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REVIEW

Liver immunology and herbal treatment

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Abstract

Beyond the metabolic functions, the liver recently has been defined as an organ of immune system (IS), which have central regulatory role for innate and adaptive immunity. The liver keeps a delicate balance between hepatic screening of pathogenic antigens and immune tolerance to self-antigens. Herbal treatments with immunological effects have potential to alter this hepatic immune balance towards either therapeutic side or diseases side by inducing liver injury *via* hepatotoxicity or initiation of autoimmune diseases. Most commonly known herbal treatments, which have therapeutic effect on liver and IS, have proven *via in vitro*, *in vivo*, and/or clinical studies were summarized in this review.

Key words: Herbal treatments; Hepatic immunology; Drug induced liver injury; Adaptive immunity; Innate immunity

Core tip: Herbal treatment is the mother of modern medicine. The ancient habit of treating diseases with plants still goes on as either primary or complementary to conventional medical treatment. The other side of medallion is the fact that the liver is number one target organ for herbal toxicity. Furthermore, liver has been recently defined as an active organ of immune system, which have central regulatory role on innate and adaptive immune response. The delicate homeostasis between immediate and efficient defense against threats (immune surveilance of antigens) without triggering harmful immune response towards self-structures (periferal immune tolerance to self antigens) is controled by liver. Herbal formulas are not a single plant extract, but is an interacting mixture of ingredients that determines the final clinical outcome as therapeutic and hepatotoxic effect. This review aimed to drive attention on both potentials of herbals from the point of immunology, in order to initiate a motivation for feature studies defining

the mechanisms of immunological interaction between herbals and liver.

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INTRODUCTION

Herbal treatment is the mother of modern medicine. Because of cultural, economical and practical reasons, the ancient habit of treating diseases with plants still goes on as either primary or complementary to conventional medical treatment. The other side of medallion is the fact that the liver is number one target organ of herbal and dietary supplements (HDS) induced toxicity. The Food and Drug Administration (FDA) under the Dietary Supplement Health and Education Act (DSHEA) regulate herbal treatments since 1994[1]. The submissions of new herbal products to FDA require the dose and list of ingredients to be written on its bottle, however, documentation of safety and efficacy is not need to be reported. Furthermore, HDS can be obtained without prescription, medical advice or monitoring. Although the actual size of the problem is not well defined, HDS-induced hepatotoxicity accounts for 20% of cases of hepatotoxicity in the United States and the rates differ from 2.5% in India to 70% in Singapore[2].

The liver is prone to drug-induced liver injury (DILI) because of its functions on metabolizing chemicals and regulating immune response. DILI can develop either by dose related direct drug toxicity, or - much more commonly - as idiosyncratic reactions due to individual susceptibility to ingredients. The complex composition of HDS eases the both direct toxicity and idiosyncratic reactions during their metabolism in liver. Idiosyncratic DILI (IDILI) is in most instances characterized by a mild injury (ALT < 3 times upper normal limit) which normalized with continuous drug treatment. This phenomenon of clinical adaptation is a biochemical adaptive response of organelles such as endoplasmic reticulum and mitochondria metabolizing chemicals. It is hypothesized that defective clinical adaptation mechanisms result in severe IDILI with jaundice and liver failure, in < 0.1% population with susceptible human leucocyte antigen (HLA) type. Microbiota is the ecological community of commensal, symbiotic and pathogenic microorganisms that literally share our body space[3]. The microbiota of gut also determinates IDILI susceptibly by regulating hepatic immune-tolerance though lipopolysaccharides (LPS) induced T-cell response in liver. According to theory, haptens of metabolized chemicals covalently bind to proteins, and become antigenic peptides. While 70%-90% of population has immune tolerance, the rest develop adaptive immune response due to their susceptible type HLA and/or dysfunctional microbiota. If biochemical adaptation mechanisms cannot control this initiated mild injury, acute liver failure develops[4]. There are supporting evidences to this theory. The many top IDILI drugs are antibiotics changing the normal microbiota. The "immune check point therapy" for cancer treatments are FDA approved antibodies that aim to inhibit T cell immune-tolerant states, such as ipilimumab (anti-CTLA4), pembrolizumab (anti-PD-1) and nivolumab (anti-PD-1). The major side effect of these treatments is to make individuals to susceptible to haptens (so inducing IDILI) and auto-antigens (so inducing autoimmunity).

This review aimed to drive attention on both therapeutic mechanisms and hepatotoxic potentials of herbal treatments from the point of immunology. After defining basic immune system (IS), we summarized the role of liver as an immune regulatory organ and then the herbal treatments with therapeutic potential on liver.

IS

IS has evolved to recognize and eliminate internal insults (*i.e.*, cancer cells) or external invading pathogens (*i.e.*, infections) by developing local or systemic response. IS composed of "classical lymphoid organs"; thymus, bone marrow, spleen, tonsils, lymph nodes and "peripheral immune organs"; skin, respiratory and gut mucosa-associated lymphoid tissues, adrenal glands. Additionally, the gut and liver are recently defined as active organs of IS. Although the primary functions of liver parenchymal cells are methabolical, they also carry out essential immune tasks. Beside all metabolic functions, liver has important role as an organ of IS.

IS begins to develop during intrauterine life. However, maturation of IS depends on antigenic stimulations from environment. The gut microbiota is initiated by maternal microorganisms gained during passage through birth channel and it dynamically change according to external conditions. The gut microbiota is necessary for proper "education" of IS. Although IS completes its maturation around teenage, lifelong antigenic stimulations from microbiota is needed for normal functioning of IS. Epidemiological observations and then, experimental data from germ-free animals leads to "hygiene hypothesis" and its modern extension called "microflora hypothesis". According to these hypotheses, the higher levels of cleanliness and decreased exposure to microorganisms (driven by factors such as antibiotic use, xenobiotics, infection, or diet) during early childhood disrupt maturation if IS. In other words, the dysbiotic gut microbiota, which arisen during critical window of IS maturation, turns the differentiation of naïve immune gut dentritic cells (DCs) from generation of Treg (regulatory) cells by tolerogenic DCs into generation of effector T cells by immunogenic DCs. This shifts TH response from Th1 type (IFN-γ mediated) to Th2 type (IL-4 mediated). As a result, the risk for autoimmune and allergic diseases increases[5,6].

By definition IS has 2 parts; the innate IS and the adaptive IS. Although this division simplifies the understanding of immune processes, IS orchestrates

whole immune cells during local or systemic responses.

Innate IS

The innate IS initiates first defense against insults, and is characterized by its ability to distinguish self from non-self. Its members are classic immune cells such as polymorphic nuclear leukocytes (neutrophils), monocytes, macrophages and DCs, natural killer (NK) cells, and innate lymphoid cells (ILCs), beside epithelial, endothelial and mesenchymal cells which are non-immune cells.

The inflammation during innate immune response is triggered by pattern-recognition receptors (PRRs). The 3 families of PRR, according to the structure they can recognize, are Toll-like receptors (TLRs), retinoic acid-inducible gene I-like receptors and nucleotide-binding oligomerization domain-like receptors. The cells expressing PRR can recognize conserved structures. For instance, miRNAs controls multiple immune processes such as regulating the innate immune responses of macrophages, dendritic cells and NK cells; involving in T-cell differentiation and function. Furthermore defective PRR function might lead to autoimmune or auto-inflammatory diseases, since nucleic acids (DNA and RNA) is commonly shared by the pathogen and host. Additionally, damage associated molecular patterns (DAMP), pathogen associated molecular patterns (PAMP), microbiome associated molecular patterns are the subtypes of PRR[6,7].

Mononuclear phagocyte system (MPS) composed by monocytes, macrophages, and DCs that have phenotypical and functional overlaping boundaries leading to uncertainty in differentiating them form each other. Antigen-presenting cells (APCs) express PRR and characterized by their ability to recognize, process and present antigens for activation of innate and adaptive immunity. The classical APCs include DC, monocytes and macrophages, although parenchymal cells can also act as APCs. MPS may be precursor of some APCs of liver, namely DCs and Kuppfer cells (KCs)[8].

ILCs are a recently identified family of heterogeneous variety of T cells and non-T cells, including NK cells, CD56+ Tcells, natural killer T cells (NKT), gamma/deltaT cells, mucosal- associated invariant T cells, lymphoid tissue-inducer cells and cells that produce IL-5, IL-13, IL-17 and IL-22. ILCs not only regulate innate and adaptive immune responses by promoting DC maturation into APCs, they have function in lymphoid tissue formation and the homeostasis of tissue stromal cells remodeling the tissues[9,10].

Adaptive IS

The adaptive immunity evolutionarily developed later than innate immunity in high-class vertebrates. The adaptive immune response occurs as second phase of immune response, mediated mainly by lymphocytes, and characterized by the features of antigen-specific response and memory response. It is initiated by antigen presentation lymphocytes. The main lymphoid repertoire includes T-cells, B cells. B cells produce specific antibodies in response to a specific antigen. These antibodies are crucial for T cells activation against bacterial infections and development of active immunization after vaccination[7,11]. However, the major mediator of adaptive immune response is the T cells, which control both the establishment and regulation of adaptive immunity.

T cells are identified by CD3 and T cell receptors (TCRs) positivity, and have vital importance in the adaptive and innate immunity. Conventional T cells express alpha-beta type TCR. Gamma-delta T cells are located in skin, genitourinary tract mucosa and gut, as well as liver. The naive T cells produced in bone barrow migrates to thymus and differentiate into 2 main subtypes are Th (helper) and Ts (supressor). The differentiated T cells are exported to periphery, where they become effector T cells upon activation by APCs or B cells. T cell activation requires binding of TCR to major histocompatibility complex (MHC), as well as binding of co-stimulatory molecule present on T cells to its co-receptor on APCs (*e.g*., binding of CD28 to B7). Th cells express CD4, which recognizes antigens in the context of MHC class Ⅱ, and are mainly regulatory cells. Ts are cytotoxic cells carrying CD8 receptors, which are activated by MHC class I molecules[11]. Recently, CD4+ cells have been divided into subsets according to their distinct cytokine production and function; Th1, Th2, T17, Treg, Tfh (follicular T helper). Some features of CD4+ cells are as shown on Table 1. Treg cells express CD4+CD25+ and are essential for maintaining immune homeostasis and self-tolerance. Treg cells either naturally produced from CD4+ thymocytes in the thymus or iTreg cells are induced at periphery from naive CD4+ T cells in response to the low-dose stimulation of TCR, TGF-beta and IL-2. Beside all these effector T cells, there are also memory T cells. Id3 is the key transcriptional regulator for controlling T-cell differentiation into either effector T cells or memory T cells by its action though mTORC signaling[7,9,12].

DCs are professional APC, which can recognize foreign antigens by their PRR, initiate immune response and constitute a bridge between innate and adaptive immunity. They primary screen surrounding microenvironment by antigen sampling and direct IS towards pro- or anti-inflammatory response[6]. DCs are found throughout the body as immature DCs and subdivided as plasmacytoid (or lymphoid) DCs and myeloid DCs. Plasmacytoid DCs mediate anti-viral immunity by its capability of viral recognition and type 1 interferons secretion. The myeloid DCs constitute conventional MPS derived DCs in blood, interstitial DCs in tissues, Langerhan cells in skin and monocyte-derived DCs. mDC can internalise antigens by phagocytosis, pinocytosis or receptor-mediated endocytosis. After generation of peptide by proteolytic degradation within endocytic vesicles, it complexes with newly synthesized MHC class Ⅱ molecule within endocytic compartment, and then is carried *via* the trans-Golgi network to the cell surface. The recognition and internalization of pathogens by DCs leads to maturation of them into professional APCs, which have altered adhesion molecule and chemokine receptor expression.

Table 1 Features of CD4+ cell subsets

	Th1	Th2	T17	Treg	Tfh
Produced Cytokines	IFN-gama, TNF-alpha, IL-2	IL-4, IL-5, IL-9, IL-10, IL-13	IL-17A, IL-17F, IL-21, IL-22, IL-26	TGF-beta, IL-10	CXCR5, IL-21
Immune response mediated aginst	Intracellular pathogens	Extracellular parasites, allergy, humoral response	Extracellular bacteria and fungi, autoimmunity	IgA secretion, self-tolerance	Differentiation of B cells
Master transcription factors for differentiation	T-bet	GATA-3	RORct	Foxp3	Bcl6
Effected cells	Macrophages, cytotoxic cells activated	Eosinophils, mast cells activated	Neutrophils activated	B cells activated Th1, Th2, Th17 supressed	B cells activated

IFN: Interferons; IL: Interleukin; TGF: transforming growth factor; TNF: Tumor necrosis factor.

After maturation DCs leave primary side of infection through lymphatic's to carry the internalized pathogen to secondary lymphoid organ. The professional APCs can be either immunogenic DCs which express high levels of MHC and co-stimulatory molecules, and secrete IL-12, IL-18, IL-21 and IL-23 or tolerogenic DCs having low expression levels, express inhibitory receptors, such as programmed death ligand-1, and releasing suppressive cytokines, such as IL-10, IL-27 and TGF-beta. Immunogenic DCs stimulate naïve CD4+ T cells to differentiation into effector cell mediating adaptive immunity against specific pathogen. On the other hand, if the antigenic peptid is presented to naïve CD4+ T cell by tolerogenic DCs, immune tolerance develops either at thymus or periphery. The consequen result in thymus is either T cell apoptosis or T cell maturation into natural Treg cells. The mechanisms for peripheral immune tolerance are anergy of T cells and exhaustion of T cells. The anergy arises when T cells are inactivated due to lack of co-stimulation. The exhaustion of T cells is characterized by expression of inhibitory receptors, namely pro-grammed death-1 (PD-1), cytotoxic T lymphocyte antigen-4 (CTLA4) and T cell immunoglobulin mucin-3. Interestingly in mice and humans, the lipid content of DCs in liver determines the maturation type of APCs, as lipid content decreases tolerant immune response is favoured. This phenomenon might be important for progression of simple steatosis into steatohepatitis. The relationship of autoimmune diseases with infection and environmental pollution and is very well known fact. It is though that the similarity between insulting antigen and self antigens of individuals with susceptible HLA haplotypes causes a shift during APCs maturation from tolerogenic DCs towards to immunogenic DCs, leading to differention of naive T cells into effector ratherden tolerogenic cells, and ending in loss of self-immune tolerance[1,9,11].

NK express CD56 in the absence of CD3, but NKT express both of them. NKT mediate anti-tumor effect by activating CD8+ T cells cytotoxicity or overriding the tolerogenic mechanisms through counter-regulation of Treg cells. NKT can identify glycolipid antigens and subtyped into two according to TCR expression profile. Type 1 or invariant NKT (iNKT) carry an invariant TCR alfa-chain pairing with a limited number of beta-chains, whereas type 2 NKT cells express a diverse array of TCRs that recognize CD1d which is MHC class I-like molecule. Innate T lymphocytes (ITLs) is composed of iNKT cells and gamma-delta T cells. ITLs regulate adaptive immune response through its key roles in initiation and polaration of APCs and other cells of IS. This feature of ITLs has made them target as immunomodulation for treatment of autoimmune diseases[1,9].

LIVER AS AN ORGAN OF IS

In order to keep homeostasis for survival, the immune response had to continuously adopt according to age, sex, dietary antigens, hormones (*i.e.*, pregnancy and lactation), and external stress factors such as microbiota, environmental flora or exposed chemicals[13]. Therefore IS has a dynamic nature and has a wide "range of normal". Beyond being a metabolic organ attached to gut, liver recently has been defined as central axis in IS controlling local and systemic immune reactions and tolerance. All types of liver cells have active immune function, including both parenchymal cells (hepatocytes, cholangiocytes) and non-parenchymal cells [liver sinusoidal endothelial cells (LSECs), hepatic satellite cells (HSCs) or ito cells, KCs, neutrophiles, mononuclear cells, lymphocytes (B cells, T cells, NK cells, NKT cells, ITL)]. Parenchymal cells occupy most of liver volume (78%-80%). Non-parenchymal cells and extracellular space represent the remaining 5%-6% and 14%-17%, respectively[11,14].

The unique anatomical and histological features of liver are important for its immune functions. The liver is located at the junction between systemic and portal circulation. It is supplied by approximately 1.5 L of blood every minute; 2/3 *via* the portal vein and 1/3 *via* the hepatic artery. The double blood supply carries a massive antigenic load from the gastrointestinal tract and systemic circulation to liver. The blood, coming from these two sources mixes within sinusoids, and then flows through hepatic lobule from peri-portal area towards central vein. The fenestrated structures of sinusoids enable intimate interaction of antigens and blood immune cells with hepatocytes, KCs and HSCs at space of Disse. The abundant cells of the innate and adaptive ISs are located in hepatic sinusoids, and have ability for pathogen sensing, phagocytosis, cytotoxicity, cytokine release and antigen presentation to T cells.

The antigen-rich blood passing through the liver sinusoids is "scanned" by IS, which is tightly regulated between activation and tolerance. The liver remains tolerant to harmless dietary antigens, products of commensal gut microbiota and auto-antigens, while responds to exogen toxins, a variety of blood-borne or gut originated viruses, bacteria and parasites, as well as to metastatic cells, which try to home to the liver. Therefore, immune roles of liver can be divided into 2 groups; immune surveillance and induction of peripheral immune tolerance[3,15]. Indeed, the hepatic IS plays predominantly tolerogenic role. This can clinically be observed in liver transplant patients, *e.g.*, liver allograft from major MHC or even ABO mismatched donors can be transplanted; if combined transplantation is done with organs from the same donor, non-liver allografts are more likely to be accepted; "operational tolerance", which describes a patient with clinically normal graft function without needing immunosuppression, developments in up to 50% of hepatic transplantations[16].

