibiotic course prior to CDI was amoxicillin as well. Three children did not receive antimicrobials prior to their first CDI. Two of the three children who did not receive antibiotics prior to their first CDI, were found to have an underlying gastrointestinal disease. The identification of the underlying disease changed the management of these patients. Five of the 12 children were previously healthy. The remaining children had different co-morbidities as described in Table 1 without a known history of colitis or immunodeficiency prior to referral. There were 9 patients that failed antibiotic treatment of CDI and required fecal microbiome transplant (FMT), which ultimately relieved CDI symptoms. Of these nine patients, 4 had a gastrostomy or gastrojejunostomy tube (Table 1), seven had history of antibiotic use, and 3 had history of acid suppressants.

After a thorough gastrointestinal workup, two patients were found to have eosinophilic disease, one subject had eosinophilic colitis and another subject had eosinophilic esophagitis. The child with eosinophilic colitis was placed exclusively on crystalline amino acid formula which resulted in resolution and prevention of any further CDI even after future exposure to antimicrobial therapy. One patient was found to have IBD proctitis, and CDI resolved after treatment of IBD. There were three subjects diagnosed with lactase deficiency.

One of the children treated with FMT, experienced a change in disease phenotype from *C. difficile* colitis that required hospitalization for bloody diarrhea with endoscopic confirmation of *C. difficile* colitis, to an asymptomatic *C. difficile* colonizer for 12 mo, followed by loss of colonization. No further CDI treatment was required despite the use of antimicrobial therapy for respiratory infection after FMT. From the FMT safety perspective, one subject developed transient fever for one day but was otherwise asymptomatic. Another subject developed bloating on the day of FMT. No serious adverse events were seen related to FMT.

Gut microbial profiles were examined before and after fecal transplant and compared to the donor profile. Children with recurrent CDI had very low abundance of *Bacteroidaceae* (Figure 1) prior to fecal transplant as well as low diversity of microorganisms compared to healthy donor (1.3 = 0.2 *vs* 3.2 + 0.4, Shannon diversity index, $P = 0.031$). After fecal transplant, the fecal microbial profile diversity improved. This phenomenon seemed to be durable for the twelve months following fecal transplant (Figure 2). Similarly, *Bacteroidaceae* became quite abundant after fecal transplant and this effect was seen over twelve months (Figure 1).

Table 1 Patient demographics and final diagnosis

Age of onset (yr)	Gender	# of CDIs	Medications	Co-morbidities	Devices	Prior hosp	Final diagnosis
8.6	F	> 5	PPI, EES, multiple antibiotics course including amoxicillin	DD, BPD	GT	Yes	Eosinophilic colitis
1.17	M	> 5	History of ranitidine, multiple antibiotic courses including amoxicillin	36 wk prematurity, SGA, GERD, cleft lip	GJT	Yes	
7	M	> 5	Erythromycin	CP, multiple orthopedic surgeries	GT	Yes	Lactase deficiency
10	F	3	Amoxicillin-clavulanate	None	None	No	
3	F	> 5	PPI, multiple antibiotics courses including amoxicillin	DD, renal disease, recurrent pneumonia	GT	Yes	
9	M	3	None	ASD	none	No	IBD proctitis
17	M	4	PPI, clindamycin	CP, DD	GT	Yes	
12	M	> 5	None	None	None	No	Eosinophilic esophagitis and lactase deficiency
4	F	4	Amoxicillin-clavulanate	History of UTI, hydronephrosis	None	Yes	
2	M	4	Amoxicillin	None	None	No	Lactase deficiency
2	M	3	None	None	None	No	
14	F	3	Cephalosporin	None	None	Yes	

Hosp: Hospitalization; PPI: Proton pump inhibitor; EES: Erythromycin ethylsuccinate; DD: Developmental delay; BPD: Bronchopulmonary dysplasia; SGA: Small for gestational age; GERD: Gastroesophageal reflux disease; CP: Cerebral palsy; ASD: Atrial septal defect; UTI: Urinary tract infection; GT: Gastrostomy tube; GJT: Gastrojejunostomy tube.

DISCUSSION

In adults, a recent meta-analysis showed age > 65 years, additional antibiotic use during follow-up, use of proton-pump inhibitors (PPIs), and renal insufficiency were most frequently associated with recurrent CDI[12]. There have been a few pediatric studies that describe risk factors for CDI in pediatric patients as well[1,9,11,13]. Underlying chronic medical condition, recent antibiotic use (specifically cephalosporins as described by Crews *et al*[13]), acid-suppressing agents, gastro-intestinal feeding device, and past or prolonged hospitalization increase the risk of developing CDI in pediatrics[1,9,13]. There is a paucity of data in the literature that focuses on recurrent infections. Kociolek *et al*[11] described a cohort of children who have recurrent CDI and found that the majority have malignancy or solid organ transplant, or IBD (n = 19, or 63%). Although there have been studies implicating IBD and immunosuppression increasing children's susceptibility to CDI[4,8], to our knowledge, no studies have directly linked recurrent CDI to undiagnosed underlying gastrointestinal disease.

With regards to antibiotic use in children who developed recurrent CDI, most of the children in our study received amoxicillin therapy, in contrast to the study by Crews *et al*[13] that showed more exposure to cephalosporins. Most children (two thirds), who did not receive antimicrobials prior to their first CDI were found to have an underlying gastrointestinal disease which was only identified when a work up was performed after referral to specialist. The authors recognize the limitation of the findings due to the small overall number of subjects in this sub-population.

Perhaps over one third of infants younger than 12 mo are colonized with *C. difficile*[14]. The rate of colonization then drops to 15% between ages 1-8 years and then 5% after age 8 years, similar to the rate in adults[14]. Due to the high rate of colonization in infants, patients under 12 mo of age were excluded from this study.

Five of the twelve children in our cohort had a gastrostomy or a jejunostomy

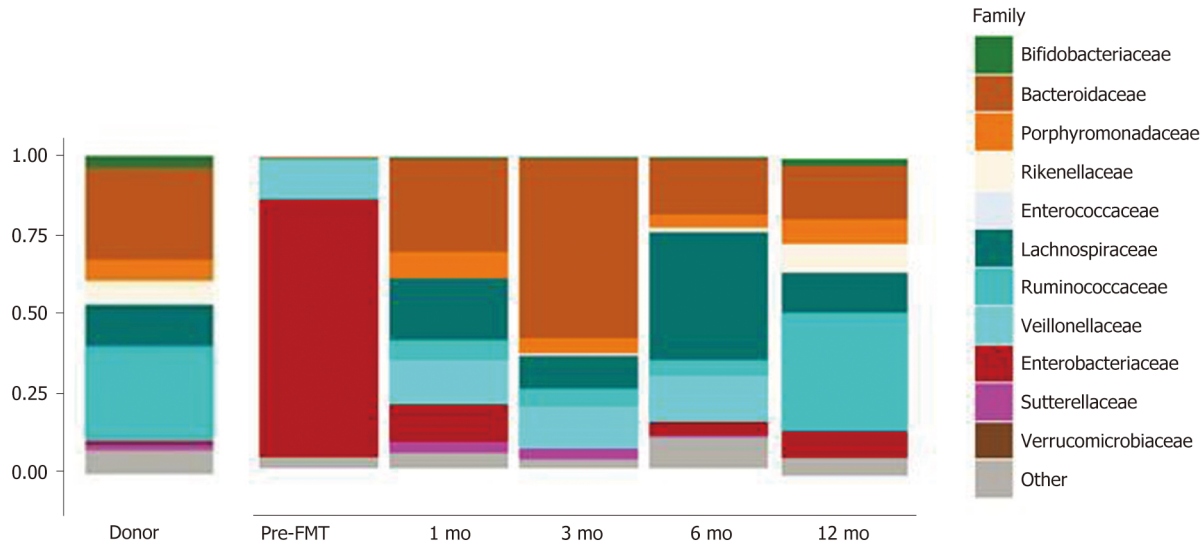


Figure 1 Bar plots depicting the percent abundance of gut microbial communities before and after fecal transplant compared to donor stools.

feeding tube, which are known to be associated with an increased risk of acquiring *C. difficile*, in adults and children[14-16]. This is likely due to spore contamination of equipment or formula, or use of formula that promotes *C. difficile* growth in the gut[14,15,17]. Most of the children in our study have been hospitalized in the past, which again would expose them to an environment that could harbor *C. difficile* spores and increasing their risk of acquiring *C. difficile*[14,18].

While there are many medical conditions known to predispose pediatric patients to CDI, such as hematopoietic stem cell transplant, IBD, cancer, fungal infections, and human immunodeficiency virus infection[14], those co-morbidities are diagnosed prior to the onset of CDI. In our study, 5 out of 12 patients had underlying pathology that was not previously identified. There have been many single study reports of other medical conditions that are associated with CDI[14], such as cystic fibrosis[19], Hirschsprung's[20], and Henoch-Schonlein purpura[21]. In our study, two patients had eosinophilic disease, which has not been described in prior studies as an association or risk factor for CDI. The discovery and treatment of an underlying colitis, namely eosinophilic colitis and IBD proctitis, resulted in prompt resolution of the recurrence of CDI.

Three of the twelve subjects were diagnosed with lactase deficiency by disaccharidase assay. Since there is overlap in symptoms with CDI and lactase deficiency, namely diarrhea and abdominal pain, the discovery and treatment of lactase deficiency allowed optimizing management and more appropriate assignment of symptoms to the correct underlying disease. However, as expected, management of lactase deficiency did not result in resolution of CDI recurrence. FMT in both subjects resulted in prompt resolution of symptoms.

All the subjects receiving FMT had resolution of symptoms for at least one year. One subject became an asymptomatic colonizer of *C. difficile* after FMT. The colonization was seen for 12 mo followed by resolution of colonization.

FMT in this patient population appeared to be highly effective and safe. Fecal transplant resulted in improved gut microbial diversity and abundance of *Bacteroides*, which appeared to be durable and seen to persist for at least twelve months. The overall numbers are small and more research will be necessary to confirm these observations.

In this subset population, it is recommended that children with recurrent CDI who do not have immunodeficiency or identified IBD be evaluated by a provider who can investigate the presence of an underlying gastrointestinal disease. In about one third of these subjects, a gastrointestinal disorder may be discovered that can impact the management of recurrent infection.

In conclusion, there are likely risk factors that are still unknown that can predispose to CDI. Pediatric patients that have more than one episode of CDI recurrence have an increased likelihood of underlying gastrointestinal pathology especially if there has been no prior use of antimicrobials and should be investigated so that proper treatment can be offered. Fecal microbial transplant is a highly effective and safe therapy for these children and results in durable changes in the gut microbiome.

