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The mechanisms and strategies to protect from hepatic ischemia-reperfusion injury

J. LI[1], R.-J. LI[2], G.-Y. LV[3], H.-Q. LIU[1]

[1]Department of Anesthesiology, The First Hospital of Jilin University, Changchun, China
[2]Department of Hand Surgery, The First Hospital of Jilin University, Changchun, China
[3]Department of Hepatobiliary and Pancreatic Surgery, The First Hospital of Jilin University, Changchun, China

Abstract. – Hepatic ischemia-reperfusion injury is a major cause of post-operative hepatic dysfunction and liver failure after transplantation. This review summarizes the mechanisms of ischemia-reperfusion injury and analyzes the protective strategies based on the recent developments in the field. Development of hepatic ischemia-reperfusion injury is associated with metabolic acidosis, calcium overloading, and changes of mitochondrial membrane permeability. Hypoxia-induced activation of Kupffer cells results in generation of reactive oxygen species (ROS). These processes lead to activation of inflammation and immune responses that involve multiple cells and signaling molecules and result in increased level of apoptosis and necrosis. Generation of ROS is one of the major risk factors in the hepatic ischemia-reperfusion injury. A number of methods aimed to reduce the oxidative stress have been investigated, and some of them have been applied clinically. The methods mainly rely on the activation of pro-survival genes and associated mechanisms capable of reducing the level of ROS and inflammation at pre-treatment and post-conditioning stages. Potential benefits of these clinical approaches have been discussed here.

Key Words:
Hepatic ischemia-reperfusion injury, Liver transplantation, Oxidative stress, Reactive oxygen species, Inflammation.

Introduction

Ischemia reperfusion injury (IRI) is referred to as the condition of hypoxia-mediated cellular damage to an organ resulting in paradoxical exacerbation following oxygen restoration[1]. It involves a process that is dynamic in nature including two interrelated stages of inflammation-mediated reperfusion injury and local ischemic insult[2]. The existence of this concept has been observed in different organ systems like heart, kidney, central nervous system, liver, lung, skeletal muscle and the intestine[3]. Under extreme conditions, IRI-mediated inflammatory response leads to multiple organ dysfunction syndrome (MODS) or systemic inflammatory response syndrome (SIRS)[4,5].

In clinical practice, hepatic IRI remains a critical as well as frequent complication that compromises the functioning of liver thereby increasing the chances of post-operative mortality and morbidity, complicating recovery and overall outcome. The liver is an organ that depends greatly on the supply of oxygen due to its high requirements of energy, and hence is susceptible to anoxic or hypoxic conditions[6,7]. Hepatic IRI has been classified into cold and warm ischemia. The occurrence of cold ischemia has been linked with the preservation and storage of organ prior to transplantation[8]. Warm ischemia is associated with shock, trauma, setting of transplantation, and electric liver surgery, where there may be a temporary interruption of blood supply[9]. It can also be contributed by Budd-Chiari syndrome, sinusoidal obstruction and toxic liver injury[10]. A variety of factors seemingly leads to the development of hepatic IRI that includes activation of Kupffer cells (KC), upregulation of pro-inflammatory cytokines, oxidative stress and so on[11], each contributing to the overall pathophysiology in varying extents. Therefore, it becomes a really tough job to target a specific mechanism or mediator in order to achieve effective protection in hepatic IRI and numerous experimental as well as clinical research strategies for reducing it have been studied extensively[12]. However, the promising results ensuing from basic research are not always fit to be applied in the clinical perspective. In addition, since the liver functions as a sort of biochemical factory for the organism,

Corresponding Author: Huan-Qiu Liu, MD; e-mail: lhuaqi@126.com

along with its physiological and anatomical position, it is highly vulnerable to ischemia.