Immune surveillance function of liver

The liver relies on its strong immunity for its immediate and efficiend defense against potentially toxic agents without triggering harmful immune response towards self-structures. Liver, primarily hepatocytes, synthesizes the major amount of proteins involved in local and systemic immune responses. These proteins are called acute phase reactants such as fibrinogen, proteinase inhibitors, complement proteins, PRRs [*e.g.*, C reactive protein, lipopolysaccharide (LPS)-binding protein, peptidoglycan-recognition protein, soluble CD14], opsonizing proteins (*e.g.*, mannose-binding lectin, serum amyloids), cytokines [*e.g.*, IL-6, tumor necrosis factor-alpha (TNF-alpha) and transforming growth factor-beta (TGF-beta)], and hepcidin. The acute phase reactants function during innate immune response, mediate inflammation as well as tissue repair and regeneration. Their expression in hepatocytes is controlled by liver-enriched transcription factors (*e.g.*, HNFs, C/EBPs), pro-inflammatory cytokines (*e.g.*, IL-6, IL-22, IL-1β, TNF-α), and downstream signaling pathways (*e.g.*, STAT3, NF-kappa B)[7,15].

Neutrophils are short-lived, circulating, phagocytic cells, which are recruited to site of infection by cytokines and chemokines, mainly IL-1 and IL-8. They are the first responders to infections and act by three main mechanisms; phagocytosis (requiring opsonization), generation of reactive oxygen species and degranulation (releasing enzymes and antimicrobial peptides), and formation of neutrophil extracellular traps (NETs). NETs are another mechanism of microbe killing. Nuclear DNA ligated with various microbicidal proteins released by activated neutrophils forms these webs. Under normal conditions the liver have few neutrophils, but they rapidly accumulate following necrosis. Neutrophils can rapidly shift their adhesive mechanisms in order to reqrude and form NETs in liver as a response to both endotoxin and bacteria[14].

Macrophages have been classified as classically activated macrophages (M1, secretes TNF-α, IL-1, IL-6, IL-8 and IL-12) or alternatively activated macrophages (M2, secretes IL-10 and TGF-β) based on their cytokine secretory patterns and proinflammatory *vs* immunoregulatory activity which however, are interchangeable functional states depending on the microenvironment the macrophages encounter[6,11]. KCs are fixed macrophages specialized at eliminating insoluble waste by phagocytosis and capable of processing and presenting antigens to T cells and participate in the regulation of the adaptive immune response. KCs reside on intravascular side of LSECs, and capture bacteria and able to bind component 3b under shear conditions while flowing through sinusoids. The role of KCs in microbial killing depends on the nature of the pathogen and on the recruited immune cells to the inflammation side. The characteristic feature of PRRs expression in liver is their constitutive expression and continuous low-level stimulation by endotoxins from gut. TLR4 is an PRRS expressed on all liver cells and it binds and clears endotoxins, and so initiates secretion of pro-inflammatory and anti-inflammatory cytokines[7].

Although, more than 80% of the CD3+ T cells are alpha-betaT-cells, the liver is also enrichmented by NKs and unconventional lymphocytes (NKT and gamma-delta T cells). The gamma-deltaT cells is 5 times higher in the liver (15%) then the periphery[4].

Hepatocytes, which constitutively express intercellular adhesion molecule-1, can directly interact with T cells through the fenestrations of LSECs. IFN-gamma primes hepatocytes to APCs by dose dependently enhancing HLA expression; from moderate HLA class I expression to enhanced HLA class Ⅱ expression at low to high IFN-gamma levels. Hepatocyte primed naïve T cells either become effector T cells or undergoes apoptosis in the absence of co-stimulatory signals. On the other hand, cholangiocytes are relatively spared form antigenic stimulation from blood, but not from those one secreted into bile. Cholangiocytes can express TLRs, HLA class I at a low frequency and co-stimulatory molecules. Hepatotropic viruses (*i.e.*, CMV) enhances HLA class I expression without inducing HLA class Ⅱ. In pathological conditions such as that of PBC, cholangiocytes act as APC by overexpress HLA class Ⅱ, as well as CD80 and CD86 co-stimulatory molecules. The limited experimental data supports that HSCs have capacity to act as APCs. The presentation of lipids to T-cells and NKT cells by HSCs can cause activation or tolerance in IS depending on co-stimulation[4,7].

Liver mediated peripheral immune tolerance

Besides conferring strong local innate immunity, the liver regulates immune homeostasis as being a major site for induction of T cell mediated local and systemic adaptive immune response. Both resident and transiting T and B cells scattered throughout the parenchyma and the portal tracts become important effector cells of defensive adaptive immune in liver after activation by APCs. Both hepatic parenchymal and non-parenchymal cells can act as APCs depending on the stimulus and special cytokine milieu. The classical hepatic APCs are

DCs and reticulo-endothelial system (including KCs and LSECs). However, hepatocytes and cholangiocytes become non-conventional APCs by expressing MHC Ⅱ, if there is under pathological insult or persistent inflammation. Classical hepatic APCs constitutively express MHC class Ⅰ-Ⅱ, co-stimulatory receptors and molecules that promote antigen uptake (*e.g.*, mannose and scavenger receptors). Under the physiological liver conditions, DCs are at immature developmental status and there is high production an anti-inflammatory cytokine (IL-10, TGF-b, TNF-a and prostaglandins) from reticulo-endothelial cells. This reduces capacity of APCs to activate effector T cells and lead to generation of anergic T cells and Treg cells. In other words, the tolerogenic nature of the liver by preferentially suppressing adaptive immunity is created by APCs, which kill or suppress effector CD4+ and CD8+ T cells and induce maturation of naïve T cells into Treg cells. Since diversion of portal flow results in the loss of immune-tolerance, it is hypothesized that physiological concentration of endotoxin is essential for maintain hepatic immune tolerance. LPS from gut microbiota drained to the liver by portal vein, modulates LSECs mediated CD4+ T cell activation by inducing secretion of IL-10 from LSECs and by down-regulating expression of MHC class Ⅱ, CD80 and CD86 on LSEC. Another proposed mechanism for hepatic induction of peripheral immune-tolerance is clonal deletion/apoptosis of antigen-specific T cell at liver. HSCs may have a role in creation of tolerogenic micro-environment. HSCs have a capacity to serve as APCs, expand Treg cells, and promote T cell apoptosis (*via* B7-H1, PDL-1) or inhibit cytotoxic CD8+ T cells[4,9,15].

The local and systemic self-tolerance can be over-ridden and so autoimmune diseases can be initiated by several pathological immune mechanisms developed in liver. First of all, pathological antigen presentation might generate of auto reactive T cells and B cells due to defective clonal deletion (apoptosis of antigen-specific T cells). Similarly ILCs also switch on the autoimmunity by promoting antigen presentation with classical APCs, by releasing cytokines that polarize immune response towards effector T cells. ILCs may also be important mediators autoimmune liver injury by killing hepatocytes and/or bile duct epithelial cells. Pathological endotoxemia caused by dysbiotic microbiota may switch immune response from Th2 to Th1 predominance. Treg cells regulate both innate and adaptive immunity through regulation of CD4+ cells, KCs and LSECs. Therefore, defective function of Treg cells impairs hepatic immune tolerance leading to autoimmune hepatitis[4,7].

SPECTRUM OF IMMUNE HOMEOSTASIS IN LIVER

Hepatic IS is always active, regardless the overall response outcome. The nature of insult to liver and spectrum of activated cells determines the clinical picture. Healthy individuals have balanced immune surveillance of pathogens together with immune tolerance towards self-antigens. The over immune tolerance in liver leads to chronic infections with viruses or hepatic metastasis of cancer cells. In contrast the over activation of hepatic immune response causes fulminant hepatitis, allograft rejection or autoimmune diseases.

Hepatic immune hemoastasis is continuously re-balanced during clinical courses of cirrhosis. Patients with compensated cirrhosis have hyperactivated IS depending on underlying etiology of the liver diseases. Hepatic decompensation is associated with increased intestinal permeability. The episodic translocation of gut microbiota and their endotoxins into portal circulation triggers systemic and hepatic inflammation. PAMRs recognizing LPS, lipopeptides, glycopolymers, flagellin and bacterial DNA/RNA, activate innate and adaptive immunity. The released pro-inflammatory cytokines and chemokines cause hepatic injury and activation of DAMP. The viscous cycle between members of PRR, namely PAMP and DAMP exhausts IS and so, switches immune response from a predominantly "pro-inflammatory" to wards "immunodeficient" status. This very late stage of cirrhosis is clinically defined as acute- on-chronic liver failure (ACLF). The immune deficient state in ACLF patients is called cirrhosis associated immune deficiency[15].

HERBAL TREATMENTS WITH POTENTIAL THERAPEUTIC EFFECT ON LIVER

Herbal treatments are very often multifaceted blends of slightly processed medicinal plants, parts of the plants or products of the medicinal plants, which are traditionally accepted, comparatively low side-effects, and naturally compatible with the human body. Herbal remedies are applied for the treatment of a variety of indications and disorders, including hepatic as well as immunological problems (Table 2). Since scientific studies on herbal treatments have shown that they might effect cytokine and immunoglobulin secretion, cellular co-receptor expression, histamine release, lymphocyte proliferation, and cytotoxic activity, thus, herbal preparations might modify immune functions. In this study, literature was surveyed based *on in vitro*, *in vivo* and clinical studies on hepatoprotective, as well as immunostimulant and/or immunomodulator effective medicinal plants, which are lead by ethnopharmacological data. Table 2 was established, which covers common name, scientific name, effective part, known phytochemical content of the plant, ethnopharmacological/clinical effects, and medicinal preparation with their corresponding references. Due to complexity of the herbal treatments, the complete scientific data on mechanism of action is lacking, although clinical outcomes of herbal treatment are promising and leading the researchers to take on demanding scientific studies on the immune activity of herbal remedies (Table 3). The most applied medical plants in herbal treatments were selected and their mechanism of action on liver diseases and on immun system were searched to establish the Table 3. Flavonoid

Table 2 Plants are effective on liver disorders and immune system

Common name	Scientific name	Effective part	Phytochemical content	Preparation on liver disorders[1]	Ref.
Chaff-flower	*Achyranthes aspera* L.	Whole plant	Ecdysterone, achyranthine, betaine, pentatriaontane, 6-pentatriacontanone, hexatriacontane and tritriacontane	Natrossil natiris	[28-30]
Fennel	*Foeniculum vulgare* Mill.	Root	Coumarins (bergapten, isopimpinellin, anthotoxin), flavonoids (quercetin, rutin)	Presselin dyspeptikum presselin, bupleurum compound phytomedicine, epagest lampugnani	[18,25,31]
Korean Ginseng, Chinese Red Ginseng	*Panax ginseng* Mey.	Root	Polysaccharides, saponins, ginsenoside	Tripid teguhsindo	[32-34]
Yarrow	*Achillea* sp.	Flower	Volatile oils, flavonoids, terpenoids, alkaloids, saponins, sesquiterpenlactones	Liv-52 drops, cheiranthol klein	[35-38]
Carqueja	*Baccharis trimera* (Less) DC	Epigeous part	Flavonoids, diterpenoids	Boldina Plata	[39-41]
Chicory	*Cichorium intybus* L.	Aerial part, root, leaf	Saccharides, methoxycoumarin cichorine, flavonoids, essential oils, anthocyanins	Natusor hepavesical soria natural, Liv-52 drops	[42-46]
Globe artichoke	*Cynara cardunculus var. scolymus* L.	Leaf	Sesquiterpenes lactones (cynaropicrin), flavonoids (cynaropicrin), phenolic acids (mainly caffeic acid derivatives)	Livstim mediherb, livton complex mediherb, lorbihepatic bioquimico, olocynan makros, rapacholin C herbapol wroclaw, farmasa, sylicynar herbapol poznan, alcafelol luper, bagohepat bago, armstrong, benevolus schwabe, boldina plata, cinarepa cristalfarma, colachofra EMS, cynarex roux-ocefa, herbapol wroclaw, cynarzym N altana, digestron loprofar, epagest lampugnani, figatil catarinense, salus, hecrosine B12 ortoquimica, hepatofalk falk, jurubileno ibefar	[47,48]
Pale purple coneflower	*Echinacea pallida* (Nutt.) Nutt. *Echinacea angustifolia* (DC.) Hell. *Echinacea purpurea* (L.) Moench	Whole plant	Alkamides, polysaccharides, glycoproteins, cichoric acid (a derivate of caffeic acid)	Andrographis complex mediherb, kalbe, hepatin lapi, imudator pyridam, herbal cleanse vitaplex	[49-52]
Faise daisy	*Eclipta alba* (syn *E. prostrata* L.)	Aerial part	Tannins, flavonoids, coumestans, saponins, alkaloids	Dipana promed	[53,54]
Chamomile	*Matricaria chamomilla* L.	Flower	Coumarin (herniarin and umbelliferone), phenylpropanoids (chlorogenic acid and caffeic acid), flavonoids (apigenin, apigenin-7-o-glucoside, luteolin, luteolin-7-o-glucoside, quercetin, rutin, naringenin), blue essential oils	Presselin dyspeptikum presselin, cholesol herbapol wroclaw, gotas digestivas bunker	[42,55-57]
Milk thisle	*Silybum marianum* (L.) Gaertn.	Seed	Polyphenolic flavonoids(silymarin, isosilyins, silibinins, silydianin silychristin)	Liverine cardinal, livermin korean ginseng, liverton siffra, livosil-b centaur, livstim mediherb, livton complex mediherb, lomacholan lomapharm, phytohepar steigerwald, poikicholan lomapharm, prol procare, samarin berlin pharm, schwohepan S schworer, silegon teva, silibene merckle, silicur hexal, silimalon nikkho, silimarin benedetti, silimarit bionorica, silimax filofarm, silirex lampugnani, siliver farmasa, silliver abbott, silmar hennig, silvaysan sanum-kehlbeck, silybon micro, silygal ivax, silyhexal hexal, sily-sabona sabona, mepha, sivylar ranbaxy, sylicaps herbapol lublin, sylicynar herbapol poznan, sylimarol herbapol pruszkow	[18,19,58,59]

				Syliverin aflofarm, sylivit herbapol poznan, solas, vionin nf tempo scan pacific, alepa duopharm, apihepar madaus, *vıa* tris, aptivium liver support cynergen, ardeyhepan emonta, ardeypharm, bibol leloup hexa, bilisan duo repha Bioglan liver-vite bioglan, bupleurum complex mediherb, bupleurum compound phytomedicine, carsil sopharma, cefasliymarin cefak, cheiranthol klein, soho capsule/syrup, depatox progen, durasilymarin merck dura, eleparon sankyo, epagest lampugnani, flavobion zentiva, hegrimarin strathmann, strathmann, hepabene ratiopharm, merckle, ratiopharm, hepabesch strathmann, hepadigenor baliarda, hepaduran v otw, loges Capsule, hepamax dankos, hepa-merz sil merz, hepar-pasc pascoe, heparsyx n syxyl, heparviton bode, tempo scan pacific, hepatin lapi, kalbe, falk, darya-varia, hepatos, Yung shin, heplant spitzner, herbal liver formula faulding, worwag, legalon-madaus, laragon roemmers, ıfet, leveron vesco, limarin serum ınstitute, herbal cleanse vitaplex	
Dandelion	*Taraxacum officinale* G.	Root	Sesquiterpenes, saponins, phenolic compounds, flavonoids, sugars	Naturica DFM ıkapharmindo, livstim mediherb, livton complex mediherb, berberis complex blackmores, cholesol herbapol wroclaw, cinarepa cristalfarma, hepatofalk falk, herbal cleanse vitaplex	[60-63]
Radish	*Raphanus sativus* L.	Leaf, root	Flavanoids, terpenoids, alkaloids, saponins, sterols	Rapacholin AC herbapol wroclaw, rapacholin c herbapol wroclaw	[64-67]
Caper	*Capparis spinosa* L.	Root bark	Sugars (glucose, arabinose, mannose, galactose), lipid, volatile oils	Liv-52 drops	[68-71]
Kinkéliba	*Combretum micranthum* G. Don	Leaf	catechins, glycosylflavones, flavans, galloylated c-glycosylflavone derivatives, flavan-piperidine alkaloid	Tisane mediflor N°5 hepatique	[72-74]
Arjuna	*Terminalia arjuna* (Roxb.) Wight and Arn.	Bark	Arjunolic acid, tomentosic acid, arjunin, *β*-sitosterol, ellagic acid, leucodelphinidin, tannins	Liv-52 drops	[75-77]
Coffee senna	*Cassia occidentalis* L.(*Senna occidentalis*)	Leaf	Anthraquinones, saponins, sterols, triterpenes, quinines, tannins, flavonoids	Tisane mediflor N°5 hepatıque, Liv-52 drops	[78-80]
Liquorice, Licorice	*Glycyrrhiza glabra* L.	Root	Triterpene saponins, flavonoids, isoflavonoids and chalcones, glycyrrhizic acid	Tisane mediflor N°5 hepatıque, neo-minophagen C dexa, torii, curliv soho, soho capsule/syrup	[18,26,81]
Holy basil	*Ocimum sanctum* Linn.	Leaf	Volatile oils (eugenol, euginal, urosolic acid, carvacrol, linalool, limatrol, caryophyllene, methyl carvicol), anthocyans, alkaloids, flavonoids, tannins, carbohydrates,xylose, polysaccharides	Andrographis complex mediherb	[22,82-85]
Rosemary	*Rosmarinus officinalis* L.	Leaf	Diterpenoids, triterpenoids, phenolic acids, and flavonoids, carnosic acid, carnosol, rosmarinic acid	Tisane mediflor N°5 hepatıque, natusor hepavesical soria natural, cinarepa cristalfarma	[86-88]
Red sage, Danshen	*Salvia miltiorrhiza* Bunge.	Root	Tanshinones (tanshinone ı, tanshinoneıı, cryptotanshinone) miltirone and salvianolic acid a, b	Bupleurum complex mediherb	[18,89,90]
Common mallow	*Malva sylvestris* L.	Leaf	Amino acids/protein derivatives, flavonoids, mucilages, terpenoids, phenol derivatives, coumarin	Tisane mediflor N°5 hepatıque	[91-93]
Tinospora, Guduchi, Giloya	*Tinospora cordifolia* (Willd.) Hook. f. and Thoms. (Guduchi)	Root, stem	Flavonoids, alkaloids, sesquiterpenes, diterpenes arabinogalactan, syringine, cordiol, cordioside, cordifoliosides (a and b), berberine, tinosporine, giloin, giloinin	Dipana promed	[22,33,94-96]

