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# Ischemia-Reperfusion Injury Associated with Liver Transplantation in 2011: Past and Future

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## 1. Introduction

Liver transplantation has evolved as the therapy of choice for patients with end-stage liver disease. However, the waiting list for liver transplantation is growing at a fast pace, whereas the number of available organs is not growing at a proportional rate. The potential use of steatotic livers for transplant, one of the most common types of organs from marginal donors, has become a major focus of investigations. However the clinical problem is still unresolved since steatotic livers are more susceptible to ischemia-reperfusion (I/R) injury and, when used, have poorer outcome than non-steatotic livers. Indeed, the use of steatotic livers for transplantation is associated with increased risk of primary non-function or dysfunction after surgery. Therefore, minimizing the adverse effects of I/R injury could improve outcomes in steatotic liver surgery, increase the number both of suitable transplantation grafts and of patients who successfully recover from liver transplantation.

The present review focuses on the complexity of hepatic I/R injury, summarizing conflicting results obtained from the literature about the mechanisms responsible for it. We also review the therapeutic strategies designed in past years to reduce I/R injury, attempting to explain why most of them have not been applied clinically. Finally, we will consider new potential protective strategies that have shown promising results for I/R injury with the potential to increase the number of liver suitable for liver transplantation.

## 2. Hepatic ischemia-reperfusion injury associated with liver transplantation. An unresolved problem in clinical practice

Liver transplantation (LT) dates back to 1963, when Thomas Starzl carried out the first transplant on a child suffering from biliary atresia. LT has evolved as the therapy of choice for patients with end-stage liver disease. However, I/R injury, inherent in every LT, is the main cause of both initial poor function and primary non-function of liver allograft. The latter is responsible for 81% of re-transplantations during the first week after surgery (Clavien et al., 1992; Jaeschke, 1996). I/R injury is a phenomenon whereby cellular damage in a hypoxic organ is accentuated following the restoration of oxygen delivery (Jaeschke, 1998; Teoh et al., 2003; Jaeschke, 2003). In the liver, this form of injury was recognized as a

clinically important pathological disorder by Toledo-Pereyra et al. in 1975 during studies of experimental LT. However, it was not until the mid-1980s that the term reperfusion injury was generally used in the literature on LT (Teoh et al., 2003).

A variety of clinical factors including starvation, graft age, and steatosis contribute to enhance liver susceptibility to I/R injury, further increasing the patient risks related to reperfusion injury (Shah & Kamath, 2003). In clinical LT, starvation of the donor, due to prolonged intensive care unit hospitalization or lack of an adequate nutritional support, increases the incidence of hepatocellular injury and primary nonfunction (Massip-Salcedo et al., 2007).

The waiting list for LT is growing at a fast pace, whereas the number of available organs is not growing at a proportional rate. The shortage of organs has led centers to expand their criteria for the acceptance of marginal grafts, which show poor tolerance to I/R (Busuttil & Tanaka, 2003). Some of these include the use of organs from aged donors, non-heart-beating donors (NHBD), and grafts such as small-for-size or steatotic livers. However, I/R injury is the underpinning of graft dysfunction that is seen in the marginal organ (Busuttil & Tanaka, 2003). The fundamental problem with NHBD organs is the prolonged warm ischemia before cold preservation (Reddy et al., 2004). Controlled NHBDs provide organs that are far less prone to ischemic damage and tend to offer superior posttransplant function (Busuttil & Tanaka, 2003). The use of uncontrolled NHBDs is associated with a very high risk of primary nonfunction (Reddy et al., 2004).

One of the benefits of reduced-size grafts from living donors is a graft of good quality with a short ischemic time, this latter being possible because live donor procurements can be electively timed with recipient procedure (Farmer et al., 2001). On the other hand, the major concern over application of living-related liver transplantation for adults is graft-size disparity. The small graft needs regeneration to restore the liver/body ratio. It is well known that I/R significantly reduces liver regeneration after hepatectomy (Franco et al., 2004).