It has been widely accepted by medical workers and patients as well as the general public that liver transplantation is, at present time, the only effective approach to treat patients with end-stage liver disease. However, liver dysfunction and failure after transplantation remain potential risks that may affect transplant survival and the life quality of patients. Studies have shown that hepatic ischemia-reperfusion injury is a major cause of post-operative hepatic dysfunction and liver failure after transplantation[13]. Thus, the mechanisms of ischemia-reperfusion injury and approaches to its prevention have been the focus of attention of the researchers in recent years. The injury may develop after organ transplantation, tumor resection or cardiopulmonary resuscitation, or as a result of trauma and shock. In this review, we will summarize the mechanisms of pathophysiology of hepatic ischemia-reperfusion injury and analyze the protective strategies; both current and future options to prevent hepatic IRI, with a special focus on the recent developments in the field.

Proposed Mechanisms of Hepatic-Ischemia Reperfusion Injury

Several cellular and functional changes take place during an event of ischemia that facilitates cellular injury[14]. As for instance, de-regulation of calcium hoemostatis and ATP depletion are caused due to reduction in oxidative phosphorylation[15]. In addition, H+/Na+ homeostasis perturbations, de-energization of mitochondria, swelling of the KC and sinusoidal endothelial cells (SEC) are caused as a result of oxygen deprivation to hepatocytes during ischemia[16]. Reactive oxygen species (ROS) producing KC activation, upregulation of proinflammatory adhesion molecules, chemokines and cytokines causing neutrophil accumulation-mediated injury to cells, and inducible nitric oxide synthase (iNOS) upregulation are all critical factors that contribute to the damage associated with inflammation[17]. Mentioned below are the major hepatic IRI mediators and mechanisms.

Metabolic Acidosis

Metabolic acidosis occurs when the body produces too much acid or when the kidneys are not removing enough acid from the body. This is the basic mechanism involved in the ischemia-reperfusion injury[18]. Metabolic acidosis develops during ischemia and hypoxia. Acidosis is a result of anaerobic glycolysis that leads to the rapid depletion of ATP from liver and consequent production of large quantities of lactic acid and ketone bodies. When the pH in tissues is lowered, phospholipases and proteolytic enzymes become suppressed thus reducing cell damage. However, once the pH of the body rises rapidly during the perfusion period, the activity of these enzymes increases. This leads to elevated level of apoptosis and necrosis and results in the development of ischemia-reperfusion injury symptoms.

Calcium Overloading

Calcium overloading theory is a further development of the metabolic acidosis theory outlined above. Under normal physiological conditions, liver cells maintain intracellular Ca^{2+} homeostasis through Na^{+}/K^{+} and H^{+}/Ca^{2+} exchanging systems, membrane calcium pumps, as well as through active membrane transport of calcium ions. The intracellular Ca^{2+} concentration is kept at a relatively low level by maintaining the large Ca^{2+} concentration gradient. The maintenance of low Ca^{2+} concentration within these cells is vital for the body's normal physiological function[19]. When ischemia-reperfusion injury occurs, intracellular ATP level is decreased leading to the decrease of activity of ATP-dependent Na^{+}/K^{+}-ATPase embedded in the cell membrane. Subsequently, this results in the increase of intracellular Na^{+} concentration and induction of the reverse transport of Na^{+}/Ca^{2+}, leading to a migration of large number of calcium ions inside the cells. In addition, ischemia and hypoxia cause increased permeability of cell membranes which results in a further increase of Ca^{2+} migration into the cell. Ischemia and hypoxia also damage the structure of mitochondria and affect their functions which, in turn, lead to the release of large number of Ca^{2+} from endoplasmic reticulum and cause intracellular calcium overloading[20]. This overloading result in the activation of various enzymes, such as xanthine dehydrogenase, phospholipase, calcium-dependent protease, which further compromise the integrity of cell membrane, structure of cytoskeleton and connections inside the cells. Besides, calcium overloading interferes with mitochondrial oxidative phosphorylation, thus, causing cell metabolic disturbances. Calcium overloading can also trigger the activation of Kupffer cells and mediates hepatocyte damage through the release of large amounts of toxic agents.

