Advances in Medicine and Medical Research
www.manspub.com
Adv Med Med Res, 2018; 1(1):2-18
ISSN 2535-2210
Open Access
Review Article

Hepatic Ischemia-Reperfusion Injury: Pathogenesis and Pharmacological Treatment

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History
Date submitted: Dec 11, 2017
Accepted: Jan 28, 2018
Published online: Feb 5, 2018

Available online at
www.manspub.com

ABSTRACT

Hepatic ischemia reperfusion injury (R/I) is a hepatic pathophysiologic process occurs post liver transplantation surgery. It also comprises complex systemic process affecting multiple tissues and organs. Hepatic I/R has serious impact on liver function, even producing irreversible failure, which may trigger multiple organ dysfunction. Many factors, including anaerobic metabolism, mitochondrial damage, oxidative stress and secretion of reactive oxygen species (ROS), intracellular calcium overload, cytokines and chemokines produced by Kupffer cells (KCs) and neutrophils are involved in the pathogenesis of hepatic I/R processes. There are many treatment options to combat hepatic I/R injury but none has shown clear beneficial clinical evidence. The purpose of this review is to provide insights into the mechanisms of hepatic I/R injury, indicating the potential factors/signaling pathways involved in this event and available therapeutic approaches that may help to improve controlling hepatic I/R during liver surgery.

Key words: ischemia/reperfusion; Kupffer cells; oxidative stress; mitochondrial damage; NF-kB; tumor necrosis factor.

Cite as: Abdel-Ghany SA, Elashry HA, Ameen MM, Abdel-Rahman KM, Yassin AA. Hepatic ischemia-reperfusion injury: Pathogenesis and pharmacological treatment. *Adv Med Med Res.* 2018;1(1):2-18.

INTRODUCTION

Ischemia and reperfusion (I/R) is a pathological condition characterized by an initial shortage of blood flow to an organ followed by the subsequent restoration of perfusion and concurrent re-oxygenation. Occlusion of the arterial blood flow, such as that happens as a result of an embolus, causes tissue hypoxia with subsequent metabolic and pathological changes. Surprisingly, restoration of blood supply and reoxygenation is commonly associated with activation of several pathways that lead to exacerbation of inflammatory response and profound tissue damage (called 'reperfusion injury') [1]. I/R injury is responsible for pathological events in a wide range of conditions; the most extensively studied of which is cardiac arrhythmia and arrest associated with myocardial ischemia and infarction [2]. Other forms of tissue injury are associated with ischemia of multiple organs and subsequent reperfusion injury when blood flow is restored. Moreover, the untoward effects of I/R are not only restricted to the specific tissue/organ undergoing the initial ischemia but also extend to include injury to other organs distant to the ischemic tissue as a result of mediator release into the bloodstream draining the revascularized tissues and subsequent transport to remote organs, so-called distant or remote organ injury. This phenomenon was studied in most tissues including gut [3], lung [4], liver [5], kidney [6], skeletal muscle [7], and heart [8].

The liver is an example the organs that is frequently exposed to I/R injury during variety of conditions such as tumor resection surgery and liver transplantation. Liver

transplantation is the standard of care in patients with end-stage liver disease (ESLD) and those with tumors of hepatic origin [9]. In Egypt, hepatitis C virus (HCV), and its long-term complications, is a major endemic health problem leading to ESLD. An Egyptian demographic health survey conducted in 2008 concluded that 14.7% of the population have been infected, making this the highest prevalence in the world [10], with much higher rates at around 26% and 28% in the Nile Delta and Upper Egypt respectively [11-12]. The vast majority of these patients eventually require liver transplantation. In our University of Mansoura, Egypt, the Gastrointestinal Surgery Center is leader in liver transplantation surgery. On Wednesday 1st March 2017, a ceremony was organized by Mansoura University to celebrate Case No. 500 for liver transplantation program. Adding that two cases are currently being transplanted weekly and there are more than 200 cases on waiting lists, and that the university seeks life for every patient [13].

Organ shortage has prompted the ultimate care of donors' livers during the transplantation surgery through avoiding prolonged periods of ischemia. However, liver ischemia is unavoidable issue during transplantation surgery making the organ particularly susceptible to I/R injury as a result of cellular damage during procurement, preservation and surgery. Undoubtedly, I/R injury might lead to poor early graft function and primary nonfunction, and furthermore, to both acute and chronic graft rejection. So, effective therapeutic strategies are mandatory to prevent acute complications resulting from I/R injury during hepatic resection and transplantation, and to improve the survival of usable donor grafts.

Development of effective therapeutic strategies to prevent hepatic I/R injury depends on our understanding of the mechanisms and pathogenesis of I/R injury which, unfortunately, remains not well-understood. Several factors/downstream signaling pathways have been implicated in the hepatic I/R injury process, including oxidative stress, anaerobic metabolism, mitochondria, intracellular calcium overload, liver Kupffer cells (KC) and neutrophils, and cytokines and chemokines [14].

The overall aim of this review, however, is to provide insights into the mechanisms of liver I/R injury, indicating the potential role of oxidative stress, inflammatory cytokines, and the role of nuclear factor kappa beta (NF-κB) in the pathogenesis of liver I/R injury, and the protective factors/pathways that may help to improve therapeutic regimens for controlling hepatic I/R injury during liver transplantation surgery. We take into consideration the major role of calcium ions and the renin-angiotensin system (RAS) in the process of I/R injury.

Types and stages of liver I/R injury

Ikeda and co-workers had previously described two major types of liver injury that are attributable to ischaemia–reperfusion in the rat model, the 'warm' and the 'cold' types [15]. The 'warm' I/R injury, with the hepatocellular damage as the prominent feature, develops in situ during liver transplantation surgery or during various forms of shock or trauma, and might lead to liver or even multiorgan failure. The 'cold' I/R injury, with damage to hepatic sinusoidal endothelial cells (SEC) and disruption of the microcirculation as the prominent features, occurs during ex vivo preservation and is usually coupled with warm I/R injury during liver transplantation surgery.

Although initial cellular targets of the two I/R injury types might be different, they do share a common mechanism in the disease etiology; that is, local inflammatory innate immune activation. The activation of liver KC and neutrophils, the production of cytokines and chemokines, the generation of reactive oxygen species (ROS), increased expression of adhesion molecules and infiltration by circulating lymphocytes and/or monocytes are immunological cascades present in both types of I/R injury [16]. Schematic representation of these stages is presented in Fig 1.

