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FACTORS IN THE PATHOPHYSIOLOGY OF THE LIVER ISCHEMIA-REPERFUSION INJURY

Eduardo E. Montalvo-Jave[1,2,3], **Tomas Escalante-Tattersfield**[1], **Jose A. Ortega-Salgado**[1], **Enrique Piña**[2], and **David A. Geller**[4]

1 *Department of Surgery, Faculty of Medicine and National Autonomous, University of Mexico (UNAM). Mexico City, Mexico and "Hospital General de Mexico OD"*

2 *Department of Biochemistry, Faculty of Medicine and National Autonomous, University of Mexico (UNAM). Mexico City, Mexico and "Hospital General de Mexico OD"*

3 *Transplantation Biology Program. Department of Surgery. Mayo Clinic. Rochester, Minnesota. USA*

4 *Department of Surgery, Thomas E. Starzl Transplantation Institute, University of Pittsburgh, PA. USA*

Abstract

Hepatic ischemia-reperfusion injury is commonplace in liver surgery, particularly in hepatic transplantation, hepatic resection, and trauma. The signaling events contributing to local hepatocellular damage are diverse and complex, and involve the interaction between hepatocytes, sinusoidal endothelial cells, Kupffer cells, as well as infiltrating neutrophils, macrophages, and platelets. Signaling mediators include cytokines, reactive oxygen and nitrogen species, calcium, complement, and several transcription factors. The purpose of this review article is to summarize the factors that contribute to the pathophysiology of hepatic ischemia-reperfusion injury.

Keywords

Liver; ischemia-reperfusion; IR injury; nitric oxide; NO; iNOS; adenosine; cytokines. Endothelial and Kupffer cells; ROS

Introduction

Interruption of an organ's blood flow, with its subsequent lack of oxygen and nutrient supply, is an inherent phenomenon during diverse surgical procedures. In liver surgery, there are clinical situations in which the ischemic periods can be particularly long, such as during the resection of large hepatic tumors, management of hepatic trauma of diverse origins, vascular reconstructions, and liver procurement for transplantation.[1–3] Once the blood flow and oxygen supply are reestablished, reperfusion enhances the injury caused by the ischemic period, aggravating the damage caused at the cellular level.[4, 5] This phenomenon, known as ischemia-reperfusion (IR) injury, impacts directly on liver viability, especially during transplantation

*Correspondence to David A. Geller, MD, FACS. Thomas E. Starzl Transplantation Institute, University of Pittsburgh, 3459 Fifth Avenue, MUH 7 South, Pittsburgh, PA 15213. E-mail: David A. Geller (gellerda@msx.upmc.edu), Correspondence to: Eduardo E. Montalvo MD. FACS. Departamento de Cirugía. Facultad de Medicina. UNAM. México. Circuito Universitario s/n. Delegación Coyoacan, 04510 Mexico City, México. E-mail: Eduardo Montalvo (montalvoeduardo@hotmail.com).

and liver surgery.[3, 6] During an ischemic period, several functional changes occur at the cellular level that promote cell injury. A decrease in oxidative phosphorylation results in ATP depletion and derangements in calcium homeostasis.[7]

The deleterious effects of ATP catabolism modification are further enhanced by the production of several substances, including reactive oxygen species (ROS), cytokines, adhesion molecules, and vasoactive agents (endothelin and thromboxane-A2). These alterations are accompanied by a decrease of cytoprotective substances including nitric oxide, prostacyclin and others.[8] Hepatic cell death occurs due to both necrosis and apoptosis. [9]