Donor age of more than 70 years was found to be associated with lower patient and graft survival (Busuttil & Tanaka, 2003, Casillas et al., 2006). Additionally these donors also have an increased incidence of steatosis, which may potentiate cold preservation injury (Busuttil & Tanaka, 2003). Steatotic livers are one of the most common types of organs from marginal donors. The present review will focus on this type of liver grafts. Among other factors, unhealthy lifestyles associated with the consumption of alcohol and inappropriate diets have increased the proportion of patients with steatotic livers.

Hepatic steatosis is a major risk factor for liver surgery and transplantation, and fatty livers are unsuitable for many reasons. Operative mortality associated with steatosis exceeds 14%, compared with 2% for healthy livers, and the risks of primary non-function and dysfunction after surgery are similarly higher (Casillas et al., 2006; Selzner et al., 2000). Thus, hepatic steatosis is the major cause of graft rejection after LT and exacerbates the organ shortage problem (Fernández et al., 2004). Therefore, minimizing the adverse effects of I/R injury could increase the number of both grafts suitable for transplantation and patients who successfully recover from LT. The first step towards achieving this objective is a full understanding of the mechanisms involved in I/R injury.

### 3. Complexity of hepatic ischemia-reperfusion injury

A large number of factors and mediators play a part in liver I/R injury (Banga et al., 2005; Casillas et al., 2006; Fan et al., 1999; Jaeschke, 2003; Lentsch et al., 2000). The relationships between the signalling pathways involved are highly complex and it is not yet possible to describe, with absolute certainty, the events that occur between the beginning of reperfusion and the final outcome of either poor function or a non-functional liver graft.

Figure 1 shows some of the mechanisms involved in the pathophysiology of I/R injury. Due to the complexity of hepatic I/R injury, the present review summarizes the established basic concepts of the mechanisms and cell types involved in this process. The lack of oxygen to hepatocytes during ischemia causes mitochondrial deenergization, ATP depletion, alterations of  $H^+$ ,  $Na^+$ ,  $Ca^{2+}$  homeostasis that activate hydrolytic enzymes and impair cell volume regulation and sinusoidal endothelial cells (SEC) as well as Kupffer cells (KC) swelling (Massip-Salcedo et al., 2007). This fact together with the imbalance between nitric oxide (NO) and endothelin (ET) production, contributes to narrowing of the sinusoidal lumen and thus to microcirculatory dysfunction. Capillary narrowing also contributes to hepatic neutrophil accumulation (Peralta et al., 1996; Peralta et al., 2000a). Concomitantly, the activation of KC releases reactive oxygen species (ROS) and proinflammatory cytokines, including tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin-1 (IL-1) (Bilzer & Gerber, 2000; Lentsch et al., 2000). ROS can also derive from xanthine deshydrogenase/xanthine oxidase (XDH/XOD). Cytokines release throughout the induction of adhesion molecules (intercellular cell adhesion molecule [ICAM] and vascular cell adhesion molecule [VCAM]) and chemokines promote neutrophil activation and accumulation, thereby contributing to the progression of parenchymal injury by releasing ROS and proteases (Jaeschke, 1998, 2003; Lentsch et al., 2000). Besides, IL-1 and TNF- $\alpha$  recruit and activate CD4 $^+$  T-lymphocytes, which produce granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon gamma (INF- $\gamma$ ) and tumor necrosis factor beta (TNF- $\beta$ ). These cytokines amplify KC activation and TNF- $\alpha$  and IL-1 secretion and promote neutrophil recruitment and adherence into the liver sinusoids (Casillas et al., 2006; Selzner, 2003). Platelet activating factor (PAF) can prime neutrophils for superoxide generation, whereas leukotriene B4 (LTB4) contributes to the amplification of the neutrophil response (Jaeschke, 1998, 2003) (see Fig. 1).