Most studies elucidated the mechanistic insights into the stages of liver I/R injury had been conducted on transgenic knockout models and in an in situ model of segmental hepatic warm I/R in mice [14, 17]. A clinically more relevant mouse model, combining cold and warm I/R injury components followed by liver transplantation, has only been established by Shen and co-workers in 2005 [18]. These studies have distinguished two distinct stages of liver I/R injury, with unique mechanisms of hepatic damage. The initial insult is an ischemic injury results from glycogen consumption, lack of oxygen supply which lead to mitochondrial dysfunction and ultimately to ATP depletion and hepatocyte cell death. The second is a reperfusion injury, which follows the ischemic injury and results primarily from an intense inflammatory immune response that includes both direct and

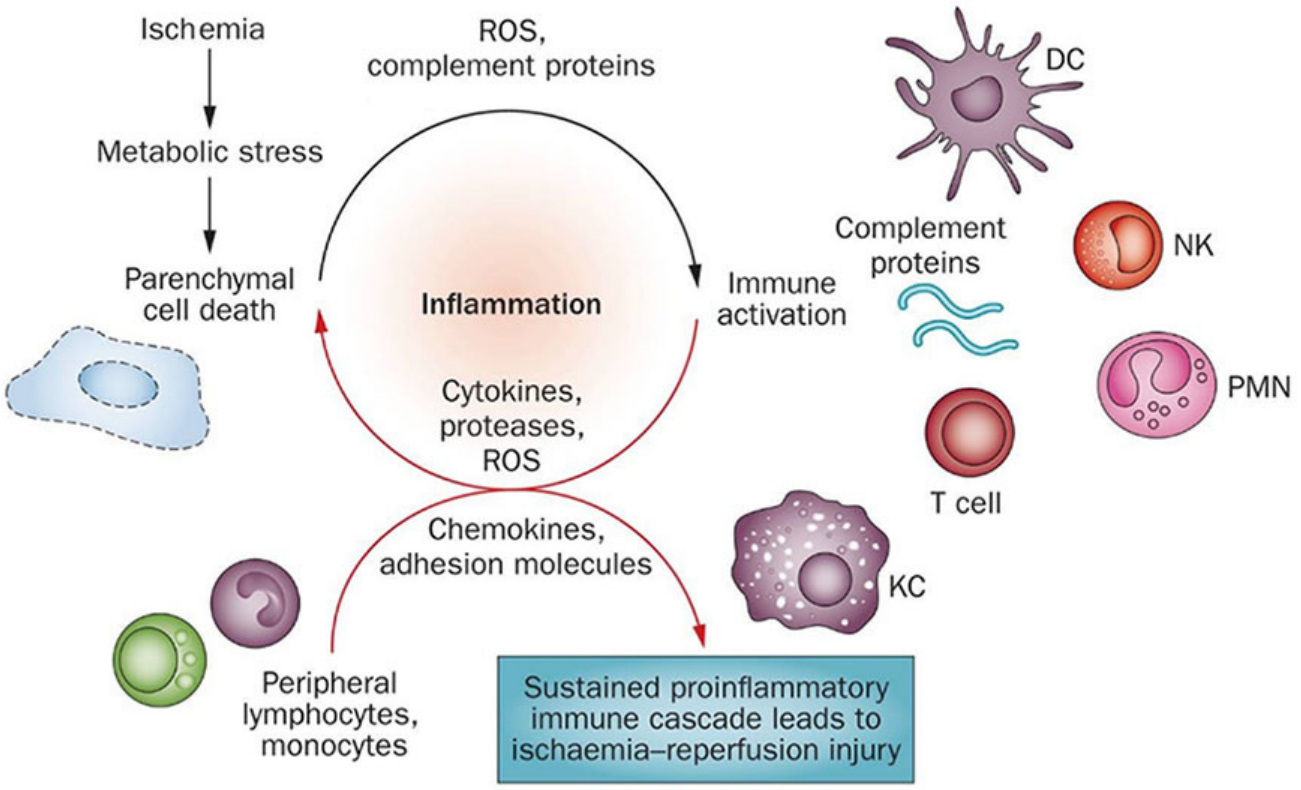

Fig 1. The distinct stages of liver ischemia–reperfusion injury. Ischemic injury, a localized process of hepatic metabolic disturbances, results from glycogen consumption, lack of oxygen supply and ATP depletion. The cell death, activation of complement and mitochondrial reactive oxygen species (ROS) production all contribute to liver immune activation after reperfusion, which involves multiple liver cell types, including Kupffer cells (KC), natural killer (NK) T cells, and polymorphonuclear cells (PMNs). The ischemia–reperfusion-activated proinflammatory immune cascade sustains itself by recruiting peripheral immune cells from the circulation, and is responsible for the ultimate liver reperfusion injury [Reproduced from Zhai et al., 2012].

indirect cytotoxic mechanisms. This inflammatory immune response activated by I/R promptly converts the immunologically quiescent liver into an inflammatory organ; even in a sterile environment [1, 17, 18]. This inflammatory immune response is critical in hepatic I/R injury as prevention of local immune activation have been shown to ameliorate I/R injury [19]. Therefore, good understanding the scenario of immune activation is important for discovering novel therapeutic targets that would be able to block the proinflammatory mechanisms whilst sparing the anti-inflammatory mechanisms.

Types of cell injury and death

There is evidence that hepatocytes and SEC are the two main cell types that are directly injured in both cold and warm I/R injury. Hepatocytes are more sensitive to warm ischemic injury (37 °C), while SEC are more sensitive to cold ischemia (4 °C) that occur in cold preservation of donor liver grafts before transplantation. Exclusive pathological injury of single cell type is not known [17].

Although the causes of cell death are variable, the mode of hepatic cell death typically follows one of two patterns. The first leads to a pathologic pattern of necrosis, the process of which is called "oncosis" or "oncotic necrosis". Oncotic necrosis is typically the consequence of acute metabolic distress resulting from IR. Apoptosis, in contrast, represents the implementation of a death program often initiated by quite specific stimuli [20]. Apoptosis leads to the systematic resorption of individual cells that minimizes inflammatory responses and leakage of cellular components into the extracellular space. Apoptosis and necrosis are usually considered separate issues, but an alternate view is emerging that apop-

tosis and necrosis are frequently the consequence of the same initiating factors and signaling pathways. Rather than being separate entities, apoptosis and necrosis in their pure form may represent extremes on a continuum of cell death [21]. However, the relative contribution of necrotic and apoptotic cell death to the overall liver injury during hepatic I/R is still controversial. Apoptosis is an energy dependent process, so theoretically when there is excessive depletion of ATP, necrosis should predominate. Also, necrosis takes longer time to become evident, normally more than 3 h. This is challenging to demonstrate experimentally in vivo, as tissue ATP before and after reperfusion would need to be measured as well as the altered metabolic activity of the cell. Many of the same factors and pathways are involved in both types of cell death, so there is much overlap. A new term "necrapoptosis" [22] or "necroptosis" [23] has been coined to describe a process that begins with a common death signal and culminates in either cell lysis (necrotic cell death) or programmed cellular resorption (apoptosis), depending on factors such as the decline of cellular ATP levels.