Changes in Mitochondrial Membrane Permeability

The changes in mitochondrial membrane permeability can trigger the overflow of apoptotic signals within the cytoplasm, leading to programmed cell death or necrosis. This chain of events at the cellular level links mitochondrial dysfunction with the development of ischemia-reperfusion injury. Mitochondrial respiratory chain produces a certain amount of peroxide in normal physiological conditions. Respiratory chain function becomes impaired as a result of hypoxia, generating larger quantities of peroxides and triggering the body's inflammatory response[21].

Depletion of ATP

The fundamental stress of ischemic and anoxic injury is the failure of oxidative phosphorylation-mediated aerobic ATP formation. The significance of the depletion of ATP in the chain of events leading up to necrotic death is showed by the capability of glycolytic substrates in rescuing sinusoidal endothelial cells and hepatocytes from lethal cellular injury[22]. However, the hepatocytes are not protected by glucose form anoxic injury as they lack hexokinase. For anaerobic glycolysis, glycogen serves as an excellent substrate[23]. On the other hand, fructose inhibits toxic chemical-induced death of the hepatocytes; this suggests that toxic cell killing targets mitochondria[24]. In addition, mitochondrial respiration and oxidative phosphorylation during the event of anoxia get completely inhibited.

Kupffer Cell Activation

Kupffer cells, the resident liver macrophages, constitute the liver sinusoids together with other cells such as sinusoidal endothelial cells, hepatic stellate cells, liver-specific natural killer cells and dendritic cells. These cells play an important role in the development of hepatic ischemia-reperfusion injury. In early reperfusion, the Kupffer cells change their metabolic behavior and generate a lot of reactive oxygen species due to ischemia and hypoxia[25]. In addition, during the early reperfusion stage, the Kupffer cells become activated by signal transduction mechanisms of Toll-like receptor 4 (TLR4), as well as by TLR4 complement system. This results in the generation of large amounts of pro-inflammatory cytokines such as tumor necrosis factor (TNF) and interleukin-1 (IL-1). These cytokines play a very important role in mediating the hepatic ischemia-reperfusion injury[26]. Oxygen free radicals (OFR) and cytokines released by activated Kupffer cells facilitate the removal of protein-polysaccharide complexes from the vascular endothelial surface and increase the exposure of adhesion molecules on the surface of endothelial cells[27]. This, in turn, promotes adhesion of neutrophils and platelets to the sinusoidal endothelial cells, thereby, increasing the endothelial cell damage and eventually causing severe disruptions of microcirculation and increase of tissue ischemia[28].

Activation of Neutrophils and Lymphocytes

Cell apoptosis caused by inflammation is an important factor during the development of ischemia-reperfusion injury as it leads to tissue damage. Active and pro-inflammatory factors generated by ischemia and reperfusion can bind to receptors of neutrophils and lymphocytes, thus, causing aggregation of neutrophils and platelets and lymphocyte activation. The activation of neutrophils and cytokines can lead to the development of ischemia-reperfusion injury mainly through the following mechanisms: (1) Oxidative enzymes induce respiratory surging and generate a lot of reactive oxygen species (ROS) and a variety of enzymes including elastase, cathepsin G, heparanase, collagenase and hydrolytic enzymes which inflict damage to cell membranes, mitochondrial membranes, membrane proteins and nucleic acids[29]. (2) In ischemia-reperfusion, a large number of proteoglycan complexes are shed from the surface of blood vessel walls, leading to the activation of intercellular adhesion molecules on the endothelial cell surface. These molecules include intercellular adhesion molecule-1 (ICAM-1), vascular adhesion molecule-1 (VCAM-1), L-selectin (L-S) and integrins, and their expressions are up-regulated in endothelial cells, neutrophils and platelets. In the early perfusion, the activated neutrophils get adhered to the surface of endothelial cells, which not only cause obstructions to microcirculation, but also lead to physical damage to the tissues due to invasion of the neutrophils from the surface of endothelial cells into endothelium. Moreover, this process results in the shedding of proteoglycan complexes from the vessel surface, which increases the permeability of blood vessels for macromolecules, proteins and cells. This leads to tissue edema and even necrosis due to the action of inflammatory cells on the underlying tissues. (3) A

large number of monocytes and macrophages, especially $CD4^+$ T cells, get activated at early stages of ischemia-reperfusion injury. The $CD4^+$ T cells amplify the inflammatory response, further aggravating the damage to tissues and cells. Although lymphocytes normally play protective role in inflammation, in ischemia-reperfusion injury, the inflammation becomes magnified and causes serious tissue damage if ischemia continues for too long[30].