Boldo, Boldu, Boldus	*Peumus boldus* Molina	Leaf	Alkaloids (isoquinoline-boldine, isoboldine, 6a,7-dehydroboldine, isocorydine, isocorydine-n-oxide, norisocorydine, laurolitsine, laurotetanine, n-methyllaurotetanine, reticuline, (-)-pronuciferine, sinoacutine), flavonoids, volatile oil, coumarin, resin, tannin)	Tisane mediflor N°5 hepatıque, natrossil natiris, natusor hepavesical soria natural, prinachol zurita, farmasa, alcafelol luper, berberis complex blackmores, boldina plata, boldopeptan neo quimica, colachofra ems, cynarzym N altana, eparema nycomed, figatil catarinense, gotas digestivas bunker, jurubileno ıbefar	[97-99]
Greater celandine	*Chelidonium majus* L.	Aerial parts	Isoquinoline alkaloids, such as sanguinarine, chelidonine, chelerythrine, berberine and coptisine, (-)-turkiyenine)	Livstim mediherb, livton complex mediherb, natusor hepavesical soria natural, schwohepan S schworer, berberis complex blackmores, chelicur hasco-lek, cynarzym N altana, hepatofalk falk, falk, darya-varia	[100-105]
Gale of the wind	*Phyllanthus niruri* L.	Whole plant	Flavonoids, alkaloids, terpenoids, lignans, polyphenols, tannins, coumarins and saponins	Natrossil natiris, meprofarm, dipana promed, gramuno graha, hepimun landson, ımudator pyridam	[106-108]
Kutki	*Picrorhiza kurroa* Royle ex Benth.	Rhizome, root	Iridoid glycoside (picrovil)	Dipana promed	[109,110]
Rhubarb	*Rheum emodi*	Rhizome	Anthraquinone (rhein, chrysophanol, aloe-emodin, emodin, physcion, and their glycosides) and stilbene (picetannol, resveratrol and their glycosides), flavonoids, glycosides, tannins, volatile oils, saponins	Natrossil natiris, boldopeptan neo quimica, eparema nycomed, SIT, hepatofalk falk	[111]
Magnolia-vine, Schisandra	*Schisandra chinensis* (Turcz.) Baill.	Fruit	Dibenzocyclooctadiene derivative lignans (or schisandra lignans), organic acids (citric, malic, fumaric and tartaric acid), sugars, vitamic C, vitamin E, phenolic acids, tannins, phytosterols, essential oil	Curliv soho, soho capsule/syrup, hepacell medikon, hepamax dankos	[31,112,113]
European black nightshade	*Solanum nigrum* L.	Whole plant	Glycoalkaloids, glycoproteins, polysaccharides, polyphenolic compounds (gallic acid, catechin, protocatechuic acid (pca), caffeic acid, epicatechin, rutin, naringenin)	Liv-52 drops, dipana promed	[114-117]
French tamaris	*Tamarix gallica* L.	Aerial parts	Tannin, tamarixin, tamauxetin, troupin, 4-methylcoumarin and 3,3′-di-o-methylellagic acid, tannic acid, 4-methylcoumarin and 3,3′-di-o-methylellagic acid	Liv-52 drops	[118,119]
Turmeric	*Curcuma longa* L.	Rhizome	Curcuminoid	Meprofarm, tripid teguhsindo, turmerik knop, aptivium liver support cynergen, chelicur hasco-lek, galena, ıvax, cinarepa cristalfarma, depatox progen, heparviton bode, tempo scan pacific, kalbe, hepatin lapi, falk	[22,31,120]
Ginger	*Zingiber officinale* Roscoe	Rhizome	Volatile oils, pungent phenol compounds [sesquiterpenoids,beta-sitosterol palmitate, isovanillin, glycol monopalmitate, hexacosanoic acid 2,3-dihydroxypropyl ester, maleimide-5-oxime, p-hydroxybenzaldehyde adenine, 6-gingerol, 6-shogaol, 1-(omega-ferulyloxyceratyl) glycerols]	Presselin dyspeptikum presselin, dipana promed, herbal cleanse vitaplex	[121-127]

[1]Martindale W, Sweetman SC. Martindale: The complete drug reference. Pharmaceutical press, 2007.

derivatives such as silybin, silymarin, obtained from milk-thistle [*Silybum marianum* (L.) Gaertn.] decreased alkaline phosphatase (completely) and gamma-glutamyl transpeptidase (partially) in CCl_4 induced liver damage[17]. Moreover, it has been claimed that Silymarine containing preparations are the principal therapeutic of choice in liver diseases caused by oxidative stress. Many studies have proven that plant phyto compound Silymarin has medical applications to cure (alcoholic and non-alcoholic) fatty liver, cirrhosis, ischaemic injury, drug and chemically-induced hepatic toxicity, radiation toxicity, viral and toxic hepatitis by means of its anti-oxidative, anti-lipidperoxidative, anti-fibrotic, anti-inflammatory, liver regenerating and immunomodulating effects. Several studies have identified that continuous usage of Silymarin has significantly proved to increase the survival period of patients with alcohol-caused liver cirrhosis and primary liver cancer[18] (Figure 1). Scientific studies also

Table 3 Commonly used plants on liver disorders and their effect mechanisms

Scientific name	Effect/mechanism on liver	Effect/mechanism on immun system	Ref.
Curcuma longa L.	Acute liver damage by chemicals, *e.g.*, ethanol, CCl_4, Dimethylnitrosamines	Immunostimulant, immunomodulatory The extract of the rhizome *C. longa* increased both Th1 (IL-2 and IFN gamma) and Th2 (IL-10) cytokines indicating its dual immune functions. NR-INF-02 significantly increased the IL-2 and IFN gamma levels in Con A stimulated splenic lymphocytes. The above results indicated that NR-INF-02 showed a specific immunity response by stimulating both Th1 and Th2 cells Polysaccharide fraction of the rhizome showed potent immunostimulatory activity towards proliferation of splenocytes cell number and IL-10 secretion. Polysaccharides might be contributing to this proliferative and cytokine release property in murine splenocytes Hot water extracts of the rhizome showed that the high polarity fraction exhibited stimulatory effects on PBMC. The cytokine productions (TGF-beta, TNF-alpha, GM-CSF, IL-1alpha, IL-5, IL-6, IL-8, IL-10, IL-13, *etc.*) have been modulated by a polysaccharide-enriched fraction.The proportion of CD14 positive stained PBMC was increased by the fraction Anti-HIV-1 and HIV-2	[18,22-24]
Foeniculum vulgare Mill.	Oxidative stress of the liver bacterial and viral infections anti-inflammatory, acute hepatotoxicity	Immunomodulatory Antimicrobial, antifungal	[18,25]
Glycyrrhiza glabra L.	Cirrhosis fibrosis chronic viral hepatitis B and C	Immunomodulatory Leukocyte count and phagocytic index (carbon clearance) was increased significantly with the treatment of water extract of *G. glabra* root. Zinc (45 mg/kg) in combination with ALE (0.75 g/kg) showed highly significant increase of leukocyte count and phagocytic index	[18,26,27]
Silybum marianum (L.) Gaertn.	Oxidative stress inflammation and fibrosis alcohol-induced cirrhosis mushroom poisoning viral hepatitis	Immunomodulatory Flavonoids from *S. marianum* normalize immunoregulatory defects *via* restoration of the cellular thiol status. T-cell activation (CD69), along with a significant decrease in TNF	[18,19]

IFN: Interferons; TNF: Tumor necrosis factor; IL: Interleukin; TGF: transforming growth factor; HIV: Human immunodeficiency virus; PBMC: Peripheral blood mononuclear cells; GM-CSF: Granulocyte-macrophage colony-stimulating factor; ALE: Aqueous liquorice extract.

have shown that, both silybin and silymarin normalized immunoregulatory failures by restoration of the cellular thiol status, T-cell activation (CD69), together with a substantial decrease in TNF[18,19]. Effects of the selected herbal medicines on immune and liver were summarized in Figure 1. Another study demonstrated that an edible plant Artichoke (*Cynara scolymus* L.) prevented CCl_4 and oxidative stress-induced hepatotoxicity and it protected the liver[20]. Inulin, obtained from artichoke, stimulates components of the IS[21]. The extract of the rhizome Turmeric (*Curcuma longa* L.), which has hepatoprotective plant, amplified both Th1 (IL-2 and IFN gamma) and Th2 (IL-10) cytokines signifying its dual immune roles. Polysaccharide fraction of this rhizome showed potent immunostimulatory action in the direction of proliferation of splenocytes cell number and IL-10 secretion. Polysaccharides of the plant extract might be causative of these proliferative and cytokine release assets in murine splenocytes. In different studies have been shown that the cytokine productions (TGF-β, TNF-α, GM-CSF, IL-1α, IL-5, IL-6, IL-8, IL-10, IL-13, *etc.*) have been modulated by polysaccharide-enriched fractions[18,22-24]. Fennel (*Foeniculum vulgare* Mill.) and liquorice (*Glycyrrhiza glabra* L.) have also shown immunomodulatory and hepatoprotective effects[18,25-27].

Herbal drugs are composed of complex mixtures of phytochemicals, unlike conventional and plant originated single compound drugs, which are composed of known chemical constituents and are precisely quantified. For that reasons studying the clinical effects of individual chemical constituents separately will not be accurate, due to various reasons, such as the synergistic or inhibiting effects of phytochemicals on each other and neutralization of harmful chemicals in the mixture by other compounds, which provides a flawless combination for therapeutic purposes. Aspects such as, absorption, distribution intrinsic concentration and metabolism of the drug should be known precisely to determine the dosage, safety margin and length of treatment. Moreover, future research should include characterization of multifactorial mechanisms of action, elucidation of adverse effects and well-designed clinical trials in pediatrics and geriatrics as well.

CONCLUSION

Scientists as well as immunologists, who study herbal treatments in hepatic diseases most be ready to face challenges and opportunities. *In vivo* and clinical molecular researches on immunomodulatory, immunoenhancing, immunostimulant effects of herbal treatments will offer novel perceptions into IS and immunotherapy. Not only single plant extract, but the interactions of the ingredients in a given herbal treatment formula determines the final clinical picture by finely tuning the balance between therapeutic effect and hepatotoxicity. Feature studies

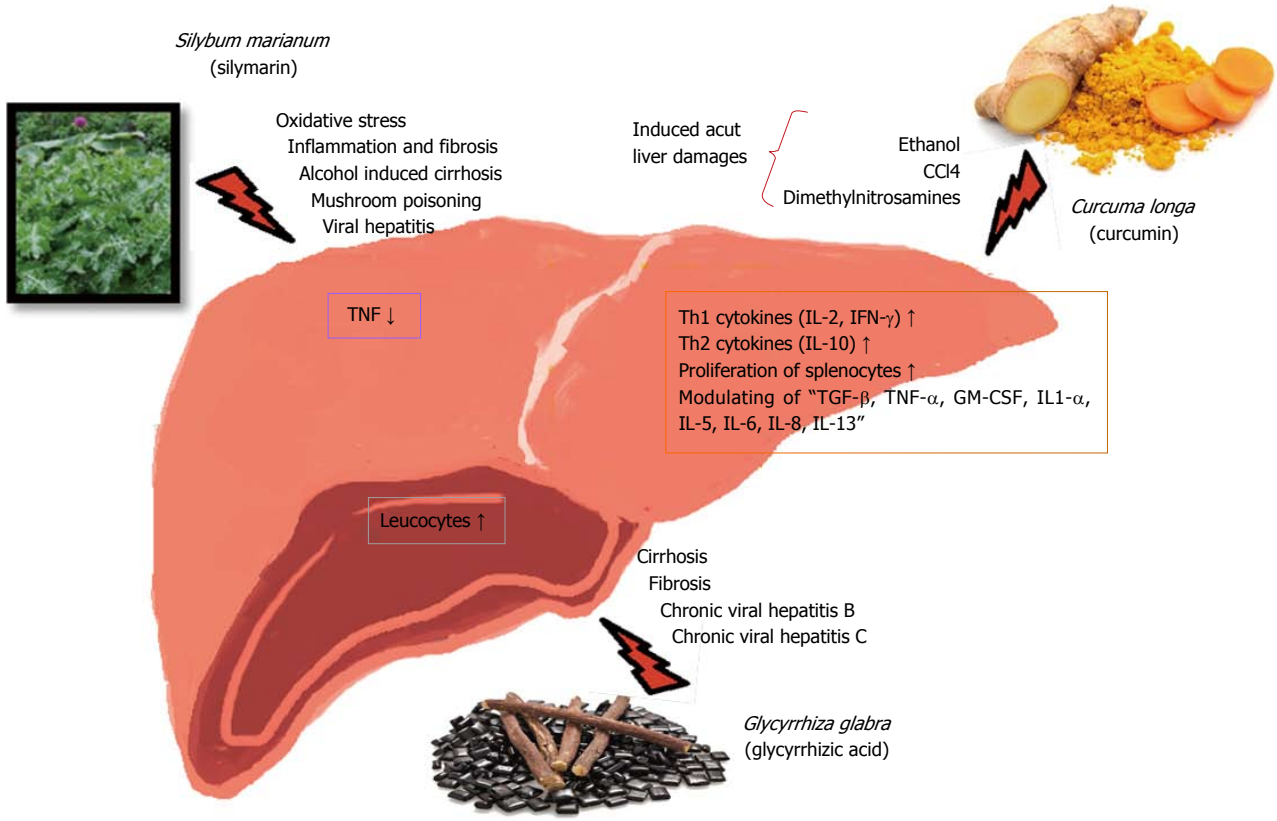

Figure 1 Immunological action mechanismes of some herbals on the liver. IFN: Interferons; TNF: Tumor necrosis factor; IL: Interleukin; TGF: Transforming growth factor.

must pricely define the interaction between the liver as an organ regulating local and systemic immune response and complex action mechanisms of herbal treatment.

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MINIREVIEWS

Risk factors and outcomes associated with alcohol relapse after liver transplantation

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Abstract

Alcoholic liver disease (ALD) is the second most common indication for liver transplantation (LT) in the United States and Europe. Unlike other indications for LT, transplantation for ALD may be controversial due to the concern for alcohol relapse and non-compliance after LT. However, the overall survival in patients transplanted for ALD is comparable or higher than in patients transplanted for other etiologies of liver disease. While the rate of alcohol use after liver transplantation does not differ among various etiologies of liver disease, alcohol relapse after transplantation for ALD has been associated with complications such as graft rejection, graft loss, recurrent alcoholic cirrhosis and reduced long-term patient survival. Given these potential complications, our review aimed to discuss risk factors associated with alcohol relapse and the efficacy of various interventions attempted to reduce the risk of alcohol relapse. We also describe the impact of alcohol relapse on post-transplant outcomes including graft and patient survival. Overall, alcohol liver disease remains an appropriate indication for liver transplantation, and long-term mortality in this group of patients is primarily attributed to cardiovascular disease or *de novo* malignancies rather than alcohol related hepatic complications, among those who relapse.

Key words: Cirrhosis; Relapse prevention; Recidivism

Core tip: There are no established risk factors or scoring systems to predict alcohol relapse after transplantation for alcoholic liver disease. Studies regarding the "6-mo rule" demonstrated heterogeneous findings, suggesting that this rule is not a reliable predictor of relapse.

Comorbid psychiatric conditions, lack of social support, and tobacco use are consistently associated with alcohol relapse. Scoring systems have been proposed, but have not been validated. Alcohol relapse may be associated with graft rejection and graft loss, though reduction in long-term survival may be attributed to cardiovascular disease and *de-novo* malignancies rather than alcohol-related hepatic complications.

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INTRODUCTION

Alcohol use disorder affects nearly 10% of the general population in both the United States and Europe and is one of the most frequent causes of liver cirrhosis in the Western world[1]. After hepatitis C virus (HCV) infection, alcoholic liver disease (ALD) is the second most common indication for liver transplantation (LT) in the United States and Europe[2,3]. According to the OPTN/SRTR 2015 annual report, 21% of liver transplantation was for alcoholic liver disease[4].

Unlike other indications for LT, transplantation for ALD may be controversial because of the concern regarding relapse and medication non-compliance after transplantation[5]. The exact proportion of ALD patients who drink alcohol after LT is unclear and is reported to range anywhere between 7%-95%[6-8]. The broad range of percentages reported in the literature is because there are no standardized definitions for alcohol relapse[6-8]. Interestingly, the rate of alcohol use after LT does not differ between patients transplanted for other etiologies of liver disease, though recipients transplanted for ALD tend to drink in greater quantities[9,10]. In terms of patterns of alcohol use, there are varying frequencies given the different definitions and follow-up periods, but in general approximately 12%-33% of liver recipients for ALD relapse to abusive or harmful amounts of drinking[11-14] and 6%-26% relapse to occasional slips after transplantation[12,14,15]. Furthermore, the overall survival rate for patients transplanted for ALD is comparable or higher than those of patients transplanted for non-ALD[2,3,10,16]. Still, separate studies have identified harmful and excessive amounts of alcohol use to be associated with increased rates of graft rejection and failure[10,15,17-19]. Due to these potential adverse complications, our aim was to discuss risk factors associated with alcohol relapse after transplantation, the efficacy of interventions attempted to prevent relapse, and the post-transplant outcomes associated with alcohol relapse[5].