The present review will present data from the literature about the possible sources of ROS, NO effects, mechanisms, and the role of some pro-inflammatory mediators such as TNF- $\alpha$ , and transcription factors, for example, nuclear factor kappa B (NF $\kappa$ B). These data will provide a better explanation on why hepatic I/R injury remains an unresolved problem in the clinical practice.

#### 3.1 Mechanisms responsible for ROS production

The source of ROS in hepatic I/R has long been controversial. As regards the mechanisms responsible for ROS production, experiments with XDH/XOD inhibitors such as allopurinol suggest that this system is the main ROS generator in hepatocytes and it has also been implicated in LT-related lung damage (Casillas et al., 2006; Fernández et al., 2002). However, results obtained in experimental models of the isolated perfused liver have underestimated the importance of the XDH/XOD system, and suggest that mitochondria could be the main source of ROS (Jaeschke & Mitchell, 1989). On the other hand, some data challenge the

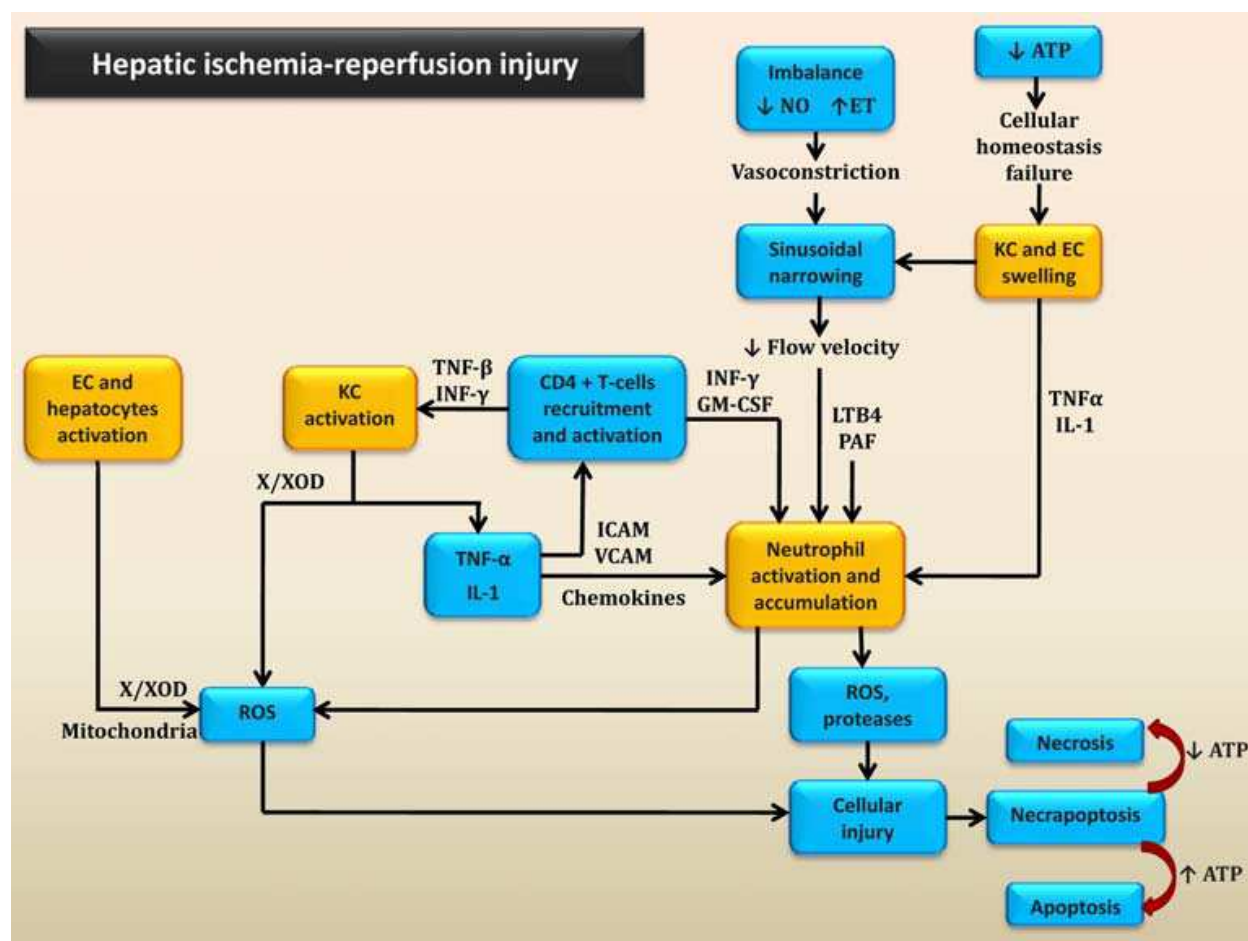


Fig. 1. Summary of the mechanisms involved in hepatic ischemia-reperfusion injury. (Bilzer & Gerber, 2000; Casillas et al., 2006; Jaeschke, 1998, 2003; Lentsch et al., 2000; Massip-Salcedo et al., 2007 ; Peralta et al., 1996, 2000a; Selzner, 2003)

pathophysiological relevance of intracellular oxidant stress during reperfusion (Grattagliano et al., 1999; Metzger et al., 1988). Grattagliano et al., 1999, demonstrated that mitochondria do not seem to actively participate in the reperfusion-induced oxidative stress. In addition, studies by Jaeschke et al. and Metzger et al. showed that the increased vascular oxidant stress after 30 and 60 min of ischemia was attenuated by inactivation of KC but not by high dose of allopurinol (Metzger et al., 1988). Interestingly, ROS release by KC occurs via the XDH/XOD system (Wiezorek et al., 1994). The conversion from XDH to XOD following cold storage is very slow in endothelial cells and hepatocytes, but much faster and higher in KC (Wiezorek et al., 1994). However, the KC function in I/R injury is still an area of active investigation. The elimination of KC did not modify the deleterious effects of I/R and the activation of neutrophils is not essential for reoxygenation injury (Imamura et al., 1995; Teoh et al., 2003). Clearly, then, there is a range of potentially conflicting results with regard to the mechanisms responsible for ROS generation in liver I/R injury. For instance, in our opinion, in