Cytokines and Complements

A variety of cytokines are involved in the pathophysiology of hepatic ischemia-reperfusion injury. The most important factors include tumor necrosis factor-α, platelet activating factor (PAF), interleukins IL-1, IL-10, etc. These cytokines cause liver injury through autocrine, paracrine and humoral mechanisms, all of which constitute the generalized response of inflammatory system. Inflammation and immune responses stimulate the production of TNF-α through the action of different types of lymphocytes. TNF-α plays important role in mediating leukocyte activation and chemotaxis, as well as in inducing the Kupffer cells to produce oxygen free radicals. The role of TNF-α in the inflammatory response caused by ischemia-reperfusion injury involves three major mechanisms: (1) activation of T and B lymphocytes, enhancement of their cytotoxicity, induction and up-regulation of the cellular adhesion molecule expression on the surface of neutrophils and sinusoidal endothelial cells (SEC) which promotes the adhesion of leukocyte to endothelial cells[31]; (2) strengthening of antigen expression of endothelial MHCI, promotion of the production of PAF, IL-1 and other inflammatory mediators by endothelial cells, activation of leukocytes and facilitation of thrombosis; (3) increase of mitochondrial membrane permeability, activation of Kupffer cells to produce peroxide residues, and induction of macrophages to release IL-1, IL-6, IL-8 and other cytokines, which result in aggravated liver damage after reperfusion[32]. IL-1 can further induce the production of ROS and induce Kupffer cells to produce TNF-α and promote leukocyte aggregation following ischemia-reperfusion[33]. PAF is another important cytokine, derived from the sinusoidal endothelial cells and is activated by Kupffer cells. PAF not only activates platelets and assists the endothelial cell adhesion, but also activates neutrophils on the sinusoidal endothelial cells to produce large amounts of oxygen free radicals. The complement system also plays a very important role during ischemia and reperfusion. There are about 30 components of complement system in the plasma that can bind to cell surface and get involved in the inflammatory response after ischemia-reperfusion[34].

Oxygen Free Radical

OFRs play an important role in ischemia-reperfusion injury. During ischemia, the oxygen content is insufficient in the tissues, and reperfusion causes an explosive increase in the level of oxygen free radicals within a very short interval of time[31]. The xanthine oxidase system, macrophage system and mitochondrial respiratory chain start to produce large amounts of oxygen free radicals after hepatic ischemia-reperfusion. Besides, endogenous antioxidants such as superoxide dismutase become inactivated or depleted, thus leading to reduced elimination of oxygen free radicals and further increasing the OFR level in the body during ischemia and hypoxia[35,36]. Oxygen free radicals universally affect proteins, lipids, nucleic acids and other biologically active molecules in the body. It is believed that four major mechanisms can lead to OFR-mediated damage to the liver cells: (1) OFRs can cause change in membrane fluidity and permeability though oxidation of cellular components and membranes; (2) OFRs mediate lipid peroxidation, generating a variety of highly toxic lipids or directly damaging the cells; (3) OFRs directly affect the sinusoidal endothelial cells of the liver by increasing platelet and neutrophil adhesion to the sinusoidal endothelial cell surface, which results in the disruption of microcirculation; (4) OFRs directly oxidize the double-stranded structure of nuclear DNA in liver parenchyma, causing DNA damage and mutations. This leads to the hepatocellular necrosis and results in damage to the structure and function of liver.

