Ischemia reperfusion-facilitated sinusoidal endothelial cell injury in liver transplantation and the resulting impact of extravasated platelet aggregation

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Summary

Background The exact sequence of events leading to ultimate hepatocellular damage following ischemia/reperfusion (I/R) is incompletely understood. In this article, we review a mechanism of organ dysfunction after hepatic I/R or immunosuppressive treatment, in addition to the potential of liver sinusoidal endothelial cell (LSEC) protection and antiplatelet treatment for the suppression of hepatocellular damage.

Methods A review of the literature, utilizing PubMed-NCBI, was used to provide information on the components necessary for the development of hepatocellular damage following I/R.

Results It is well-established that LSECs damage following hepatic I/R or immunosuppressive treatment followed by extravasated platelet aggregation (EPA) is the root cause of organ dysfunction in liver transplantation. We have classified three phases, from LSECs damage to organ dysfunction, utilizing the predicted pathogenic mechanism of sinusoidal obstruction syndrome. The first phase is detachment of LSECs and sinusoidal wall destruction after LSECs injury by hepatic I/R or immunosuppressive treatment. The second phase is EPA, accomplished by sinusoidal wall destruction. The various growth factors, including thromboxane A2, serotonin, transforming growth factor-beta and plasminogen activator inhibitor-1, released by EPA in the Disse’s space of zone three, induce portal hypertension and the progression of hepatic fibrosis. The third phase is organ dysfunction following portal hypertension, hepatic fibrosis, and suppressed liver regeneration through various growth factors secreted by EPA.

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Conclusion We suggest that EPA in the space of Disse, initiated by LSECs damage due to hepatic I/R or immunosuppressive treatment, and activated platelets may primarily contribute to liver damage in liver transplantation. Endothelial protective therapy or antiplatelet treatment may be useful in the treatment of hepatic I/R following EPA.

Keywords Ischemia/reperfusion · Extravasated platelet aggregation · Sinusoidal endothelial damage · Antiplatelet agents · Endothelial protection

Introduction

Ischemia/reperfusion (I/R) injury to the liver is a major complication of hemorrhagic shock, liver resection, and transplantation [1]. Although the sequence of events that leads to extent of hepatocellular damage after hepatic I/R is incompletely understood, liver sinusoidal endothelial cells (LSECs) may loosen from their tethers to the space of Disse in zone 3 or even detach completely [2].

Hepatic I/R is considered as a biphasic phenomenon. Cellular damage due to hypoxia and a lack of biomechanical stimulus is exacerbated upon the restoration of oxygen delivery and shear stress [3]. Although the initial ischemic insult to the liver is tolerable, this first step triggers essential molecules in the induction of the more devastating reperfusion injury. Early phases of reperfusion are characterized pathologically by endothelial cell swelling, vasoconstriction, neutrophil entrapment, and platelet aggregation within the sinusoids—resulting in failure of the microcirculation [4]. Simultaneously, nitric oxide (NO) levels are markedly reduced and there is an imbalance between endothelin-1 and NO production from NO synthase (NOS). This leads to vasoconstriction of the sinusoids [5]. As such, vasoconstriction of the sinusoids gives rise to narrowing of the sinusoidal lumen with consequential decreased leukocyte velocity. The frequency of leukocyte-endothelial cell contact is elevated, promoting leukostasis. Flow is hindered in the sinusoidal network of the hepatic microcirculation due to stagnant leukocytes, unable to completely occlude the sinusoidal lumen [6]. Microcirculatory failure leads to aggravated and prolonged ischemia. Hypoxic regions of the liver heighten the degree of necrosis, Kupffer cell activation, and induce further cytokine and reactive oxygen species release. This creates a cycle of excessive inflammatory response, reactive oxygen and nitrogen species production, and further oxidative tissue injury [7].