order to clarify the importance of XDH/XOD versus mitochondria it should be taking into account that there are differences in the experimental models evaluated, including the times of ischemia. In this line, XDH/XOD play a crucial role in hepatic I/R injury only in conditions in which significant conversion of XDH to XOD occurs (80-90% of XOD) such as 16 h of cold ischemia. However, this ROS generation system does not appear to be crucial at



shorter ischemic periods such as 6 h of cold ischemia (Fernández et al., 2002). Thus, even after prolonged periods of ischemia, where a significant conversion of XDH to the XOD occurs, this enzyme may only play a minor role compared with mitochondria (Jaeschke & Mitchell, 1989). In contrast with the experimental studies, the clinical reports suggest that 45-65% XOD was sufficient to induce hepatic damage (Pesonen et al., 1998). In addition, the drugs used for inhibiting XDH/XOD should be considered, since, for example allopurinol, seems to have more than one mechanisms of action. It is not only a potent inhibitor of XOD, but it may also improve ischemia-induced mitochondrial dysfunction (Casillas et al., 2006; Jeon et al., 2001). In fact, evidence for reduced mitochondrial dysfunction after high doses of allopurinol was shown in a warm hepatic I/R model (Jeon et al., 2001). Similarly, in assessing the relative contribution of intracellular versus vascular oxidant stress to hepatic I/R injury, it should also be noted that oxidative stress in hepatocytes and the stimulatory state of KC after I/R depend on the duration of ischemia, and may also differ between ischemia at 4°C and that at 37°C, which probably leads to different developmental mechanisms of liver damage (Casillas et al., 2006). The differences in KC function in liver I/R injury cannot be attributed to the type experiment, since most authors used an *ex vivo* model of perfused rat liver. Nor could they be explained by differences in the times of cold ischemia, since the results obtained following the same ischemic period (24 h) were completely opposed (Imamura et al., 1995). The type of drug used for KC inactivation is the most probable explanation, since most of the studies implicating KC as main source of ROS used gadolinium chloride (GdCl<sub>3</sub>) (Schauer et al., 2001; Zhong et al., 1996) whereas those that did not implicate KC used liposome-encapsulated dichloromethylene diphosphate (Imamura et al., 1995). Indeed, differences in the properties and action mechanisms of these two KC inhibitors have been reported.

