Review

EASL EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER | JOURNAL OF HEPATOLOGY

Hepatic ischemia and reperfusion injury: Effects on the liver sinusoidal milieu

Carmen Peralta[1], Mónica B. Jiménez-Castro[1], Jordi Gracia-Sancho[2,*]

[1]*Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Centro de Investigación Biomédica en Red en Enfermedades Hepáticas y Digestivas (CIBEREHD), Barcelona, Spain;* [2]*Barcelona Hepatic Hemodynamic Laboratory, IDIBAPS, CIBEREHD, Barcelona, Spain*

Summary

Ischemia-reperfusion injury is an important cause of liver damage occurring during surgical procedures including hepatic resection and liver transplantation, and represents the main underlying cause of graft dysfunction post-transplantation. Cellular and biochemical processes occurring during hepatic ischemia-reperfusion are diverse and complex, and include the deregulation of the healthy phenotype of all liver cellular components. Nevertheless, a significant part of these processes are still unknown or unclear. The present review aims at summarizing the current knowledge in liver ischemia-reperfusion, but specifically focusing on liver cell phenotype and paracrine interaction deregulations. Moreover, the most updated therapeutic strategies including pharmacological, genetic and surgical interventions, as well as some of the scientific controversies in the field will be described. Finally, the importance of considering the subclinical situation of liver grafts when translating basic knowledge to the bedside is discussed.

Deregulation of hepatic cell phenotype due to ischemia-reperfusion injury

Ischemia-reperfusion (I/R) injury is an important cause of liver damage during surgical procedures such as hepatic resection and liver transplantation. I/R injury is a biphasic phenomenon whereby cellular damage due to hypoxia and lack of biomechanical stimulus is accentuated upon restoration of oxygen delivery and shear stress. The signaling events contributing to local hepatocellular damage are diverse and complex, and involve the interaction between hepatocytes, liver sinusoidal endothelial cells (LSEC), Kupffer cells (KC), hepatic stellate cells (HSC), as well as infiltrating neutrophils, macrophages, and platelets [1–4]. It is important to note that I/R injury represents the main reason of liver graft dysfunction post-transplantation, independently of liver basal characteristics, being even more relevant when using organs from extended-criteria donors.

Hepatocytes are very much negatively affected by I/R, especially when ischemia is normothermic. Most early changes in the anoxic hepatocytes occur in the mitochondria. The lack of O_2, as a terminal electron carrier for the mitochondrial respiratory chain, immediately interrupts the electron flow causing the respiratory chain to become reduced. Since mitochondria are no longer accepting electrons from substrates, a reduction in pyridine nucleotides occurs, resulting in an increase in the intracellular NADH/NAD+ ratio. The abruption of oxidative phosphorylation rapidly leads to cellular ATP depletion, acceleration of glycolysis, increased formation of lactate, and alterations on H^+, Na^+, and Ca^{2+} homeostasis, altogether inducing serious deleterious effects on the hepatocyte. Ischemia also leads to a considerable increase in cAMP, which is an important factor in glucose metabolism. cAMP, through the action of cAMP-dependent protein kinase, leads to the phosphorylation/deregulation of key enzymes involved in the control of carbohydrate metabolism [5,6]. Reperfusion injury mainly derives from toxic reactive oxygen species (ROS) generated upon reintroduction of O_2 to ischemic tissues. ROS are produced from both intracellular and extracellular sources, being the mitochondria their major source in liver cells [7] (Fig. 1).

Liver sinusoidal endothelial cells (LSEC) form the vascular wall of the hepatic sinusoid, lack an organized basal membrane, and the cytoplasm of these flattened cells is penetrated by open fenestrations that form clusters called sieve plates, making the hepatic microvascular endothelium discontinuous [8]. LSEC play important protective roles controlling vascular homeostasis, inflammation, vascular tone, and toxicants clearance. Thus, maintenance of a healthy LSEC phenotype is indispensable to minimize any type of liver injury.

