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Research review

Rodent models of hepatic ischemia–reperfusion injury: time and percentage-related pathophysiological mechanisms

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ABSTRACT

Ischemia and reperfusion (IR) injury remains one of the major problems in liver surgery and transplantation, which determines the viability of the hepatic tissue after resection and of the grafted organ. This review aims to elucidate the mechanisms involved in IR injury of the liver in rodent experimental studies and the preventative methods and pharmacologic agents that have been applied. Many time- and percentage-related liver IR injury rodent models have been used to examine the pathophysiological mechanisms and the parameters implicated with different morbidity, mortality, and pathology findings. The most preferred experimental rodent model of liver IR is the induction of 70% IR for 45 min, which is associated with almost 100% survival. In this model, plasma levels of several parameters such as alanine transaminase, aspartate aminotransferase, gamma-glutamyltransferase, endothelin-1, malonodialdehyde, tumor necrosis factor α, interleukin 1b, inducible nitric oxide synthase, and caspases are increased. The increase of caspases is associated with the initiation of hepatic cellular apoptosis. The main injuries observed 24 h after reperfusion are nuclear pyknosis, cytoplasmic hypereosinophilia, severe necrosis, and loss of intercellular borders. Both ischemic pre- and post-conditioning preventative methods and pharmacologic agents are successfully applied to alleviate the IR injuries. The selection of the time- and percentage-related liver IR injury rodent model and the potential preventative method should be related to the clinical question being answered.

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1. Introduction

Ischemia and reperfusion (IR) injury remains one of the major problems in liver surgery and transplantation, which determines the viability of the hepatic tissue after resection and of the grafted organ. Ischemia of the liver occurs from a temporary interruption of blood flow lasting for a short time, as in tumor resection, repair of trauma or can last up to several hours (cold ischemia) as in cases of liver transplantation. The ischemic insult causes detrimental cellular mechanisms that are augmented by the subsequent restoration of blood flow after surgery or transplantation of the organ, culminating in the manifestation of IR injury. Many investigations have shed light on the complex pathophysiological mechanisms of liver IR–induced damages that include the release of tissue enzymes, oxidative modification of essential proteins and lipids, and a dysregulated inflammatory response, which ultimately leads to reduction of the regeneration of hepatocytes, tissue necrosis, and apoptosis [1]. During ischemia, glycogen, oxygen, and adenosine triphosphate (ATP) depletion result in cellular metabolic disturbances, whereas the reperfusion injury acts on the hepatocytes both in a direct and indirect way [2]. In severe cases, the inflammatory response after IR may even provoke systemic inflammatory response syndrome or multiple organ dysfunction syndrome [3]. The first state of hypoxic damage occurs in the pericentral region of the hepatic lobules. If severe enough, pericentral liver hypoxia leads to ischemic hepatitis.

IR injury is more pronounced in fatty than in normal livers and has been associated with increased mitochondrial dysfunction, predominant cellular necrosis compared with apoptosis, and decreased ability of the liver to produce ATP [4]. Several mechanisms are considered to be responsible for the increased sensitivity of fatty livers to IR injury than normal livers, which are beyond the scope of this review.

In general, liver injury after warm IR is distinguished in two phases. The initial phase (<2 h after reperfusion) is associated with oxidative stress. Activated endothelial cells of the microcirculation produce less nitric oxide (NO) and more reactive oxygen species (ROS), which cause direct hepatocellular injury [3,5]. The following imbalance between superoxide and NO in endothelial cells results in the release of inflammatory mediators [3]. The late phase of liver injury (6–48 h) is an inflammatory disorder mediated by recruited neutrophils. Activated neutrophils damage hepatocytes through the release of ROS, elastase, cathepsin G, heparinase, collagenase, and hydrolytic enzymes that are likely to be directly cytotoxic to hepatocytes [5]. Dying cells seem to undergo mainly necrosis during IR, whereas <2% of sinusoidal endothelial cells (SEC) and hepatocytes are considered to be apoptotic [5,6]. Specifically, necrosis affects extensive areas of parenchymal cells consistent with the massive release of alanine transaminase (ALT) in serum, indicating that necrosis is the main cause of liver injury during IR.

1.1. *Early phase*

In the early phase, the so-called “pH paradox” is observed. During ischemia, intracellular pH falls. Thus, hepatocytes are protected against the onset of cell necrosis, whereas restoration of a normal pH during reperfusion of ischemic cells accelerates cell death. The onset of the mitochondrial permeability transition (MPT) results in mitochondria becoming freely permeable to solutes and loss of cell viability. ROS, high concentrations of Ca^{2+}, and oxidant chemicals induce MPT, whereas Mg^{2+}, low pH, and cyclosporin A block it. Furthermore, glycine is known to protect liver, kidney, and other cells against cell death in various models of hypoxia and ATP depletion. Moreover, glycine protects SEC against pH-dependent posthypoxic and reperfusion injury [5].

1.2. *Intermediate phase*

The intermediate phase is characterized by oxidative stress with a predominance of pro-oxidants over antioxidants. During ischemia, hypoxanthine accumulates intracellularly and converts into toxic ROS on reperfusion [7]. If oxidative stress exceeds a certain threshold, impairment of cellular function or irreversible damage and cell death occur. All cells produce ROS, but the main sources are the mitochondrion, followed by phagocytic cells, neutrophils, and monocytes. Although neutrophils are a significant source of ROS, they do not seem to play an essential role in reoxygenation injury. Monocytes produce large quantities of extracellular ROS when stimulated to phagocytise. Inflammatory cells primarily produce superoxide, whereas activated Kupffer cells (KC) and neutrophils generate tumor necrosis factor (TNF), interleukin (IL)-1, NO, hypochlorous acid and leukotrienes; each of these may exert pro-oxidant effects [5]. ROS induce cellular damage by lipid peroxidation of the cellular membrane, activation of leucocytes, chemotaxis, rise of leukocyte adhesion molecules, and cytokine gene expression [7].

During this phase, several cytokines and chemokines are implicated, such as

a TNF-α, which is a pleiotropic cytokine produced by numerous cell types in response to various inflammatory and immunomodulatory stimuli. The underlying mechanisms by which TNF-α induces hepatocellular injury are not fully defined. TNF-α may provoke direct toxicity to mitochondria causing apoptotic or necrotic cell death. TNF-α also stimulates KC to generate more TNF-α.
b IL-1, which is known to promote production of ROS and to upregulate free radical production by neutrophils. IL-1 is speculated to enable TNF-α generation, whereas, in turn, TNF-α may also provoke IL-1 release. Similarly, IL-6 is also released during IR, from KC under hypoxia, from cultured lymphocytes, endothelial cells, and myocytes [5]. Particularly, IL-6 treatment has been found to have protective effects against warm IR injury in rodents [8].
c Cytokine-induced neutrophil chemoattractant may be one of the crucial factors that link the early phase of KC activation and the late phase of IR injury mediated by neutrophils.

Additionally, NO is a pluripotent gaseous free radical that acts as either a primary mediator of liver cell injury or as part of a protective mechanism against deleterious stimuli. NO in the