Definitions

There are no standardized definitions or classification criteria to describe alcohol consumption after transplantation. Terms that have been used in the literature include recidivism or relapse[7,15-17,19]. Quantification of alcohol consumption after LT can also be described using terms such as abstinence, occasional slip, harmful drinking and excessive drinking, though the definitions of these terms are variable (Table 1)[8,20,21]. Lucey *et al*[22] defines harmful drinking as consumption of 4 or more drinks in one day or drinking for 4 or more days in succession, whereas a slip is defined as consumption of a limited amount of alcohol, followed by immediate measures to re-establish abstinence. De Gottardi *et al*[11] defined harmful drinking as alcohol consumption greater than 40 g/d that was associated with the presence of alcohol-related damage, such as histologic features of alcoholic liver injury on biopsy. The Diagnostic and Statistical Manual of Mental Disorders Version Ⅳ defined alcohol abuse as meeting one of the following criteria during a 12 mo period: Use which causes failure to fulfill major role obligations at work, school or home, use which causes a hazardous situation, use which causes legal problems or use continuing in the setting of recurrent social or interpersonal problems[23,24]. Faure's study used the World Health Organization definition where excessive alcohol consumption was > 20 g and > 30 g/d for women and men[10].

"The 6 mo rule"

Many centers require 6 mo of abstinence to be listed for liver transplantation. The 6 mo rule has two presumed purposes: To allow patients to recover from their liver disease and preclude the need for liver transplantation and to identify patients who are likely to remain abstinent after liver transplantation[1]. Nonetheless, there are conflicting findings as to whether this length of abstinence is needed to reduce the risk of relapse[11,25-27]. There have been several studies which have found that duration of abstinence less than 6 mo is associated with alcohol use and harmful drinking (Table 2)[11,28,29]. Additionally, Tandon *et al*[30] calculated that for every additional month of pre-LT abstinence there was a 5% decrease in the adjusted relapse rates. This is contrasted by other studies that have shown that the 6-mo rule is not a strong indicator of future drinking[26,27,31]. Based on the conflicting outcomes, the 6-mo rule may not reliably predict post-transplant relapse.

Furthermore, achieving 6 mo of abstinence is not always feasible, particularly for patients with severe alcoholic hepatitis that is refractory to treatment[32,33]. In fact, certain professional societies suggest that the 6-mo rule should not be required in patients where the expected mortality of the disease would not allow for a 6-mo waiting period[1,18,34]. Additionally, survival outcomes are superior among patients with severe alcoholic hepatitis that is refractory to corticosteroids and subsequently undergo OLT, as compared to those receiving standard of care[34-37]. As demonstrated by Mathurin *et al*[34] patients with severe alcoholic hepatitis who underwent OLT had a significantly greater cumulative 6 mo survival of 77% compared to

Table 1 Definitions of alcohol use after liver transplantation

Study	Term	Definition
Lucey *et al*[21]	Harmful drinking	Consumption of 4 or more drinks in one day or drinking for 4 or more days in succession
	Occasional slip	Consumption of a limited amount of alcohol, followed by immediate procedures to re-establish abstinence
De Gottardi *et al*[11]	Harmful drinking	Consumption greater than 40 g/d that is associated with the presence of alcohol-related damage, such as histologic features of alcoholic liver injury on biopsy
Diagnostic and Statistical Manual of Mental Disorders Version Ⅳ	Alcohol abuse	Meeting one of the following criteria during a 12 mo period: Use which causes failure to fulfill major role obligations at work, school or home, use which causes a hazardous situation, use which causes legal problems or use continuing in the setting of recurrent social or interpersonal problems
World Health Organization	Occasional consumption	Men: < 20 g/d Women: < 30 g/d
	Excessive consumption	Men: > 20 g/d Women: > 30 g/d

23% for controls who did not receive transplantation (P < 0.001).

PATIENT FACTORS ASSOCIATED WITH RELAPSE

Age

Like the 6-mo rule, age has an inconsistent association with alcohol relapse after LT. A few studies have found that younger age is associated with alcohol relapse after LT and that the category of patients that relapsed were significantly younger compared to those that did not[14,26,38]. One study found that age < 45 years was associated with increased risk of relapse and another found an association between relapse and age < 40 years[15,38]. These findings are contrasted by other studies that found no association between age and alcohol relapse[8,27]. Furthermore, two larger studies determined that age is not an independent risk factor associated with alcohol relapse[11,15]. Based on the heterogeneity of these findings, we believe that age is not a reliable predictor of risk of alcohol relapse.

Social support

Lack of social support is an extrinsic factor that has consistently been associated with an increased risk of relapse for patients transplanted for ALD[13,15,31,39]. ALD patients who resumed alcohol use post-LT were more likely to be divorced or separated from their partners compared to those that remained abstinent, and multiple studies found that the lack of a spouse or life partner is a predictor of alcohol relapse[8,13,15,31]. One study also suggested that marriage is protective against binge drinking[13]. Therefore, it is important to ensure that patients with ALD have a strong support system during LT evaluation.

Comorbid psychological conditions

The presence of psychiatric comorbidities or previous diagnosis of a mental illness has been found to be an important intrinsic risk factor for increased risk of relapse after LT[11,13,31]. Multivariate analysis showed that a pre-LT diagnosis of a psychiatric disorder (anxiety or depressive disorder) at the time of listing was independently associated with a significantly increased risk of harmful levels of alcohol relapse, which is defined as consumption of greater than 40 g/d[11]. Another study also determined that a prior diagnosis of a mental illness was significantly associated with harmful drinking, which was defined in the study as consumption of greater than 140 g of ethanol per week[31]. Furthermore, prior treatment for co-morbid psychiatric disorders is a potential risk factor for alcohol relapse[40]. Evaluation for comorbid psychiatric conditions during the LT evaluation period may potentially help identify ALD patients that are at higher risk of both alcohol relapse and harmful drinking after transplantation.

Employment

In a cross-sectional study of organ transplant patients, only 37.5% of liver transplant patients were employed post-transplant[41]. Furthermore, among liver transplant recipients, those transplanted for ALD are significantly less likely to be employed both before and after transplant compared to transplant recipients for non-ALD[9]. A total of 29% of transplant recipients with ALD and 59% of those with non-ALD worked pre-transplantation, *vs* 33% of those with ALD *vs* 80% of non-ALD at 3 years post-transplantation (P < 0.00001)[9]. Furthermore, ALD patients that were previously employed were less likely to return to work compared to patients transplanted for non-ALD[8]. Despite the low proportion of ALD patients that work pre and post-transplant, employment status does not appear to be significantly associated with the risk of alcohol relapse after transplantation[8,26,27,31].

Cigarette smoking

Studies have found cigarette smoking to be associated with alcohol relapse after transplant for alcoholic cirrhosis[17,31,40,42]. Kelly *et al*[31] demonstrated in univariate analysis that pre-transplant tobacco use was a predictor of harmful alcohol drinking in the post-transplant period. This was not a significant finding when subjects were

Table 2 Risk factors associated with alcohol relapse

Risk Factor	Ref.	Study design	Sample size	Results
Abstinence less than 6 mo pre-LT	Perney *et al*[26] (2005)	Retrospective	*n* = 61	Associated with severe relapse to heavy drinking[1]
	De Gottardi *et al*[11] (2007)	Retrospective	*n* = 387	Associated with relapse
	Pfitzmann *et al*[15] (2007)	Retrospective	*n* = 300	Associated with relapse
	Tandon *et al*[30] (2009)	Retrospective	*n* = 171	For every 1-mo increment increase in pre-transplant abstinence, there was a 5% decrease in the adjusted relapse rate
	Karim *et al*[29] (2010)	Retrospective	*n* = 80	Associated with relapse and is an independent risk factor for relapse
	Satapathy *et al*[42] (2015)	Retrospective	*n* = 148	Associated with alcohol relapse
	Osorio *et al*[28] (1994)	Retrospective	*n* = 43	No association
	Jauhar *et al*[27] (2004)	Retrospective	*n* = 112	No association
	Björnsson *et al*[8] (2005)	Retrospective	*n* = 103	No association
	Addolorato *et al*[25] (2013)	Retrospective	*n* = 55	No association
	Egawa *et al*[40] (2014)	Retrospective	*n* = 140	No association
Abstinence < 1 yr pre-LT	Kelly *et al*[31] (2006)	Retrospective	*n* = 100	No association with harmful relapse[2]
	Gedaly *et al*[79] (2008)	Retrospective	*n* = 142	Independent predictor of relapse
Age	Perney *et al*[26] (2005)	Retrospective	*n* = 61	Alcohol relapse group was younger compared to the non-relapse group
	Pfitzmann *et al*[15] (2007)	Retrospective	*n* = 300	Age < 40 yr of age was associated with relapse, but was not an independent risk factor
	Karim *et al*[29] (2010)	Retrospective	*n* = 80	Age < 50 yr of age approached clinical significance for alcohol relapse
	Rice *et al*[14] (2013)	Retrospective	*n* = 300	Alcohol relapse group was younger compared to the non-relapse group
	Grąt *et al*[38] (2014)	Retrospective	*n* = 97	Younger age < 45 associated with relapse
	Satapathy *et al*[42] (2015)	Retrospective	*n* = 148	Older patients had lower likelihood of alcohol relapse
	De Gottardi *et al*[11] (2007)	Retrospective	*n* = 387	Age > 50 yr associated with relapse
	Jauhar *et al*[27] (2004)	Retrospective	*n* = 112	No association
	Björnsson *et al*[8] (2005)	Retrospective	*n* = 103	No association
Social support	Kelly *et al*[31] (2006)	Retrospective	*n* = 100	Lack of partner associated with harmful alcohol relapse[2]
	Pfitzmann *et al*[15] (2007)	Retrospective	*n* = 300	Absence of life companion associated with increased risk of alcohol relapse
	DiMartini *et al*[13] (2006)	Prospective	*n* = 167	Marriage is protective against binge use
	Rodrigue *et al*[39] (2013)	Retrospective	*n* = 118	Limited social support associated with alcohol relapse
	Egawa *et al*[40] (2014)	Retrospective	*n* = 140	Marital status associated with alcohol relapse and harmful relapse[3]
	Satapathy *et al*[42] (2015)	Retrospective	*n* = 148	Support from immediate family (spouse, parent or child) was highly correlated with reduced risk of alcohol relapse
Marital status	Björnsson *et al*[8] (2005)	Retrospective	*n* = 103	No association
Psychiatric condition	De Gottardi *et al*[11] (2007)	Retrospective	*n* = 387	Associated with relapse
	Karim *et al*[29] (2010)	Retrospective	*n* = 80	Associated with relapse
	Kelly *et al*[31] (2006)	Retrospective	*n* = 100	Previous diagnosis of a mental illness associated with harmful drinking[2]
	DiMartini *et al*[13] (2006)	Prospective	*n* = 167	History of depressive disorder associated with alcohol relapse
	Egawa *et al*[40] (2014)	Retrospective	*n* = 140	A history of treatment for psychological diseases other than alcoholism before LT is associated with risk of alcohol relapse but not harmful drinking[3]
	Jauhar *et al*[27] (2004)	Retrospective	*n* = 112	Comorbid psychiatric condition had no association with relapse
Employment	Jauhar *et al*[27] (2004)	Retrospective	*n* = 112	No association
	Perney *et al*[26] (2005)	Retrospective	*n* = 61	No association
	Kelly *et al*[31] (2006)	Retrospective	*n* = 100	Previous occupation not associated with harmful drinking
	Egawa *et al*[40] (2014)	Retrospective	*n* = 140	Post-LT occupational status not associated with alcohol relapse
	Satapathy *et al*[42] (2015)	Retrospective	*n* = 148	Employment status at time of transplant was not associated with alcohol relapse
Cigarette smoking	Pageaux *et al*[17] (2003)	Retrospective	*n* = 128	Occasional and heavy drinkers were more likely to be cigarette smokers compared to abstinent patients
	Kelly *et al*[31] (2006)	Retrospective	*n* = 100	Median cigarette use per day was higher in harmful alcohol relapse group
	Rodrigue *et al*[56] (2013)	Retrospective	*n* = 118	Associated with alcohol relapse
	Egawa *et al*[40] (2014)	Retrospective	*n* = 140	Cigarette smoking after LT associated with alcohol relapse
	Satapathy *et al*[42] (2015)	Retrospective	*n* = 148	Active cigarette smoking at time of LT associated with alcohol relapse
Non-compliance with clinic visits	Egawa *et al*[40] (2014)	Retrospective	*n* = 140	Associated with alcohol relapse and harmful relapse[3]
Pre-LT substance abuse or alcohol treatment	DiMartini *et al*[13] (2006)	Prospective	*n* = 167	Prior alcohol rehabilitation was associated with relapse
	Gedaly *et al*[79] (2008)	Retrospective	*n* = 142	Participation in rehabilitation was associated with relapse
	Jauhar *et al*[27] (2004)	Retrospective	*n* = 112	Substance abuse treatment before LT had no association with relapse
	Björnsson *et al*[8] (2005)	Retrospective	*n* = 103	No association

[1]Alcohol consumption of more than 21 units per week for males and 14 units per week for females; [2]Alcohol consumption greater than 140 g of ethanol per week; [3]Alcohol consumption greater than 40 g per day that was associated with the presence of alcohol-related damage. LT: Liver transplantation.

divided into no smoking, prior smoking or active smoking categories[31]. Additionally, ALD patients who drank both occasionally and heavily after LT were more likely to be smokers compared to those who remained abstinent[17]. Independent of alcohol relapse, cigarette smoking is an important risk factor for recipient morbidity and mortality[20,31,43,44]. Long-term consequences of cigarette smoking include hepatic artery thrombosis, cardiovascular disease and new onset malignancy of the aerodigestive tract[43,44]. History of tobacco use was also found to be associated with poorer survival after LT from cardiovascular disease or *de novo* non-hepatic cancer[20,31,43,44].

Noncompliance with clinic visits

Egawa *et al*[40] found noncompliance with clinic visits after LT, defined as 3 absences without notice, to be associated with both alcohol relapse and harmful drinking. In the study population, most patients underwent living donor liver transplantation, due to scarcity of deceased donors in Japan[40]. Furthermore, a cross-sectional study found that those who missed clinic appointments had lower adherence to immunosuppressive medications after liver transplant for any etiology ($P < 0.001$). In the study, non-adherence to immunosuppressive medications was liberally defined as any missed doses of transplant medications[45]. This finding is significant because strict adherence to immune suppressant agents is a very important factor in long-term outcome after liver transplant[46]. In multivariate analysis, missing physician appointments was the only independent factor associated with non-adherence to immune suppressants. Survey respondents who missed clinic visits were more than 4.7 times as likely to be non-adherent with immune suppressants compared to those who did not miss clinic visits (OR = 4.7, 95%CI: 1.5-14.7, $P = 0.008$[45]).

HCV infection

HCV infection and ALD often co-exist and approximately 8%-10% of liver transplantation performed was for mixed HCV and ALD cirrhosis[47]. Aguilera *et al*[48] compared post-transplantation outcomes among patients transplanted for alcoholic cirrhosis, mixed alcoholic cirrhosis and HCV and HCV alone. Interestingly, there was no significant difference in rate of alcohol relapse between the mixed HCV and alcoholic cirrhosis group (8%) and the alcoholic cirrhosis group (18%). Alcohol relapse also does not affect liver histology or liver functions tests differently in recipients with concomitant HCV *vs* ALD alone. Additionally, rates of rejection and graft loss were not significantly different between the mixed HCV and ALD and ALD groups. While recurrence of HCV is a major cause of reduced survival in patients transplanted for HCV cirrhosis, 5-year survival was comparable between the mixed HCV and ALD group (73%) and alcoholic cirrhosis group (76%)[49,50]. Though further studies are warranted, based on these studies, presence of HCV does not appear to result in greater risk of alcohol relapse or worse post-transplantation outcomes.

Scoring systems to predict alcohol relapse

The two main scoring systems in the literature for alcohol relapse after LT are the High Risk Alcoholism Relapse (HRAR) Scale and the Alcohol Relapse Risk Assessment (ARRA). The High Risk Alcoholism Relapse Scale was designed and piloted in the male veteran population and consists of 3 variables: Duration of heavy drinking, number of drinks per day and number of prior alcoholism inpatient treatment experiences[51]. Each item is scored 0-2 and possible score ranges from 0 to 6. A HRAR score greater than 3 is associated with high risk of alcohol relapse[11].

The HRAR Scale has yet to be validated and thus far two studies did not find the HRAR score to be associated with post-OLT alcohol use[40,52]. In terms of the ARRA, this tool found 9 domains to be significantly predictive of alcohol relapse. This scoring system includes both intrinsic and extrinsic risk factors of alcohol relapse. The intrinsic factors include low motivation for alcohol treatment and poor stress management skills. The extrinsic factors include limited social support, engagement in social activities with exposure to alcohol and lack of nonmedical behavioral consequences. The remaining factors are absence of hepatocellular carcinoma, dependence on tobacco and ongoing alcohol use after diagnosis of liver disease. Groups in ARRA Ⅲ and Ⅳ (with 4-6 and 7-9 out the 9 factors) had significantly higher rates of alcohol relapse and were more likely to return to pre-transplant levels of drinking[39]. The ARRA scale has not been validated by other studies.

The Stanford Integrated Psychosocial Assessment for Transplant (SIPAT) was developed from a comprehensive literature review of psychosocial factors found to predict outcomes in liver, lung and heart transplant patients[53]. The SIPAT has been evaluated by one prospective study in liver, lung, kidney and heart transplant recipients. While mortality and organ failure was not associated with SIPAT scores, secondary medical and psychosocial outcomes such as rejection episodes, hospitalizations, infections and psychosocial decompensation were predicted by SIPAT[54]. The SIPAT has not yet been studied separately in liver transplant patients. In conclusion, there are no validated scoring systems to predict risk of alcohol relapse after LT at this time.