Keywords: Transplantation; KLF2; Endothelium; Hepatocyte; LSEC.
Received 30 April 2013; received in revised form 17 June 2013; accepted 18 June 2013
* Corresponding author. Address: Barcelona Hepatic Hemodynamic Laboratory, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), C/Rosselló 153, 4th floor, room 4.5, 08036 Barcelona, Spain. Tel.: +34 932275400x4306; fax: +34 932279856.
E-mail address: jgracia@clinic.ub.es (J. Gracia-Sancho).
Abbreviations: I/R, ischemia-reperfusion; LSEC, liver sinusoidal endothelial cells; KC, Kupffer cells; HSC, hepatic stellate cells; NAD, nicotinamide adenine dinucleotide; ROS, reactive oxygen species; NO, nitric oxide; ET, endothelin; TXA_2, thromboxane A2; KLF2, Kruppel-like factor 2; eNOS, endothelial nitric oxide synthase; TNF, tumour necrosis factor; IL, interleukin; INF-γ, interferon-gamma; ICAM-1, intracellular adhesion molecule-1; MIP-2, macrophage inflammatory protein-2; ENA-78, epithelial neutrophil activating protein-78; CINC, cytokine-induced neutrophil chemoattractant-1; GM-CSF, granulocyte-macrophage colony-stimulating factor; $GdCl_3$, gadolinium chloride; NFκB, nuclear factor kappa B; HO-1, heme oxygenase-1; PPAR, peroxisome proliferator-activated receptor.