A common event leading to both apoptosis and necrosis is mitochondrial permeability transition (MPT) pore formation (discussed below) and dysfunction, although the mechanistic basis of mitochondrial injury may vary in different settings. Prevention of these modes of cell death is an important target of therapy, but controversies still exist regarding which mode of cell death predominates in various forms of liver disease and injury. Resolution of these controversies may come with the recognition that apoptosis and necrosis frequently represent alternate outcomes of the same cellular pathways to cell death, especially for cell death mediated by mitochondrial MPT pore. An understanding of processes leading to liver cell death will be important for development of effective treatments to prevent hepatocellular death following hepatotoxins or I/R injury and prevent liver cell failure due to various pathologies [23].

Different assay methods have been utilized to identify and quantify apoptosis, including assay for various proapoptotic proteins such as caspase-3 which is thought to be a specific indicator of apoptosis, and Bax. The Terminal Deoxynucleotidal Transferase dUTP Nick End Labeling (TUNEL) procedure is a very common method for examining DNA fragmentation that results from apoptosis. Although TUNEL is a very effective technique for apoptosis assay, one major limitation of this approach is that TUNEL detects apoptotic cells at the latest stage in the process. Varying levels of apoptosis and necrosis have been described in the literature for different ischemia reperfusion models, but these conclusions on the different degrees of apoptosis versus necrosis need to be interpreted carefully as most assay methods for apoptosis are relatively nonspecific [24].

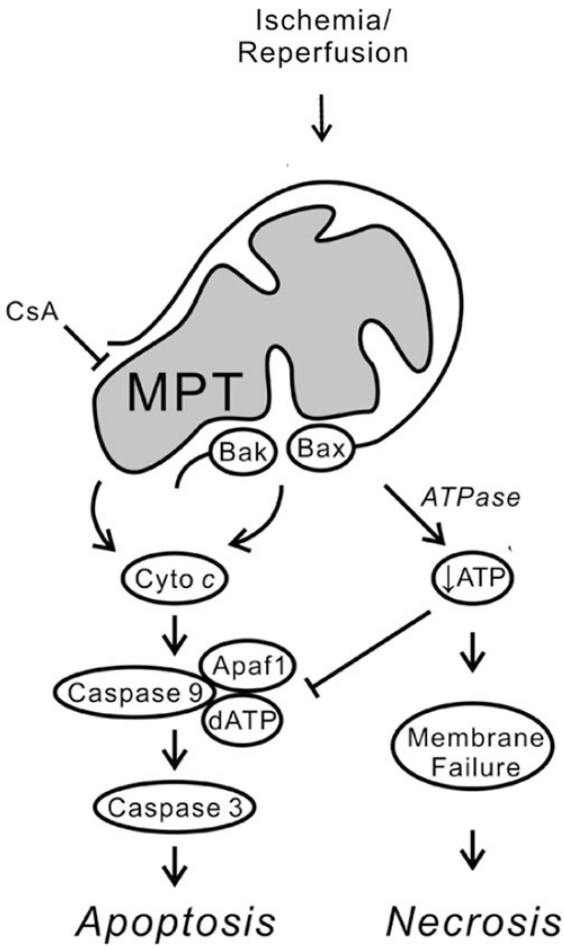

Fig 2. Necrosis and apoptosis as alternate outcomes of mitochondrial permeabilization. Ischemia/reperfusion, hepatotoxins and other stresses converge on mitochondria to induce membrane permeabilization. Permeabilization may involve formation of channels in the outer membrane or induction of a cyclosporin (CsA) sensitive MPT followed by mitochondrial swelling and outer membrane rupture. Other stresses act directly to cause the MPT. After membrane permeabilization, cytochrome c is released to the cytosol and activates in sequence caspase 9 and caspase 3 in a reaction requiring Apaf1 and ATP. With severe and pervasive mitochondrial dysfunction, ATP decreases in part due to activation of the mitochondrial uncoupler-stimulated ATPase. With ATP depletion, caspase activation is blocked, and necrosis occurs instead [From Malhi et al., 2006].

Mechanisms underlying liver I/R injury

The mechanisms of liver I/R injury have been extensively studied, but nevertheless remain generally unclear [25].

The factors/pathways implicated in the hepatic I/R injury process include anaerobic metabolism and acidosis, mitochondrial damage, oxidative stress, disrupted intracellular calcium overload, liver KC and neutrophils, and cytokines and chemokines. More importantly, an effective prevention or treatment method remains unavailable.

Anaerobic metabolism

Liver I/R injury exerts broad spectrum metabolic effects on the body. Immediately after arterial blood has been cut off the liver, the metabolic pattern is shifted from aerobic to anaerobic leading to stopping of mitochondrial oxidative phosphorylation of the hepatocytes and accumulation of acidic metabolites, such as H+ ions, lactic acid and ketone bodies [25]. When mitochondrial ATP is considerably depleted, all intracellular ATP-dependent metabolic and enzymatic functions are gradually ceased off. However, after the pH restore to normal values with reperfusion, activation of intracellular pH-dependent proteases and phospholipases takes place leading to more worsening of tissue damage. This is called the pH paradox [17]. The toxicity resulting from acidic environment and low ATP results also in disturbance of Na+ and calcium homeostasis, impairment of the cellular functions, signaling interactions, and sodium/potassium ATPase (Na+/K+-ATPase), leading finally to mitochondrial damage and cell death [26].

Mitochondrial dysfunction

The mitochondria are the place where oxidative phosphorylation mainly takes place. Many studies have shown that mitochondrial dysfunction plays a critical role in the process of hepatocyte damage after I/R injury [27, 28, 29]. Opening of a non-specific pore in the mitochondrial inner membrane that is called the mitochondrial permeability transition (MPT) pore is a critical event in the progression of cell death in response to I/R injury (also known as mitochondrial permealization). During the ischemic period, the inner MPT pore is kept quiescent being inhibited by the low pH. However, upon reperfusion, the huge increases in mitochondrial Calcium, coupled with excess formation of ROS, induce opening of the MPT pore [27]. The pore can pass any molecule < 1500 Dalton, and therefore, H+ ions can pass back into the matrix through this channel, thereby inhibiting electron transport and ATP synthesis [30]. In addition, water molecules enter the mitochondria under osmotic gradient causing the mitochondria to swell and even rupture which leads eventually to hepatocyte cell death. The extent to which pores remain open correlates directly with the duration of ischemia and inversely with the recovery of cells. Agents or protocols that protect against MPT pore opening such as cyclosporin A [31], melatonin [32] and edaravone [33] were shown to provide mitochondrial protection against reperfusion injury. In a recent review, Bernardia and Di Lisa have recently discussed the molecular mechanism of the mitochondrial permealization and its significance in cell response to ROS and ischemic injury [34].