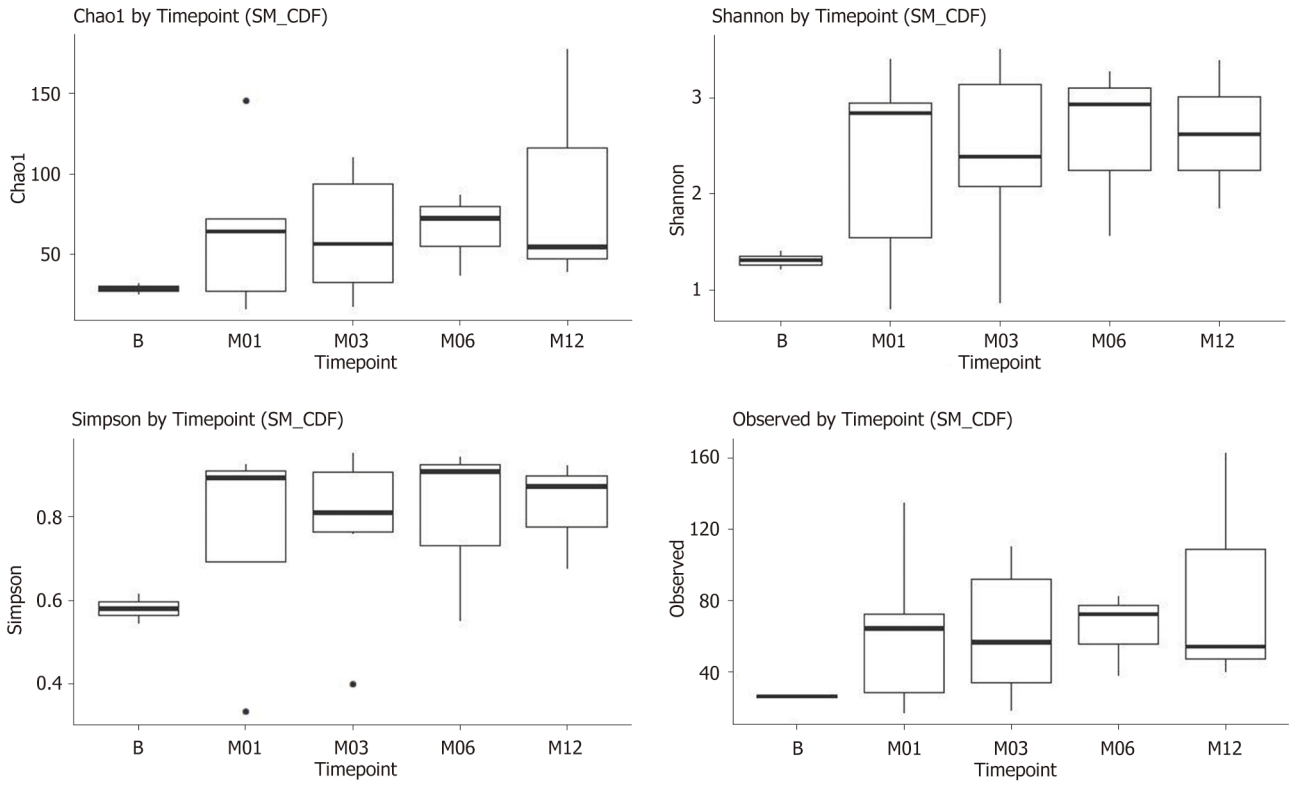


Figure 2 Diversity index at baseline and 1, 3, 6 and 12 mo after fecal transplant, showing consistent increase in diversity compared to baseline.

ARTICLE HIGHLIGHTS

Research background

Childhood recurrent *Clostridium difficile* infections (CDI) may be difficult to control and may represent an unknown underlying pathology. Recurrence often occurs in immunodeficiency disorders and inflammatory bowel disease (IBD).

Research motivation

There have been multiple studies showing correlation between certain risk factors predisposing to the development of CDI. Risk factors such as acid suppressing agents, especially H2 receptor antagonists, exposure to antibiotics and immunosuppressants, comorbidities such as cancer, cystic fibrosis and IBD, and hospitalization have been known to increase the incidence of CDI for some time. These studies are charged with the task of understanding the risk for developing the infection in general, however, there is a paucity of studies that describe a select population of children that have *recurrence* of this infection. While community acquired CDI is more common in pediatrics than adults, recurrent CDI is not common in children.

Research objectives

The main objectives of this report are understanding CDI in a very unique population of children who are not immunocompromised and do not have any identified IBD. This study describes important discoveries of unidentified underlying gastrointestinal conditions which may not be recognized unless the child is adequately evaluated by a specialist in the field. The study also describes the success, and the durable gut microbial changes after fecal microbial transplant in this population. These discoveries contribute to the successful outcome in management of these subjects by identifying and addressing the underlying disease.

Research methods

Pediatric patients with recurrent CDI, defined as two or more distinct episodes of CDI associated with diarrhea or bloody diarrhea who were referred for evaluation to pediatric gastro-enterology service were identified. Subjects younger than one year and older than twenty-one years of age were excluded. All subjects with known immunosuppression or IBD prior to referral were excluded. Subjects had been followed up for at least one year.

Research results

We have observed 12 children in succession. All patients received CDI antibiotics prior to referral. Five of the 12 patients had previously undiscovered potential pathologies, including eosinophilic colitis and IBD. After the treatment of basal colitis, the symptoms of CDI disappear and there is no need for CDI treatment. Nine patients required fecal microbial transplantation for antibiotic CDI failure, which is safe and effective (100% efficacy) for preventing recurrence.

Intestinal microbial changes following fecal transplantation are characterized by a significant and sustained increase in diversity and the abundance of *Bacteroides*.

Research conclusions

Children with recurrent CDI deserve a through gastrointestinal workup as they may frequently have an underlying disease which can contribute to the management of the condition. When medical therapy fails in this population, fecal microbial transplant is a safe and durable therapy. Children with recurrent CDI may have unidentified gastrointestinal disease contributing to the recurrence of the infection. Children with recurrent clostridium difficile frequently have an unidentified gastrointestinal disorder, which when identified and addressed, can help with management of clostridium difficile recurrence.

Research perspectives

Children with recurrent CDI need a thorough gastrointestinal workup to optimize their care and management. Future research should focus on individualized medicine and targeting underlying disease on a case by case basis.

REFERENCES

1 **Brown KE**, Knoderer CA, Nichols KR, Crumby AS. Acid-Suppressing Agents and Risk for Clostridium difficile Infection in Pediatric Patients. *Clin Pediatr (Phila)* 2015; **54**: 1102-1106 [PMID: 25644650 DOI: 10.1177/0009922815569201]

2 **Deshpande A**, Pant C, Anderson MP, Donskey CJ, Sferra TJ. Clostridium difficile infection in the hospitalized pediatric population: increasing trend in disease incidence. *Pediatr Infect Dis J* 2013; **32**: 1138-1140 [PMID: 23546535 DOI: 10.1097/INF.0b013e3182948518]

3 **Pant C**, Deshpande A, Gilroy R, Olyaee M, Donskey CJ. Rising Incidence of Clostridium difficile Related Discharges among Hospitalized Children in the United States. *Infect Control Hosp Epidemiol* 2016; **37**: 104-106 [PMID: 26424193 DOI: 10.1017/ice.2015.234]

4 **Nylund CM**, Goudie A, Garza JM, Fairbrother G, Cohen MB. Clostridium difficile infection in hospitalized children in the United States. *Arch Pediatr Adolesc Med* 2011; **165**: 451-457 [PMID: 21199971 DOI: 10.1001/archpediatrics.2010.282]

5 **Khanna S**, Baddour LM, Huskins WC, Kammer PP, Faubion WA, Zinsmeister AR, Harmsen WS, Pardi DS. The epidemiology of Clostridium difficile infection in children: a population-based study. *Clin Infect Dis* 2013; **56**: 1401-1406 [PMID: 23408679 DOI: 10.1093/cid/cit075]

6 **Davidovics ZH**, Michail S, Nicholson MR, Kociolek LK, Pai N, Hansen R, Schwerd T, Maspons A, Shamir R, Szajewska H, Thapar N, de Meij T, Mosca A, Vandenplas Y, Kahn SA, Kellermayer R; and the FMT Special Interest Group of the North American Society of Pediatric Gastroenterology Hepatology, Nutrition, the European Society for Pediatric Gastroenterology Hepatology, Nutrition. Fecal Microbiota Transplantation for Recurrent Clostridium difficile Infection and Other Conditions in Children: A Joint Position Paper From the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition and the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition. *J Pediatr Gastroenterol Nutr* 2019; **68**: 130-143 [PMID: 30540704 DOI: 10.1097/MPG.0000000000002205]

7 **Sammons JS**, Toltzis P. Pitfalls in Diagnosis of Pediatric Clostridium difficile Infection. *Infect Dis Clin North Am* 2015; **29**: 465-476 [PMID: 26188603 DOI: 10.1016/j.idc.2015.05.010]

8 **Hourigan SK**, Chirumamilla SR, Ross T, Golub JE, Rabizadeh S, Saeed SA, Elson CO, Kelly CP, Carroll KC, Oliva-Hemker M, Sears C. Clostridium difficile carriage and serum antitoxin responses in children with inflammatory bowel disease. *Inflamm Bowel Dis* 2013; **19**: 2744-2752 [PMID: 24145927 DOI: 10.1097/01.MIB.0000435434.53871.36]

9 **Samady W**, Pong A, Fisher E. Risk factors for the development of Clostridium difficile infection in hospitalized children. *Curr Opin Pediatr* 2014; **26**: 568-572 [PMID: 25032717 DOI: 10.1097/MOP.0000000000000126]

10 **McFarland LV**, Ozen M, Dinleyici EC, Goh S. Comparison of pediatric and adult antibiotic-associated diarrhea and Clostridium difficile infections. *World J Gastroenterol* 2016; **22**: 3078-3104 [PMID: 27003987 DOI: 10.3748/wjg.v22.i11.3078]

11 **Kociolek LK**, Palac HL, Patel SJ, Shulman ST, Gerding DN. Risk Factors for Recurrent Clostridium difficile Infection in Children: A Nested Case-Control Study. *J Pediatr* 2015; **167**: 384-389 [PMID: 26001313 DOI: 10.1016/j.jpeds.2015.04.052]

12 **Deshpande A**, Pasupuleti V, Thota P, Pant C, Rolston DD, Hernandez AV, Donskey CJ, Fraser TG. Risk factors for recurrent Clostridium difficile infection: a systematic review and meta-analysis. *Infect Control Hosp Epidemiol* 2015; **36**: 452-460 [PMID: 25626326 DOI: 10.1017/ice.2014.88]

13 **Crews JD**, Anderson LR, Waller DK, Swartz MD, DuPont HL, Starke JR. Risk Factors for Community-associated Clostridium difficile-associated Diarrhea in Children. *Pediatr Infect Dis J* 2015; **34**: 919-923 [PMID: 26164847 DOI: 10.1097/INF.0000000000000767]

14 **Pant C**, Deshpande A, Altaf MA, Minocha A, Sferra TJ. Clostridium difficile infection in children: a comprehensive review. *Curr Med Res Opin* 2013; **29**: 967-984 [PMID: 23659563 DOI: 10.1185/03007995.2013.803058]

15 **Bliss DZ**, Johnson S, Savik K, Clabots CR, Willard K, Gerding DN. Acquisition of Clostridium difficile and Clostridium difficile-associated diarrhea in hospitalized patients receiving tube feeding. *Ann Intern Med* 1998; **129**: 1012-1019 [PMID: 9867755 DOI: 10.7326/0003-4819-129-12-199812150-00004]

16 **Yokohama S**, Aoshima M, Asama T, Shindo J, Maruyama J. Clostridium difficile-associated enteric disease after percutaneous endoscopic gastrostomy. *J Gastroenterol* 2009; **44**: 121-125 [PMID: 19214673 DOI: 10.1007/s00535-008-2283-5]