### 3.2 Mediators and transcription factors in I/R injury

#### 3.2.1 Nitric oxide

It is difficult to distinguish between beneficial and harmful mediators in I/R injury. Some authors have found that NO exerts a beneficial effect on I/R injury in different organs, tissues and cells, whereas other studies report no effect or even a deleterious action of NO (Peralta et al., 2001a). In our opinion, in addition to the differences in animal species, experimental models of hepatic I/R tested, and the dose and timing of administration of the different pharmacological modulators of NO, these differential effects of NO could be explained, at least partially, by the different source of NO. In this context, some studies suggest that although endothelial NO synthase (eNOS)-derived NO production is protective in I/R, inducible NO synthase (iNOS)-derived NO production may contribute to I/R injury. This may be a function of the NO generation kinetics of the two isoforms in I/R. The basal, low-level NO generation by the constitutively expressed eNOS isoform may abrogate the microcirculatory stresses of engraftment and reperfusion. In contrast, iNOS-derived NO cannot be generated until several hours after stimulation because of requirements for transcriptional induction of this isoform. Excess NO production may no longer be of microcirculatory benefit at this later time (Shah & Kamath, 2003). Furthermore, the excessive levels of iNOS-derived NO production may be detrimental through the generation of NOS-derived superoxide production or the generation of peroxynitrite. Additionally, whether NO is cytoprotective or cytotoxic in hepatic I/R injury may be determined at apoptosis (Casillas et al., 2006). For example, NO may promote apoptosis by inducing cytochrome c

(Cyt c) release and caspase activation (Chung et al., 2001). However, NO may also upregulate the anti-apoptotic protein Bcl-2 (Genaro et al., 1995). In addition, to understand the different results in relation with the action mechanisms of NO, it is important to clarify whether the NO source is endogenous or exogenous. In this regard, although the beneficial role of endogenous NO could be related to an attenuation of leukocyte accumulation, the exogenous supplementation of NO did not modify this parameter but was associated with an inhibition of endothelin release (Peralta et al., 2001a).

### 3.2.2 TNF and NFκB

Differential effects of NO mentioned above have also been reported for other mediators involved in hepatic I/R injury. According to the cell type and experimental or pathologic conditions, TNF- $\alpha$  is protective or injurious to the liver in the context of I/R injury. TNF- $\alpha$  may stimulate cell death or it may induce hepatoprotective effects mediated by antioxidant, antiapoptotic, and other anti-stress mediators coupled with a pro-proliferative biologic response (Casillas et al., 2006). For example, although the deleterious effect of the TNF- $\alpha$  in local and systemic damage associated with hepatic I/R is well established (Peralta et al., 1999), this mediator is also a key factor in hepatic regeneration (Teoh et al., 2003), an important process in reduced-size LT. Conversely, a study by our group found no correlation between TNF- $\alpha$  levels and liver regeneration in reduced-size LT (Franco et al., 2004), while Tian *et al.*, 2006, linked disruption of TNF- $\alpha$  release to lower hepatic injury and increased liver regeneration. These divergent results about the role of TNF- $\alpha$  in liver regeneration could be explained by different TNF- $\alpha$  inhibitors or animal species utilized in these experiments as well as differences in the experimental models of LT used, including the times of cold ischemia. These differential effects observed for TNF- $\alpha$  can also be extrapolated to transcription factors.