INTERVENTIONS TO PREVENT RELAPSE

Relapse prevention and psychosocial therapy

Studies have been conducted regarding relapse prevention before and after OLT. Erim *et al*[55] conducted a study that demonstrated that patients who received 6 mo of pre-LT psycho-educational therapy had significantly less alcohol recidivism during the pre-transplant waiting period. Björnsson *et al*[8] evaluated the effectiveness of active addiction treatment prior to transplant and demonstrated that active addiction treatment during the

pre-LT period may reduce the risk of relapse after LT by more than 50% (from 48% to 22%). In the study, 19 out of 40 (48%) patients transplanted before the start of structured management had resumed alcohol compared to 13 (22%) out of 58 after this intervention that did not (P = 0.002). No treatment was offered in the post-operative period. In a retrospective study, Addolorato *et al*[25] evaluated the use of an alcohol addiction unit (AAU) that was integrated within the transplant center. Post-LT patients either followed up with an addiction specialist at the transplant center or were offered addiction counseling by a provider outside the transplant unit. Patients who followed up in the AAU received multimodal treatment with counseling and pharmacologic treatment. Counseling involved 30-min sessions that emphasized craving evaluation and identification of risk factors for alcohol relapse. Out of 92 cirrhotic liver transplant recipients the alcohol relapse rate was remarkably lower in recipients managed by the alcohol addiction unit within the transplant center (16.45%) compared to patients managed by psychiatrists not affiliated to liver transplant units (35.1%).

Rodrigue *et al*[56] found that patients who had received substance abuse treatment before LT did not differ in alcohol relapse compared to patients who did not (30% *vs* 39%, P = 0.20). Interestingly, he discovered that patients who received substance abuse treatment both before and after transplant had significantly lower rates of alcohol relapse (16% *vs* 41%) compared to patients who received substance abuse treatment only before transplant (45%) or those who did not receive any substance abuse treatment (41%). While more studies are needed to evaluate relapse prevention strategies, follow-up with addiction specialists integrated with a transplant unit and a combination of pre and post-transplant interventions may be more efficacious[56].

Pharmacological interventions

Several medications are approved for alcohol dependence, but only baclofen has been studied in a randomized control trial (RCT) in patients with alcoholic cirrhosis[57,58]. Baclofen is a gamma amino butyric acid receptor agonist that works by reducing craving for alcohol. In a RCT, a total of 84 patients with both alcohol use dependence and liver cirrhosis were randomized to receive baclofen 10 mg three times daily or placebo for 12 wk. Baclofen demonstrated significant efficacy in promoting alcohol abstinence and reducing alcohol relapse. There were no serious side effects reported and no patients discontinued the medication during the study[58]. Furthermore, the baclofen study group displayed a significant decrease in alanine aminotransferase, gamma-glutamyl transferase, bilirubin and international normalized ratio values compared to placebo. It is theorized that the improvement in liver function tests was due to the significant reduction of alcohol intake in the baclofen group[58]. Baclofen has yet to be studied in the decompensated patient and post-LT population.

Other drugs that are currently approved for alcohol dependence include disulfiram, naltrexone and acomprosate, however these have not been studied in the post-transplant population. Additionally, both disulfiram and naltrexone are not ideal options for ALD patients due to their risk of hepatotoxicity[59-62].

Disulfiram was one of the first drugs approved for alcohol dependence and is an irreversible inhibitor of aldehyde dehydrogenase (ALD)[60,63,64]. If alcohol is consumed while taking disulfiram, acetaldehyde levels will increase and result in a disulfiram reaction of hypotension, flushing, nausea and vomiting that may deter patients from drinking alcohol[63]. Naltrexone is an antagonist of κ-and μ-opioid receptors and increases dopamine release in the mesolimbic system, which may help reduce alcohol craving[65]. The long acting intramuscular formulation of naltrexone may be less hepatotoxic because it does not undergo first pass metabolism by the liver, but both the oral and intramuscular formulations currently carry a black-box warning for liver damage[59,62]. Another anti-craving medication, acamprosate, is an N-methyl-D-aspartate glutamate receptor antagonist with an unclear mechanism of action. It is not metabolized by the liver and is not associated with liver toxicity[66]. Furthermore, a preliminary study suggested that 1 d of administration was well tolerated in patients with Child-Pugh class A and B cirrhosis[67]. More studies are needed to establish its efficacy in patients transplanted for alcohol liver disease and its safety profile with repeated administration.

Other promising pharmacologic agents to reduce alcohol relapse include topiramate and ondansetron[59]. Topiramate is only partially metabolized by the liver (22%) and is primarily excreted by the kidneys[68]. Ondansetron is a serotonin (5-HT3) receptor antagonist that is thought to downregulate dopaminergic neurons, reducing the reward pathway for alcohol[69]. It has been shown to be more effective than placebo in increasing total days of abstinence and percentage of abstinent days[70]. Its major side effect was QT prolongation, which was a dose related complication[71]. More studies are needed to evaluate the efficacy and safety profiles of topiramate and ondansetron in post-liver transplant patients[68,70].

Consequences of alcohol use on allograft outcomes

Graft rejection, graft loss and recurrent alcohol cirrhosis are feared complications of alcohol relapse after transplant for ALD patients. It has been suggested that alcohol relapse may lead to reduced compliance associated with a significantly increased graft rejection rate[14,17,72]. Pageaux *et al*[17] demonstrated that that while there was no significant difference in graft rejection rates between abstinent, occasional drinkers or heavy drinkers, the rejection episodes observed in the heavy drinker category were related to poor compliance to immunosuppressant medications. Therefore, alcohol consumption after LT may be a marker of medication non-adherence and can potentially predict risk of graft rejection. Overall, graft

loss from recurrence of ALD is uncommon, but multiple studies have shown that alcohol use after transplant is associated with an increased risk of graft loss and advanced allograft fibrosis[14,17,72-74]. In a study by Rice *et al*[14] any alcohol relapse increased the risk of graft failure, but upon subdivision by drinking pattern, a single slip or intermittent relapse was not associated with graft failure, but continuous heavy drinking was significantly associated with decreased graft survival. In terms of histopathology, patients with alcohol relapse were more likely to have advanced fibrosis (stage 3 or higher) compared to those that remained abstinent[14]. In the study, 20.8% of patients had a single slip and 33.3% of patients relapsed to continuous heavy drinking[14]. Multiple studies have demonstrated that patients with heavy post-transplant drinking were more likely to have more fatty changes and severe fibrosis[17,48]. Still, these histologic findings may also be explained by nonalcoholic hepatitis, given the fact that metabolic syndrome is common among post-LT patients[75].

Survival

The overall survival rates of patients transplanted for ALD are comparable or higher than the survival rates of patients transplanted for other etiologies[2,3,10,16]. According to an article by Dumortier, survival after liver transplant for ALD is 92.6% at 1 year, 88.5% at 3 years, 84.3% at 5 years and 73.4% at 10 years, which is comparable to that of patient's transplanted for other etiologies of cirrhosis[20]. While occasional slips are not associated with reduced survival, relapse to abusive or harmful levels of drinking is associated with increased mortality in ALD patients[15]. Interestingly, mortality after LT for ALD is rarely due to recurrent alcoholic cirrhosis. According to DuMortier *et al*[20], only 3% of deaths were related to alcohol cirrhosis after transplant and only 0.7% of the patients transplanted for alcoholic cirrhosis died from recurrent alcoholic cirrhosis. This finding was consistent with another study where only 1 (1%) death was related to alcohol relapse whereas the majority of deaths were attributed to cancer[27]. Björnsson *et al*[8] also found that deaths in the group of patients that resumed alcohol use were not directly related to alcohol use. While alcohol use itself does not reduce post-transplant survival, recurrent alcoholic cirrhosis does significantly reduce post-transplant survival. One-, 5-, 10- and 15-year survival was 100%, 87.6%, 49.7% and 21.0%, respectively, for patients with recurrent alcoholic cirrhosis *vs* 100%, 89.4%, 69.9% and 41.1%, respectively, for the patients without recurrent alcoholic cirrhosis ($P < 0.001$)[76]. Furthermore, Cuadrado *et al*[72] found no difference in 1 or 5 year survival in those who were abstinent *vs* those with alcohol relapse, but the study did find a remarkably worse 10 year survival in patients with alcohol use of more than 30 g/d (45.1% *vs* 85.5%). This difference in long-term mortality did not appear to be related to liver failure, graft rejection, infection rate or metabolic disturbances, but was attributed to a higher frequency of deaths from *de novo* malignancy and cardiovascular events[72]. Therefore, the major long-term causes of mortality in patients transplanted for ALD appear to be due to cardiovascular disease and *de novo* malignancy rather than related to alcohol use[10,20,38,72,76].

CONCLUSION

Overall, ALD is a good indication for liver transplantation. Patients transplanted for ALD have comparable survival rates to patients transplanted for other etiologies of liver disease[2,3,10,16].

Based on this review article, consistent predictors of alcohol relapse include comorbid psychiatric conditions, social support and tobacco use[11,13,15,29,31,40,77,78]. While the 6-mo rule is a common prerequisite for LT listing, it is not a reliable predictor of alcohol relapse[8,27,28]. It is also not feasible for some patients, particularly those with severe alcoholic hepatitis that is refractory to medical management[34]. Furthermore, scoring systems to predict relapse such as the HRAR and ARRA have been proposed but have yet to be validated by other studies.

Additionally, participation in an addiction unit integrated within a transplant center was found to be efficacious in reducing alcohol relapse after LT, but further studies are still needed to reproduce this finding[25]. Rodrigue *et al*[56] did not find pre-LT treatment of substance abuse disorders to significantly impact relapse post-LT, but patients who received both pre-and post-transplant substance abuse treatment were significantly less likely to drink post-transplant. Therefore, continuous addiction treatment may play an important role in this population.

Multiple drugs have been approved for alcohol dependence, but the majority has not yet been studied in patients transplanted for ALD[57,58]. Baclofen appears to be the most promising pharmacologic agent in promoting abstinence post-transplant and was shown to have a good safety profile in patients with advanced liver disease. Further research is needed to determine whether baclofen can reduce alcohol relapse in ALD patients in the post-transplant period. Acamprosate, topiramate and ondansetron are also promising agents because of their lower risk of hepatotoxicity, but further research is needed[59,66,67].

Lastly, alcohol relapse is associated with increased rates of graft rejection[14,17,72]. This is thought to be due to the association between alcohol use and non-adherence to immunosuppressive agents[14,17,72]. While occasional slips do not impact graft loss, a harmful or excessive amount of alcohol use post-LT has been found to be associated with an increased rate of graft loss and advanced fibrosis[14,17,48]. Heavy drinkers were also noted to have more fatty changes and steatohepatitis compared to those who remained abstinent, though this finding may be confounded by nonalcoholic steatohepatitis[14,17,72,73,75]. Overall, survival in ALD patients is comparable or higher compared to those transplanted for other etiologies of liver disease[2,3,10,16]. Long-term survival at 10 years was found to be significantly lower in those

that resumed alcohol use, but this was attributed to mortality from *de novo* malignancies and cardiovascular events rather than due to liver failure[72,75].

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ORIGINAL ARTICLE

Basic Study

Angiotensin Ⅱ or epinephrine hemodynamic and metabolic responses in the liver of L-NAME induced hypertension and spontaneous hypertensive rats

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Abstract

AIM

To study hepatic vasoconstriction and glucose release induced by angiotensin (Ang) Ⅱ or Epi in rats with pharmacological hypertension and spontaneously hypertensive rat (SHR).

METHODS

Isolated liver perfusion was performed following portal vein and vena cava cannulation; Ang Ⅱ or epinephrine (Epi) was injected *in bolus* and portal pressure monitored; glucose release was measured in perfusate aliquots.

RESULTS

The portal hypertensive response (PHR) and the glucose release induced by Ang Ⅱ of L-NAME were similar to normal rats (WIS). On the other hand, the PHR induced

by Epi in L-NAME was higher whereas the glucose release was lower compared to WIS. Despite the similar glycogen content, glucose release induced by Ang Ⅱ was lower in SHR compared to Wistar-Kyoto rats although both PHR and glucose release induced by Epi in were similar.

CONCLUSION

Ang Ⅱ and Epi responses are altered in different ways in these hypertension models. Our results suggest that inhibition of NO production seems to be involved in the hepatic effects induced by Epi but not by Ang Ⅱ; the diminished glucose release induced by Ang Ⅱ in SHR is not related to glycogen content.

Key words: Epinephrine; Liver perfusion; Spontaneously hypertensive rat; Glucose; Angiotensin Ⅱ; L-NAME

Core tip: Angiotensin (Ang) Ⅱ and epinephrine (Epi) induce hemodynamic and metabolic responses in a normal liver. These responses are altered in different ways in two models of hypertension. We observed that inhibition of NO production seems to be involved in the hepatic hemodynamic and metabolic effects induced by Epi but not by Ang Ⅱ. Furthermore, diminished glucose release induced by Ang Ⅱ in spontaneously hypertensive rat is not related to glycogen content, but might be due to the glycogen phosphorylase activation by Ang Ⅱ.

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INTRODUCTION

The renin-angiotensin-aldosterone system (RAAS) regulates blood pressure homeostasis and vascular injury and repair responses. This system has been associated with diverse physiological functions, but also with inflammation, fibrosis, and target-organ damage. Local forms of the RAAS have been described in many tissues[1-5]. The importance of RAAS in the pathophysiology of hypertension has been observed in brain, heart, adrenal glands, vasculature, and kidney[6-9].

Several components of RAAS are present in the liver, which synthesizes angiotensinogen, a glycoprotein that contains the sequence of angiotensin in its amino-terminal portion. Angiotensin converting enzyme (ACE) is a carboxypeptidase present primarily in the perivenous region. Besides converting angiotensin (Ang) Ⅰ in Ang Ⅱ, it is the major kininase involved in bradykinin degradation in the liver[10]. In 1976, Borges *et al*[11] showed that both Ang Ⅰ and Ang Ⅱ infused into the portal vein of a rat induced hypertensive effect, and they also demonstrated for the first time the conversion of Ang Ⅰ into Ang Ⅱ by the rat liver. This hypertensive response induced by Ang Ⅱ is mediated by AT1 receptor because when losartan was co-infused with Ang Ⅱ into the liver portal vein it abolished the hypertension response[12]. Captopril infusion prevented pressor action of Ang Ⅰ, thus the PHR previously attributed to Ang Ⅰ is actually a result of its conversion to Ang Ⅱ by hepatic ACE. This conversion is rapid, but the portal hypertensive action after Ang Ⅰ *in bolus* injection is significantly delayed compared to Ang Ⅱ injection[13]. Metabolic effects induced by Ang Ⅱ, such as glucose release and O_2 consumption, are only diminished in the presence of losartan, which demonstrates that these effects are partially dissociated on bivascular liver perfusion. Therefore, another receptor besides AT1R might also be involved on these Ang Ⅱ hepatic effects[12,14].

ACE inhibition or blockade of angiotensin receptors are widely used in clinical medicine in the treatment of hypertension. The role of the hepatic RAAS has been associated with fibrosis and cirrhosis, and its resulting portal hypertension. Up-regulation of hepatic ACE, ACE2 and AT1R was observed in animal models of fibrosis and cirrhosis by bile duct ligation or carbon tetrachloride induction[15-17]. Ang Ⅱ, *via* AT1R, stimulates activation of quiescent stellate cells, activates myofibroblasts proliferation, and promotes the release of inflammatory cytokines, as well as the excessive deposition of extracellular matrix components[18].

The catecholaminergic sympathetic nervous system is another common system with metabolic (glucose and lactate release as well as oxygen consumption increase) and hemodynamic (vasoconstriction) effects. This system plays a key role in blood pressure homeostasis and normal metabolism and participates in the pathophysiology of many diseases. The liver contains abundant sympathetic innervation derived from the hepatic nerve plexus, and circulating catecholamines regulate liver tone[19]. The presence of the α1- and β-adrenergic receptors on hepatocytes was demonstrated in various species like catfish, goldfish, and rats[20-22]. In fed state, epinephrine (Epi) promotes hepatic glucose production by activation of glycogenolysis and, in fasted state, Epi accelerates gluconeogenesis[23].

In patients with essential hypertension, plasma levels of norepinephrine are significantly elevated and the increased sympathetic activity is accompanied by diastolic and systolic pressure increases. Neuroadrenergic factors may contribute to the maintenance and progression of hypertensive state as well as its development[24]. A correlation between the RAAS and the sympathetic nervous system has also been described. The latter is activated by Ang Ⅱ and plays a fundamental role in the homeostasis of blood pressure control[25]. The multifactorial etiology of hypertension has led researchers to postulate, over time, various experimental models, each one involving one or more mechanisms, contributing to the assembly of a human essential hypertension "mosaic". A pharmacological hypertension model is the blockade

of nitric oxide synthesis. Biancardi *et al*[26] showed that vasoconstriction in response to L-NAME by the sympathetic tone plays an important role in the initiation and maintenance of hypertension. The RAAS also contributes to high blood pressure in animals chronically treated with L-NAME. Chronic treatment with ACE inhibitors or AT1 blockers is able to prevent the onset of, or reverse, a hypertension and renal injury already established, indicating a involvement of RAAS in the genesis and maintenance of this hypertension[27]. A spontaneously hypertensive rat (SHR) is the widely used genetic hypertension model that presents elevated sympathetic activity[28]. Although these animals are generally considered to be characterized by a low activity of circulating RAAS[29], some studies indicate that treatment with ACE inhibitors or AT1 receptor blockers or both reduces cardiac or renal dysfunction or both of these dysfunctions in SHRs[30-32].

Although the liver is not a target organ in physiopathology of hypertension, the presence of AT1 receptor and ACE may still indicate unknown specific roles. Sympathetic hyperactivity was described in most models of hypertension[28] but little is known about the consequences of this hyperactivity in the liver. Therefore, the aim of this work was to evaluate the hepatic response to Ang Ⅱ and Epi in hypertension models. Using the isolated rat liver perfusion, we studied the vasoconstrictor hepatic effect as well as metabolic (glucose release) effect of Ang Ⅱ and Epi in two different hypertension experimental models: One genetic (SHR) and one pharmacological (systemic inhibition of NO synthase).