17 **O'Keefe SJ**. Tube feeding, the microbiota, and Clostridium difficile infection. *World J Gastroenterol* 2010; **16**: 139-142 [PMID: 20066732 DOI: 10.3748/wjg.v16.i2.139]

18 **Morinville V**, McDonald J. Clostridium difficile-associated diarrhea in 200 Canadian children. *Can J Gastroenterol* 2005; **19**: 497-501 [PMID: 16107901 DOI: 10.1155/2005/326296]

19 **Pohl JF**, Patel R, Zobell JT, Lin E, Korgenski EK, Crowell K, Mackay MW, Richman A, Larsen C, Chatfield BA. Clostridium difficile Infection and Proton Pump Inhibitor Use in Hospitalized Pediatric

Cystic Fibrosis Patients. *Gastroenterol Res Pract* 2011; **2011**: 345012 [PMID: 22144994 DOI: 10.1155/2011/345012]

20 **Thomas DF**, Fernie DS, Bayston R, Spitz L, Nixon HH. Enterocolitis in Hirschsprung's disease: a controlled study of the etiologic role of Clostridium difficile. *J Pediatr Surg* 1986; **21**: 22-25 [PMID: 3511213 DOI: 10.1016/s0022-3468(86)80644-3]

21 **Boey CC**, Ramanujam TM, Looi LM. Clostridium difficile-related necrotizing pseudomembranous enteritis in association with Henoch-Schonlein purpura. *J Pediatr Gastroenterol Nutr* 1997; **24**: 426-429 [PMID: 9144126 DOI: 10.1097/00005176-199704000-00012]

World Journal of Meta-Analysis

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

World J Meta-Anal 2019 July 31; 7(7): 358-372

DOI: 10.13105/wjma.v7.i7.358

ISSN 2308-3840 (online)

META-ANALYSIS

Effect of dl-3-n-butylphthalide on infarction volume in animal models of ischemic stroke: A meta-analysis

Di Luan, Zheng-Yu Wu, Yuan-Xiang Zhang, Li-Li Yuan, Yang Xu, Zhao-Hu Chu, Ling-Song Ma, Ya-Ping Wang, Shou-Cai Zhao

ORCID number: Di Luan (0000-0002-8832-7555); Zheng-Yu Wu (0000-0002-0416-0760); Yuan-Xiang Zhang (0000-0002-0317-9435); Lili Yuan (0000-0002-5497-6338); Yang Xu (0000-0001-8778-3885); Zhao-Hu Chu (0000-0002-8103-5533); Ling-Song Ma (0000-0001-9035-0159); Ya-Ping Wang (0000-0002-1753-0863); Shou-Cai Zhao (0000-0001-8555-4835).

Author contributions: Luan D, Wu ZY, Zhang YX, Yuan LL, and Zhao SC conceived and designed the study; study searching was done by Luan D and Wu ZY. Further, studies screening and selection was done by Luan D and Wu ZY independently and disagreement was resolved by Zhao SC, Xu Y, Chu ZH, and Ma LS. Luan D and Wu ZY performed data extraction independently. Wang YP did analysis in supervision of Zhao SC. All authors were involved in interpretation of the results. The final draft of this manuscript was done by Luan D and read and approved by all the authors.

Supported by the National Natural Science Foundation of China, NO. 81701161.

Conflict-of-interest statement: The authors deny any conflict of interest.

PRISMA 2009 Checklist statement: The manuscript is designed as per PRISMA 2009 guideline and checklist is provided as supplementary file.

Di Luan, Li-Li Yuan, Yang Xu, Zhao-Hu Chu, Ling-Song Ma, Ya-Ping Wang, Shou-Cai Zhao, Department of Neurology, Yijishan Hospital affiliated to Wannan Medical College, Wuhu 241000, Anhui Province, China

Zheng-Yu Wu, Yuan-Xiang Zhang, Department of Clinical Pharmacy, Yijishan Hospital affiliated to Wannan Medical College, Wuhu 241000, Anhui Province, China

Corresponding author: Shou-Cai Zhao, MD, Doctor, Professor, Teacher, Department of Neurology, Yijishan Hospital affiliated to Wannan Medical College, Wuhu 241000, Anhui Province, China. neurozsc@wnmc.edu.cn
Telephone: +86-18505539779

Abstract

BACKGROUND

Ischemic stroke is a frequently-occurring disease in the elderly and characterized by high morbidity and mortality. Dl-3-n-butylphthalide (NBP), a synthetic compound based on natural celery seeds, has potential therapeutic effects on cerebral ischemia, brain trauma, memory impairment, and epilepsy.

AIM

To evaluated the effect of NBP on infarct volume in experimental ischemic stroke.

METHODS

Twenty one relevant literatures were included from the PubMed, EMBASE, Web of Science, Chinese National Knowledge Infrastructure, VIP information database, and Wanfang database, and data on the effect of dl-3-n-butylphthalide on infarction volume in the middle cerebral artery occlusion model were extracted. Statistical analysis was performed using standard mean difference with random effects model of Revman 5.3.

RESULTS

The data of meta-analysis of the 21 studies had suggested that NBP reduced the cerebral infarction volume of middle cerebral artery occlusion model animals compared to the control group significantly [SMD: -3.97, 95%CI: -4.71 to -3.23, $P < 0.01$; heterogeneity: $\chi^2 = 59.09$, df = 20 ($P < 0.01$); $I^2 = 66$ %].

CONCLUSION

NBP was effective in experimental ischemic stroke.

Key words: Butylphthalide; Animal model; Ischemic stroke; Meta-analysis

Manuscript source: Unsolicited manuscript

Received: May 10, 2019
Peer-review started: May 14, 2019
First decision: July 26, 2019
Revised: July 27, 2019
Accepted: July 29, 2019
Article in press: July 29, 2019
Published online: July 31, 2019

P-Reviewer: Tang Y
S-Editor: Wang JL
L-Editor: A
E-Editor: Wu YXJ

Core tip: The systematic review of animal research is of great significance in drug development. This study reports for the first time a systematic review and meta-analysis of the effects of butylphthalide on the volume of cerebral infarction in experimental ischemic stroke.

Citation: Luan D, Wu ZY, Zhang YX, Yuan LL, Xu Y, Chu ZH, Ma LS, Wang YP, Zhao SC. Effect of dl-3-n-butylphthalide on infarction volume in animal models of ischemic stroke: A meta-analysis. *World J Meta-Anal* 2019; 7(7): 358-372
URL: https://www.wjgnet.com/2308-3840/full/v7/i7/358.htm
DOI: https://dx.doi.org/10.13105/wjma.v7.i7.358

INTRODUCTION

Ischemic stroke is a frequently-occurring disease in the elderly and characterized by high morbidity and mortality[1,2]. The current treatment includes drug-based thrombolysis and interventional therapy in acute stage, however there were many inherent limitations of it[3,4]. To date, more than 1000 clinical trials of potential neuroprotective drugs have been verified to be failures[5].

Dl-3-n-butylphthalide (NBP), a synthetic compound based on natural celery seeds, has potential therapeutic effects on cerebral ischemia, brain trauma, memory impairment, and epilepsy, of which the injectable formulations have been approved for the treatment of acute ischemic stroke in China[6]. NBP protects the integrity of cerebrovascular structures[7], promotes the formation of collateral circulation, accelerates the proliferation of neonatal capillary[8,9], and increases the cerebral blood perfusion[10]; by targeting mitochondria, it improves neuronal energy metabolism[11], reduces oxidative stress damage and neuronal apoptosis[12]. The systematic review of animal research is of great significance in drug development[13]. To this end, we conducted a meta-analysis of preclinical studies to evaluate the efficacy and the mechanisms of NBP for experimental ischemic stroke.

MATERIALS AND METHODS

Literature search strategies

This meta-analysis was based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement[14]. All Chinese and English literatures before August 2018 on the effects of NBP for experimental ischemic stroke were searched in the six databases, which included PubMed, EMBASE, Web of Science, Wanfang database, VIP Chinese Journal Service Platform database and China National Knowledge Infrastructure database. Furthermore, to further confirm the relevant literature, we searched the list of references for potential publications. In the retrieval of the Web of Science, PubMed and EMBASE databases, only one keyword of "butylphthalide" was retrieved. In the searching of other databases, the following search strategy: "butylphthalide" AND "cerebral ischemia OR brain ischemia OR cerebral infarction OR brain infarction OR stroke OR cerebral ischemia/reperfusion OR cerebral I/R" were performed.

Inclusion and exclusion criteria

The inclusion criteria must be met the follows: (1) The experimental ischemic stroke model was established by the adoption of middle cerebral artery occlusion (MCAO); (2) The intervention group used NBP and the control group applied blank or non-functional solvent; and (3) The cerebral infarct volume was included in the study results and the unit of infarct volume was "%", and the calculation formula was (infarction volume / whole brain volume) × 100%. The exclusion criteria were followed: (1) The intervention group was not administered NBP or the intervention group was taken NBP with other medicines concomitantly; (2) The animal models was not adopt for proceeding to MCAO; (3) Without control group; (4) Repeating literature; and (5) The data were not available.

Literature screening and data extraction

By reading the title, abstract and full text according to the inclusion and exclusion criteria, the literature and extracted data were screened independently and cross-checked by the first author and the second author. When there was a disagreement, the point must be reached through the discussion panel which consisted of all authors, and the final conclusion was determined by the corresponding author.

Extracting the following data from the included literature: (1) The year of publication and the name of the first author; (2) The species, age, weight, gender, anesthesia methods, and model types of experimental animals (transient MCAO or permanent MCAO); (3) Therapeutic dose, route of administration, time of onset of treatment, and duration of treatment of the interventions; (4) The mean value and standard deviation of the cerebral infarction volume; and (5) The potential therapeutic mechanism of NBP for ischemic stroke.

Moreover, to study the multiple doses and multiple time points on effects of NBP, the final experimental data using the highest dose was extracted; If the volume of cerebral infarction cannot be obtained directly from the original text, the author of the literature is contacted by e-mail to get complete data, and if not, calculation was performed by using digital scale software.

Quality assessment

The risk of bias tool of the Systematic Review Centre for Laboratory animal Experimentation's was applied to assess the methodological quality of the included studies[15]: (1) Baseline characteristics: The strain, gender, age, weight, anesthesia methods, name and anesthetic dose of the experimental animals were involved; (2) Allocation concealment: The experimental animals were grouped randomly; (3) Sequence generation: The generation of allocation sequence was random; (4) Random housing: The living environment and feeding conditions of each group of animals were in the conformity with those of each group; (5) blindly feeding: The blind method was adopt for the breeder; (6) Random outcome assessment: Evaluate the outcomes stochastically on the premise of random selection of animals; (7) Result evaluator blindly: The blind method adopted by the outcome evaluator; (8) Outcome data completely: All data of animals were included in the final analysis; (9) No selective outcome reporting: No report bias; and (10) No other sources of bias: There were no other factors that contributed to the risk of high bias.

Statistical analysis

The Revman 5.3 software was used to analyze all data, the cerebral infarction volume was considered as continuous data, and the standard mean difference with random effects model were used to assess the combined effect sizes.

RESULTS

Study inclusion

A total of 5149 relevant literatures were retrieved from six databases, in them, there were 4630 duplicates and irrelevant were excluded, resulting in 519 literatures. After reading the titles and abstracts of the enrollment articles, 449 were rejected due to review comments, reviews, case reports, clinical trials and editorials literatures. By reading the full text of the remaining 70 articles, 49 articles were eliminated as the animal model was not established by the MCAO method and the intervention drug was not NBP monotherapy; there was no control group and the infarct volume calculation formula did not meet the inclusion criteria. Ultimately, 21 eligible articles were identified[8,16-35] (Figure 1).