MICROCIRCULATORY FAILURE

During the ischemic period, the lack of energetic substrate interferes with active transmembrane transport, producing edema in Kupffer cells (KC) and endothelial cells (EC).[10] Loss of the delicate equilibrium between nitric oxide (NO) and endothelin (ET) induces vasoconstriction and narrowing of the sinusoidal lumen, compromising leukocyte flow and bringing them in close contact with the capillary wall.[11] The increase in contact between leukocytes and EC promotes leukotaxis, and although not occluding the capillary lumen completely, the trapped leukocytes interfere with the flow of blood through the sinusoidal capillaries.[12–14] Platelet aggregation within the hepatic sinusoids further aggravates the turbulent flow rate through the partially occluded capillaries.[15] On reperfusion of the ischemic liver, the collapse of the microcirculation maintains areas of ischemic liver parenchyma, in a phenomenon known as “no-reflow”.[8, 16, 17] In addition to the microcirculatory failure, the activation of KC and neutrophils leads to the synthesis of inflammatory cytokines, further aggravating the severity of the ischemic injury. The cytokines most frequently implicated in IR injury are the tumor necrosis factor-alpha (TNF-α), interleukins 1 (IL-1) and 6 (IL-6), prostaglandins (PG), and ROS, especially superoxide (O_2^-) and hydrogen peroxide (H_2O_2).[18–23] Several relevant factors and mediators such as NO are involved in the ischemic injury of the liver. NO is synthesized from L-arginine by the action of nitric oxide synthase (NOS). NO is an important mediator of immunomodulation, neurotransmission, and platelet aggregation.[24] Within EC, NO triggers cGMP to reduce the vascular tone and act as a vasodilator,[24,25] NO can mediate the intensity of the IR injury by modulating neutrophil adhesion, platelet aggregation, and stellate (Ito) cell relaxation.[4, 15, 26] Stellate cells contract when exposed to endothelin-1 (ET-1), whereas sodium nitroprusside (NO donor) induces their relaxation.[27] Therefore, one of the mechanisms involved in IR injury is loss of the equilibrium between ET and NO levels during reperfusion.[27, 28] At the beginning of reperfusion, NO levels decrease and ET levels increase, favoring microcirculatory vasoconstriction.[29, 30] Ischemia reduces intracellular NADPH and oxygen (factors necessary for NO synthesis) and induces the release of arginase,[31] producing and important reduction in NO synthesis, with a significant increase in degradation of its precursor L-arginine.[31, 32] Both endogenous and exogenous NO protect hepatocytes and EC against IR injury, apparently by vasodilation, and by inhibiting the expression of adhesion molecules (E-selectin) within the sinusoidal lumen.[25, 26] In order to produce significant amounts of NO in response to a specific stimulus such as IR, the inducible nitric oxide synthase enzyme (iNOS) is synthesized *de novo*, a process that takes 4 to 6 hours.[24, 33] Blockade of the L-arginine/NO synthase pathway has been shown to worsen hepatic apoptosis and liver transplant preservation injury.[34, 35] Augmenting graft iNOS expression with adenoviral iNOS transduction has also been shown to improve liver transplant preservation injury an improve survival in severe preservation injury.[35] In clinical practice, an increase in NO concentration, as well as the reduction of ET, have been shown to decrease the severity of IR injury.[36]

FACTORS INVOLVED

CELL TYPES

Kupffer cells—During the initial stages of reperfusion KC are activated, producing morphologic changes that cause them to protrude into the sinusoids, contributing to the reduction of blood flow within the sinusoidal lumen.[18, 37] Activated KC release a large amount of both proinflammatory (TNF-α, IL-6, IL-1, and prostaglandins) and anti-inflammatory mediators (IL-10, IL-13), as well as ROS.[22, 23] Some studies show that IR injury can be attenuated or aggravated by the suppression or potentiation of KC activity, respectively.[37, 38] Cold liver preservation induces strong KC activation;[39] modulation of KC activity can therefore attenuate the IR damage in transplanted organs and consequently improve their survival.