MATERIALS AND METHODS

Animals

Adult male Wistar EPM-1 rats (WIS), SHRs (bred by the Central Animal House of the Federal University of São Paulo - UNIFESP), and Wistar Kyoto (WKY) rats (bred by Central House of the University of de São Paulo - USP) aged 12-16 wk were used. The animals were housed in a conditioned environment and were fed a standard laboratory diet (Purina) and water ad libitum. This study was conducted according to the International Guiding Principles for Biomedical Research Involving Animals[33] and was approved by the Ethics in Research Committee of UNIFESP (CEP 1455/09).

Experimental groups

After one week of acclimatization, two experimental groups were studied: (1) L-NAME, pharmacologic induced model of hypertension: Wistar EPM-1 rats received N^G-nitro-L-arginine methylester (0.5 mg/mL) in drinking water for 10 d and were compared to healthy, Wistar EPM-1 rats; and (2) SHRs were compared to WKY rats.

Indirect systolic blood pressure

Body weight and tail indirect systolic blood pressure (SBP) were recorded weekly. SBP was measured by tail-cuff plethysmography (NIBP Controller, ADInstruments, Australia) in unanesthetized rats that were placed in a warm cupboard (45 ℃) for 15 min. SBP values for individual rats were obtained from the average of 3-4 consecutive measurements and were considered valid only when these readings did not differ by more than 5 mmHg. Procedure was performed at least 48 h before the perfusion experiments to minimize the influence of animal stress on our results. Upon confirmation of animal hypertension, perfusion of rat liver *in situ* was conduct as previously described[34].

Glycemia and insulinemia

Blood samples were collected from the abdominal aorta before portal vein cannulation. They were centrifuged at 3000 rpm to remove red cells, and serum was stored at -20 ℃. Glucose was determined by enzymatic method (Glucose PAP kit, Labtest Diagnóstica, Sao Paulo, Brazil) and the concentration of insulin was determined using a direct ELISA kit specific for rat and mouse analysis (Millipore, United States).

In situ rat liver perfusion

Monovascular rat liver perfusion was performed as previously described[34]. Briefly, the rat was anesthetized with urethane, 1.3 g/kg, *i.p.* (Sigma Chemical Co., United States), and hemoglobin-free, nonrecirculating liver perfusion was performed. Abdominal and thoracic cavities were opened and the portal vein (entry *via*) and the vena cava (exit *via*) cannulated. The perfusion fluid was Krebs/Henseleit-bicarbonate buffer, pH 7.4, containing 1 mg/mL BSA (Sigma Chemical Co., United States) saturated with an oxygen/carbon dioxide mixture (95/5%). Fluid was pumped in a constant flow (3-4 mL/min.g liver) through a temperature-regulated membrane oxygenator (37 ℃) prior to entering the liver *via* the portal vein. The oxygen uptake in the outflowing perfusate was monitored continuously with a polarigraphic type of probe (Delta OHM HD2109.2, Italy) adequately positioned in a chamber at the exit of the perfusate. Liver viability was evaluated by bile production and oxygen consumption. The portal pressure was measured by using a vertically positioned, graduated fluid-filled column attached before the afferent cannula open to the atmospheric. After 20 min of stabilization previously determined (glucose release and portal pressure), 2 nmol Ang Ⅱ (Sigma Chemical Co., United States) or 40 nmol Epi (Sigma Chemical Co., United States) was injected *in bolus* into the portal vein cannula. Aliquots of perfusate were collected (0 and every 30 s until 5 min and 6, 8 and 10 min) for glucose determination.

Portal pressure

Portal pressure was recorded during all experiments (0, 15, 30 and 45 s and 1-10 min). The portal pressure increase was determined over the basal pressure and the maximum increase measured. The portal hypertensive response (PHR; the area under the curve) was calculated

Table 1 Serum parameters and glycogen content

Group	Glycemia (mg/dL)	*n*	Insulinemia (ng/mL)	*n*	Glycogen content (mg/100 mg liver)	*n*
WIS	75.4 ± 4.2	9	2.1 ± 0.4	12	2.9 ± 0.2	10
L-NAME	80.7 ± 7.5	8	2.0 ± 0.4	12	2.3 ± 0.2	10
WKY	76.9 ± 4.0	9	3.8 ± 0.6	12	2.8 ± 0.2	10
SHR	86.2 ± 4.0	8	2.7 ± 0.4	13	2.8 ± 0.2	10

Serum and liver fragment for glycogen content measurement were collected before the liver perfusion experiment. Values are expressed as mean ± SEM. Student's *t*-test; L-NAME *vs* WIS and SHR *vs* WKY. WIS: Similar to normal rats; SHR: Spontaneously hypertensive rat; WKY: Wistar Kyoto.

from the graphic: Portal pressure increase *vs* time after agonist injection and expressed as cmH_2O.min.

Metabolic effects

Metabolic effects were evaluated on the basis of oxygen consumption and glucose release by perfused liver. Oxygen consumption was calculated from input-output differences expressed as μmol O_2 consumed/min.g liver. Glucose released was determined in perfusate aliquots using an enzymatic method (Glucose PAP kit, Labtest Diagnóstica, Sao Paulo, Brazil) and expressed as μmol glucose released/min.g liver. This parameter was also used to assure the liver viability. The amount of glucose released was calculated (area under the curve) from the graphic: Glucose increase *vs* time after agonist injection and expressed as μmol/min.g liver.

Glycogen

In order to avoid loss of the liver glycogen content during the 30 min of perfusion, a fragment of caudate lobe was removed after a rapid exsanguination at the beginning of the perfusion procedure. Quantification of the glycogen was based on the extraction of the polysaccharide with an alkaline solution (30% KOH) and its conversion into glucose during the reaction of the exergonic homogenized with a solution of sulfuric acid and anthrone[35]. The concentration of glycogen (expressed as mg/100 mg liver) was determined from a glucose standard curve. Furthermore, liver fragments were removed at the end of the experiment and processed by the company Histotech Teaching Blades (http://www.histotech.com.br/site/). The histological analysis of liver glycogen was performed using the periodic acid-Schiff (PAS) staining.

Statistical analysis

The results are expressed as mean ± SEM. Comparisons were performed by using Student's *t*-test and a value of $P < 0.05$ was adopted as the level of significance. Analysis was performed using Graph Pad Prism 5.0 program.

RESULTS

Hypertension animal model characterization

Arterial blood pressure of SHR and rats submitted to drug hypertension (L-NAME) was evaluated before the perfusion experiments. The tail systolic blood pressure (mmHg) of L-NAME (169.1 ± 4.8; $n = 12$) and SHR groups (180.2 ± 5.9; $n = 10$) were higher (*t*-test, $P < 0.001$) when compared to WIS (126.4 ± 2.9; $n = 9$) and Wistar Kyoto (127.0 ± 2.0; $n = 15$), respectively. The glycemia and insulinemia of the rats used in the experiments are shown in Table 1; values of glycemia of normotensive animals were taken as the reference value. The glycemia of both the L-NAME and SHR groups was similar when compared to their respective control groups. The insulinemia of all groups were within normal range (0-118 pmol/L)[36] without difference between groups.

The perfusion experiments were performed in the morning when the animals, which have nocturnal habits, were in a well-fed state confirmed by hepatic glycogen content. No difference in liver glycogen content among groups (Table 1) was found. At the end of perfusion another fragment of the liver was removed for histological analysis for glycogen content (PAS staining) and compared to the perfused livers of animals left for 24 h of fasting. We observed that even after 30 min of perfusion, the hepatic glycogen of all groups was noticeably higher than in fasted animals (Figure 1).

Liver viability

To ensure liver viability during the period of liver perfusion experiment (approximately 30 min), bile production and oxygen consumption were monitored. The bile was collected before and after injection of Epi or Ang Ⅱ. As the bile production before and after agonist injection were similar, the arithmetic average was used for statistical analysis. The bile production (mL/min.g liver) was similar among groups (WIS: 1.2 ± 0.1, $n = 16$; L-NAME: 1.2 ± 0.1, $n = 15$; WKY: 1.1 ± 0.1, $n = 14$; SHR: 1.1 ± 0.1, $n = 13$). The oxygen consumption was observed throughout the perfusion period ensuring the functioning of the organ. The basal oxygen consumption (μmol/min.g liver) of SHR (2.5 ± 0.1, $n = 14$) was lower (*t*-test, $P = 0.0151$) when compared to WKY (3.2 ± 0.2, $n = 16$). This parameter on L-NAME (3.1 ± 0.2, $n = 15$) was similar to WIS (3.2 ± 0.1; $n = 17$). After agonist injection, oxygen consumption was maintained but no standard response was observed: It remained the same in some experiments and increased in others. As the perfusion fluid did not contain glucose, its release was observed from the beginning of the experiment. Basal glucose release was similar in all groups (Figure 2A and B); after agonists injection its release continued throughout the entire experiment, ensuring hepatic viability.

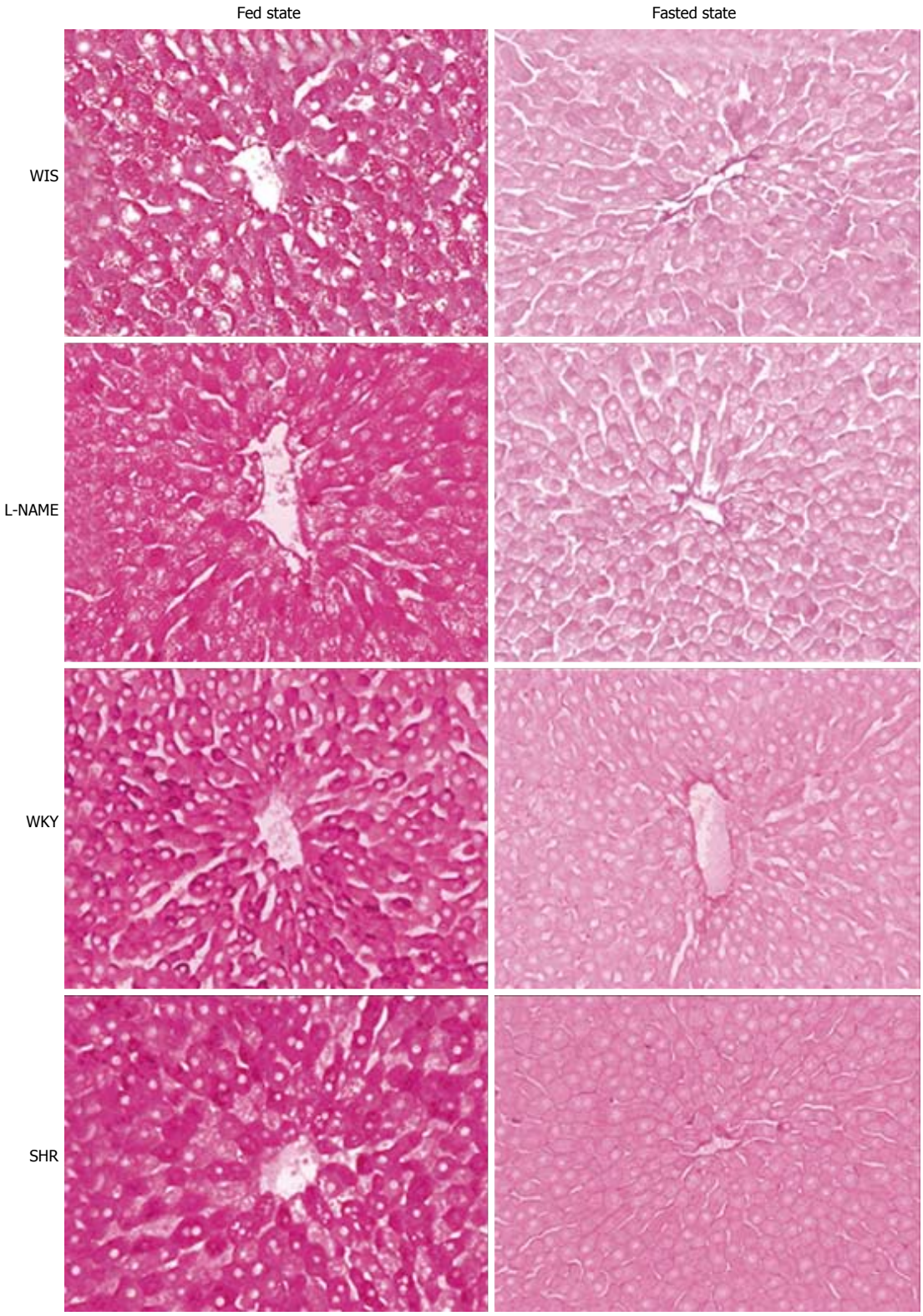

Figure 1 Hepatic glycogen. Periodic acid Schiff's staining of cross-section of perfused livers from fed or 12 h fasted rats. Fragments taken after 30 min of perfusion. Increase 200 ×. WIS: Similar to normal rats; SHR: Spontaneously hypertensive rat; WKY: Wistar Kyoto.

Glucose release induced by Ang Ⅱ or Epi

Following Ang Ⅱ injection, the amount of glucose released (Figure 2A and B) from the L-NAME group was similar compared to the WIS group, whereas the amount released from SHR livers was lower than its WKY control group (Table 2).

The glucose release induced by epinephrine is shown in Figure 2C and D; the amount released (AUC) from the L-NAME group (4.2 ± 0.4) was lower when compared to its WIS control group (7.5 ± 0.9), whereas the SHR

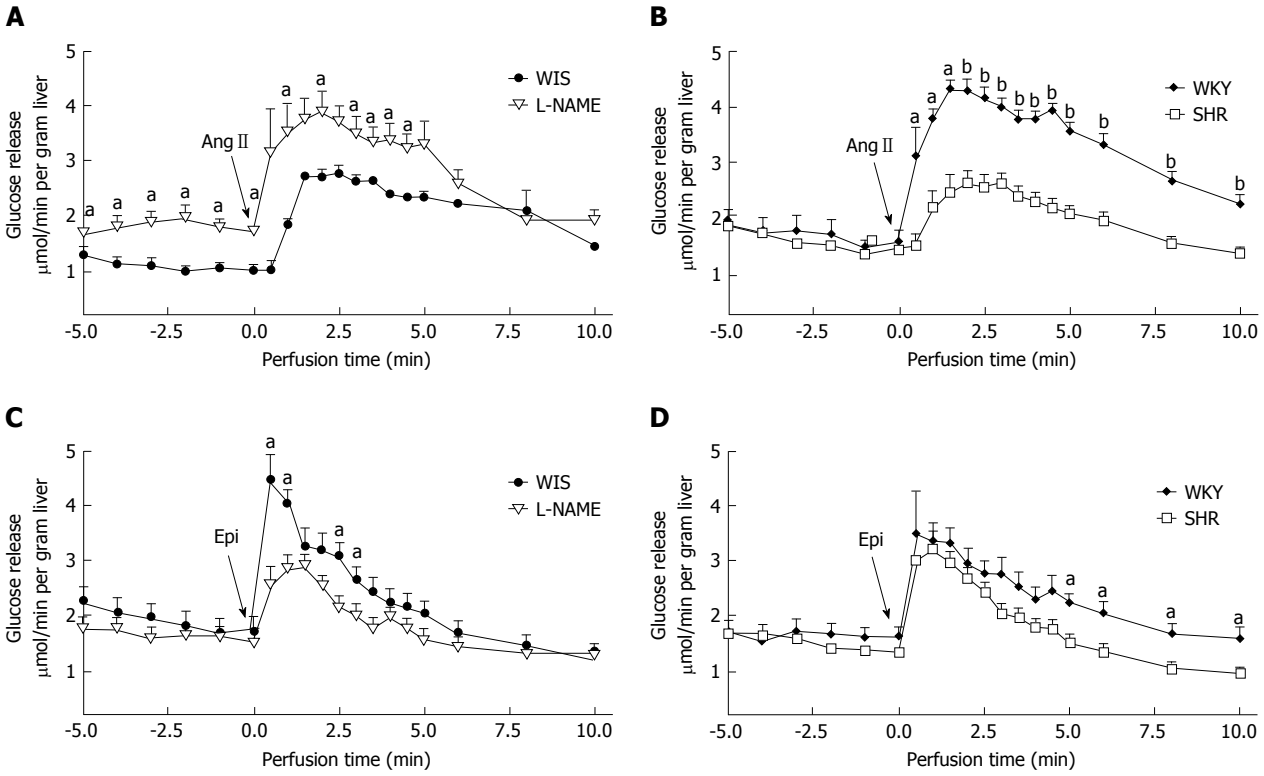

Figure 2 Glucose release induced by angiotensin Ⅱ and epinephrine. Livers were perfused with Krebs-Henseleit-bicarbonate buffer and after stabilization 2 nmol Ang Ⅱ (A, B) or 40 nmol Epi (C, D) was injected *in bolus* into afferent cannula and this moment was considered as time 0 min. Glucose release was determined in perfusate aliquots collected during all experiments. Student's *t*-test; [a]*P* < 0.05 and [b]*P* < 0.0001 compared with respective controls for each time point. WIS: Similar to normal rats; SHR: Spontaneously hypertensive rat; WKY: Wistar Kyoto; Ang: Angiotensin; Epi: Epinephrine.

group was similar to the WKY group (Table 2).

PHR to Ang Ⅱ or Epi

Basal portal pressure (before agonist injection) was similar in all groups. Ang Ⅱ (2 nmol) or Epi (40 nmol) was injected in portal vein and both agonists promoted portal vasoconstriction. Despite a 20-fold difference in agonists doses, the maximum portal pressure increase (cmH_2O) induced by Ang Ⅱ and Epi was similar in among groups (Ang Ⅱ: WIS: 7.9 ± 1.2, *n* = 7; L-NAME: 7.6 ± 1.1, *n* = 7; WKY: 10.5 ± 0.3, *n* = 7; SHR: 6.5 ± 1.2, *n* = 10; Epi: WIS: 6.1 ± 0.7, *n* = 10; L-NAME: 8.9 ± 0.7, *n* = 8; WKY: 7.9 ± 0.7, *n* = 8; and SHR: 6.2 ± 0.5, *n* = 6).