Study characteristics

A total of 21 articles were collected, and among which, 5[24,25,32,33,35] were published in Chinese and the remaining were in English. A total of 314 animals were included in 21 studies to investigate the effect of NBP on the volume of cerebral infarction in the experimental ischemic stroke model. A total of 314 animals, including 159 in the experimental group and 155 in the control group were involved in 21 studies to investigate the effect of NBP on the volume of cerebral infarction in the experimental ischemic stroke model. Moreover, in 21 articles, there were 15 studies were performed on SD rats (15/21, 71.4%), 3[25,29,35] on Wistar rats (3/21, 14.3%), one[22] on C57BL6/J mice (1/21, 4.8%), one[16] on CD1 mice (1/21, 4.8%), one[31] on 129S2/Sv mice (1/21, 4.7%); meanwhile, twenty studies of them were applied males (20/21, 95.2%), and both males and females (1/21, 4.8%) one were adopted in one study[18]. As for anesthesia method, intraperitoneal injection of chloral hydrate in animals was used in the study of fourteen(14/21, 66.7%), injection of sodium pentobarbital in the abdominal cavity was adopted in one[19] study (1/21, 4.8%), ketamine and xylazine

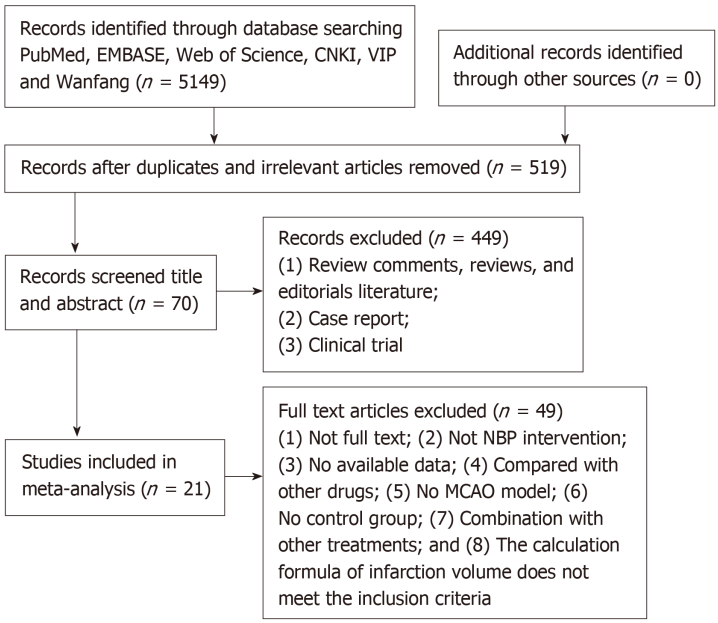


Figure 1 Literature inclusion flow chart. NBP: Dl-3-n-butylphthalide; MCAO: Middle cerebral artery occlusion.

injection into the peritonealcavity in anesthesia of experimental animals were employed in one[29] study (1/21, 4.7%), isoflurane inhalation anesthesia was accepted in the study of one[22] (1/21, 4.8%), and only one study[34] was adopted both atropine sulfate to reduce airway secretions, and inhalation anesthesia with isoflurane (1/21, 4.7%), in addition, three studies[17,18,33] that were not mentioned the anesthesia methods (3/21, 14.3%). Meanwhile, the animal models used in the fifteen studies were transient MCAO (tMCAO) (15/21, 71.4%), and a permanent MCAO (pMCAO) were proceeded in six studies (6/21, 28.6%)[16,25,29,33,35]. To detect infarction volume, there were 19 and 2[8,22] studies adopting the 2,3,5-triphenyltetrazolium chloride and cresyl violet as the staining agents respectively (Table 1).

Study quality

Of the 21 studies, five studies got 7 points, four studies got 6 points, eight studies got 5 points, two studies got 4 points, and two studies got 3 points. None of the studies described blind feeding and random outcome assessment; the result evaluator blindness was described only in two studies[8,32]; all studies described the data of baseline characteristics; two studies[18,29] have found other sources of bias; no incomplete outcome data, and no selective outcome reporting were described in 11 and 17 studies, respectively (Table 2).

Effectiveness

The data of Meta-analysis of the 21 studies had suggested that NBP reduced the cerebral infarction volume of MCAO model animals compared to the control group significantly [SMD: -3.97, 95%CI: -4.71 to -3.23, $P < 0.01$; heterogeneity: $\chi^2 = 59.09$, df = 20 ($P < 0.01$); $I^2 = 66$ %] (Figure 2). Moreover, the data of meta-analysis of fifteen studies adopting the tMCAO model also had verified that NBP reduced infarct volume significantly [SMD: -3.67, 95%CI: -4.52 to -2.82, $P < 0.01$; heterogeneity: $\chi^2 = 42.34$, df = 14 ($P < 0.01$); $I^2 = 67\%$] (Figure 3A). The same is true of studies using the pMCAO model [SMD: -4.70, 95%CI: -5.92 to -3.47, $P < 0.01$; heterogeneity: $\chi^2 = 9.26$, df = 5 ($P = 0.10$); $I^2 = 46\%$] (Figure 3B). To analyzed the effects of the NBP on the volume of cerebral infarction with pre- or post-administrated NBP in proceeding the MCAO model, the data had showed that both the pre-administration [SMD: -3.93, 95%CI: -5.51 to -2.36, $P < 0.01$; heterogeneity: $\chi^2 = 25.58$, df= 6 ($P < 0.01$); $I^2 = 77\%$] (Figure 4A) and the post-administration [SMD: -3.62, 95%CI: -4.32 to -2.92, $P < 0.01$; heterogeneity: $\chi^2 = 17.92$, df = 11 ($P = 0.08$); $I^2 = 39\%$] (Figure 4B) all reduced the infarct volume of the model animals. A funnel plot was adopted to evaluate publication bias and a slight bias was found (Figure 5A).

DISCUSSION

Table 1 Characteristics of the included studies

Study (yr)	Species / Age, Sex / Weight, Number (Control group / Experimental group)	Anesthetic	Model	Intervention dose administration method time point / duration	Measurement of infarction volume	Outcome index	*P*-values
Qin *et al*[8], 2018	SD rats/8 weeks	5 % chloral hydrate	tMCAO 2 h	90 mg/kg, daily	Cresyl violet	1 Infarction volume	$P < 0.05$
	Male/250-300 g	400 mg/kg	Reperfusion 7 d	Gavage	Image J	2 Ameliorate body weight loss	$P < 0.05$
	6/6	Intraperitoneally		Postoperative / 7 d		3 Improve neurological behavior scores	$P < 0.05$
						4 Reduce brain atrophy volume	$P < 0.01$
						5 Upregulate *PTGIS*, *PTGES*; downregulate *TBXAS 1*	$P < 0.05$
						6 Prevent *REN*,*AGT*, *ACE 1*, *AGTR 1*; upregulate *RoA*	$P < 0.05$
						7 Increase the diameter of middle cerebral artery	$P < 0.05$
Zhao *et al*[16], 2018	CD 1 mice / 10-12 wk	10 % chloral hydrate	pMCAO 24 h	120 mg/kg	2% TTC	1 Infarction volume	$P < 0.05$
	Male/27-30 g	35 mg/g				2 Improve neurological behavior scores	$P < 0.05$
	10/10	Intraperitoneally				3 Decrease the water content of brain	$P < 0.05$
						4 Decrease the permeability of blood-brain barrier	$P < 0.05$
						5 Decrease pinocytotic vesicles of capillary endothelial cells	
						6 Downregulate *MMP 9*	$P < 0.05$
						7 Upregulate *Claudin 5*, *VEGF*, *GFAP*, *Nrf 2* and *HO 1*	$P < 0.05$
Wang *et al*[18], 2018	SD rats/unknown	Unknown	tMCAO 2 h	1 mg/kg	2% TTC	1 Infarction volume	$P > 0.05$
	Male and female/250-280 g		Reperfusion 48 h	Intravenously	Image Pro Plus	2 Improve neurological behavior scores	$P > 0.05$
	8/12			postoperative/4 h and 24 h			
Yan *et al*[17], 2017	SD rats/adult	Unknown	tMCAO 2 h	75 mg/kg, daily	2% TTC	1 Infarction volume	$P < 0.01$
	Male/180-220 g		Reperfusion 24 h	Gavage		2 Decrease the water content of brain	$P < 0.05$
	8/8			Preoperative/7 d		3 Decrease the permeability of blood-brain barrier	$P < 0.01$

						4 Decrease cell apoptosis	
						5 Decrease ROS,cleaved caspase-3, p-p38; increase SOD	$P < 0.01$
						6 Decrease MDA, p-JNK	$P < 0.05$
Zhang *et al*[19], 2016	SD rats/unknown	Sodium pentobarbital	tMCAO 1 h	4.5 mg/kg	TTC	1 Infarction volume	$P < 0.01$
	Male/250-320 g	50 mg/kg	Reperfusion 24 h	Intraperitoneally		2 Improve neurological behavior scores	$P < 0.001$
	8/8			Postoperative/-		3 Decrease the water content of brain	$P < 0.05$
						4 Upregulate *HGF*; downreglate *TLR4*	$P < 0.001$
Yin *et al*[20], 2016	SD rats/unknown	Chloral hydrate	tMCAO 2 h	80 mg/kg, daily	2% TTC	1 Infarction volume	$P < 0.001$
	Male/280-320 g	300 mg/kg	Reperfusion 24 h	Gavage	Image Pro Plus	2 Improve neurological behavior scores	$P < 0.05$
	6/6	Intraperitoneally		preoperative/7 d		3 Decrease the water content of brain	$P < 0.01$
						4 Decrease MDA	$P < 0.01$
						5 Increase SOD	$P < 0.05$
						6 Increase GSH-Px	$P < 0.001$
Hua *et al*[21], 2015	SD rats/57-61 days	Chloral hydrate	tMCAO 2 h	60 mg/kg	2% TTC	1 Infarction volume	$P < 0.05$
	Male/250-280 g	300 mg/kg	Reperfusion 24 h	Gavage	Image Pro Plus	2 Improve neurological behavior scores	$P > 0.05$
	6/6	Intraperitoneally		Postoperative/-		3 Decrease the water content of brain	$P < 0.01$
						4 Decrease MDA; increase GSH, SOD, Nrf 2, Trx, Bcl-2	$P < 0.01$
						5 Decrease NF-κB p65; increase Txnip	$P < 0.05$
Lu *et al*[22], 2014	C57BL6/J/ dult	Isoflurane	tMCAO 0.75 h	10 mg/kg	Cresyl violet	1 Infarction volume	$P < 0.05$
	Male/ nknown	Initiated 3 %	Reperfusion 23 h	Intravenous	Image J	2 Improve neurological behavior scores	$P < 0.05$
	10/10	Maintained 1.5 %		Preoperative/-		3 Decrease MMP 9; increase TIMP 1	$P < 0.01$
						4 Increase SBP, p-ERK	$P < 0.01$
Wang *et al*[23], 2013	SD rats/unknown	Chloral hydrate	tMCAO 2 h	80 mg/kg, daily	TTC	1 Infarction volume	$P < 0.05$
	Male/280-320 g	300 mg/kg	Reperfusion 24 h	Gavage		2 Improve neurological behavior scores	$P > 0.05$
	6/6	Intraperitoneally		preoperative/7 d		3 Decrease the water content of brain	$P > 0.05$
Wang *et al*[24], 2013	SD rats/unknown	10 % chloral hydrate	tMCAO 2 h	80 mg/kg, daily	2% TTC	1 Infarction volume	$P < 0.05$
	Male/250 ± 20 g (n = 30)	3 mL/kg	Reperfusion 24 h	Gavage		2 Downregulate *GRP78*, *CHOP*	$P < 0.05$