Neutrophils—Activated neutrophils contribute to IR damage through the release of ROS and several proteases.[40] Neutrophils accumulate in the liver at the initial stages of reperfusion, and their adhesion to EC is mediated by the interaction between selectins and integrins expressed in the neutrophil membrane, and intercellular adhesion molecules (ICAM) expressed on EC.[41, 42] IR increases ICAM-1 expression in hepatic EC, probably through TNF-α and IL-1 synthesis.[43, 44] In fact, increased ICAM-1 expression has been associated with acute liver rejection,[45, 46] and neutralization of ICAM-1 decreases the severity of IR injury.[47, 48]

Recent studies propose that NK-T cell and T cells also play an important role in hepatic IR injury.[49] Resident lymphocytes found within the liver include conventional alphabeta TCR cells as well as unconventional NK and gammadelta T cells. These lymphocytes can alter inflammation through the secretion of soluble mediators such as cytokines and chemokines or through cognate interactions in an antigen-dependent manner. Expression of these mediators will then result in the recruitment of more lymphocytes and neutrophils.[50]

Platelets—Platelets adhere to the hepatic sinusoids and induce programmed EC death upon reperfusion of transplanted organs.[51] Platelets synthesize and release several factors that play an important role in the liver IR and hepatic regeneration.[52] These include cytokines, growth factors such as transforming growth factor-β (TGF-β), serotonin, and calpain. Platelet-derived serotonin has recently been shown to promote tissue repair after normothermic hepatic ischemia in mice. [53] In human platelets, calpain activation is dependent on fibrinogen binding to integrin and subsequent platelet aggregation, suggesting a potential role for this protease in the regulation of post-aggregation responses.[54] Platelets also produce NO that leads to the production of peroxynitrite, which acts as a potent inductor of programmed cell death in EC.[55, 56]

MEDIATORS

Cytokines—Cytokines play a relevant role in IR injury, both by starting and maintaining the inflammatory response, as well as modulating its severity.[57, 58] The substances most studied in this context are the TNF-α and interleukins IL-1 and IL-6. These cytokines have large proinflammatory activity, inducing IL-6 and IL-8 synthesis[59] and lower anti-inflammatory IL-10 levels.[60, 61] IL-8 is a potent neutrophil chemotactic and activating factor, and correlates with the neutrophil infiltration in an IR model.[61] The expression of adhesion molecules (β2-integrins and selectins) also promote leukocyte-EC interaction.[43] These factors, together with chemokines and complement factors, recruit polymorphonuclear leukocytes (PMN)[8] that infiltrate the liver, perpetuating and amplifying the ischemic injury by releasing additional ROS, TNF-α, and diverse proteases.[62] TNF-α by itself produces leukocyte chemotaxis and activation,[19] and induces ROS production by KC.[63] In turn, IL-1 induces TNF-α synthesis by KC and induces neutrophil recruitment, which in turn produce ROS.[20, 64] Both TNF-α and

IL-1 levels are increased during hepatic IR injury,[39,45] and their neutralization decreases the intensity of IR injury.[20, 64] Recent studies have confirmed the relationship between IL-1 and neutrophil recruitment within hepatic tissue after IR, but its relationship to the extent of hepatocellular injury remains unclear.[65]

Reactive oxygen species (ROS)—Aerobic metabolism releases ROS, which under normal circumstances are neutralized through diverse antioxidant mechanisms.[66] Under stress conditions, the balance between ROS and antioxidants shifts towards the former, resulting in oxidative stress and cytotoxicity.[40, 67]

Some of the processes involved both directly and indirectly in IR injury by ROS synthesis include the transformation of xanthine dehydrogenase into xanthine oxidase (an oxygen-dependant process that produces uric acid, releasing the ROS superoxide and hydrogen peroxide),[68] induction of NADPH oxidase by activated KC and neutrophils (ROS production is blocked when NADPH oxidase is inhibited), and NO production and its conversion to peroxynitrite (both considered reactive nitrogen species).[56, 67] Within the liver, the cytotoxic effects of ROS translate into nitrosylation of iron-sulfur groups and tyrosine residues, inactivation of the heme group, and lipid peroxidation.[5, 56]

Because of the potential inhibition of ROS by antioxidant agents, several studies have focused in modulating the severity of IR injury with different mechanisms, including pharmacologic α-tocopherol,[69] allopurinol,[70, 71] N-acetylcysteine,[72] and enzymatic superoxide dismutase (SOD)[73] and catalase[73, 74] therapies. Endogenous antioxidant levels decrease significantly during reperfusion.[69, 75] Therefore, the administration of exogenous antioxidants, particularly in the early stages of reperfusion, could significantly decrease the severity of IR damage in transplanted livers.