Xanthine Oxidase

Xanthine oxidase is an important mediator in the formation of intracellular ROS. It is believed that xanthine oxidase plays a role in hepatic ischemia-reperfusion injury[37]. Recent studies have confirmed that allopurinol, an inhibitor of xanthine oxidase, has protective effects in ischemia-reperfusion injury[38]. However, the clinical applications of such inhibitors are limited at present time. Major stumbling blocks to such interventions include the slow conversion of xanthine dehydrogenase to xanthine oxidase in the condi-

tions of ischemia, and the fact that production of reactive oxygen components also depends on the presence of xanthine and hypoxanthine.

Volatile Anesthetics and Ischemia-Reperfusion Injury

The effects of volatile anesthetics on ischemia-reperfusion injury have been under investigation for many years. The volatile anesthetics, such as halothane, isoflurane, sevoflurane, when used in during pre-conditioning or post-treatment, were shown to inhibit the adhesion between polymorphonuclear cells and vascular endothelial cells after ischemia-reperfusion injury, thereby, reducing the inflammatory reactions[39].

Concentration Imbalance Between Endothelin (ET) and Nitric Oxide (NO)

Endothelin (ET), a peptide secreted by vascular endothelial cells, has been shown to possess a strong vasoconstriction effect. There are two reasons for the increased ET level after ischemia-reperfusion. One is the increased concentration of calcium ions that causes the secretion of endothelin by endothelial cells during hypoxic ischemic stage. The other is that the gut-derived liver endotoxins arriving through the portal vein may also stimulate the endothelial cells to produce ET during the reperfusion period. High concentration of ET results in microvascular contractions, microcirculation disturbances, and tissue damage[26]. Nitric oxide (NO) is derived from L-arginine and produced by the enzyme NO synthase (NOS). NO causes vasodilation, inhibition of the leukocyte adhesion to endothelial cells, inhibition of platelet aggregation, and has the antagonistic effect to ET. Besides, NO reduces the adhesion of neutrophils to vascular endothelium, mainly by reducing the expression of P-selectin and ICAM-1. The loss of concentration balance between endothelin and nitric oxide leads to further contraction of microvasculature and disturbance of microcirculation. Besides, even when the blood flow in the ischemic area is restored, the microcirculation remains affected[40].

Apoptosis and Necrosis

ATP and other cellular energy sources are in short supply in organs and tissues during the ischemic phase, resulting in dysfunctions of dynamic equilibrium in most of the cells and affecting signal transduction, cell proliferation and apoptotic cycle. The shortage of ATP production affects the ATP-hypersensitive Na^+/K^+-ATPase and causes dysfunctioning of channels. As a result intracellular Na^+ is not effectively pumped out of the cells and lead to cell edema[41]. In addition, toxic metabolites produced by the cells in the ischemic period prompts the retention of water in the cells causing their swelling. If the ischemic time exceeds 24 hours, the damage to the ATP biosynthesis processes in the reperfusion period becomes irreversible, leading to cell death via necrosis or apoptosis[42]. The mechanisms of apoptosis and necrosis in the liver during ischemia-reperfusion injury still remain to be fully investigated. However, these mechanisms are linked to the processes of inflammation taking place during the reperfusion phase.

Protective Approaches and Strategies for Hepatic Ischemia-Reperfusion Injury

Generation of ROS is one of the most important risks in the hepatic ischemia-reperfusion injury. A number of methods aimed to reduce the oxidative stress have been investigated in the ischemia-reperfusion injury models, some of which have been applied clinically[21].

Commonly Used Approaches

The commonly used and predominant methods for the prevention of hepatic IRI include surgical and pharmacological interventions.

Surgical Methods

Surgical methods have been routinely used in the treatment of ischemia reperfusion injury. Various nonselective or selective occlusion techniques are needed to be employed during liver resection so as to reduce the loss of blood; with which post-operative outcomes are associated. The various occlusion techniques include the total hepatic vascular exclusion (THVE), intermittent or continuous Pringle Maneuver (PM) and the segmental or hemi-hepatic occlusion of the hepatic artery or portal vein[43]. Unfortunately, these methods often give rise to negative outcomes like hepatic IRI[10]. Instead of using continuous PM, intermittent PM use can reduce hepatic IRI to a great extent as it appears to be well tolerated[44]. The optimal ischemic intervals are still debated and numerous studies have tried to define the optimal PM cycle. Although there is ongoing debate about the optimal ischemic interval, in all it can be of 30 minutes duration followed by reperfusion for 5 minutes[43]. As per the finding of der Broek et al[45], no significant difference could