Oxidative stress

A substantial body of literature has shown that oxidative stress plays a crucial role in I/R injury [35-37]. Many highly reactive ROS molecules including superoxide anions, hydroxyl radicals, and peroxide hydrogen are generated during the period of hepatic I/R injury. These reactive molecules attack many cellular organelles, proteins, lipids, and nucleic acids leading to enzyme inhibition, mitochondrial instability and formation of deleterious lipid peroxides. ROS can also damage endothelial cells and destroy the integrity of the microcirculatory system. There are thought to be three main pathways for the generation of ROS: conversion of xanthine dehydrogenase to xanthine oxidase (XO) during ischemia, activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, and uncoupling of the mitochondrial electron transport chain. Although hepatocytes can directly generate ROS, physiologically KC are thought to be the main source of ROS in the early stages of liver I/R injury with natural killer T-cells being the main source later, and neutrophils being the main source in the very later stages [38-39]. Many studies have shown that antioxidants, such as superoxide dismutase, catalase, glutathione, α-tocopherol, or herbal antioxidants exerted some protection against I/R injury induced by ROS [35-36]. On the other hand, recent researches have investigated the role of nuclear transcription factors controlling the function and expression of genes coding for natural antioxidant defense components in combating ischemia–reperfusion-induced oxidant stress [40]. Individual oxidation stress-related gene armamentarium has been also studied in hepatic I/R injury models [41].

Microcirculatory dysfunction

Microcirculatory disturbance plays an important part in

hepatic I/R injury. Reduction in sinusoidal caliper and diminished blood flow are among the earliest changes in reperfusion injury. This results from a combination of direct damage to liver SEC, together with vasoconstriction and expression of adhesion molecules with accumulation of platelets and leucocytes. Two of the key vasoactive players that maintain sinusoidal vascular tone are the vasoconstrictor endothelin-1 (ET-1), and the vasodilator and inhibitor of platelet aggregation, nitric oxide (NO). In the early stages of liver I/R injury, there is a relative excess of ET-1 [17].

Liver transplantation in pigs has provided evidence that after reperfusion KC activation leads to increased release of ET-1 which binds to endothelin-A receptors on SEC and hepatocyte, thereby reducing hepatic micro and macro-circulation resulting in accelerated liver injury. The activation of this pathway is associated with increased expression of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and endothelial nitric oxide synthase (eNOS) [42]. Some studies have shown that carbon monoxide and NO, which are all vasodilators, are likely play a role in reducing the severity of liver I/R injury [25, 43].

Cytosolic calcium overload

Physiologic intracellular calcium concentration is about 10–100 nM, which is 10,000 folds less than the extracellular concentration. This gradient is essentially maintained by four mechanisms: (1) the ATP-dependent transmembrane efflux pump; (2) the Na+/K+ mediated Na+/calcium exchange pump; (3) the ATP-dependent calcium stores present in the endoplasmic reticulum; and (4) the mitochondrial oxygen-dependent calcium pump [44]. Under ischemic conditions, the absence of oxygen stops oxidative phosphorylation and diminishes ATP, thereby, the energy source by which three of the four mentioned mechanisms that preserve hepatocellular calcium homeostasis is depleted. This, in turn, leaves nonselective cell membrane calcium entry channels unopposed, resulting in increase in calcium entry [45]. Mitochondrial sequestration, the last of the four mechanisms, subsequently overloads the mitochondria with calcium causing more inhibition of oxidative phosphorylation as oxygen levels are further depleted [46]. In addition, and as a consequence of ischemia, the cytosolic pH falls due to accelerated anaerobic glycolysis. In an attempt to reestablish normal pH, hepatic cells extrude H+ ions in exchange for Na+ via a NHE. The Na+ ions are then exchanged for calcium by another Na+/Calcium exchanger causing a net increase in cytosolic calcium [47]. Furthermore, endogenous release of cytosolic calcium stored in the endoplasmic reticulum is also enhanced through activation of ryanodine receptors [48]. Upon reperfusion, there is neutrophil accumulation and release of ROS and inflammatory cytokines by the activated neutrophils and KC which further devastate the cellular damage and capillary degradation. Prior to the reestablishment of oxygen, the hydrolysis of ATP is believed to accumulate hypoxanthine, a precursor of free-radical formation. In addition, the increased intracellular calcium from the ischemic phase promotes the production of conversion of xanthine dehydrogenase into XO, a proteolytic enzyme involved in free-radical synthesis. Thus, during reperfusion the re-oxygenation of cells causes (1) XO-induced production of ROS; and (2) restoration of ATP levels, which, in turn, allows active uptake of calcium by the mitochondria, resulting in massive calcium overload and destruction of the mitochondria.

Normally the level of calcium inside the mitochondria is low, but as cytoplasmic calcium increases, the mitochondria can act as a buffering box for the excess calcium [48]. During overload, however, the mitochondrial Na+/Calcium exchanger is overwhelmed, allowing mitochondrial levels to become high enough to trigger activation of the MPT pore in the inner mitochondrial membrane with subsequent cell death by apoptosis or necrosis [27]. The rise of cytosolic calcium associated with I/R injury can also induce pathological activation of Calcium/CaM-dependent protein kinase II, PKA, and PKC [23] and activate calcium dependent calpain family of proteases that can degrade a number of intracellular proteins [49]; all of these events join forces to cell death and organ dysfunction. Studies have also shown that a period of I/R alters the redistribution of calcium between cellular and subcellular compartments leading to release of unwanted autophagosomes, plasma membrane crumbling, mitochondrial dysfunction, and nuclear condensation [50].

Calcium channel blockers (CCBs) include phenylalkylamines (e.g. verapamil), dihydropyridines (e.g. nifedipine, amlodipine, and nimodipine), and benzothiazepines (e.g. diltiazem). calcium channel blockers of dihydropyridine group, such as nimodipine, are derived from the molecule dihydropyridine and often used to reduce systemic vascular resistance and arterial pressure, and to prevent cerebral vasospasm [51].