Complement system—Activation of the complement system has been demonstrated during IR. The complement system consist of about 30 soluble and membrane bound protein can be activated by any one of three pathways, the antibody-dependent classical pathway, the alternative pathway, or mannose-binding lectin (MBL) pathway.[76] Activated complement acts both directly through the formation and deposition of membrane attack complexes,[77] and indirectly by stimulating the production of chemotactic agents and proinflammatory cytokines, resulting in migration and adhesion of leukocytes and neutrophil recruitment within the sinusoids.[78, 79] Complement inhibitors have been shown to be effective in reducing pathology of various organ-specific I/R injuries. For example, a partial IR rat model was used to investigate the efficacy of a small molecule C5a receptor antagonist against hepatic I/R injury. This antagonist ameliorated neutrophil infiltration, liver injury, and mortality.[80] However, only a few complement inhibitors such as the small molecule C5a receptor antagonist, and recombinant sCR1 or C5 antibody are currently suitable for clinical testing in humans. [81]

Calcium—Calcium was one of the first factors implicated in IR, by modulating the severity of IR with Ca^{2+} channel blockers. During IR, Ca^{2+} is essential for the activation of calcium-dependent phospholipases, nucleases, and proteases, and it plays a key role in the interruption of oxidative phosphorylation by decreasing ATP levels.[82] Modulation of mitochondrial calcium management has also been shown to attenuate hepatic warm ischemia-reperfusion injury. [89]

Adenosine—Adenosine is an endogenous compound that is produced by the enzymatic metabolism of ATP, ADP, and AMP. At high concentrations, it confers certain protection against ischemia by inhibiting platelet aggregation,[83] neutrophil activation,[84] and ET and ROS production while enhancing NO production.[84, 85] During liver ischemia reperfusion, adenosine and inosine are released from the liver, which in turn contributes to homeostasis by

releasing glucose from the hepatic glycogen through stimulation of A_3 adenosine receptors.[86] With reperfusion, inosine can be washed out of the organ,[87] thus eliminating completely its protective effect. Inosine, when converted to hypoxanthine and xanthine, is also involved in ROS.[88]

Molecular mechanisms involved in liver ischemia reperfusion—When the liver is subjected to an ischemic insult, the alterations induced by oxidative stress can exceed the compensatory capacity of the liver, producing cell death. The ischemic event can reprogram gene expression of the surviving cells, initiating cellular mechanisms that allow them to regenerate and remodel. ATP is depleted during the ischemic period, and then liver injury is further exacerbated during reperfusion. One of the important transcription factors involved in mediating hepatic IR injury is nuclear factor kappa B (NF-κB).[89–91]

NF-κB is normally found in the cytoplasm attached to the inhibitory protein IκB.[92] During oxidative stress, IκB is degraded, allowing for the translocation of NF-κB to the nucleus.[92, 93] When activated, NF-κB induces the synthesis of iNOS, cytokines (TNF-α), chemokines, and adhesion molecules (ICAM-1).[27, 93] The most important mechanism for NF-κB activation is ROS production, particularly hydrogen peroxide (H_2O_2),[94] whereas the administration of antioxidants decreases its activation. NF-κB is activated during two different stages of IR, with different actions: at an early stage (from 30 min to 3 h of reperfusion), it induces an increase in the expression of proinflammatory cytokines (IL-1β, IL-6, and TNF-α). At a later stage (9 to 12 h after reperfusion), it acts as an anti-inflammatory agent.[95] Other genes that may participate in IR include those of ET-1, NOS-3, heme-oxygenase, and those of the heat stress factor proteins.[96] ROS have been documented to either activate or modulate all these pathways.