be found between the ischemic intervals of 30 minutes and 15 minutes considering hepatocyte injury, liver functioning, median blood loss and morbidity. THVE offers lesser loss of blood during operation; however, it is associated with a number of adversities including increased operative time, hemodynamic tolerance, increased post-operative hospital stay as compared to that in PM[46,47]. Hypothermic perfusion, done with cytoprotective solutions along with cooling of the organ surface locally is used to increase ischemic tolerance[48]. Parenchymal hypothermia has been proven experimentally to be able to decrease inflammatory response and oxidative stress associated with IRI, thereby, allows for more complex hepatectomies[49,50].

Pharmacological Interventions

Since, the pathophysiology of hepatic IRI involves a number of mediators and mechanisms, pharmacological interventions of varied types are being tested so as to inhibit the phenomenon. In liver resection, methylprednisolone, ulinastatin, glucose and trimetazidine may render protection against IRI[51]. However, due to the lack of adequate clinical trials, their routine use during controlled liver resections for reducing IRI remains a controversial issue. In experimental liver IRI, prednisolone acts as an anti-inflammatory agent that reduces both apoptotic cell count as well as inflammatory markers[52]. It also resulted in reduced post-operative complications as well as hospital stay; significantly decreasing the levels of inflammatory markers such as C-reactive protein (CRP) and IL-6 and bilirubin[53]. Nilotinib, a receptor tyrosine kinase inhibitor was found to reduce both activation of liver p38 mitogen-activated protein kinase (MAPK) and Jun N-terminal kinases (JNK) in mice and, thus, can help in liver IRI attenuation in humans[54].

Future Promising Interventions

Although surgical and pharmacological interventions are commonly used in the treatment of hepatic IRI, there are a number of complications associated with both the methods. The fact that severe complications can take place through hepatic IRI-mediated cell damage during or after liver resections or liver transplantation highlights the requirement for further research into this field so that the undesirable effects can be prevented. Accordingly, in recent times, numerous methods have been devised and developed that can result in the protection of the liver from IRI. Most of these results are obtained from animal models of hepatic IRI, and the data from large clinical trials are being awaited for the application of these results into clinical practice[55]. Some of these interventions have been discussed here.

Pro-survival Genes and Antioxidants

An important group of endogenous genes in organisms referred to as the pro-survival genes play a number of vital roles in them. Pro-survival genes can be activated by ischemic injury and can eliminate the reactive oxygen species generated by oxidative stress in the body[31,56]. The majority of such genes are under the control of Nrf2 protein. When the body confronts oxidative stress or hypoxic injury, Nrf2 is activated, enters into the nucleus and regulates the transcription and expression of other genes. A variety of proteins *in vivo* are regulated by Nrf2. They include NADPH: quinone oxidoreductase 1, heme oxygenase-1 (HO-1), glutamate cysteine ligase (Gcl), microsomal epoxide hydrolase, glutathione-S transferase and sulfiredoxin 1[3]. All these enzymes are regulated by Nrf-2, thereby rendering protective effects on ischemia-reperfusion injury caused by oxidative stress[57].

Glutathione (GSH): Elevated level of reduced glutathione in the hepatic cells represents an effective way of antagonizing oxidative stress induced by ischemia-reperfusion injury and scavenging the ROS within the cells. Increased intracellular concentration of reduced glutathione leads to its continuous release into the inner walls of the vessels. The ROS produced by Kupffer cells are subsequently removed[58]. It has been confirmed experimentally that infusion of N-acetylcysteine during or before the ischemia-reperfusion injury development can reduce the release of ROS by the maintenance of adequate glutathione concentration[59]. N-acetylcysteine has been shown to render a protective effect against acute hepatic ischemia-reperfusion injury in mice[60]. N-acetylcysteine has also recently been used in phase IV clinical trials[21] which revealed that it can reduce the expression of biochemical markers caused by partial hepatectomy. Although no significant impact on the long-term prognosis was observed, the studies related to the protective effect of GSH in hepatic ischemia-reperfusion injury might bring important results in the future.