LSECs, which lack an organized basal membrane, form the vascular wall of the hepatic sinusoid. Fenestrations penetrate the cytoplasm of these flattened cells forming clusters, called sieve plates, which render the hepatic microvascular endothelium discontinuous [8]. LSECs play an integral protective role in maintaining vascular homeostasis, inflammation, vascular tone, and toxicant clearance. Thus, the preservation of a healthy LSEC phenotype is fundamental to minimize any type of liver injury [3]. Damage to the endothelium, following hepatic I/R injury, is apparent within LSECs and hepatocytes, indicated by the deposition of fibrinogen and erythrocyte congestion resulting in enlarged sinusoids. Sloughed LSECs, red cells, and stellate cells embolize downstream, leading to venous occlusions that progress to disrupt the normal liver architecture and achieve centrilobular necrosis [9]. In the late phases of the disease, fibrosis and occlusion of the terminal venules develop, leading to hepatic failure and possibly death [10].

Sinusoidal obstruction syndrome (SOS), previously known as veno-occlusive disease, commences with sustained LSECs injury, resulting in bleeding in the space of Disse and centrilobular hemorrhagic necrosis. The fundamental cause, moreover, is damage around the centrilobular area, including the sinusoid, by acute cellular rejection, antibody-mediated rejection or hepatic I/R injury [11]. SOS is a life-threatening syndrome that results from sinusoidal congestion and is characterized by hepatomegaly, ascites, portal hypertension, weight gain and jaundice [12].

Platelets play an important role in hepatocellular damage. Furthermore, platelets have been suggested to be involved in the inflammatory response of hepatic I/R injury in various organs [13]. They are able to roll and adhere to postreperfusion endothelium in a P-selectin-dependent manner [14, 15]. Platelets accumulate in the posts ischemic microvasculature early after reperfusion via P-selectin-ligand interactions. Platelet recruitment and subsequent activation might play an important role in the pathogenesis of hepatic I/R injury [14]. Platelet aggregation also correlated with reperfusion injury, thrombocytopenia and early graft dysfunction in liver transplantation [16]. As such, persistent thrombocytopenia after reperfusion is an unfavorable indicator for early liver graft dysfunction [17].

We previously reported that platelet aggregation in the space of Disse along with the sinusoid and platelets phagocytosis by hepatocytes were observed in the allograft tissue of a living donor liver transplantation recipient with thrombocytopenia, who encountered a complication of SOS [18]. Therefore, LSECs damage after hepatic I/R or immunosuppressive treatment followed by extravasated platelet aggregation (EPA) is the root cause of organ dysfunction in liver transplantation.

This manuscript will review the role of platelet aggregation and possible prevention for hepatocyte injury in hepatic I/R. The PubMed database was utilized in searching for published literature in this area.

Platelets and hepatic ischemia/reperfusion injury

Whereas many of the mechanisms underlying the hepatic I/R-induced inflammatory response still remain unknown, growing evidence suggests a role for platelets in the pathogenesis of posts ischemia reperfusion injury [14]. Ischemia leads to the accumulation and activation...
Ischemia reperfusion-facilitated sinusoidal endothelial cell injury in liver transplantation

Platelets release potent proinflammatory chemokines and modulate leukocyte function [26]. Activated platelets release growth factors, such as thromboxane (TX) A2, serotonin, vascular endothelial growth factor (VEGF)-A, transforming growth factor (TGF)-β and plasminogen activator inhibitor (PAI)-1. TXA2 is a strong vasoactive metabolite of arachidonic acid with powerful proaggregatory and proinflammatory properties. TXA2 is a vasoconstrictor that increases portal venous resistance [27] and causes portal hypertension. Serotonin is well-known released from platelets on damage to the blood vessels walls. It acts as a potent vasoconstrictor. Although VEGF-A acts as a vasodilator under ordinary circumstances, it acts paradoxically as a vasoconstrictor in patients with endothelial failure [28]. Bevacizumab, an antibody against VEGF-A, protects against liver injury associated with SOS [29]. PAI-1 suppresses fibrinolysis and the progression to fibrosis in the tissue microenvironment. In addition, PAI-1 acts as a negative regulator of hepatocyte proliferation by inhibiting urokinase-type plasminogen activator (u-PA), which activates hepatocyte growth factor [30, 31]. TGF-β, a major antiproliferative factor for hepatocytes, stimulates collagen synthesis through activated hepatic stellate cells (HSCs) [32].