	5/5	Intraperitoneally		Preoperative / 7 d			
Pan *et al*[25], 2013	Wistar rats/unknown	10 % chloral hydrate	pMCAO 24 h	0.8 g/kg, daily	2% TTC	1 Infarction volume	$P < 0.05$
	Male/200-250 g	3 mL/kg		Gavage	Biosens Digitan Image	2 Decrease the water content of brain	$P < 0.05$
	8/8	Intraperitoneally		Preoperative/14 d		3 Decrease Smac, S100B	$P < 0.05$
Zhang *et al*[26], 2013	SD rats/unknown	6 % chloral hydrate	tMCAO 2 h	200 mg/kg	2% TTC	1 Infarction volume	$P < 0.01$
	Male/250 ± 20 g		Reperfusion 24 h	Intraperitoneally	Image Pro Plus	2 Improve neurological behavior scores	$P < 0.01$
	8/8	Intraperitoneally		Postoperative/-		3 Increase VEGF	$P < 0.01$
Wang *et al*[27], 2012	SD rats/unknown	chloral hydrate	tMCAO 2 h	90 mg/kg, daily	2% TTC	1 Infarction volume	$P < 0.05$
	Male/250-300 g	300 mg/kg	Reperfusion 3 d	Gavage		2 Improve neurological behavior scores	$P < 0.05$
	10/10	Intraperitoneally		Postoperative/3 d		3 Decrease the water content of brain	$P < 0.05$
						4 Decrease the ratio of TXB_2 : 6-keto-$PGF_{1\alpha}$	$P < 0.05$
Wu *et al*[28], 2012	SD rats/unknown	Chloral Hydrate	tMCAO 2 h	80 mg/kg, daily	2% TTC	1 Infarction volume	$P < 0.001$
	Male/250-280 g	350 mg/kg	Reperfusion 24 h	Gavage		2 Improve neurological behavior scores	$P < 0.001$
	6/6	Intraperitoneally		Preoperative/7 d		3 Decrease the water content of brain	$P > 0.05$
						4 Decrease MDA; increase SOD	$P < 0.001$
Zhang *et al*[29], 2012	Wistar Kyoto rats/3 mo	Ketamine, xylazine	pMCAO 7 d	80 mg/kg, daily	2% TTC	1 Infarction volume	$P < 0.01$
	Male/unknown	75 mg/kg, 10 mg/kg		Gavage	Image Pro Plus		
	6/6	Intraperitoneally		Postoperative/7 d			
Zhao *et al*[30], 2012	SD rats/unknown	Chloral hydrate	tMCAO 2 h	50 mg/kg, daily	2% TTC	1 Infarction volume	$P < 0.05$
	Male/250-300 g	300 mg/kg	Reperfusion 3 d	Gavage		2 Improve neurological behavior scores	$P < 0.05$
	6/6	Intraperitoneally		Postoperative / 3 d			
Li *et al*[31], 2010	129S2/Sv/adult	4 % chloral hydrate	pMCAO 24 h	100 mg/kg	2% TTC	1 Infarction volume	$P < 0.05$
	Male/20-25 g			Intraperitoneally		2 Decrease cleaved-caspase 3; caspase 9, p-JNK; p-p38	$P < 0.05$
	10/10	Intraperitoneally		Postoperative 1 h/-		3 Reduce mitochondrial release of cytochrome c and AIF	$P < 0.05$
Cao *et al*[32], 2009	SD rats/3-4 mo	10 % chloral hydrate	tMCAO 2 h	25 mg/kg, twice a day	TTC	1 Infarction volume	$P < 0.01$
	Male/280-350 g		Reperfusion 3 d	Gavage	Image Pro Plus	2 Improve neurological behavior scores	$P < 0.05$
	5/5	Intraperitoneally		Postoperative/3 d		3 Upregulate *VEGF*, *bFGF*	$P < 0.05$
Li *et al*[33], 2008	SD rats/3-4 mo	Unknown	pMCAO 3 d	25 mg/kg, twice a day	TTC	1 Infarction volume	$P < 0.05$

	Male/280-350 g			Gavage	Image Pro Plus	2 Improve neurological behavior scores	$P < 0.05$
	5/5			Postoperative/3 d		3 Upregulate *VEGF*, *bFGF*	$P < 0.05$
Zhang *et al*[34], 2006	SD rats/unknown	3 % isoflurane	tMCAO 2 h	10 mg/kg	4% TTC	1 Infarction volume	$P < 0.001$
	Male/270-330 g	Endotracheal intubation	Reperfusion 24 h	Intravenously	SPOT Biometrics	2 Improve neurological behavior scores	$P < 0.01$
	10/10			intraoperative / -			
Lin *et al*[35], 1996	Wistar rats/unknown	Chloral hydrate	pMCAO 24 h	240 mg/kg	4% TTC	1 Infarction volume	$P < 0.001$
	Male/250-350 g			Gavage		2 Improve neurological behavior scores	$P < 0.001$
	8/8			Postoperative/-			

PTGIS: *Prostacyclin synthase*; *PTGES*: *Prostaglandin E synthase*; *TBXAS 1*: *Thromboxane A2 synthase 1*; *REN*: *Renin*; *AGT*: *Angiotensinogen*; *ACE 1*: *Angiotensin converting enzyme 1*; *AGTR 1*: *Angiotensin II receptor type 1*; tMCAO: Transient middle cerebral artery occlusion; pMCAO: Permanent middle cerebral artery occlusion; TTC: 2,3,5-triphenyltetrazolium chloride; *MMP 9*: *Matrix metallopeptidase 9*; *VEGF*: *Vascular endothelial growth factor*; *GFAP*: *Glial fibrillary acidic protein*; *Nrf 2*: *NF-E2-related factor 2*; *HO 1*: *Heme oxygenase 1*; ROS: Reactive oxygen species; SOD: Superoxide dismutase; MDA: Malonaldehyde; *HGF*: *Hepatocyte growth factor*; *TLR4*: *Toll like receptor 4*; GSH-Px: Glutathione peroxidase; GSH: Glutathione; Trx: Thioredoxin; Txnip: Thioredoxin-interacting protein; TIMP 1: Tissue inhibitor of metalloproteinase 1; SBP: Spectrin breakdown product; *GRP 78*: *Glucose-regulated protein 78*; *CHOP*: *C/EBP-homologous protein*; Smac: Second mitochondria-derived activator of caspases; S100B: S100 calcium binding protein B; TXB2: Thromboxane B2; 6-keto-PGF1α: 6-keto-prostaglandin F1α; AIF: Apoptosis-inducing factor; *bFGF*: *Basic fibroblast growth factor*.

Summary of evidence

The preclinical meta-analysis study evaluated the effects of NBP on infarct volume in experimental ischemic stroke, which was based on experimental data from 314 animals in five Chinese literatures and sixteen English literatures. The evidence obtained from present study suggest that NBP might play potential neuroprotective roles for ischemic stroke by increasing cerebral blood flow, enhancing mitochondrial function, protecting integrity of the structure and function of blood-brain barrier, and developing anti-inflammatory and antioxidant stress.

Limitations

First, the absence of relevant literatures in other languages other than Chinese and English, may lead to selective bias. Second, none of study provides sample size calculations, blind feeding, and random outcome assessment. Third, the lack of negative research may result in an overestimation of the efficacy of NBP. Fourth, cerebral infarction is usually accompanied by other conditions, such as old age, hypertension, hyperlipidemia, diabetes, heart disease and so on[36-39]. We did not analyze the effects of NBP on cerebral infarction when the accompanying situation occurred. Fifth, one study [18] using female animals does not rule out estrogen neuroprotection, that has been reported[40]. One study[29] of anesthetic drugs containing ketamine did not eliminate its neuroprotection, that has been reported in preclinical and clinical studies[41,42].

Potential neuroprotective mechanisms

Summarizing the included literatures, we have found that NBP plays a neuroprotective role in experimental ischemic stroke by acting on multiple targets (Figure 5B). We have drawn a conclusion of the underlying mechanisms as follows: (1) Increase blood supply to brain tissue in the ischemic area: Dilate the middle cerebral artery[8]; regulate the expression of *REN, AGT, ACE 1, AGTR 1, RoA, PTGIS, PTGES* and *TBXAS 1* in ischemic brain tissue [8], decrease TXB2 and 6-keto-PGF1α ratio[27], and reduce thrombosis; (2) Promote angiogenesis: Increase VEGF and bFGF in ischemic brain tissue[16,26,32,33]; (3) Anti-inflammatory: Inhibition of TLR4/NF-κB signaling pathway[19,21]; decrease the expression of S100B in ischemic brain tissue[25]. (4) Protect the structure and function of the blood-brain barrier: Modulate the expression of *MMP-9* and *claudin-5* in ischemic brain tissue[16,22]; up-regulate the expression of *GFAP* in ischemic brain tissue, stabilize astrocytes[16]; increase the expression of TIMP1 and decrease the expression of SBP in ischemic brain tissue[22]; (5) Antioxidative stress: Enhance Nrf-2/HO-1 signaling pathway[16]; reduce the expression of ROS and MDA in ischemic brain tissue; increase the expression of SOD, GSH-px, GSH, Trx and Txnip in ischemic brain tissue[17,20,21,28]; (6) Protect the structure and function of mitochondria: Increase the expression of Bcl-2 in ischemic brain tissue[21]; reduce the expression of

Table 2 Risk of bias of the included studies

Study (yr)	A	B	C	D	E	F	G	H	I	J	Score
Qin *et al*[8], 2018	√	√	√				√	√	√	√	7
Zhao *et al*[16], 2018	√	√	√						√	√	5
Wang *et al*[18], 2018	√		√	√				√	√		5
Yan *et al*[17], 2017	√			√					√	√	4
Zhang *et al*[19], 2016	√			√					√	√	4
Zhao *et al*[16], 2018	√	√	√						√	√	5
Yin *et al*[20], 2016	√	√	√					√	√	√	6
Hua *et al*[21], 2015	√	√	√	√				√	√	√	7
Lu *et al*[22], 2014	√	√	√	√				√	√	√	7
Wang *et al*[23], 2013	√	√	√						√	√	5
Wang *et al*[24], 2013	√	√	√					√		√	5
Pan *et al*[25], 2013	√	√	√					√		√	5
Zhang *et al*[26], 2013	√	√	√						√	√	5
Wang *et al*[27], 2012	√	√	√						√	√	5
Wu *et al*[28], 2012	√	√	√						√	√	5
Zhang *et al*[29], 2012	√	√	√	√				√	√		6
Zhao *et al*[30], 2012	√	√	√	√				√	√	√	7
Li *et al*[31], 2010	√								√	√	3
Cao *et al*[32], 2009	√	√	√	√			√	√		√	7
Li *et al*[33], 2008	√	√	√	√				√		√	6
Zhang *et al*[34], 2006	√	√	√	√					√	√	6
Lin *et al*[35], 1996	√								√	√	3

A: Baseline characteristics; B: Allocation concealment; C: Sequence generation; D: Random housing; E: Blind feeding; F: Random outcome assessment; G: Result evaluator blind; H: No incomplete outcome data; I: No selective outcome reporting; J: No other sources of bias.