The hepatic portal pressure increase after bolus injection of Ang Ⅱ was normalized after about 10 min of perfusion (Figure 3A and B). The curve prolife of portal pressure of L-NAME and SHR groups was similar to their control groups (WIS and WKY, respectively). The PHR induced by Ang Ⅱ in both L-NAME and SHR was similar when compared to their WIS and WKY control groups, respectively (Table 3). The effect of Epi in portal pressure was more transient than Ang Ⅱ. Following Epi injection, the portal pressure increase was normalized after about 5 min (Figure 1). The PHR induced by Epi in the L-NAME group was higher when compared to the WIS group. On the other hand, no difference in PHR of SHRs existed compared to the control WKY group (Table 3).

DISCUSSION

All key components of the RAAS are present in the normal liver and are up-regulated in response to chronic liver injury, with growing evidence that the intrahepatic RAAS plays important roles in both the pathophysiology of portal hypertension and liver fibrosis[18]. The use of ACE/Ang Ⅱ/AT1R axis inhibitors associated with ACE2/Ang (1-7)/Mas axis activation is a promising strategy-serving regimen to prevent and treat chronic liver diseases as well as acute liver injury[37]. Hepatic glucose metabolism can be modulated by NO directly inhibiting glycogen synthesis and gluconeogenesis, and indirectly inhibiting glycogen breakdown *via* the secretion of other intrahepatic mediators[38,39].

In the liver, both Ang Ⅱ and Epi cause vasoconstriction and glucose release. Although the liver is not considered the target organ in hypertension pathophysiology, it is an important metabolic regulator organ. To study hepatic effects of Ang Ⅱ and Epi, we used two different experimental models of hypertension: Pharmacological (systemic inhibition of NO synthase) and genetic (SHR). Chronic oral administration of L-NAME promotes a rapid deployment of hypertension in the first days of treatment that is largely mediated by the RAAS. The rats treated with ACE inhibitors, such as captopril and enalapril, or with AT1 receptor antagonists, such as losartan, restore blood pressure to near normal levels[40,41]. In our study,

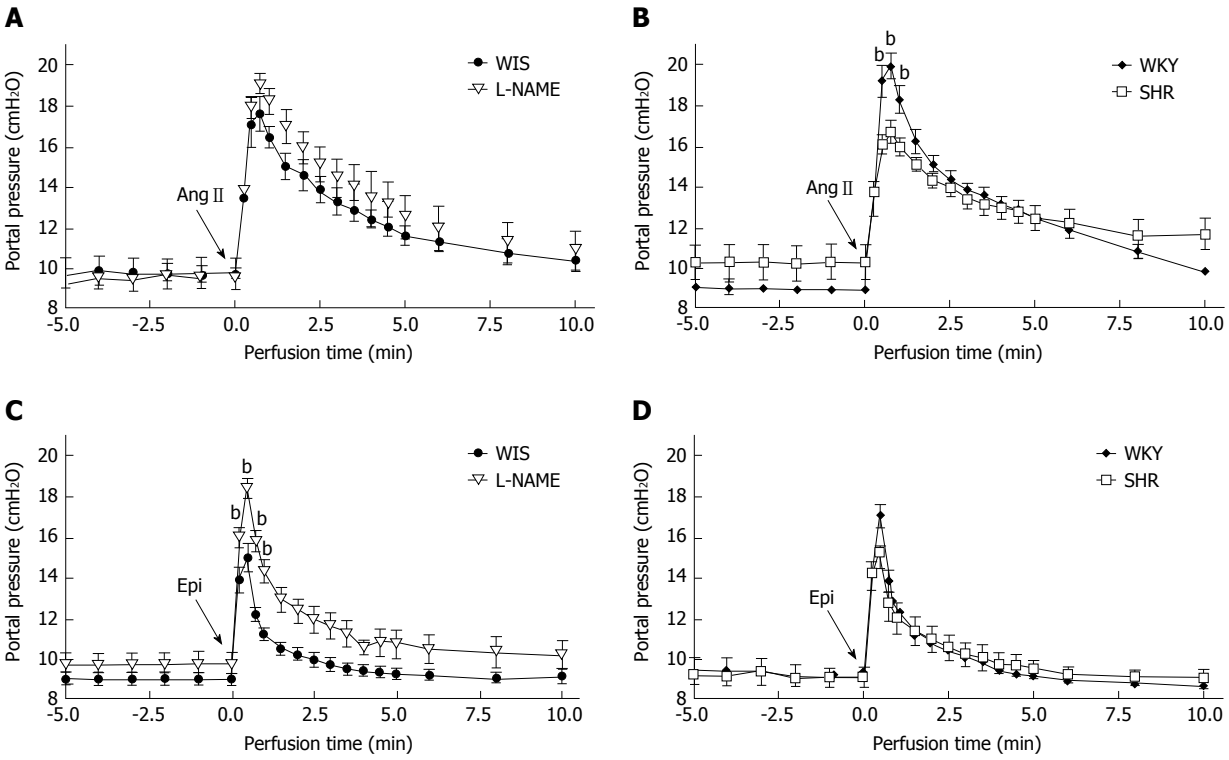

Figure 3 Portal pressure induced by angiotensin Ⅱ or epinephrine. Livers were perfused with Krebs-Henseleit-bicarbonate buffer and after 20 min stabilization, 2 nmol Ang Ⅱ (A, B) or 40 nmol epinephrine (C, D) was injected *in bolus* into afferent cannula and this moment was considered as time 0 min. The portal pressure was continuously monitored by water manometer attached to the circuit before the cannula. Student's *t*-test; $^{b}P < 0.0001$ compared with respective controls for each time point. WIS: Similar to normal rats; SHR: Spontaneously hypertensive rat; WKY: Wistar Kyoto; Ang: Angiotensin; Epi: Epinephrine.

Table 2 Glucose release induced by angiotensin Ⅱ or epinephrine

Group	Glucose released μmol/min.g liver			
	Angiotensin Ⅱ	*n*	Epinephrine	*n*
WIS	11.3 ± 0.9	7	7.5 ± 0.9	10
L-NAME	11.2 ± 1.5	7	4.2 ± 0.4[d]	8
WKY	16.4 ± 1.5	7	8.0 ± 0.9	8
SHR	5.42 ± 0.6[b]	10	5.9 ± 0.7	6

The amount of glucose (area under the curve) was calculated from the curve glucose release increase *vs* time after agonist injection. Student's *t*-test; $^{b}P < 0.0001$ and $^{d}P = 0.002$ compared with respective control (L-NAME *vs* WIS and SHR *vs* WKY). WIS: Similar to normal rats; SHR: Spontaneously hypertensive rat; WKY: Wistar Kyoto.

Table 3 Portal hypertensive response to angiotensin Ⅱ or epinephrine

Group	Portal hypertensive response cmH2O.min			
	Angiotensin Ⅱ	*n*	Epinephrine	*n*
WIS	26.4 ± 3.2	7	8.2 ± 0.8	10
L-NAME	38.1 ± 4.8	7	18.5 ± 1.9[b]	8
WKY	29.0 ± 1.1	7	10.0 ± 1.1	8
SHR	25.9 ± 3.7	10	10.5 ± 1.1	6

The portal hypertensive response (PHR; area under the curve) was calculated from portal pressure increase curve *vs* time after agonist injection and expressed as cmH2O.min. Student's *t*-test; $^{b}P < 0.0001$ compared with respective control (L-NAME *vs* WIS and SHR *vs* WKY). WIS: Similar to normal rats; SHR: Spontaneously hypertensive rat; WKY: Wistar Kyoto.

10 d of L-NAME treatment were sufficient to induce a high level systolic blood pressure. On the other hand, the SHR strain is the most widely used phenotypic experimental model in hypertension research with specific potential in the study of polygenic hypertension, being associated with cardiac hypertrophy, heart failure, and renal dysfunction. Hepatic functions are also altered at the molecular level in this model of primary hypertension[42].

Treatment with L-NAME did not affect fasting glucose levels but reduced significantly insulin levels in blood and increased insulin sensitivity of rats[43]. Gouveia *et al*[44] described increased glycemia and insulinemia values for fasted or fed SHRs. We observed normal glycemia and insulinemia in both hypertension models in fed state, which contrasts with the studies that show changes in these metabolic parameters. The discrepancy may be due to the metabolic states of the animals in the studies.

Tarsitano *et al*[43] described how prolonged treatment (2-8 wk) with NO synthase inhibitor enhanced hepatic glycogen levels. In our study, as the treatment with L-NAME was only for 10 d, the amount of liver glycogen was similar to the WIS group. This short period of treatment might not have been enough to observe possible changes in the glycogen content. Chronic or acute administration of an inhibitor of NO synthesis (L-NAME or L-NNMA) was shown to alter systemic RAAS, decreasing plasma level Ang Ⅱ as well as renin activity[45]. Nevertheless, hepatic glucose release profile induced by Ang Ⅱ in chronically treated L-NAME animals was similar to the control, which suggests that NO is not involved in the glucose release

after induction.

Interestingly, in the L-NAME group, the glucose release induced by Epi was lower than in the control group, suggesting that this effect may be related to the inhibition of NO synthesis. In cultured rat hepatocytes, Hodis *et al*[46] observed that glycogenolysis occurs *via* α-adrenergic stimulation and signaling cascade that involves the production of NO. Similarly, our results suggest that the chronic inhibition of NO synthase might inhibit hepatic glycogenolysis, which in turn decreases the release of glucose in the perfusate during the experiment. Therefore, the differences in glucose release following the L-NAME treatment evidenced that the increase in hepatic glycogenolysis was probably mediated by NO when activated by Epi but not by Ang Ⅱ.

In the SHR group, it was described that muscle glycogen content was lower, but livers presented similar levels of glycogen in the fed and fasted states[44]. Likewise, we found similar amounts of liver glycogen in the SHR and WKY groups. Despite this similarity, after Ang Ⅱ *in bolus* injection, glucose released was lower in the SHR group compared to the control group. This result suggests that glucose release is not necessarily related to glycogen content, but may be due to a possible difference in glycogen phosphorylase activation by increased $[Ca^{2+}]_i$ induced by Ang Ⅱ[47]. On the contrary, in this hypertension model, glucose release induced by Epi was similar when compared to the control.

Both Ang Ⅱ and Epi are potent physiological vasoconstrictors. We observed that although these agonists led to similar maximum increases of the portal pressure, Ang Ⅱ promoted a higher PHR, even using doses 20-fold lower. These response differences may be related to the prolonged responses induced by Ang Ⅱ in the liver or with the amount of Ang Ⅱ receptor *vs* Epi receptor. An enhanced Ang Ⅱ-mediated vasoconstriction was observed in healthy elderly individuals and this apparent increase is due, at least in part, to the potentiation of α-adrenergic vasoconstriction. These findings suggest that cross-talk between RAAS and adrenergic systems may be an important regulator of resting vascular tone and muscle blood flow with advancing age[48]. Cross-talk between the α1-adrenergic receptor (α1R) and AT1R potentially exists on two levels: Receptor heterodimerization between α1R and AT1R and second messenger level[49].

No difference in the PHR of Ang Ⅱ in the pharmacologic hypertensive model was found, which suggests no changes in the expression of hepatic AT1 receptor. Our result contrasts with AT1R up-regulation described in the L-NAME model in other tissues such as the aorta[50], adrenals[51] and heart[52].

On the other hand, in L-NAME-treated animals, Epi induced increased PHR. It was shown that in rats, chronic inhibition of NO synthase produces endothelial dysfunction, increased vascular response to adrenergic stimulation, and perivascular inflammation[53]. NO is also involved in regulation of sympathetic nerve activity in human skin and muscle cells[54]. Therefore, this increased hypertensive effect in the liver of L-NAME-treated rats may be related to increased sympathetic vascular activity. The disparity between the effects of portal vasoconstriction (higher) and glucose released (lower) in the L-NAME group is a further indication that these effects might be dissociated in two components: One with direct action in the hepatocyte and the other as a presinusoidal response.

We also observed similar vasoconstrictor effect of Ang Ⅱ in the SHR group. Although in this strain, higher levels of *AT1R* gene expression was described in brain regions involved in arterial blood pressure control[55]. Despite widely described sympathetic hyperactivity in this model[56-58], in this work, PHR to Epi on SHRs was similar to the control group.

In conclusion, Ang Ⅱ and Epi responses are altered in different ways in these two models of hypertension. Our results suggest that inhibition of NO production seems to be involved in the hepatic hemodynamic and metabolic effects induced by Epi but not by Ang Ⅱ. Furthermore, diminished glucose release induced by Ang Ⅱ in SHR is not related to glycogen content, but to the glycogen phosphorylase activation by Ang Ⅱ, that is under investigation.

COMMENTS

Background

In a normal liver, angiotensin (Ang) Ⅰ is rapidly converted in Ang Ⅱ by hepatic angiotensin converting enzyme, and Ang Ⅱ promotes hypertensive response mediated by the AT1 receptor. Besides this hemodynamic effect, Ang Ⅱ induces metabolic effects (glucose release and O_2 consumption). Epinephrine promotes hepatic metabolic (glucose and lactate release and O_2 consumption increase) as well as hemodynamic (vasoconstriction) effects. It has also been described as a correlation between the renin-angiotensin-aldosterone system (RAAS) and the sympathetic nervous system; the latter is activated by Ang Ⅱ and plays a fundamental role in the homeostasis of blood pressure control. In hypertension, sympathetic hyperactivity is described but little is known about this hyperactivity in the liver. The hepatic response to Ang Ⅱ and Epinephrine in hypertension has not been studied yet. Therefore, the relevance of this study is to understand the hepatic effects of these hormones in two different hypertensive models.

Research frontiers

The RAAS and the catecholaminergic system are present in the normal liver. The interaction of RAAS with the catecholaminergic sympathetic nervous system in the liver of hypertensive animals might bring to light relevant aspects of the relationship among metabolic disorders such as hypertension, type II diabetes, obesity, and hypertriglyceridemia.

Innovations and breakthroughs

No descript of hemodynamic and metabolic effects of the two hormones Ang Ⅱ and Epi exists in the literature on RAAS and the catecholaminergic system in the livers of hypertensive rats. This is the first study evaluating hemodynamic and metabolic effects of the two hormones Ang Ⅱ and Epi. Inhibition of NO production in the L-NAME model increased hepatic hemodynamic and metabolic effects induced by Epi but not by Ang Ⅱ. Furthermore, diminished glucose release induced by Ang Ⅱ in SHRs is not related to glycogen content. Therefore, the hepatic effect of Ang Ⅱ or Epi is different depending on the pathophysiology of systemic arterial hypertension

Applications

Although not target organs in hypertension, RAAS and sympathetic nervous system are overexpressed, elucidating the hepatic role of these systems, which

can bring knowledge about metabolic-related comorbidities and therapeutics.

Terminology

The portal hypertensive response represents the area under the curve and was calculated from the graphic: Portal pressure increase (cmH_2O) *vs* time after agonist injection (min) and expressed as $cmH_2O.min$. It considers not only the perfusion pressure increase but the effect of the agonist over time.

Peer-review

In this paper, authors give some new information about the effects of Epi and Ang Ⅱ on glucose release, finding that inhibition of NO production seems to be involved in the hepatic hemodynamic and metabolic effects induced by Epi but not by Ang Ⅱ.

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ORIGINAL ARTICLE

Retrospective Study

Use of aspartate aminotransferase to platelet ratio to reduce the need for FibroScan in the evaluation of liver fibrosis

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Abstract

AIM

To evaluate the performance of aspartate aminotransferase to platelet ratio (APRI) score against FibroScan in predicting the presence of fibrosis.

METHODS

Data of patients who concurrently had APRI score, FibroScan and liver biopsy to assess their hepatitis C virus (HCV) and hepatitis B virus (HBV) over 6 years were retrospectively reviewed and details of their disease characteristics and demographics were recorded. Advanced fibrosis was defined as $\geqslant$ F3.

RESULTS

Of the 3619 patients (47.5 ± 11.3 years, 97M:36F) who had FibroScans and APRI for HCV and HBV, 133 had concurrent liver biopsy. Advanced liver fibrosis was found in 27/133 (20%, F3 = 21 and F4 = 6) patients. Although APRI score (P < 0.001, AUC = 0.83) and FibroScan (P < 0.001, AUC = 0.84) predicted the presence of advanced fibrosis, the sensitivities and specificities were only modest (APRI score: 51.9% sensitivity, 84.9% specificity; FibroScan: 63% sensitivity, 84% specificity). Whilst 13/27 (48%) patients with advanced fibrosis had APRI $\leqslant$ 1.0, no patients with APRI $\leqslant$ 0.5 had advanced fibrosis, with

100% sensitivity. The use of APRI $\leqslant$ 0.5 would avoid the need for FibroScan in 43% of patients.

CONCLUSION

APRI score and FibroScan performed equally well in predicting advanced fibrosis. A proposed APRI cut-off score of 0.5 could be used as a screening tool for FibroScan, as cut-off score of 1.0 will miss up to 48% of patients with advanced fibrosis. Further prospective validation studies are required to confirm this finding.

Key words: Liver fibrosis; Aspartate aminotransferase to platelet ratio; Utilization; FibroScan

Core tip: This is the first study to show that an aspartate aminotransferase to platelet ratio (APRI) score of 0.5 could potentially be used as a screening tool to predict the need for FibroScan in patients with hepatitis C or hepatitis B. Our study showed that an APRI score of 0.5 could reduce the need for FibroScan in 43% of the study cohort with high sensitivity.