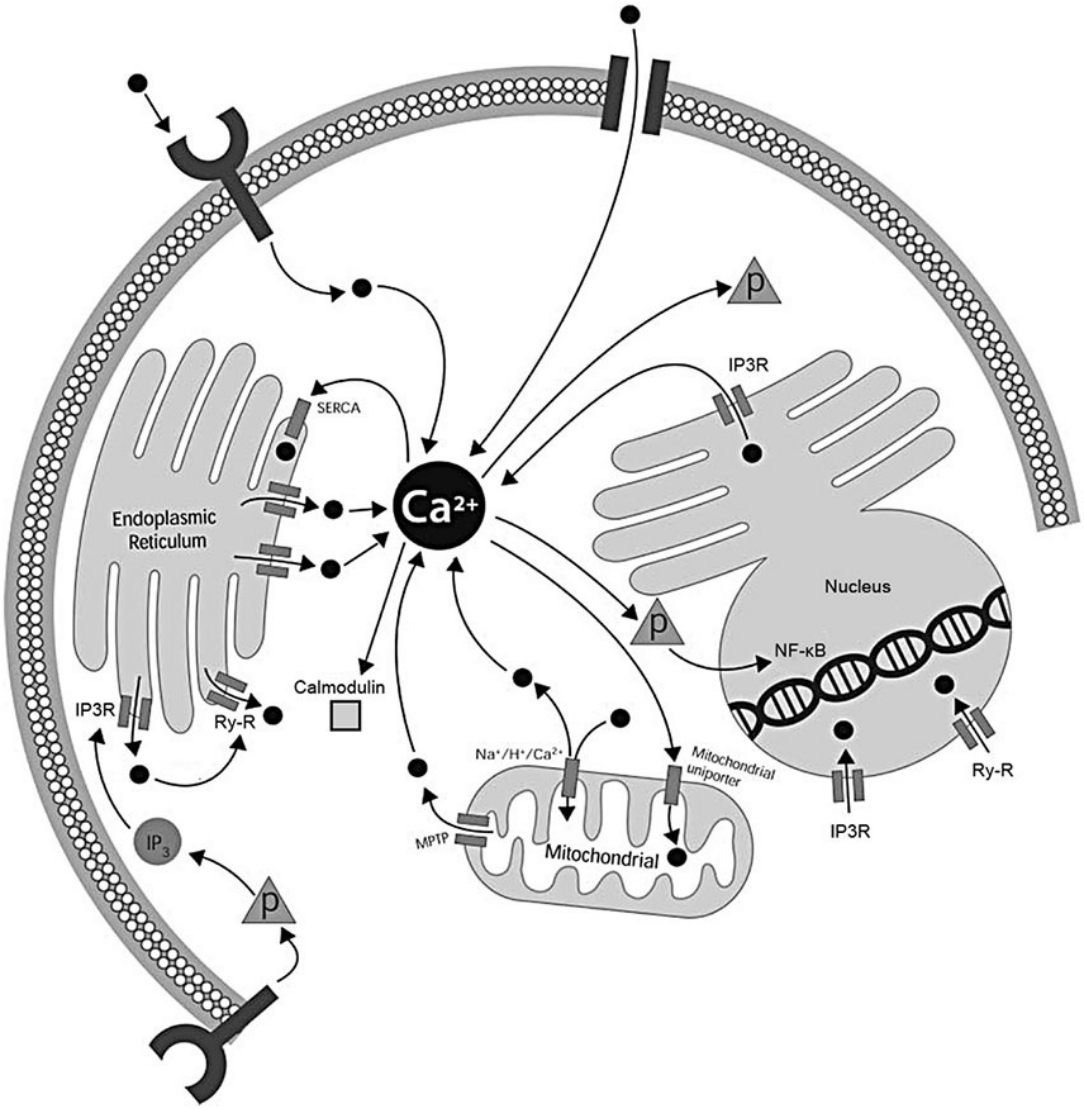

Fig 3. Overview of calcium-mediated intracellular signaling pathways. Calcium initially enters the cell through transmembranous channels and receptors prior to pooling the cytoplasm where free calcium resides. The plasma membrane calcium-entry pathways include calcium store-operated channels, receptor-activated channels, and ligand-gated channels. Calcium outflow across the plasma membrane is controlled by Ca2+/Mg2+ ATPase and Na+/Ca2+ exchanger. Located in the cytoplasm, calcium-dependent proteins, such as PKA, PKC, calpain, and calmodulin (collectively represented by triangular P). Mitochondrial calcium uptake is mediated by its uniporters, whereas outflow is managed by MPT pore and Na+/Ca2+ and Ca2+/H+ antiporters. Calcium is released from endoplasmic reticulum by IP3R and RyR. Nuclear transcription factors such as NF-κB are upregulated by cytoplasmic proteins [Reproduced from Chang et al., 2010].

Several reports have suggested that different CCBs improve survival in hepatic I/R injury owing to their ability to inhibit the increase of cytosolic calcium in hepatocytes during I/R and prevent mitochondrial toxicity [52-53]. In one study supporting this assumption, amlodipine prevented hepatocyte cellular damage and preserved mitochondrial integrity by inhibiting the calcium channels of hepatocytes in rat liver I/R injury [53]. Other reports, however, suggest that the protective role of CCBs is not only due to preventing an increase in hepatic calcium concentration in parenchymal cells, but also seem to protect the liver from ischemic injury by acting on sinusoidal lining or vascular cells rather than on parenchymal cells [54].

Hepatic tissue blood flow has been considered to play an important role in hepatic I/R events, and in liver transplantation, graft microcirculation failure is considered a major determinant of postischemic liver injury [16]. A previous study reported that amlodipine attenuated hepatic I/R injury by cytoprotective effects on parenchymal and non-parenchymal hepatocytes during both preservation and reperfusion

by improvement of microvascular circulation and inhibition of mediator release [55]. Chin and co-workers have also reported that the protective effect of the calcium antagonist diltiazem in liver I/R injury was mainly due to improvement in microvascular circulation [56]. Other calcium channel antagonist verapamil was demonstrated to have a modest protective effect in hepatic I/R injury mainly by prevention early generation of ROS [57].

On the other hand, systemic hypertension is a common complication after liver transplantation. Several studies have reported that about 50% or more of the liver transplant recipients develop hypertension [58]. Due to their significant vasodilatory effects attenuating the vasoconstriction and endothelin-1 induced by immunosuppressant treatment, CCBs have been commonly used for the treatment of hypertension in these patients [58]. According to a review published in 2011 on the management of hypertension in liver transplant patients, dihydropyridine CCBs, such as nifedipine, isradipine, or amlodipine, are preferable due to their least interaction with the cytochrome P450 enzyme system and, therefore, carry the minimal risk of potential disruption of immunosuppressant levels. The authors also concluded that for the treatment of de novo hypertension after liver transplantation, dihydropyridine CCBs should be used as first line drugs due to their potent vasodilatory effects [59].

Role of Kupffer cells (KC) and neutrophils

Liver inflammation can be induced by I/R but it is typically described as sterile inflammation compared to inflammation induced by invading pathogens. Similar to the response to invading microorganisms, the sterile inflammation induced by I/R injury is characterized by the production of cytokines, chemokines, and other proinflammatory stimuli by mainly hepatic KCs and neutrophils [23, 60]. The KCs mainly mediate liver ischemic injury in the earlier stage of reperfusion (within 2 h) through synthesizing and librating ROS and the pro-inflammatory cytokines TNF-α and IL-1to cause further activation of neutrophils and enhance the expression of the adhesion molecules intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) in liver SEC. This further promotes the adhesion, migration, and recruitment of neutrophils in the subsequent stage of reperfusion with release of potent oxidants, inflammatory cytokines, and proteases resulting in extensive collateral damage to liver cells [61].