Smac in ischemic brain tissue[25]; reduce mitochondrial release of cytochrome C and AIF[31]; and (7) Anti-apoptosis: Reduce the expression of cleaved caspase-3, p-p38 and p-JNK in ischemic brain tissue[17,31]; increase the expression of HGF and p-ERK in ischemic brain tissue[19,22]; decrease the expression of GRP78 and CHOP in ischemic brain tissue, and inhibit endoplasmic reticulum stress-induced apoptosis[24].

Implications

Animal experiments are an important link between basic research and clinical experiments. The results have reference value for the next step in designing and implementing clinical research. Compared with clinical research, the principles of randomization and blindness are theoretically easier to be implemented in animal experiments. Animal research is important for comprehending disease mechanisms, and high-quality preclinical research is also critical for translational medicine[43,44]. Therefore, to obtain more accurate and less biased experimental data, designing animal programs should follow the guidelines all the time[15,45], calculate sample size in the beginning, apply applicable animals, use appropriate anesthetic drugs, adopt random feeding, and blind models during the experiment, and employ random outcome measurements at the time of evaluation.

Similar to artemisinin, NBP is also a plant-derived drug approved for the treatment of acute ischemic stroke in China. We envision that NBP promote to treat more patients in the world, like artemisinin, and it requires a large number of randomized, double-blind, and multi-center clinical trials in terms of safety and efficacy.

In conclusion, we conducted the first preclinical systematic review and meta-analysis of the effects of NBP on experimental ischemic stroke, and found that NBP was effective in experimental ischemic stroke.

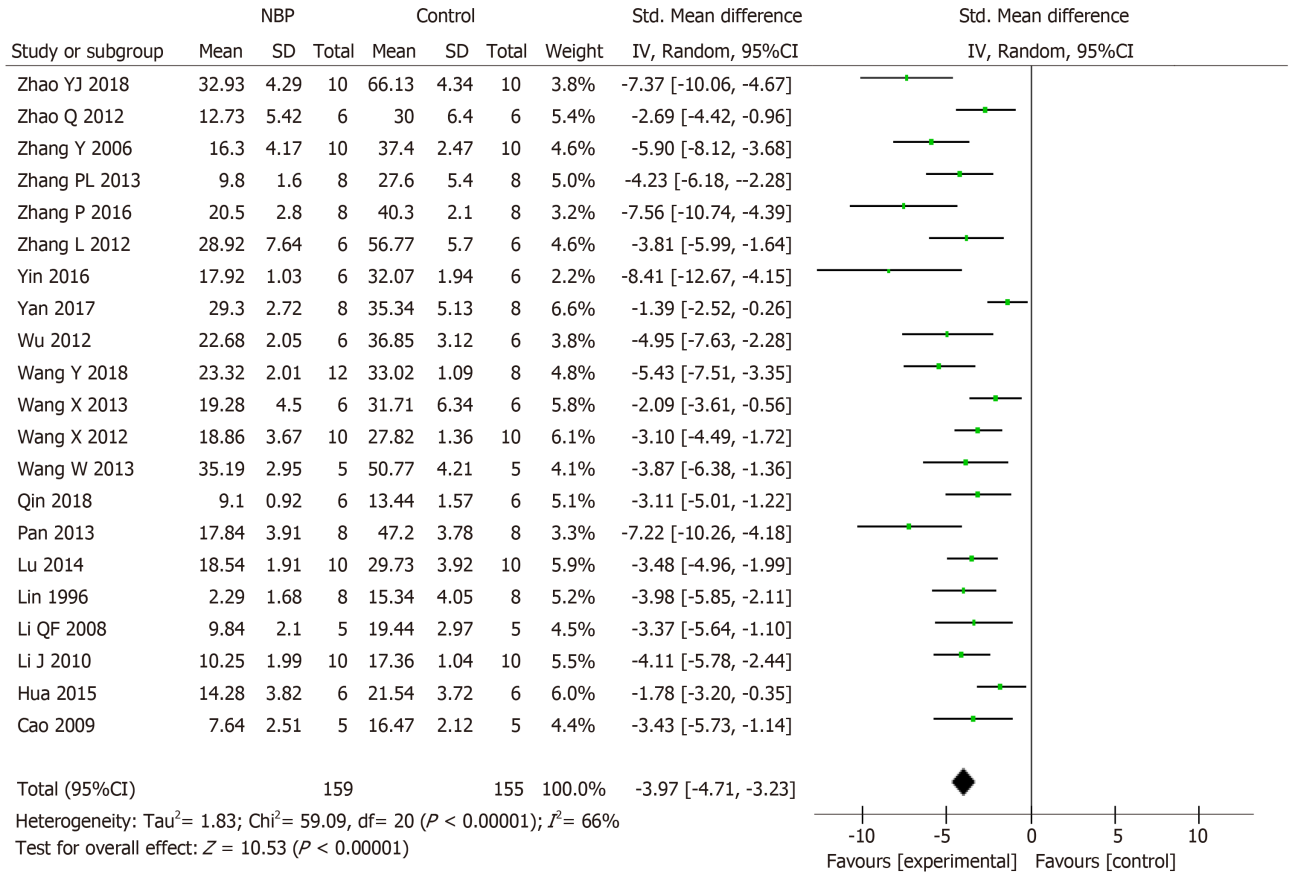
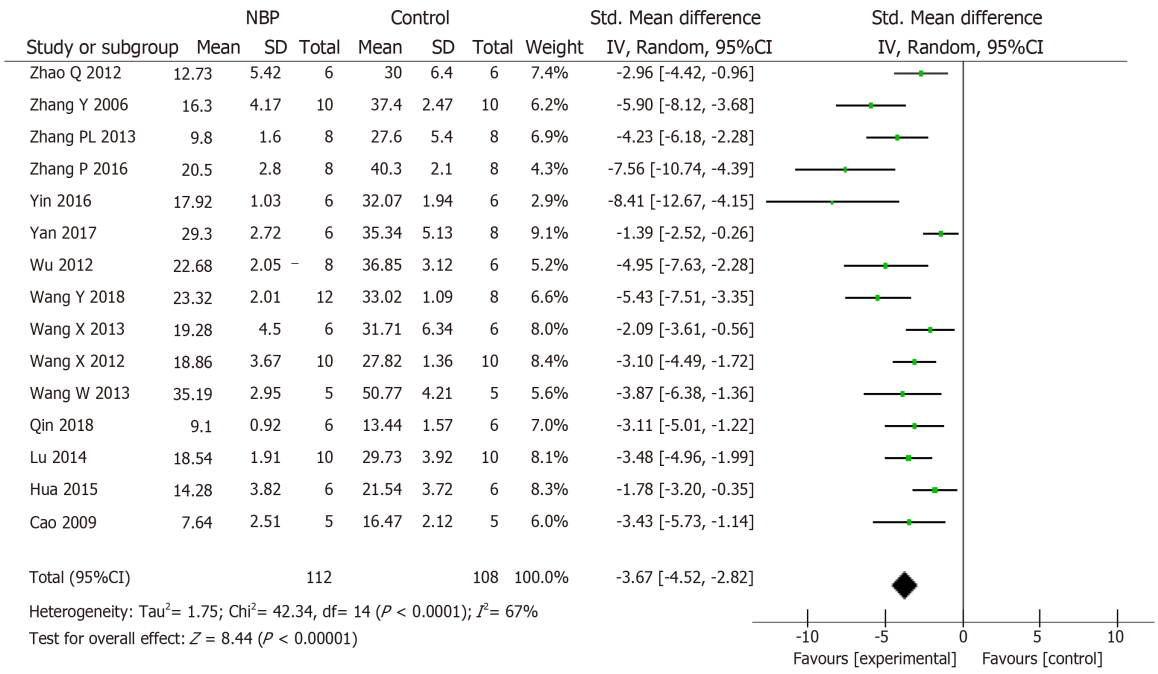


Figure 2 The forest plot: Effects of dl-3-n-butylphthalide for decreasing the cerebral infarction volume compared with control group.

A



B

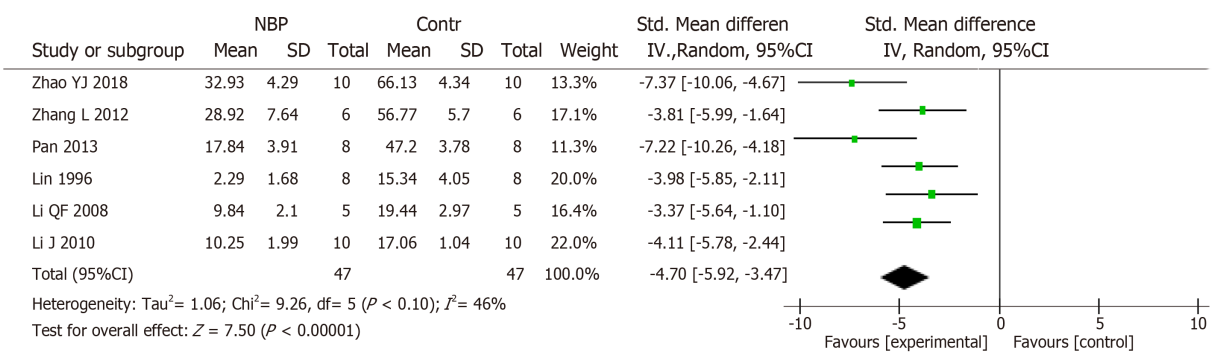


Figure 3 The forest plot: Effects of dl-3-n-butylphthalide for decreasing the cerebral infarction volume compared with control group in transient (A) and permanent (B) middle cerebral artery occlusion model, respectively.