Superoxide Dismutase (SOD): Catalase and superoxide dismutase (SOD) are the first two antioxidant enzymes that have been proven to be effective in the pre-conditioning of ischemia-reperfusion injury. The protection mechanism and the role of SOD have been intensively investigated over the years. The studies demonstrated that bioavailability of natural superoxide dismutase is quite low. Therefore, even high doses of intravenous SOD can only play a partial role in protecting the organs against ischemia-reperfusion injury[61]. Currently, researchers are continuing the attempts to improve the bioavailability of SOD by modifying its structures. Although the protective effect of genetically modified SOD against ischemia-reperfusion injury has been confirmed in animal experiments, the trials on human subjects are still in their infancy.

Heat shock protein (HSP): Heat shock proteins, also known as the stress response proteins, are expressed in the body at the stage of stress response when the level of reactive protein is increased. Most of the heat shock proteins are controlled by Nrf2. Heat shock protein HSP32 is also known as heme oxygenase-1 (HO-1). This is a well-studied protein which has protective effects on ischemia-reperfusion injury. The protective mechanism of HSP32 includes two aspects. The protein induces the formation of bilirubin which has antioxidant function. In addition, the byproduct formed in this process, carbon monoxide, can induce the formation of p38, a key protein which has ischemia-protective properties[62]. HSP70 is another Nrf-2-inducible protein from the heat shock family which has been proven to possess liver protective effects. HSP70 can increase survival and protection against IRI injury in the liver[63]. Currently, HSP32 is considered as the most promising candidate for treatment of hepatic ischemia-reperfusion injury among all the heat shock proteins.

Ischemic Pre-conditioning and Post-Conditioning

A simple and brief treatment of ischemic organ is essential before ischemia-reperfusion is performed on the tissues. This procedure is called ischemic pre-conditioning. Ischemic pre-conditioning is one of the commonly used and well-studied organ protection measures. The potential mechanisms of ischemic pre-conditioning include the lowering of the level of oxidative stress generated on the pre-conditioning stage, the activation of pro-survival protein p38, the induction and generation of antioxidant proteins HO-1, as well as increased cell proliferation post-injury[64]. Stimulation of these mechanisms at the pre-treatment stage greatly decreases the possibility of oxidative stress and inflammation when the body goes through the ischemia-reperfusion injury. The protection mechanisms involved in pre-conditioning and post-conditioning methods are similar to each other. The pro-survival PI3K/Akt pathway is activated *in vivo* when the body experiences ischemic injury[65], subsequently the antioxidant enzyme superoxide dismutase is induced again and the production of vasodilator nitric oxide is increased[66]. Because of the increased vasoconstriction during ischemia-reperfusion, NO is beneficial by facilitating hepatic blood flow during reperfusion[67].

In order to avoid further damage of the organs, researchers have tried a variety of methods to simulate the process of ischemia-reperfusion injury to protect the organs. For instance, it is possible to adjust the intracellular concentration of certain protective substances to simulate the ischemic pre-conditioning process[68]. The substances most commonly used to this end include the adenovirus 2A receptor agonist[69] and atrial natriuretic peptide[70]. The mechanism of ischemic pre-conditioning may primarily be attributed to the enhancement of the body's antioxidant capacity against ischemia-reperfusion injury[71]. More importantly, pre-conditioning can enhance the cellular response to stress and make subsequent treatment possible. The protective role of inhalation of volatile anesthetics in ischemic-reperfusion injury has attracted a lot of research interest in the field of organ protection in recent years. Both isoflurane[72] and sevoflurane can reduce hepatic ischemia-reperfusion injury, while sevoflurane can reduce the release of transaminase following hepatic ischemia-reperfusion, thus improving the patient outcomes[73].