Neutrophil infiltration occurs as a result of inflammatory responses to necrotic cells and release of mediators and ROS by KCs and blocking of KCs activation by the use of gadolinium chloride or methyl palmitate could reduce acute liver cell injury caused by I/R [62]. The sequestration of innate immune cells occurs primarily during reperfusion, which restores the delivery of oxygen and neutrophils to the tissues. Although essential to support cellular metabolism, the flux of oxygen into the previously ischemic tissues fires the formation of ROS by enzymes such as XO and NADPH oxidase by the invading neutrophils which will then direct their cytotoxic armory at parenchymal cells causing reperfusion injury that exacerbates cell damage and death caused by the previous ischemia [60].

Cytokines and downstream signaling

The complex relationship between cytokines and chemokines in liver I/R injury is not fully understood. The activation of KCs leads to the release of wide array of pro-inflammatory cytokines such as TNF-α, IL-1β, IL-6, platelet-activating factor (PAF) and other chemokines. Anti-inflammatory mediators are also released by activated KCs which consist of prostaglandins (PG), IL-4, IL-10 and IL-13 [63]. Serum level of TNF-α was found to rise within 30 min of reperfusion and persists for up to 8 h [64]. The major effect of TNF-α is to induce liver cell injury through neutrophil activation, ROS production, and mitochondrial toxicity [65]. TNF-α triggers expression of nuclear factor kappa beta (NFκB) and IL-1 which in turn upregulates the production of TNF-α in a positive feedback loop. Both TNF-α and IL-1β also increase expression of adhesion molecules in hepatic SEC. The role of these cytokines in hepatic I/R injury is further confirmed by experiments which show that their suppression by monoclonal antibodies and receptor antagonists attenuates liver I/R injury [66].

Interferon (IFN)-β is a cytokine which is implicated throughout the reperfusion stage in liver I/R injury as shown from studies performed on knockout mice. The damaging effects of IFN-β are mediated by binding to IFN receptor subtype 1. Animal models deficient of this receptor showed modest hepatic injury after I/R [67]. Studies on animal knockout models have also confirmed the release of IFN-γ by the natural killer T cells (NKT) during the early period of reperfusion and its contribution to liver cell damage [68]. Activation of toll-like receptor 4 (TLR4) on the surface of NKT

cells during liver I/R stimulates the release of both IFN-β and IFN-γ. The adverse effect of PAF in liver I/R injury was also demonstrated in rats pretreated with a specific PAF receptor antagonist. These rats exhibited suppression of the increased TNF-α and neutrophil attracting chemokines [69].

Not all inflammatory cytokines appear to be injurious in hepatic I/R injury. In agreement with this fact, Camargo and co-workers have early shown that IL-6 appears to play a protective role as evidenced by the finding of that I/R injury in livers of IL-6 knockout mice was more severe than in wild type mice. Administration of recombinant IL-6 to the knockout mice before ischemia restored the mild type injury patterns seen in wild type mice [70]. There is some evidence that IL-4, IL-10 and IL-13, all of which are anti-inflammatory cytokines, may also play a protective role in I/R injury. An early study conducted on mice showed that IL-10 suppressed expression of TNF-α, NF-κB activation, and significantly reduced liver neutrophil recruitment, edema and cell injury following partial hepatic I/R injury [71]. A recent study performed with cerebral ischemia in IL-4 knockout mice which showed that IL-4 partially protects against ischemic brain injury in mice [72]. Chemokines, like cytokines, are a family of locally produced factors, but chemokines are smaller molecules, which act locally at site of inflammation to guide leukocyte chemotaxis. The chemokine, known as CXCL-10, was shown to contribute to liver I/R injury 1h after reperfusion onwards in association with activation of KCs and neutrophils and increased TNF-α and IL-1 release [73]. In agreement with this finding, a study conducted in 2009 has shown that activation of chemokine receptor type 2 contributes to liver injury during I/R and increase neutrophil recruitment [74].

Role of NF-κB

NF-κB is a pleiotropic transcription factor which is present in almost all cell types and is present as a homo- or heterodimeric complex formed by the Rel-like domain-containing proteins RELA/p65, RELB, NFKB1/p105, NFKB1/p50, REL and NFKB2/p52 and the heterodimeric p65-p50 complex appears to be most abundant one. The dimers bind at kappa-B sites in the DNA of their target genes and the individual dimers have distinct preferences for different kappa-B sites that they can bind with variable affinity and specificity. Different dimer combinations act as transcriptional activators or repressors. NF-κB is controlled by various mechanisms of post-translational modification and subcellular compartmentalization as well as by interactions with other cofactors or corepressors [75].

NF-κB is best known for its function in the expression of immunoregulatory genes relevant in many biological processes such as inflammation, immune response, cell differentiation and growth, tumorigenesis and apoptosis. In particular, NF-κB plays a pivotal role in regulating the transcription of cytokines TNF-α, adhesion molecules, and other cytokines and chemokines. In resting cells, NF-κB is retained in an inactive state in the cytoplasm by direct binding to an inhibitor of NF-κB (I-κB) protein. In response to inflammatory stimuli, including ROS and TNF-α, I-κB proteins are phosphorylated and degraded. Degradation of I-κB proteins unmasks the nuclear localization sequence of NF-κB subunits, allowing NF-κB to translocate to the nucleus and bind specific promoter elements and induces gene transcription [76].

During the reperfusion period, activated neutrophils infiltrate tissues and adhere to hepatic SEC through adhesion molecules, resulting in localized release of proteases, ROS and various cytokines and inflammatory mediators that activate NF-κB and contribute to tissue injury. The activation of NF-κB in rats subjected to warm liver I/R injury was shown to occur within 1–2 h after the initiation of reperfusion and decreases after 4 h. NF-κB furthermore upregulates the expression of cytokines, like TNF-α, and of ICAM-1 and VCAM-1, enhancing the recruitment of neutrophils. TNF-α and many other cytokines in addition of being triggered by NF-κB but can also directly upregulate it, thus establishing a positive feedback loop that can amplify the inflammatory response and extend the duration of inflammation. The damage in I/R injury therefore spreads throughout the entire tissue [77]. Reported results suggest that decreased NF-κB activation and subsequent reduction in TNF-α level are important in the protective mechanism of hepatic I/R injury. Inhibition of XO derived ROS generation in activated neutrophils by allopurinol was also shown to markedly diminish NF-κB activation and attenuate hepatic [78] and renal [79] I/R injury in rat models.