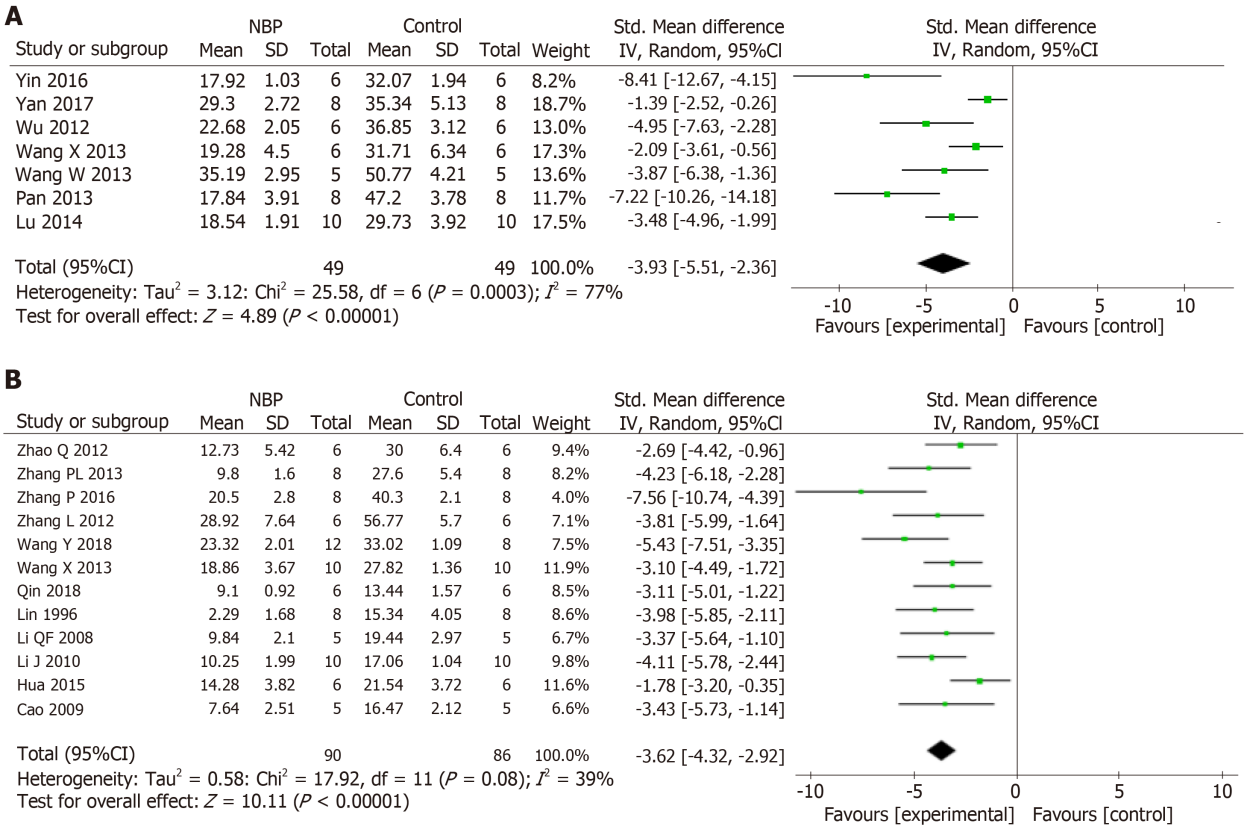


Figure 4 The forest plot: Effects of dl-3-n-butylphthalide for decreasing the cerebral infarction volume compared with control group in pre (A) and post-administration (B) model, respectively.

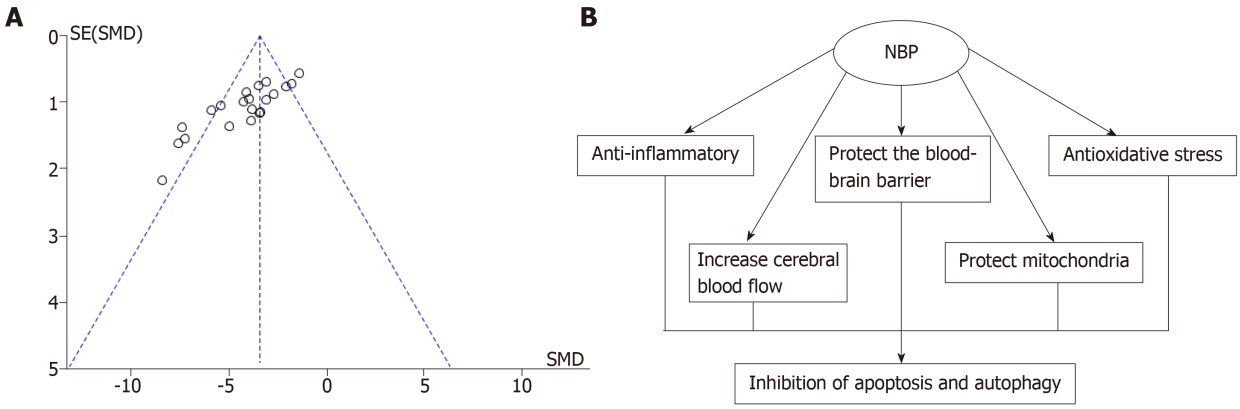


Figure 5 The funnel plot evaluation publication bias and underlying mechanism of dl-3-n-butylphthalide in neuroprotection. A: The funnel plot evaluation publication bias for the dl-3-n-butylphthalide on infarction volume; B: The underlying mechanism of dl-3-n-butylphthalide in neuroprotection.

ARTICLE HIGHLIGHTS

Research background

Ischemic stroke is a frequently-occurring disease in the elderly and characterized by high morbidity and mortality. Dl-3-n-butylphthalide (NBP), a synthetic compound based on natural celery seeds, has potential therapeutic effects on cerebral ischemia, brain trauma, memory impairment, and epilepsy. The systematic review of animal research is of great significance in drug development.

Research motivation

There are many studies on the therapeutic effects of NBP in the middle cerebral artery occlusion model, and there is controversy about whether NBP reduces the volume of cerebral infarction.

Research objectives

To evaluated effect of NBP on infarct volume in experimental ischemic stroke.

Research methods
We searched Chinese and English databases to screen NBP-related literature. Data such as cerebral infarction volume and potential therapeutic mechanisms were extracted. The risk of bias tool of the Systematic Review Centre for Laboratory animal Experimentation's was applied to assess the methodological quality of the included studies. Data analysis was performed by Revman 5.3 software.

Research results
The data of meta-analysis of the 21 studies had suggested that NBP reduced the cerebral infarction volume of middle cerebral artery occlusion (MCAO) model animals compared to the control group significantly. Moreover, the data of meta-analysis of fifteen studies adopting the tMCAO model also had verified that NBP reduced infarct volume significantly. The same is true of studies using the pMCAO model. To analyze the effects of the NBP on the volume of cerebral infarction with pre- or post-administrated NBP in proceeding the MCAO model, the data had showed that both the pre-administration and the post-administration all reduced the infarct volume of the model animals.

Research conclusions
NBP was effective in experimental ischemic stroke.

Research perspectives
Animal experiments are an important link between basic research and clinical experiments. The results have reference value for the next step in designing and implementing clinical research. Compared with clinical research, the principles of randomization and blindness are theoretically easier to be implemented in animal experiments. Animal research is important for comprehending disease mechanisms, and high-quality preclinical research is also critical for translational medicine. Therefore, to obtain more accurate and less biased experimental data, designing animal programs should follow the guidelines all the time, calculate sample size in the beginning, apply applicable animals, use appropriate anesthetic drugs, adopt random feeding, and blind models during the experiment, and employ random outcome measurements at the time of evaluation.

REFERENCES

1 **Feigin VL**, Norrving B, Mensah GA. Global Burden of Stroke. *Circ Res* 2017; **120**: 439-448 [PMID: 28154096 DOI: 10.1161/CIRCRESAHA.116.308413]

2 **Writing Group Members**. Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, Das SR, de Ferranti S, Després JP, Fullerton HJ, Howard VJ, Huffman MD, Isasi CR, Jiménez MC, Judd SE, Kissela BM, Lichtman JH, Lisabeth LD, Liu S, Mackey RH, Magid DJ, McGuire DK, Mohler ER 3rd, Moy CS, Muntner P, Mussolino ME, Nasir K, Neumar RW, Nichol G, Palaniappan L, Pandey DK, Reeves MJ, Rodriguez CJ, Rosamond W, Sorlie PD, Stein J, Towfighi A, Turan TN, Virani SS, Woo D, Yeh RW, Turner MB; American Heart Association Statistics Committee; Stroke Statistics Subcommittee. Heart Disease and Stroke Statistics-2016 Update: A Report From the American Heart Association. *Circulation* 2016; **133**: e38-360 [PMID: 26673558 DOI: 10.1161/CIR.0000000000000350]

3 **de Ridder IR**, Fransen PS, Beumer D, Berkhemer OA, van den Berg LA, Wermer MJ, Lingsma H, van Zwam WH, Roos YB, van Oostenbrugge RJ, Majoie CB, van der Lugt A, Dippel DW. Is Intra-Arterial Treatment for Acute Ischemic Stroke Less Effective in Women than in Men? *Interv Neurol* 2016; **5**: 174-178 [PMID: 27781046 DOI: 10.1159/000447331]

4 **Chen HS**, Qi SH, Shen JG. One-Compound-Multi-Target: Combination Prospect of Natural Compounds with Thrombolytic Therapy in Acute Ischemic Stroke. *Curr Neuropharmacol* 2017; **15**: 134-156 [PMID: 27334020]

5 **Jickling GC**, Sharp FR. Improving the translation of animal ischemic stroke studies to humans. *Metab Brain Dis* 2015; **30**: 461-467 [PMID: 24526567 DOI: 10.1007/s11011-014-9499-2]

6 **Wang S**, Ma F, Huang L, Zhang Y, Peng Y, Xing C, Feng Y, Wang X, Peng Y. Dl-3-n-Butylphthalide (NBP): A Promising Therapeutic Agent for Ischemic Stroke. *CNS Neurol Disord Drug Targets* 2018; **17**: 338-347 [PMID: 29895257 DOI: 10.2174/1871527317666180612125843]

7 **Zhao Y**, Li J, Zhang P, Chen C, Li S. Protective effects of dl-3n-butylphthalide against diffuse brain injury. *Neural Regen Res* 2013; **8**: 2615-2624 [PMID: 25206572 DOI: 10.3969/j.issn.1673-5374.2013.28.003]

8 **Qin C**, Zhou P, Wang L, Mamtilahun M, Li W, Zhang Z, Yang GY, Wang Y. Dl-3-N-butylphthalide attenuates ischemic reperfusion injury by improving the function of cerebral artery and circulation. *J Cereb Blood Flow Metab* 2018; 271678X18776833 [PMID: 29762050 DOI: 10.1177/0271678X18776833]

9 **Zhao Y**, Lee JH, Chen D, Gu X, Caslin A, Li J, Yu SP, Wei L. DL-3-n-butylphthalide induced neuroprotection, regenerative repair, functional recovery and psychological benefits following traumatic brain injury in mice. *Neurochem Int* 2017; **111**: 82-92 [PMID: 28359729 DOI: 10.1016/j.neuint.2017.03.017]

10 **Xiong Z**, Lu W, Zhu L, Zeng L, Shi C, Jing Z, Xiang Y, Li W, Tsang CK, Ruan Y, Huang L. Dl-3-n-Butylphthalide Treatment Enhances Hemodynamics and Ameliorates Memory Deficits in Rats with Chronic Cerebral Hypoperfusion. *Front Aging Neurosci* 2017; **9**: 238 [PMID: 28798681 DOI: 10.3389/fnagi.2017.00238]

11 **Liu RZ**, Fan CX, Zhang ZL, Zhao X, Sun Y, Liu HH, Nie ZX, Pu XP. Effects of Dl-3-n-butylphthalide on Cerebral Ischemia Infarction in Rat Model by Mass Spectrometry Imaging. *Int J Mol Sci* 2017; **18**: E2451 [PMID: 29165327 DOI: 10.3390/ijms18112451]

12 **Feng L**, Sharma A, Niu F, Huang Y, Lafuente JV, Muresanu DF, Ozkizilcik A, Tian ZR, Sharma HS.