Extracellular nucleotides are released in a regulated manner either by platelets, a variety of vascular and hepatic cells in response to inflammatory stress, through cellular swelling, or with exocytosis. Levels of extracellular nucleotides are, in turn, regulated by CD39 (ectonucleoside triphosphate diphosphohydrolase-1/ENTPD1), the dominant vascular ectonucleotidase. CD39, expressed only on the luminal surface of proliferating or activated LSECs and absent in hepatocytes [21, 22], hydrolyzes the terminal phosphate of adenosine triphosphate(ATP) and ADP in an enzymatic cascade that generates adenosine monophosphate(AMP) [23]. Under normal conditions, in the absence of stressors such as hypoxia/ischemia, high shear stress, and triggers for inflammation (cytokines/chemokines), CD39 helps to maintain a homeostatic vascular environment, maintain blood fluidity and inhibit inflammation [24]. ADP, one of the most potent signals for platelet aggregation, as well as proinflammatory signals such as ATP, are released into the extracellular environment in the presence of adverse conditions, such as hepatic I/R. When this occurs, the extracellular concentration of ADP and ATP increases markedly [25]. When platelets contact collagen, they produce ADP, which further promotes platelet aggregation.

Extravasated platelet aggregation and organ dysfunction

The liver can be subjected to three forms of ischemia, namely cold (or hypothermic), warm (or normothermic),
and rewarming [34]. In transplanted liver ischemia, disruption of the endothelial wall leads to leukocyte [35] and platelet adhesion [15, 36], which induces microcirculatory disturbances [37]. Transplanted liver damage is slightly different from liver resection, due to the duration of sustained ischemia.

The subendothelial space of Disse, located between the hepatocytes and the sinusoids, contains HSCs (myofibroblasts) and a network of reticular fibers holding the hepatocytes together. Large amounts of albumin and other plasma proteins enter the space of Disse, pass through hepatocyte junctions, and form ascites containing a high concentration of protein and a low serum to ascites albumin gradient. In normal liver, the space of Disse contains a matrix of basement-membrane constituents that is not electron dense [38]; in injured tissue this may be replaced by matrix filled with fibril-forming collagens and fibronectin [39].

In the normal liver, collagen type III are concentrated in the portal tracts and around terminal hepatic veins, with occasional bundles located between hepatocytes and endothelial cells in the space of Disse. Hepatic ischemia causes endothelial cell activation following HSCs activation, increasing hepatic parenchymal and portal tract fibrosis in ischemic liver tissues [40]. Following a fibrogenic stimulus, HSCs undergo a complex process of activation in which they become transformed from quiescent to activated myofibroblast-like cells [41–43]. Activated HSCs are the primary cell type responsible for the production of collagen I. This subendothelial accumulation of collagen, termed “capillarization” of the sinusoids, is associated with clinical liver disease [44]. Fibrosis may also impede the rapid exchange of solutes between the sinusoidal space and hepatocytes [45].

Following the destruction of endothelial wall and resulting LSECs damage caused by hepatic I/R or immunosuppressive treatment, platelets enter the space of Disse (Fig. 1b) and aggregate by activated HSCs (Fig. 2). Furthermore, platelets produce platelet-derived growth factor TGF-β to activate HSCs and promote fibrosis [46]. Therefore, liver fibrosis may be caused by EPA in this pathway (Fig. 3).

The production of collagen I may be associated with increased serum alanine transaminase level and be utilized as a marker for hepatic I/R injury.