Adhesion molecules

The adhesion of neutrophils to hepatic parenchymal cells and SEC, and migration into liver tissue require sequential steps in which many adhesion molecules are involved. The

selectin category of adhesion molecules (P, E and L-selectin) are expressed by SEC early in reperfusion to mediate adhesion of platelets and neutrophils. Studies have shown that P-selectin expression is raised 20 to 30 min after reperfusion [80]. Other researchers have suggested that E-selectin expression, and not P-selectin, is required for I/R injury to occur [81]. This is followed by stronger adhesion of leukocytes on SEC by upregulation of other adhesion molecules such as VCAM-1 and ICAM-1. By doing so, they migrate across the endothelium into the liver parenchyma enhancing ROS production and degranulation of cytoplasmic vesicles containing enzymes capable to degrade extracellular matrix and hepatocytes.

Role of Renin-Angiotensin System (RAS)

The implication of the renin–angiotensin system (RAS) in cardiac and renal pathology has been subject of substantial body of research, as it may synchronize fibrosis and tissue repair, and is consequently a goal for treatment with RAS blockers. In addition to the systemic RAS, many tissues and organs can express local functional RAS components that are regulated by incentives within the same organ. Recent research has pointed at the presence of local RAS in the liver, and its role in the pathogenesis of liver disease and fibrogenesis [82]. Both local and systemic RAS appear to play bimodal role in tissue remodeling and scarring, especially after injury [83].

The 'classical' RAS includes principally angiotensin II (Ang-II), angiotensin-converting enzyme (ACE) and the Ang-II type 1 (AT1) receptor, and is responsible for, not only sodium homoeostasis and vasoconstriction by acting on vascular smooth muscle cells, but also has a role in inflammation, cytokine production, cell proliferation, and tissue repair. It is also involved in the pathogenesis of the metabolic syndrome through its effects on fat metabolism and insulin sensitivity [84]. Other components of the RAS that may mediate inflammation and fibrosis include the prorenin receptor, which is likely to have intracellular functions beyond its role as a receptor for renin and prorenin. Another 'alternative' RAS has been identified in both human and rodents. It consists of a structural homologue of ACE, named ACE2, which shares more than 40% structural similarity with ACE [85]. ACE2 is able to cleave both Ang-I to Ang-(1–9) and Ang-II to Ang-(1–7) respectively. Many effects mediated by Ang-(1–7) appear to oppose those of Ang-II and the AT1 receptors, and include vasodilation, improvement of endothelial function, inhibition of cell proliferation, and antithrombotic effects [86]. There is increasing evidence that Ang-(1–7), beside its effect on its specific Mas receptor; it may also mediate part of its effects via the AT2 receptors.

Several researches have studied the expression of RAS components in the liver both in animal models of hepatic fibrosis and in human tissue samples. In an elegant binding study, Bataller and co-workers demonstrated that hepatic stellate cells (HSC) express the AT1 receptor and that Ang-II exerted a marked concentration-dependent increase in intracellular calcium levels, cell contraction and proliferation. Paizis and co-workers demonstrated that gene expression of both ACE and AT1 receptor were upregulated in the liver by bile duct ligation, and were specifically marked in areas of active hepatic fibrogenesis [87]. In addition, Ang-II was shown to stimulate gene expression of transforming growth factor-beta (TGF-β) and fibronectin by activating AT1 receptors in KC. In human samples of advanced fibrotic liver, the expression of AT1 receptors was downregulated in hepatocytes, while it was upregulated in HSC, vascular endothelium and bile duct epithelium [88]. In addition to the above-mentioned effects of RAS in the development of liver fibrosis, results of recent research have shown that this system also plays a proinflammatory role in tissue, however, the mechanisms by which the RAS may play a role in mediating hepatic inflammation remains largely not well-understood. Several researches have shown that Ang-II stimulates expression of proinflammatory cytokines and chemokines, adhesion molecules such as ICAM-1, and activates proinflammatory transcription factors such as NF-κB [89]. In the liver, both KC and SEC have been shown to respond to Ang-II to produce proinflammatory and profibrotic cytokines, chemokines and ROS. Harrison and co-workers have demonstrated that Ang-II increases superoxide production via direct activation of NADPH oxidase [90].

Although a substantial body of data in the literature has documented the role of RAS in liver diseases, inflammation, and fibrosis, little is known about the role of RAS and/or its blockers in the pathogenesis/protection of hepatic I/R injury. The very few studies available in this topic yielded inconsistent conclusions. In rodent models of hepatic I/R injury, Guo and co-workers have shown that blocking of the systemic of RAS by captopril and lisinopril could effectively protect the liver during IR. The authors concluded that this effect was

possibly through inhibition of TNF-α and ICAM-1 [91]. In another study, lisinopril could effectively attenuate liver I/R injury in rat model. The author concluded that this effect was possibly by improving nitric oxide availability and reducing oxidative stress [92].

Treatment options to ameliorate I/R injury in liver transplantation

Pharmacological treatment is believed to be effective to reduce I/R injury in liver transplantation based on data available today from experimental research. Most of the studied agents have short-term survival benefits identified in experimental researches; therefore, the identified agents should be further evaluated in human clinical trials. A recent metanalysis study has reviewed the available pharmacological agents to reduce hepatic I/R injury in rat liver transplantation [93]; however, none of these pharmacological agents against I/R injury have become part of the clinical routine.

Antioxidants

Because of the strong evidence for the importance of ROS in hepatic I/R injury, a number of different intervention strategies have been successfully used in multiple models of warm and cold I/R injury.

Xanthine oxidase (XO)

This hypothesis was mainly based on XO being invoked as a critical source of ROS during I/R injury, and the protective effect of the XO inhibitor allopurinol [79]. Unfortunately, there are multiple reasons for the limited importance of this approach under clinically relevant conditions. First, the enzyme XO exists as xanthine dehydrogenase and the conversion to the ROS-generating oxidase requires long time of ischemia. In addition, the ROS formation depends on the substrates xanthine and hypoxanthine which are relatively fast metabolized and flushed out together with other metabolites from the liver during reperfusion.

Glutathione (GSH)

The tripeptide glutathione (GSH), present in high concentrations in hepatocytes, is a highly effective antioxidant by serving as an electron donor. Intravenous infusion of GSH has been shown to protect against reperfusion injury following rat liver transplantation [94]. On the other hand, treatment with the cysteine derivative N-acetylcysteine (NAC) can increase intracellular GSH levels and strengthen the defense arsenal when given before liver I/R. Furthermore, clinical trials have reached phase IV for the use of NAC as a protective agent against both warm and cold ischemia during liver surgeries [95]. Gene transfer of glutathione synthesis components glutamine cysteine ligase catalytic subunit, glutamine cysteine regulatory subunit, and glutathione synthase are attractive options to protect against I/R injury by increasing intracellular GSH levels [96].