TiO2-Nanowired Delivery of DL-3-n-butylphthalide (DL-NBP) Attenuates Blood-Brain Barrier Disruption, Brain Edema Formation, and Neuronal Damages Following Concussive Head Injury. *Mol Neurobiol* 2018; **55**: 350-358 [PMID: 28856586 DOI: 10.1007/s12035-017-0746-5]

13 **de Vries RB**, Wever KE, Avey MT, Stephens ML, Sena ES, Leenaars M. The usefulness of systematic reviews of animal experiments for the design of preclinical and clinical studies. *ILAR J* 2014; **55**: 427-437 [PMID: 25541545 DOI: 10.1093/ilar/ilu043]

14 **Stewart LA**, Clarke M, Rovers M, Riley RD, Simmonds M, Stewart G, Tierney JF; PRISMA-IPD Development Group. Preferred Reporting Items for Systematic Review and Meta-Analyses of individual participant data: the PRISMA-IPD Statement. *JAMA* 2015; **313**: 1657-1665 [PMID: 25919529 DOI: 10.1001/jama.2015.3656]

15 **Hooijmans CR**, Rovers MM, de Vries RB, Leenaars M, Ritskes-Hoitinga M, Langendam MW. SYRCLE's risk of bias tool for animal studies. *BMC Med Res Methodol* 2014; **14**: 43 [PMID: 24667063 DOI: 10.1186/1471-2288-14-43]

16 **Zhao YJ**, Nai Y, Ma QS, Song DJ, Ma YB, Zhang LH, Mi LX. Dl-3-n-butylphthalide protects the blood brain barrier of cerebral infarction by activating the Nrf-2/HO-1 signaling pathway in mice. *Eur Rev Med Pharmacol Sci* 2018; **22**: 2109-2118 [PMID: 29687870 DOI: 10.26355/eurrev_201804_14744]

17 **Yan RY**, Wang SJ, Yao GT, Liu ZG, Xiao N. The protective effect and its mechanism of 3-n-butylphthalide pretreatment on cerebral ischemia reperfusion injury in rats. *Eur Rev Med Pharmacol Sci* 2017; **21**: 5275-5282 [PMID: 29228445 DOI: 10.26355/eurrev_201711_13852]

18 **Wang Y**, Huang Y, Xu Y, Ruan W, Wang H, Zhang Y, Saavedra JM, Zhang L, Huang Z, Pang T. A Dual AMPK/Nrf2 Activator Reduces Brain Inflammation After Stroke by Enhancing Microglia M2 Polarization. *Antioxid Redox Signal* 2018; **28**: 141-163 [PMID: 28747068 DOI: 10.1089/ars.2017.7003]

19 **Zhang P**, Guo ZF, Xu YM, Li YS, Song JG. N-Butylphthalide (NBP) ameliorated cerebral ischemia reperfusion-induced brain injury via HGF-regulated TLR4/NF-κB signaling pathway. *Biomed Pharmacother* 2016; **83**: 658-666 [PMID: 27468961 DOI: 10.1016/j.biopha.2016.07.040]

20 **Yin W**, Lan L, Huang Z, Ji J, Fang J, Wang X, Ji H, Peng S, Xu J, Zhang Y. Discovery of a ring-opened derivative of 3-n-butylphthalide bearing NO/H2S-donating moieties as a potential anti-ischemic stroke agent. *Eur J Med Chem* 2016; **115**: 369-380 [PMID: 27031213 DOI: 10.1016/j.ejmech.2016.03.044]

21 **Hua K**, Sheng X, Li TT, Wang LN, Zhang YH, Huang ZJ, Ji H. The edaravone and 3-n-butylphthalide ring-opening derivative 10b effectively attenuates cerebral ischemia injury in rats. *Acta Pharmacol Sin* 2015; **36**: 917-927 [PMID: 26073328 DOI: 10.1038/aps.2015.31]

22 **Lu YM**, Huang JY, Wang H, Lou XF, Liao MH, Hong LJ, Tao RR, Ahmed MM, Shan CL, Wang XL, Fukunaga K, Du YZ, Han F. Targeted therapy of brain ischaemia using Fas ligand antibody conjugated PEG-lipid nanoparticles. *Biomaterials* 2014; **35**: 530-537 [PMID: 24120040 DOI: 10.1016/j.biomaterials.2013.09.093]

23 **Wang X**, Wang L, Li T, Huang Z, Lai Y, Ji H, Wan X, Xu J, Tian J, Zhang Y. Novel hybrids of optically active ring-opened 3-n-butylphthalide derivative and isosorbide as potential anti-ischemic stroke agents. *J Med Chem* 2013; **56**: 3078-3089 [PMID: 23509954 DOI: 10.1021/jm4001693]

24 **Wang W**, Kong W, Chen M, Zheng X, Zhao S, Kong X. Effects of 3-n-butylphthalide pretreatment on endoplasmic reticulum stress in rats with cerebral ischemia reperfusion. *Linchuang Shenjingbingxue Zazhi* 2013; **26**: 122-124

25 **Pan J**, Chen X, Li Q, Song D. Brain protective effect of butylpthalide preconditioning in rat with cerebral ischemics. *Linchuang Shenjingbingxue Zazhi* 2013; **26**: 44-46

26 **Zhang PL**, Lu HT, Zhao JG, Li MH. Protective effect of dl-3n-butylphthalide preconditioning on focal cerebral ischaemia-reperfusion injury in rats. *Acta Neuropsychiatr* 2013; **25**: 12-17 [PMID: 26953069 DOI: 10.1111/j.1601-5215.2012.00649.x]

27 **Wang X**, Zhao Q, Wang X, Li T, Lai Y, Peng S, Ji H, Xu J, Zhang Y. Studies on the enantiomers of ZJM-289: synthesis and biological evaluation of antiplatelet, antithrombotic and neuroprotective activities. *Org Biomol Chem* 2012; **10**: 9030-9040 [PMID: 23076046 DOI: 10.1039/c2ob26511g]

28 **Wu J**, Ling J, Wang X, Li T, Liu J, Lai Y, Ji H, Peng S, Tian J, Zhang Y. Discovery of a potential anti-ischemic stroke agent: 3-pentylbenzo[c]thiophen-1(3H)-one. *J Med Chem* 2012; **55**: 7173-7181 [PMID: 22827516 DOI: 10.1021/jm300681r]

29 **Zhang L**, Yu WH, Wang YX, Wang C, Zhao F, Qi W, Chan WM, Huang Y, Wai MS, Dong J, Yew DT. DL-3-n-Butylphthalide, an anti-oxidant agent, prevents neurological deficits and cerebral injury following stroke per functional analysis, magnetic resonance imaging and histological assessment. *Curr Neurovasc Res* 2012; **9**: 167-175 [PMID: 22621233]

30 **Zhao Q**, Zhang C, Wang X, Chen L, Ji H, Zhang Y. (S)-ZJM-289, a nitric oxide-releasing derivative of 3-n-butylphthalide, protects against ischemic neuronal injury by attenuating mitochondrial dysfunction and associated cell death. *Neurochem Int* 2012; **60**: 134-144 [PMID: 22142531 DOI: 10.1016/j.neuint.2011.11.013]

31 **Li J**, Li Y, Ogle M, Zhou X, Song M, Yu SP, Wei L. DL-3-n-butylphthalide prevents neuronal cell death after focal cerebral ischemia in mice via the JNK pathway. *Brain Res* 2010; **1359**: 216-226 [PMID: 20800583 DOI: 10.1016/j.brainres]

32 **Cao W**, De Ji Qu Z, Li Q, He L, Zhou D. Effects of dl23n2butylphthalide on the Expression of VEGF and bFGF in Transient Middle Cerebral Artery Occlusion Rats. *Sichuan Daxue Xuebao (Yixue Ban)* 2009; **40**: 403-407

33 **Li Q**, Kong S, De Ji Qu Z, He L, Zhou D. Effects of dl-3-n-butyIphthalide on Expression of VEGF and bFGF in Rat Brain with Permanent Focal Cerebral lschemia. *Sichuan Daxue Xuebao (Yixue Ban)* 2008; **39**: 84-88

34 **Zhang Y**, Wang L, Li J, Wang XL. 2-(1-Hydroxypentyl)-benzoate increases cerebral blood flow and reduces infarct volume in rats model of transient focal cerebral ischemia. *J Pharmacol Exp Ther* 2006; **317**: 973-979 [PMID: 16527903 DOI: 10.1124/jpet.105.098517]

35 **Lin JF**, Feng YP. [Effect of dl-3-n-butylphthalide on delayed neuronal damage after focal cerebral ischemia and intrasynaptosomes calcium in rats]. *Yao Xue Xue Bao* 1996; **31**: 166-170 [PMID: 9206264]

36 **Schumacher K**, Kornej J, Shantsila E, Lip GYH. Heart Failure and Stroke. *Curr Heart Fail Rep* 2018; **15**: 287-296 [PMID: 30062623 DOI: 10.1007/s11897-018-0405-9]

37 **Fan YL**, Zhan R, Dong YF, Huang L, Ji XX, Lu P, Liu J, Li P, Cheng XS. Significant interaction of hypertension and homocysteine on neurological severity in first-ever ischemic stroke patients. *J Am Soc Hypertens* 2018; **12**: 534-541 [PMID: 29678422 DOI: 10.1016/j.jash.2018.03.011]

38 **Dandapat S**, Robinson JG. Guidelines for Management of Hyperlipidemia: Implications for Treatment of Patients with Stroke Secondary to Atherosclerotic Disease. *Curr Neurol Neurosci Rep* 2016; **16**: 24

[PMID: 26838351 DOI: 10.1007/s11910-016-0621-1]
39 **Hu WS**, Lin CL. Use of the progression of adapted Diabetes Complications Severity Index to predict acute coronary syndrome, ischemic stroke, and mortality in Asian patients with type 2 diabetes mellitus: A nationwide cohort investigation. *Clin Cardiol* 2018; **41**: 1038-1043 [PMID: 29896758 DOI: 10.1002/clc.22991]
40 **Céspedes Rubio ÁE**, Pérez-Alvarez MJ, Lapuente Chala C, Wandosell F. Sex steroid hormones as neuroprotective elements in ischemia models. *J Endocrinol* 2018; **237**: R65-R81 [PMID: 29654072 DOI: 10.1530/JOE-18-0129]
41 **Zeng ZW**, Zhang YN, Lin WX, Zhang WQ, Luo R. A meta-analysis of pharmacological neuroprotection in noncardiac surgery: focus on statins, lidocaine, ketamine, and magnesium sulfate. *Eur Rev Med Pharmacol Sci* 2018; **22**: 1798-1811 [PMID: 29630129 DOI: 10.26355/eurrev_201803_14599]
42 **Bell JD**. In Vogue: Ketamine for Neuroprotection in Acute Neurologic Injury. *Anesth Analg* 2017; **124**: 1237-1243 [PMID: 28079589 DOI: 10.1213/ANE.0000000000001856]
43 **Fluri F**, Schuhmann MK, Kleinschnitz C. Animal models of ischemic stroke and their application in clinical research. *Drug Des Devel Ther* 2015; **9**: 3445-3454 [PMID: 26170628 DOI: 10.2147/DDDT.S56071]
44 **Hackam DG**, Redelmeier DA. Translation of research evidence from animals to humans. *JAMA* 2006; **296**: 1731-1732 [PMID: 17032985 DOI: 10.1001/jama.296.14.1731]
45 **Kilkenny C**, Browne WJ, Cuthill IC, Emerson M, Altman DG. Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. *Osteoarthritis Cartilage* 2012; **20**: 256-260 [PMID: 22424462 DOI: 10.1016/j.joca.2012.02.010]

Published By Baishideng Publishing Group Inc
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: bpgoffice@wjgnet.com
Help Desk: https://www.f6publishing.com/helpdesk
https://www.wjgnet.com