It is well known that NFκB can regulate various downstream pathways and thus has the potential to be both pro- and antiapoptotic (Fan et al., 1999). Currently it is not clear whether the beneficial effects of NFκB activation in protection against apoptosis or its detrimental proinflammatory role predominate in liver I/R (Fan et al., 1999). Hepatic neutrophil recruitment and hepatocellular injury are significantly reduced when NFκB activation is suppressed in mice following partial hepatic I/R (Casillas et al., 2006). However, nuclear factor kappa B (NFκB) activation is essential for hepatic regeneration after rat LT, and reduces apoptosis and hepatic I/R injury (Bradham et al., 1999). To understand the role of NFκB in the context of hepatic I/R, is important to consider the differences in animal species used, for instance, mechanisms of protection from apoptosis might be different in rats and mice (Chaisson et al., 2002). In addition, the experimental design used to evaluate the role of this transcription factor may also be important. Thus, some studies using adenoviral vector containing a repressor to prevent NFκB activation may not accurately reflect the role of NFκB signalling in regenerating liver because adenoviral vectors themselves cause increased TNF- $\alpha$  levels, DNA synthesis, and apoptosis in the liver before partial hepatectomy (Iimuro et al., 1998). Moreover, to explain these apparently controversial effects of NFκB, the pattern of NFκB activation under cold ischemia conditions should be taken into account. Takahashi *et al.*, 2002, have demonstrated in rat LT that NFκB activation during reperfusion occurs in two phases. The early peak of NFκB DNA binding was found 1-3 h after reperfusion and represents the nuclear translocation of NFκB p50/p65 heterodimers, whereas the second

peak, mainly composed of p50 homodimers, was observed at 12 h post-reperfusion. In this study, the donor liver treatment with adenovirus encoding the I $\kappa$ B super-repressor gene cannot affect the early peak of NF $\kappa$ B activation, but partially inhibited the second peak of NF $\kappa$ B DNA binding. The results indicated that, in contrast to early NF $\kappa$ B activation, inhibition of the late phase of NF $\kappa$ B activation was not associated with variations in levels of inflammatory mediators, but rather enhanced hepatocellular apoptosis (Takahashi et al., 2002), which reinforces the dual function of NF $\kappa$ B in transplanted liver. Nevertheless, this hypothesis does not fully explain the differences in the results. Indeed, Bradham *et al.*, 1999, observed a marked increase in apoptosis when NF $\kappa$ B blockade was carried out at 3 h of reperfusion, which seems to be a reperfusion time associated with the early peak of activation of NF $\kappa$ B. Of course, there are differences between Takahashi's and Bradham's studies. For example, whereas Bradham infused the adenoviral vector by endovenous injection 24 h before liver explantation, in Takahashi's study the graft was perfused with UW solution containing the adenovirus immediately before cold storage.

### 3.2.3 Neutrophil accumulation

Activation of neutrophils has been implicated in the hepatic microvascular dysfunction and parenchymal damage associated with I/R (Cutrin et al., 2002). Still, a controversial topic is the question of how neutrophils actually accumulate in the liver. The classical theory argues that the increased expression of adhesion molecules such as ICAM-1 and P-selectin plays a key role in neutrophil accumulation and the subsequent liver damage associated with I/R (Banga et al., 2005, Cutrin et al., 2002). In contrast, it has also been reported that neutrophil accumulation observed in the liver following I/R is not dependent on the up-regulation of either ICAM-1 or P-selectin (Peralta et al., 2001b).