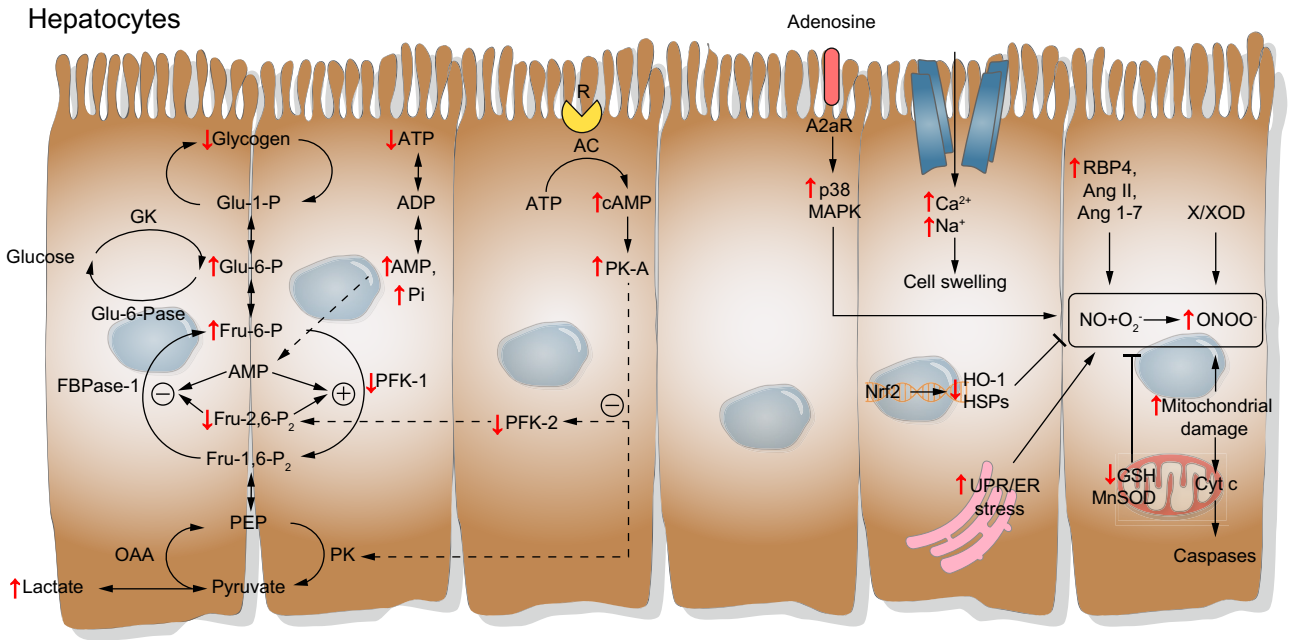

Fig. 1. Hepatocyte phenotype deregulations due to ischemia and reperfusion injury. A summary of the molecular mechanisms affecting the hepatocyte is given, the specific modifications due to I/R are indicated in red. AC, adenine cyclase; AMPK, 5′ adenosine monophosphate-activated kinase; ADP, adenine diphosphate; ATP, adenine triphosphate; A2aR, A2A adenosine receptor; Ang, angiotensin; cAMP, 3′-5′-cyclic adenosine monophosphate; ER, endoplasmic reticulum; FBPase-1, fructose 1,6-bisphosphatase; GK, glucokinase; GSH, gluthatione; HO-1, heme oxygenase-1; HSPs, heat shock proteins; MAPK, mitogen-activated protein kinase; NO, nitric oxide; Nrf2, nuclear factor (erythroid-derived 2)-like 2; O_2^-, superoxide; OAA, oxaloacetic acid; $ONOO^-$, peroxynitrite; PEP, phosphoenolpyruvate; PFK, phosphofructokinase; PK, protein kinase; RBP4, retinol-binding protein 4; SOD, superoxide dismutase; UPR, unfolded protein response; X/XOD, xanthine/xanthine oxidase.

LSEC are particularly vulnerable to I/R injury and develop serious alterations during cold storage (Fig. 2). In fact, pioneering studies by Caldwell-Kenkel and colleagues described the deregulation of LSEC viability due to I/R injury as plasma membranes discontinuation, nuclear membranes vacuolization, and cell shape rounding [9]. These very initial observations, which were usually reported at the Kupffer Cell Foundation meetings (currently the International Society for Hepatic Sinusoidal Research), have been extended during the last decades. Nowadays, it is accepted that hepatic endothelium damage occurring during cold preservation represents the initial factor leading to hepatic I/R injury, determining poor graft microcirculation, platelet activation, persistent vasoconstriction, upregulation of adhesion molecules, oxidative stress, Kupffer cell activation, neutrophil infiltration, and hepatocyte death.

During the ischemic period, the lack of energetic substrate interferes with active transmembrane transport, producing edema in KC and LSEC [10]. This fact, together with the imbalance between low nitric oxide (NO) bioavailability and exacerbated endothelin (ET) and thromboxane A2 (TXA_2) production, contributes to narrowing the sinusoidal lumen, and thus to microcirculatory dysfunction. Diminished NO levels within the liver during I/R are derived from both decreased production and increased scavenging by elevated levels of ROS, and ultimately modulate the intensity of the I/R injury by regulating neutrophil adhesion, platelet aggregation, and HSC contraction [11–13]. In addition, recent studies have demonstrated that lack of biomechanical stimuli occurring during cold preservation for transplantation markedly deteriorates LSEC protective phenotype by downregulating the expression of the transcription factor Kruppel-like Factor 2 (KLF2), which orchestrates the transcription of a variety of protective genes including the endothelial synthase of NO (eNOS), the anti-thrombotic molecule thrombomodulin, or the antioxidant transcription factor Nrf2 [4,14].

Concomitantly to LSEC deregulation, KC suffer from a profound activation process that is promoted by neighbour hepatic cells-released damage-associated molecular patterns (DAMPs) and, under conditions of sepsis or endotoxemia, also by pathogen-associated molecular patterns (PAMPs) [15,16]. Activated KC significantly increase their release of ROS and pro-inflammatory cytokines, including tumour necrosis factor-α (TNF-α), interleukin-1 (IL-1), interferon-γ (INF-γ) and interleukin-12 (IL-12) (Fig. 2) [17,18]. Both TNF-α and IL-1 upregulate Mac-1 (CD11b/CD18) adhesion proteins on neutrophils and induce IL-8 synthesis, further promoting neutrophil chemotaxis within the parenchyma [19]. Moreover, IL-1 has the potential to stimulate the release of ROS by neutrophils, which will further increase TNF-α synthesis by KC [20]. TNF-α stimulates the expression of the intracellular adhesion molecule-1 (ICAM-1) on the intraluminal side of LSEC, contributing to neutrophil rolling, binding, and parenchymal extravasation [20]. TNF-α also induces P-selectin expression in LSEC, being essential for the recruitment of neutrophils [21]. TNF-α has been shown to increase the release of other molecules, including interleukin-6 (IL-6), macrophage inflammatory protein-2 (MIP-2), epithelial neutrophil activating protein-78 (ENA-78), cytokine-induced neutrophil chemoattractant-1 (CINC), and a number of CXC motif chemokines (including CXL-1, -2, and -3). In addition, IL-1 and TNF-α recruit and activate CD4+ T-lymphocytes, which produce granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon gamma (INF-γ) and tumour necrosis factor beta (TNF-β). These cytokines amplify KC activation and promote neutrophil recruitment and adherence into the liver sinusoids [1,22,23].