

How Institut Georges Lopez Preservation Solution Protects Nonsteatotic and Steatotic Livers Against Ischemia-Reperfusion Injury

Liver transplantation has become the only curative therapy for many patients with end-stage liver disease and/or acute liver failure. However, this therapy is always hampered by the extreme sensitivity of organs to ischemia-reperfusion (I/R) injury. The process of I/R injury combines many interrelated factors and mediators that produce a cascade of events ultimately leading to graft failure. During ischemia, cellular damage is mainly due to the breakdown of energy metabolism, leading finally to lactate accumulation concomitant with derangements in calcium homeostasis. After reperfusion, liver injury is aggravated by the generation of toxic reactive oxygen species (ROS). Subsequent generation of inflammatory mediators and accumulation of neutrophils contribute to the progression of hepatic damage. Consequently, experimental and clinical studies seek to prevent liver I/R injury to preserve organ function.

It is well recognized that the success of liver transplantation strongly depends on the composition of the preservation solution. It is well known that the main goal of organ preservation is the maintenance of functional, biochemical, and morphological integrity of the graft. In situ flushing of the organs with preservation solution permits rapid cooling to 4°C. This is main strategy to minimize ischemic injury during cold static preservation. These liver grafts preservation conditions determine graft viability against normothermic reperfusion injury, which is especially aggravated with the use of steatotic livers. In the 1980s, Belzer and

Southard developed the University of Wisconsin (UW) solution that supports good graft function even after extended cold preservation. However, the conditions in which organ transplantation was practiced in the 1980s, when UW solution was first developed, have now changed extremely. The lack of organs for transplantation has obliged physicians to utilize marginal grafts, such as steatotic livers, which show poor tolerance against I/R injury. In addition, recent studies have demonstrated that UW preservation solution presents two major limitations: the presence of hydroxyethyl starch (HES), as an oncotic agent, can trigger red blood cell aggregation and the high potassium levels require a flush of the organ in the recipient before transplantation. Recently, the Institut Georges Lopez (IGL-1) solution has been proposed as an alternative to UW solution for kidney and liver transplantation. Its benefits in clinical and experimental transplantation seem to be due to replacement of HES with polyethylene glycol and a lower K₊ concentration. In this review, we have focused on possible protective mechanisms of IGL-1 against liver cold I/R injury, especially during use of marginal grafts such as steatotic livers.

IGL-1 PROTECTIVE MECHANISMS AGAINST LIVER I/R INJURY

Today, there is an increasing need for solutions that afford improved protection to livers from marginal donors. Studies from our laboratory have demonstrated that IGL-1 is superior to UW solution for rat liver preservation after 24-hour cold storage. More recently, we showed that IGL-1 solution more efficiently protected steatotic livers against cold I/R injury than UW solution. Despite these beneficial effects that initially are associated with antioxidant effects, the protective mechanisms of IGL-1 are complex. They may include the involvement of several prosurvival molecules such as nitric oxide (NO), as well as the activation and transduction of various cell signaling factors, such as hypoxia inducible factor-1 alpha (HIF-1 α) and heme oxygenase 1 (HO-1).

NITRIC OXIDE

NO, a diffusible and protective molecule, exerts vasodilatory and antioxidant properties that determine fatty liver protection. The generation of NO prevents microcirculatory dysfunction and the exacerbated oxidative stress within fatty livers undergoing I/R. We have demonstrated that steatotic livers preserved in IGL-1 solution show transient generation of NO as a consequence of activation of the constitutive endothelial NO synthase (eNOS). The addition of NO inducers, such as trimetazidine (an anti-ischemic drug), to IGL-1 solution potentiates eNOS-derived NO, enhancing protection of graft livers, confirming the direct relevance of NO for graft preservation against cold I/R injury. NO favors the stabilization of HIF-1_α during reperfusion, which is generated during cold ischemia. In addition, the benefits NO are also associated with its capacity to counteract endothelins (ET) that originate during I/R. ET are potent vasoconstrictor peptides that accumulate in the vascular space of the graft during cold storage. A correlation was observed between the duration of cold storage and the degree of ET release that is responsible for reduction of sinusoidal diameter with subsequent microcirculatory derangements, especially in the presence of steatosis. In addition, the NO enhancement induced by IGL-1 can contribute to the amelioration of the oxygenation of hepatic tissue¹⁸ and to the prevention of the impaired microcirculation due to hepatocyte fat accumulation with subsequent sinusoidal space reduction. Also, we demonstrated that livers preserved in IGL-1 solution supplemented with insulin-like growth factor (IGF-1) display increased protein kinase B or AKT (PKB/AKT) phosphorylation, which in turn, activates eNOS and NO generation. In this study, we observed a reduced release of proinflammatory cytokines, such as tumor necrosis factor (TNF- α) which mediates the progression of liver reperfusion damage. This observation was consistent with accumulating evidence of cross talk between IGF-1 and TNF- α during I/R injury. NO generated by IGF-1 inhibits TNF- α release, as similarly occurs in the hepatic protection induced by ischemic preconditioning.

OXIDATIVE STRESS AND MITOCHONDRIAL INJURY

ROS generated from both intracellular and extracellular sources during the I/R process determine progression of tissue injury. Liver mitochondria appear to be an important sources of ROS generation. Recently we demonstrated that IGL-1 was more efficient than UW solution to prevent lipid peroxidation associated with hepatic I/R. IGL-1 also prevented mitochondrial damage as evidenced by lower glutamate dehydrogenase (GLDH) activity levels. CYTOPROTECTIVE FACTORS: HIF-1_α AND HO-1 HIF-1_α, a cytoprotective transcription factor, mediates adaptive responses to changes in tissue oxygenation such as those occurring during liver preservation. Indeed, recently we demonstrated that HIF-1_α was up-regulated among liver grafts preserved in IGL-1 solution. This overexpression of HIF-1_α during cold storage was increased when IGL-1 was supplemented with trimetazidine (TMZ). Under normoxic conditions, HIF-1_α is degraded by prolylhydroxylases. We demonstrated that NO generation by the actions of TMZ and IGL-1 impair normoxic degradation of HIF-1_α, thus contributing to its accumulation and favoring enhanced expression of other cytoprotective genes such as HO-1 during liver reperfusion. It is well assumed that increases in HO-1 activity ameliorate liver graft outcomes after transplantation. In conclusion, IGL-1 protects liver grafts against cold I/R injury and ameliorates function among steatotic and nonsteatotic organs after reperfusion.