To explain the results that neutrophil accumulation is not dependent on adhesion molecules, we subscribe to the theory proposed by Jaeschke, 2003. This theory argues that although P-selectin and ICAM-1 appear to be relevant for neutrophil adherence in postsinusoidal venules, the neutrophils relevant for the injury accumulate in sinusoids, which were identified as the dominant sites for neutrophil extravasation. In these capillaries, neutrophil sequestration does not depend on B2 integrins or on ICAM-1 or selectins (Essani et al., 1998; Vollmar et al., 1995; Jaeschke et al., 1996). Thus, mechanical factors such as active vasoconstriction, vascular lining cell swelling and injury, and reduced membrane flexibility after activation of the neutrophil, appear to be involved in trapping of these leukocytes in sinusoids (Jaeschke et al., 1996). The extensive vascular injury during reperfusion eliminates, in part, the sinusoidal endothelial cell barrier and the neutrophil has direct access to hepatocytes (Jaeschke, 2003; McKeown et al., 1988). Nevertheless, even with damaged but still present EC, transmigration may still be required (Jaeschke, 1998). As a consequence, I/R injury is only moderately or not at all attenuated by anti-ICAM therapies (Farhood et al., 1995; Vollmar et al., 1995). In regard with the role of P-selectin, sinusoidal EC neither contain Weibel Palade bodies nor do they transcriptionally upregulate relevant levels of P-selectin (Essani et al., 1998). However, during I/R, a number of interventions directed against selectins reduced hepatic neutrophil accumulation and cell injury (Amersi et al., 2001). Because these findings cannot be explained by the prevention of P-selectin-dependent rolling in sinusoids, it has been suggested that most liver I/R models include some degree of intestinal ischemia, which leads to neutrophil accumulation in remote organs including the liver (Casillas et al., 2006; Kubes et al., 2002). Thus the lower number of neutrophils in



the liver when selectins are blocked may be a secondary effect due to the protection of antiselectin therapy against intestinal reperfusion injury (Kubes et al., 2002).

### 3.3 Cell death in liver transplantation

The severity of hepatocyte damage depends on the length of time the ischemia lasts. In human LT, a long ischemic period is a predicting factor for post-transplantation graft dysfunction, and some transplantation groups hesitate to transplant liver grafts preserved for more than 10 h (Fernández et al., 2002). Some studies in experimental models of LT indicate that 24 h of cold ischemia induces low survival at 24 h after LT. However, at shorter ischemic periods, LT may also result in primary organ dysfunction. The main victims of ischemic injury are the hepatocytes and SECs. These two cell types show different responses to different types of ischemia: hepatocytes are more sensitive to warm ischemia and SECs to cold ischemia (Bilzer & Gerber, 2000; McKeown et al., 1988). Although most hepatocytes remain viable after 48 h of cold preservation and reperfusion, SECs suffer severe damage following reperfusion (40% non-viable) (Caldwell et al., 1989). The result of this sinusoidal damage is the subsequent microcirculatory abnormalities upon reperfusion, resulting in hepatocyte injury and dysfunction (McKeown et al., 1988). This contributes to the development of primary nonfunction or impaired primary function after LT. However, some studies have called the importance of sinusoidal injury into question. Huet et al., 2004, have demonstrated that damage to the extracellular matrix from prolonged preservation and reperfusion appears to be the critical factor in graft failure (Banga et al., 2005). In addition, it is possible that perturbations in hepatocyte levels of adenine nucleotides during cold storage can trigger proteolytic events that contribute to damage in the liver graft and subsequently compromise hepatic functions after LT (Kukan & Haddad, 2001). Moreover, cold ischemia profoundly disturb several key hepatocellular functions, such as volume and pH homeostasis, as well as solute transport and drug metabolism, protein synthesis and mitochondrial function. This contributes to preservation injury of the liver graft. Therefore, these observations indicate that aside from reducing EC damage, LT therapy may benefit from strategies aimed at improving the maintenance of appropriate hepatocyte functions (Kukan & Haddad, 2001; Vajdova et al., 2002).