Mitochondrial Permeability (MPT) Inhibitors

The increased mitochondrial permeability is a major cause of cell death during hepatic ischemia-reperfusion injury. Oxidative stress can promote MPT, which in turn further elevates the oxidative stress level[74]. Therefore, MPT inhibitors can be effective against hepatic ischemia-reperfusion injury and reduce mitochon-

drial and intracellular ROS generation[75]. It has been experimentally confirmed that pre-treatment with antioxidants melatonin[76] or edaravone[77] can protect the mitochondrial respiratory chain and reduce swelling of mitochondria when the hepatic ischemia-reperfusion injury occurs. Edaravone is one of the antioxidant drugs that can be effective against hepatic ischemia-reperfusion injury. The drug may be useful in both cold and warm ischemia and is effective in large mammals[78]. It acts primarily through the inhibition of MPT, maintaining adequate ATP concentration during hepatic ischemia-reperfusion injury[79]. Edaravone is one of most successful drugs in the family of mitochondria-specific antioxidants and demonstrates clear protective effects in ischemia-reperfusion injury.

Nuclear Factor-kB (NF-kB)

NF-kB plays a number of roles in response to acute injury in both cold and warm hepatic ischemia. In KC, NF-kB activation stimulates cytokine expression-mediated inflammation; however, its activation may provide cellular protection in hepatocytes[80]. Since, NF-kB has multidimensional functions in reperfusion injury, several immunomodulatory therapeutic strategies revolve around it. In one such study, the receptor activator of NF-kB and its ligand RANKL were found to be crucial in response to hepatic IRI[81]. It was observed that before ischemia or during reperfusion, enhanced activation of hepatocyte NF-kB takes place following treatment with RANKL thereby significantly reducing liver cell damage.

Nitric Oxide

NO is a critical endogenous molecule implicated in IRI. The accumulating results from the available literature suggest the notion that exogenous supply of NO could be helpful in the protection of liver from IRI. But it is noteworthy to mention that NO is very unstable in nature. Therefore, it is necessary to provide NO after adhering it with another molecule such as diazeniumdiolates, S-nitrosothiols, and liver-selective NO donors[82-85].

Chinese Medicinal Herb

In recent years, an increasing number of researchers have been investigating the protective effects of herbal medicines in ischemia-reperfusion injury. Accumulating data confirmed that the majority of studied herbal medicines, such as green tea extract[86], mainly reduce the body inflammation level during the ischemia-reperfusion injury through anti-oxidative action. A number of herbs combine anti-inflammatory effects with calcium channel blocking and can also reduce the inflammation during injury[87]. However, many herbal remedies have to be used in high concentrations and require prolonged pre-treatment[88], thus, restricting their clinical usefulness.

It has been well established that large quantities of ROS are produced in the initial stages of ischemia-reperfusion injury. The generation of ROS leads to the increase of cell death via apoptosis or necrosis. Apoptotic and necrotic cells attract macrophages and neutrophils, thus, causing cell aggregation and further damage to the cells which results in continuous generation of ROS during the reperfusion period. Theoretically, the therapeutic measures targeting ROS and inflammation both at the initial stage of ischemia-reperfusion injury and after the reperfusion could have protective effects. Although the protective effect of ROS-targeting antioxidants has been observed in animal models, their exact mechanisms remain unclear, thus, limiting their clinical applications. However, with the continuing investigations on ischemia-reperfusion injury in animal models, the importance of antioxidant therapy is becoming more evident. Pre-conditioning and the pharmacological approaches appear to be the most promising treatment modalities for anti-ischemia-reperfusion injury in the future.

Conclusions

Hepatic ischemia-reperfusion injury remains an ever intriguing topic which surprises us with newer and countless interactions and mediators between them. As the list of mechanism behind hepatic IRI increase, the number of potential therapeutic strategies also goes on increasing. It is now a well established fact that combating hepatic IRI is not possible by targeting a single factor or mediator but rather multiple factors have to be considered to successfully counter the adverse effects of the problem. Hopefully, the research in this field would provide the necessary inputs for the introduction of newer and safer methods of liver protection against IRI.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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