Superoxide dismutase (SOD)

Administration of exogenous catalase or superoxide dismutase (SOD) was among earliest interventions as a pretreatment before ischemic injury. Consequentially, induction or maintenance of SOD levels has become another common explanation for mechanism of protection by numerous substances [97]. However, even intravenous administration of high doses resulted in only partial protection, largely due to poor bioavailability of the enzyme. Thus, potential antioxidant therapies using modified variants of SODs, or catalase, may be alternative option.

α-Tocopherol

α-tocopherol is a vitamin E analogue that is favorably absorbed in humans. In an early study, oral pretreatment with α-tocopherol attenuated I/R injury and lipid peroxidation and increased synthesis of ATP when compared to control mice. The effect on cellular ATP levels suggests that this treatment improved mitochondrial function. However, in a double-blind randomized placebo controlled trial, treatment with a full racemic mixture of α-tocopherol showed no reduction in clinical outcome [98]. A latest mixture composed of vitamin E, taurine, and GSH showed a reduction in hepatic I/R injury in rats [99]. This suggests that a combination of more than one antioxidant may be more effective than employing a single one.

Ischemic preconditioning

Ischemic preconditioning is probably the most common method investigated for reducing I/R injury. It relies on exposing the liver to a short period of ischemia then reperfusion before the real period of hepatic ischemia. Ischemic preconditioning has been shown to reduce inflammatory

response as well as attenuate oxidant stress [100]. The mechanisms underlying the preconditioning effect involve activation of the prosurvival p38/MAPK signaling pathway [101], induction of antioxidant stress response genes such as heme oxygenase-1, and increased hepatocyte proliferation in response to ischemic challenge [102]. There is evidence that ischemic preconditioning shows beneficial promise in the clinical settings. Ischemic postconditioning has also been shown to be protective against I/R injury and has similar mechanisms as to preconditioning, such as activation of the prosurvival PI3K/Akt signaling pathway and induction of antioxidant enzymes such as SOD and the vasodilator nitric oxide [103].

MPT pore prevention

As discussed in section 3.2., opening of MPT pore in the inner mitochondrial membrane is a critical event in mitochondrial toxicity and progression of cell death in response to I/R injury. A number of treatments have been tried to decrease MPT pore during I/R principally by preventing extracellular ROS generation, including administration of melatonin and edaravone [32-33]. Edaravone is an intravenous drug that has been used to help people recover from stroke in Japan since 2001. In May 2017, edaravone was FDA approved to treat patients with amyotrophic lateral sclerosis (ALS) in the United States [104]. Although originally designed for use in ischemic stroke, the drug inhibited MPT pore formation and exerted good preservation of mitochondrial respiration potential and a reduction in mitochondrial swelling when given before I/R of liver [33] and brain [105], most probably owing to its strong antioxidant properties.

Targeting inflammatory cytokines/chemokines

The protective roles of IL-4, IL-10, IL-12, IL-13 and IL-18, all of which are anti-inflammatory cytokines, have been investigated in animal models of liver I/R injury. A study performed on mice showed that injection of recombinant murine IL-10 after partial hepatic I/R injury suppressed expression of TNF-α, NF-κB and significantly reduced neutrophil recruitment, inflammation and hepatocellular injury. Viral gene transfer of IL-10 and injection of hypertonic saline solution which also increases IL-10 levels also reduced hepatic I/R injury. Xiong and co-workers have recently shown that IL-4 partially protects against ischemic brain injury in IL-4 knockout mice [72].

A murine model was used to explore the role of IL-12 following hepatic I/R injury. Hepatic ischemia for 90 min followed by reperfusion for 4 h resulted in hepatocyte expression of IL-12. Hepatocellular injury, neutrophil recruitment, increased TNF- α and serum ALT were markedly reduced after administration of neutralizing antibody to IL-12 [106]. The histamine agonist, dimaprit, also exerted protective effects in liver I/R injury by decreasing IL-12 level. The role of IL-13, an antiinflammatory cytokine, was also studied in murine model of hepatic I/R injury. Endogenous IL-13 decreased hepatocellular injury, neutrophil recruitment, and TNF-α level [107]. To the opposite of PAF, prostaglandin-E1 (PGE-1) was found to have protective effect in liver I/R injury. Infusion of PGE-1 was demonstrated to markedly reduce plasma alanine aminotransferase (ALT), TNF-α, ICAM-1, VCAM-1, P- and E-selectin in canine model of hepatic I/R injury [108]. A similar, but earlier, study conducted on rats confirmed the cytoprotective effect of PGE-1 and indicated that protection against I/R injury by PGE-1 occurs most probably through down-regulation of ICAM-1 [109].

Interventions inhibiting NF-κB

The NF-κB is a key regulator of inflammatory cytokines in I/R injury. As mentioned earlier it is kept quiescent in resting cells by complexing with its specific inhibitory protein I-κB. This I-κB when become phosphorylated, it degrades away unmasking the binding sequence of NF-κB to be able to bind its specific site on DNA and affect gene transcription. Some therapeutic trials relied on prevention of NF-κB activation through inhibition of I-κB degradation in the setting of experimental myocardial infarction [110]. However, this approach warrants further investigation in the setting of transplantation. Another way to inhibit I-κB breakdown would be the inhibition of proteasomes responsible for its degradation and thus, termination of function, of specifically marked proteins. In hepatic, renal, as well as cerebral ischemia the respective injury could be prevented by administration of proteasome inhibitor lactacystin or its derivative PS519. Experimental protocols analyzing the effects of gene therapy such as interference with NF-κB translocation have been also introduced. However, all these approaches have not been yet translated into larger clinical trials.

Interventions inhibiting adhesion molecules

Adhesion molecules, such as ICAM-1 and VCAM-1, are necessary for adhesion of neutrophils to liver cells and their infiltration into liver tissue. Experimental evidence suggests that a reduced expression of adhesion molecules ameliorates renal and hepatic I/R injury. In phase I and II study, an anti-ICAM-1 antisense-oligonucleotide was analyzed in order to prevent acute graft rejection. Unfortunately, the oligonucleotide did not further reduce the rate of acute rejections or improved graft survival as compared to a conventional immunosuppressive protocol [111].

TBC-1269 is an investigational inhibitor of more than one selectin member (i.e. multiselectin inhibitor) that reduced serum levels of the pro-inflammatory TNF-α and augmented levels of the anti-inflammatory cytokine IL-10 after liver I/R [112]. The multiselectin inhibitor OC-229 also provided significant protection to the ischemic liver including reduction of NF-κB activation [113]. However, while anti-adhesion molecule targeted therapies show some promise in experimental organ transplantation, more data is still needed before the clinical significance of this therapeutic intervention can be examined.

Conflict of interest

The authors declare that they have no conflict of interest.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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