Apoptosis and necrosis—During reperfusion, TNF-α and other mediators activate many of the proteins involved in apoptosis, such as the proteases caspase-3 and caspase-8, along with mitochondria cytochrome-C release to the cytoplasm.[81] The cascade of events that starts with these substances leads to DNA destruction and cell death.[56] With this in mind, it is reasonable to think that apoptosis is the final effector of cell death during IR. However, in spite of the fact that suppression of apoptosis improves survival after ischemia and decreases reperfusion damage,[97–100] some investigators argue that the predominate cell death event during IR is massive necrosis,[101] particularly in steatotic livers. In view of this controversy, Lemasters in 1999, proposed the theory of “necroapoptosis”,[102] emphasizing the main mechanisms that participate in IR at the cellular level and suggesting that both cell death mechanisms, necrosis and apoptosis, occur simultaneously during ischemia, and that they even imbricate during reperfusion. The ischemic stimulus can culminate in necrosis or in apoptosis, depending on the interaction with other determining factors, such as a significant reduction of ATP levels[103] or in the fat content of the liver.[104]

Conclusion

The cell signaling pathways and mediators of hepatic ischemia reperfusion are summarized in figure 1. In vitro and pre-clinical animal studies have led to an overall better understanding of liver anatomy, physiology, and the complex signaling events during IR injury. [105]

Application of pharmacologic, genetic, and surgical approaches to reduce hepatic IR injury have been applied and are increasingly being utilized. Therapeutic approaches include pharmacologic use of N-acetylcysteine, prostaglandins, prostacyclin, and ischemic pre-conditioning [56, 106107, 108109110] Careful liver manipulation, and efforts to minimize warm ischemia time are also important principles. Strategies to improve liver outcomes and minimize I/R injury were summarized recently in a review by Clavien and colleagues[111]

Ultimately, the goal is application to safer clinical liver surgery during hepatic resections, liver transplantation, and the operative management of liver trauma.

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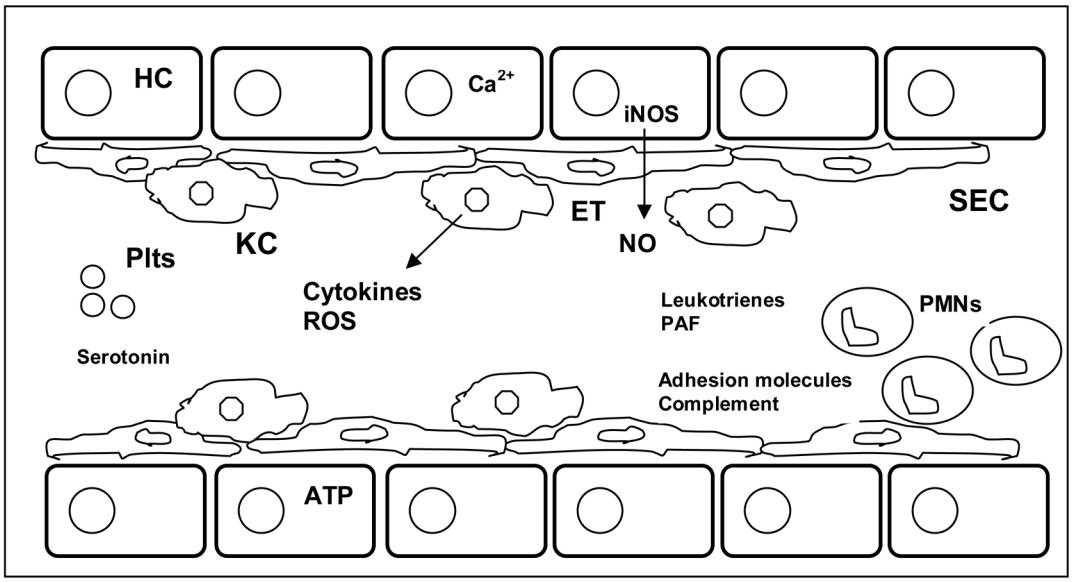

Figure 1.

Mechanisms involved in hepatic ischemia-reperfusion injury. Hepatocyte (HC), Sinusoidal Endothelial cell (SEC), Kupffer cell (KC), Neutrophil (PMN), Platelets (Plts), nitric oxide (NO), Endothelin (ET), Calcium (Ca^{2+}), Adenosine triphosphate (ATP), Platelet activating factor (PAF), Reactive oxygen species (ROS)