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INTRODUCTION

Chronic hepatitis C virus (HCV) and hepatitis B virus (HBV) are among the most common causes of liver fibrosis[1]. A determination of the degree of liver fibrosis in these patients is essential to guide management as well as for prognostication[2-6]. Liver biopsy has long been considered the gold standard for assessment of liver fibrosis[2,7,8]. However, liver biopsy is an invasive procedure that carries a 0.3%-0.6% overall risk for complications and a 0.05% mortality rate[8,9]. Several contraindications also exist which may preclude patients from having a liver biopsy, namely coagulopathy[4]. As a liver biopsy only samples approximately 1/50000 of the liver, there have been concerns with sampling errors despite an adequate number of portal tracts and sample size[7,10,11]. Intra- and inter-observer variation in histological interpretation has also been reported[12,13]. Given these limitations, much research has been dedicated to evaluating non-invasive methods to determine liver fibrosis[5,9]. Of these, the FibroScan and aspartate aminotransferase (AST) to platelet ratio (APRI) are commonly used in our hospital.

FibroScan is a novel non-invasive method that measures liver stiffness using both ultrasound and low-frequency elastic waves[14]. A recent meta-analysis showed that FibroScan had a good sensitivity, specificity and high accuracy for detecting liver cirrhosis[15]. However, invalid assessments rates have been quoted to range between 2.4% and 9.4%, mainly due to high body mass index[13].

In 2003, Wai *et al*[2] proposed a novel index APRI with an area under the receiver operating curve (AUROC) for predicting significant fibrosis and cirrhosis 0.80 and 0.89 respectively. A recent meta-analysis showed that an APRI score greater than 1.0 is able to predict cirrhosis with a sensitivity of 76% and a specificity of 72%[16]. This suggests that and APRI score of 1.0 or more would not be an ideal screening tool given it could miss a proportion of patients with cirrhosis. The aim of this study was thus, to evaluate and compare the performance of APRI score against FibroScan in predicting the presence of liver fibrosis and to determine the best APRI cut-off score which can predict the likelihood of fibrosis and the need for further assessment with FibroScan.

MATERIALS AND METHODS

Study population

A retrospective analysis was performed of all the patients with HCV or HBV, who had been referred for FibroScan to the Department of Gastroenterology and Hepatology in the Royal Adelaide Hospital, the largest tertiary referral hospital in South Australia, between January 2010 and June 2016. Inclusion criteria were infection with either HCV or HBV, a valid FibroScan assessment, a liver biopsy within 12 mo of the FibroScan and an APRI score within 6 mo of the liver biopsy. HCV was defined as a positive HCV RNA and HBV was defined as a positive hepatitis B surface antigen and HBV DNA. Exclusion criteria were the use of the XL probe, current interferon-based treatment, co-infection with human immunodeficiency virus, other causes of chronic liver disease, hepatocellular carcinoma, prior liver transplantation, blood results more than 6 mo before or after the liver biopsy, incomplete FibroScan reports and invalid FibroScan assessments. An invalid FibroScan was defined as an interquartile range of more than 30% and a success rate of less than 60%. The project was approved by The Royal Adelaide Hospital Research Ethics Committee, and all patient data were de-identified (RAH protocol approval number: R20160616).

Data collection

Detailed data was collected from FibroScan reports and electronic medical records which included age, gender, HCV or HBV, FibroScan results, FibroScan success rate, FibroScan interquartile range, AST level, platelet count and Scheuer fibrosis scores on liver biopsy reports.

The APRI score was calculated using the proposed formula:

APRI = [(AST level/ULN)/platelet count (10^9/L)] × 100 (2)

The reference value for AST used was 45 IU, which is the upper limit of normal in our laboratory. The FibroScan cut-offs used to define cirrhosis were a median of 14kPa

Table 1 Baseline characteristics of the 133 patients

Characteristic	Value
Gender	97M/36F
Age (yr)	47.5 ± 11.3
Indication for FibroScan	
HCV	79
HBV	54
Mean FibroScan score (kPa)	11.5
Mean IQR (kPa)	2.17
Mean success rate	95.6%
Mean APRI score	0.75
Mean AST level (U/L)	65.5
Mean platelet count (× 10^9/L)	214

HCV: Hepatitis C virus; HBV: Hepatitis C virus; IQR: Interquartile range; AST: Aspartate aminotransferase; APRI: AST to platelet ratio.

and 12.9 kPa for HCV and HBV respectively. Liver fibrosis based on the Scheuer fibrosis system was either no fibrosis (F0), enlarged, fibrotic portal tracts (F1), periportal or portal-portal septa but intact architecture (F2), fibrosis with architectural distortion but no obvious cirrhosis (F3) or probable or definite cirrhosis (F4)[17,18]. Advanced fibrosis was defined as F3 and F4.

Statistical analysis

Patient characteristics were expressed as mean ± SD or *n* (%). Diagnostic performances for FibroScan and APRI score were analysed separately according to sensitivity (Se), specificity (Sp), negative predictive values (NPV), positive predictive values (PPV) and AUROC.

RESULTS

Of the 3619 patients (47.5 ± 11.3 years, 97M:36F) who had FibroScans performed, 133 (3.7%) had either HCV or HBV with concurrent APRI score and liver biopsy assessment. The mean FibroScan score was 11.5 kPa and the mean APRI score was 0.75. The baseline characteristics of the 133 patients are summarized in Table 1. Histological analysis revealed that 25 (18.8%) patients were F0, 42 (31.6%) were F1, 39 (29.3%) were F2, 21 (15.8%) were F3 and 6 (4.5%) were F4. Therefore, advanced fibrosis was found in 27/133 (20%) patients.

Performance of standard FibroScan cut-offs and an APRI score of 1.0 in predicting advanced fibrosis

Although both APRI ($P < 0.001$, AUC = 0.83) and FibroScan ($P < 0.001$, AUC = 0.84) assessments were able to predict the presence advanced fibrosis (Figure 1), the Se, Sp, NPV and PPV of both APRI and FibroScan were only modest (Table 2). Overall, there was good correlation between the APRI score and FibroScan score (Figure 2).

Optimal APRI cut-off scores to predict the presence of liver fibrosis

Based on liver biopsy 9/39 (23%) patients with F2, 12/21 (57%) patients with F3 and 2/6 (33%) patients with F4 had an APRI score of 1.0 or more. Thus, the use of APRI score of 1.0 or more to screen for the need for FibroScan would have missed 13/27 (48%) patients with advanced fibrosis (F3 and F4).

Table 2 Performance indicators of aspartate aminotransferase to platelet ratio score 1.0 and FibroScan for advanced fibrosis

	APRI	FibroScan
Sensitivity	51.9%	63.0%
Specificity	84.9%	84.0%
PPV	46.7%	50.0%
NPV	87.4%	89.9%
Accuracy	78.2%	79.7%

NPV: Negative predictive values; PPV: Positive predictive values; APRI: Aspartate aminotransferase to platelet ratio.

In contrast, based on our plot chart (Figure 3), none of the patients with APRI score of 0.5 or less had F3 or F4 on liver biopsy. Using a lower cut-off APRI score of 0.5 would increase the sensitivity to 100%, but reduce the specificity to 59%. More importantly, the use of APRI score of 0.5 or less would avoid the need of FibroScan assessment in 43% of patients with HCV or HBV who were referred for the procedure.

DISCUSSION

Early and accurate assessment of the degree of liver fibrosis is essential in the management and prognostication of patients with HCV and HBV[2-6]. Given the issues associated with liver biopsy, much research has been dedicated to evaluating non-invasive methods to determine liver fibrosis[5,9]. This study focused on the performance of FibroScan as well as APRI to detect liver fibrosis as these are commonly used in our hospital.

In regards to FibroScan, the AUROC for advanced fibrosis in our study was 0.84. This is comparable to previous studies where the AUROC has ranged between 0.85 to 0.91[3,5,8]. Similarly, the AUROC of 0.83 obtained in the study for APRI was in concordance with previous reports of approximately 0.83 to 0.89[2,3,16]. Overall, our study showed that there was good correlation between FibroScan and APRI in predicting the presence of fibrosis and this is in keeping with results from previous studies[3,8].

There has been an increasing use of FibroScan in our hospital as evident by the growing number done over the past few years; 472 FibroScans in 2013, 612 in 2014 and 761 in 2015. FibroScan is painless, easy to perform and has good patient acceptance[13]. The diagnostic performance is however, influenced by high body mass index[8,13,19]. Thus, the study design excluded patients who required the use of the XL probe.

A recent systematic review looking at the cost-effectiveness of FibroScan compared to liver biopsy showed that FibroScan is economically attractive, but does incur added cost of approximately $1250 to $2922[1]. Apart from the cost, the accessibility of FibroScan may be an issue in the primary health care and resource limited setting. Thus, it would be ideal to have a less expensive,

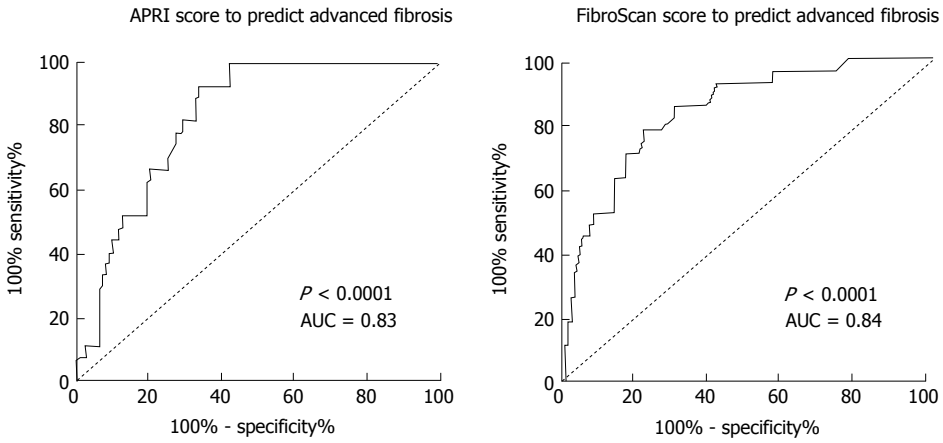

Figure 1 Area under the receiver operating curves depicting the performance of aspartate aminotransferase to platelet ratio score and FibroScan in the prediction of advanced fibrosis on liver biopsy. APRI: Aspartate aminotransferase to platelet ratio; AUC: Area under curve.

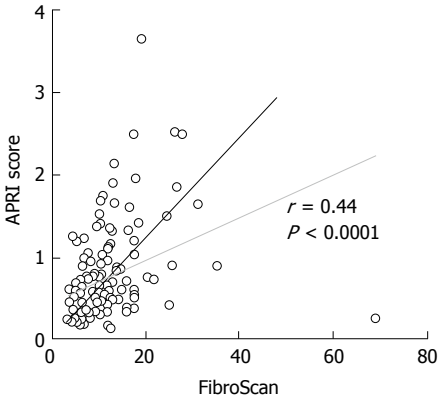

Figure 2 Correlation between aspartate aminotransferase to platelet ratio score and Z-score of FibroScan. APRI: Aspartate aminotransferase to platelet ratio.

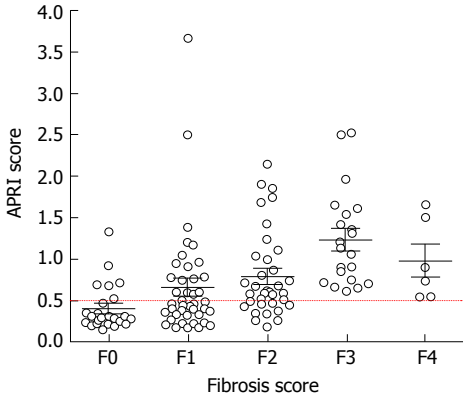

Figure 3 Plot diagram depicting the aspartate aminotransferase to platelet ratio scores in each category fibrosis scores rated on liver biopsy. APRI: Aspartate aminotransferase to platelet ratio.

non-invasive method to screen for patients who would need a FibroScan. The APRI score is an appealing tool, particularly in rural areas, given its ease of use and routine availability of the components of the score in all laboratories.

We evaluated the use of APRI score for this purpose and found that: (1) the use of the currently suggested APRI cut-off score of 1.0 or more to screen for FibroScan would have missed 13/27 (48%) patients with advanced fibrosis; and (2) the use of a lower APRI cut-off score of 0.5 will prevent this problem and avoid the need for FibroScan assessment in 43% of patients with HCV or HBV. A recent study detected the F4 cut-off value for APRI to be 0.7[20]. This cut-off would have missed 2/6 (33.3%) F4 patients in our study.

Although the proposed APRI cut-off of 0.5 would miss approximately one-third of patients with significant fibrosis (F2), this proportion would be even higher if the cut-off value of 1.0 is used. With the recent evolution in the treatment of HCV, sustained virological response is achievable in the vast majority of patients. Current guidelines recommend anti-viral therapy for all patients except those with limited life expectancy or clear contraindications[21]. The decision for treatment initiation is no longer guided by fibrosis stage except in situations where there are limitations to universal treatment of all patients, and for guiding the duration of treatment in patients with established cirrhosis. Fibrosis staging, however, remains relevant for prognostication. While the new suggested cut-off may miss patients with F2 fibrosis, it is more critical to identify patients with F3 and F4 patients who require ongoing hepatocellular cancer surveillance and screening/surveillance for varices[21]. Current guidelines do not recommend routine follow-up of patients with F0-F2 fibrosis following successful treatment of HCV, although this decision would be dependent on clinical judgement especially in patients with confounding risk factors for fibrosis progression (obesity, alcohol, *etc*).

In regards to HBV, the decision to initiate treatment is based on the disease phase (immune tolerant, immune active, immune control or immune escape) and risk of disease progression or liver related complications. This is mostly guided by ALT and HBV DNA level[22]. Liver biopsy or FibroScan is not required for make treatment decision but may be useful in patients who have elevated

DNA levels but normal ALT levels[22]. As the nature of chronic hepatitis B is dynamic, it is recommended that all patients undergo serial monitoring. Given the indices for the APRI score are routine laboratory test and will change with disease progression, this should prompt recalculation of the APRI score and re-staging of the disease by FibroScan or liver biopsy if deemed necessary.

The weakness of this study is the relatively small sample size of patients with liver biopsies. While liver biopsy has historically been considered the "gold standard" for assessment of liver fibrosis, it is imperfect with concerns with of sampling error due to patchy distribution of fibrosis, risk of complications and expense. It has now largely been replaced by non-invasive measures of fibrosis as first line/standard of care for fibrosis assessment. Consequently, the volume of liver biopsies performed in our centre and across most centres has fallen dramatically and it would no longer be considered to perform routine liver biopsies in patients with viral hepatitis. In this study, we only included patient with hepatitis B and C and the finding cannot be generalised to patients with other aetiology for their liver disease. Furthermore, differences exist between patients with hepatitis B and hepatitis C which may impact on their APRI score or FibroScan readings. High ALT levels in hepatitis B may lead to overestimation of fibrosis by FibroScan, whilst HCV-associated immune thrombocytopenia may falsely elevate the APRI score[23]. We also acknowledge that this is a retrospective study from a single centre. Intra- and inter-observer variation in histological interpretation was avoided with the use of a single pathologist who specializes in gastrointestinal pathology.

We, therefore, propose that the use of a new cut-off APRI score of 0.5 could potentially be used to predict the need for FibroScan in the evaluation of patients with viral hepatitis, which would result in significant reduction in health care cost and resources.

In the evaluation of patients with HCV or HBV, APRI score and FibroScan performed equally well in predicting advanced fibrosis. The use of APRI $\geqslant$ 1.0 to predict the need for FibroScan would miss 48% of patients with advanced fibrosis. In the current study, we found that an APRI cut off score of 0.5 is more reliable than 1.0, and able to predict the presence of advanced fibrosis in 100%. More importantly, the use of APRI score of 0.5 or more as a screening tool for advanced fibrosis can reduce the need for FibroScan in 43%. Larger prospective validation studies are warranted to confirm this finding.

COMMENTS

Background

FibroScan is a novel non-invasive method that identifies significant liver fibrosis and cirrhosis. Consequently, its use has greatly increased, posing a demand to the health care system. Aspartate aminotransferase (AST) to platelet ratio index (APRI) is a cheap, blood-test based scoring system that can predict liver fibrosis. Previous study suggested that a score of 1.0 has modest sensitivity and specificity in predicting cirrhosis. This study examined the relationship between the APRI and F-score in predicting advanced fibrosis related to viral hepatitis, and whether it can be used to predict the need of FibroScan.

Research frontiers

The focus of this study is to examine the use of APRI score to predict the need for FibroScan assessment, thus, allowing a better stratification of need and demand of FibroScan in a busy hepatology centres.

Innovations and breakthroughs

Using liver biopsy as gold standard, APRI score and FibroScan performed equally well in predicting advanced fibrosis. More important, the current study found that an APRI cut-off score of 0.5 can be used as a screening tool for FibroScan, as the previously proposed cut-off score of 1.0 missed up to 48% of patients with advanced fibrosis. The use of the newer APRI cut-off score of 0.5 resulted in the avoidance of needs for FibroScan assessment in 43% of referred patients.

Applications

APRI, therefore, should be routinely used in clinical practice and can be used a guide to perform FibroScan. This practice is likely to be cost-effective and improve the work flow of the FibroScan service.

Terminology

FibroScan is a novel non-invasive method that measures liver stiffness using both ultrasound and low-frequency elastic waves. AST to platelet ratio index (APRI) {calculated by [(AST level/ULN)/Platelet count (10^9/L)] × 100} is a scoring system that can predict the presence of advanced fibrosis with good sensitivity and specificity.

Peer-review

The manuscript is a retrospective study evaluated the performance of APRI score against FibroScan in predicting the presence of fibrosis and proposed a new-cut off score of APRI as a screening tool. This study provides a good concept and enhances utilization of APRI score.

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