Possible treatment

No standard method for treating hepatic dysfunction by hepatic I/R has yet been established. Systemic anticoagulation and thrombolytic therapies have been tested extensively [47]. Defibrotide, a polydeoxyribonucleic acid, was recently shown to have a promising response rate in patients with severe SOS [48]. Conditional to the extent of LSECs damage and EPA in the space of Disse, prophylactic administration of endothelial protective and antiplatelet agents may be effective prior to the development of irreversible damage.

Additionally, antiapoptosis induced by ischemic preconditioning can improve liver function, as well as protect LSECs [49]. Ischemic preconditioning directly protected hepatocytes after warm I/R, not via suppression of alterations in sinusoidal cells, as is present in cold hepatic I/R injury [50].
Reinforcement and protection of LSECs

Hyperglycemia, indicative of diabetes mellitus, is a major risk factor for endothelial dysfunction and vascular complications. In recent years, significant advances have been made in understanding endothelial cell dysfunction, triggered by high glucose concentration [51]. In order to prevent graft injury during perioperatively tight glycemic control, it is necessary to reduce the risk of microangiopathic organ injury by preventing endothelial cell injury [52]. The artificial pancreas might have beneficial effects in liver transplant recipients due to the hepatoprotective effects of insulin, including the stimulation of regeneration and endothelial cell protection [53]. Additionally, insulin has an antiinflammatory effect through the suppression of inflammatory cytokines (e.g., nuclear factor kappa B) [54]. To ascertain this, we applied a closed-loop glycemic control system with an artificial pancreas (STG-55, NIKKISO CO., LTD., Tokyo, Japan) to the liver transplant recipient.

In obese patients with type 2 diabetes, insulin delivery to and insulin-dependent glucose uptake by skeletal muscle are delayed and impaired. Therefore, it is pivotal to impair insulin resistance for endothelial cells. Kubota et al. [55] demonstrated that impaired insulin signaling in endothelial cells, due to reduced insulin receptor substrate two expression and insulin-induced eNOS phosphorylation, causes attenuation of insulin-induced capillary recruitment and insulin delivery, reducing glucose uptake by skeletal muscle. The use of agents such as Beraprost, a stable prostacyclin analogue (PGI2), capable of improving insulin resistance and resulting vascular endothelial function, may ultimately contribute to increasing the life expectancy of patients with peripheral artery disease [56]. To improve insulin resistance, we administer Beraprost sodium during the perioperative state.

Antiplatelet agents therapy

Phosphodiesterase 3 (PDE3) inhibitors Cilostazol and Milrinone may be appropriate, owing to their antiplatelet properties, ability to increase tolerance to hepatic I/R injury [57], and induction of immune tolerance via an enhanced regulatory T-cell response [58]. Furthermore, PDE3 inhibition regulates endothelial CD39 at a post-translational level [25]. To reaffirm such a treatment, a few experimental studies in rat models demonstrated that PDE3 inhibitors protected SOS [59], and attenuated graft injury using an orthotopic liver transplant model [60].

In clinical practice, we administered a PDE3 inhibitor with the use of local infusion therapy to living donor liver transplant recipients or patients with small remnant liver volume after a hepatectomy such as in tri-segmentectomy, with favorable outcomes [18].

Beraprost also prevents platelet aggregation by increasing cyclic AMP to effectively reduce the amount of TXA2, which has coagulant properties and is made by platelets. Beraprost is also administered as an antiplatelet agent.

Conclusion

We suggest that EPA in the space of Disse, initiated by LSECs damage due to hepatic I/R or immunosuppressive treatment, and activated platelets may primarily contribute to liver damage in liver transplantation. Endothelial protective therapy or antiplatelet treatment may be useful in the treatment of hepatic I/R following EPA.

Conflict of interest

The authors declares that there is no conflict of interests regarding the publication of this paper.

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