Apoptosis has been regarded as the fate of cells experiencing I/R injury (Sasaki et al., 1996). In this line, different studies have demonstrated apoptotic death in hepatocytes and/or SECs after both cold and warm ischemia of the rat liver (Gao et al., 1998; Kohli et al., 1999). All of the aforementioned studies (Gao et al., 1998; Kohli et al., 1999; Sasaki et al., 1996) used TdT-mediated dUTP-biotin nick and labelling (TUNEL staining) for DNA ladders to demonstrate apoptosis. However, the ability of TUNEL staining to distinguish between apoptosis and necrosis has been called into question. The activation of caspases has also been used to demonstrate apoptosis in rat SECs following cold I/R (Natori et al., 1999). Indeed, use of pan-caspase inhibitors protected rat liver SECs and hepatocytes against I/R injury after prolonged periods of both cold and warm ischemia. On the other hand, other groups oppose the view that the majority of cells undergo apoptosis in response to either warm or cold I/R injury, believing that necrosis is the principle form of cell death (Massip-Salcedo et al., 2007). They believe that in a number of studies the proportion of cells undergoing apoptosis is not of significant magnitude and that the degree of caspase activation does not correlate with the number of SECs and hepatocytes supposedly undergoing apoptosis. Thus, a controversy has emerged over the past years as to whether

necrotic or apoptotic cell death accounts for the severe parenchymal injury observed during hepatic reperfusion. Although it has long been assumed that necrosis and apoptosis are different processes this may not actually be the case. First we will briefly review some basic background information on death cell signalling pathways in hepatocytes in order to understand the shared pathway that leads to both necrosis and apoptosis.

Apoptosis occurs through two main pathways. The first, referred to as the intrinsic (mitochondrial) pathway, is typically activated by a variety of stressors such as DNA damage, p53 activation, growth factor deprivation, and metabolic disturbances (Malhi et al., 2006). The second is the extrinsic pathway that is triggered through death receptors (Malhi et al., 2006). It is well known that one of the most important regulators of intrinsic pathway is the Bcl-2 family of proteins. The Bcl-2 family includes proapoptotic members such as Bax, Bak, Bad, Bid and antiapoptotic members such as Bcl-2, Bcl-XL and Bcl-W (Ghobrial et al., 2005). Following death signal, proapoptotic proteins undergo posttranslational modifications resulting in their activation and translocation to the mitochondria. Then, the outer mitochondrial membrane becomes permeable, leading to the release of Cyt c, which promotes caspase 9 activation, which then activates caspase 3 and the final stages of apoptosis (Ghobrial et al., 2005). In the extrinsic pathway, a variety of mediators, including tumor TNF- $\alpha$ , Fas ligand, and tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) first bind to their respective death receptors, which cause receptor oligomerization and the association of various adapter proteins, including Fas-associated death domain, TNF- $\alpha$  receptor-associated death domain, and TNF- $\alpha$  receptor-associated factor. Fas-associated death domain and TNF- $\alpha$  receptor-associated death domain promote binding of procaspase 8 and its proteolytic activation to catalytic caspase 8. If sufficient amounts of caspase 8 are generated at the receptor, caspase 8 can directly activate procaspase 3. In hepatocytes the caspase 8 interacts with the intrinsic pathway and cleaves Bid, a BH3 only proapoptotic Bcl2 family member, to a truncated form, tBid. tBid translocates to mitochondria, causing mitochondrial permeabilization and release of mitochondrial effectors of apoptosis, such Cyt c (Yin, 2000) (see Fig. 2).

The mechanisms that induce the release of mitochondrial intermembrane proteins such as Cyt c remain controversial (Jaeschke & Lemasters, 2003). In hepatocytes TNF- $\alpha$  and Fas dependent signalling induce the onset of the mitochondrial permeability transition (MPT), which leads to large-amplitude mitochondrial swelling, rupture of the outer membrane, and release of Cyt c and other proteins from the intermembrane mitochondrial space (Jaeschke & Lemasters, 2003). In some models, tBid interaction with either Bax or Bak, forms channels in the mitochondrial outer membrane that release Cyt c and other proteins from the intermembrane space. If MPT onset occurs in relatively few mitochondria, the organelles become sequestered into autophagosomes for lysosomal digestion, a process that eliminates the damaged and potentially toxic mitochondria (Casillas et al., 2006; Jaeschke & Lemasters, 2003). When the MPT involves more mitochondria, mitochondrial swelling leads to outer membrane rupture and Cyt c release. Provided that ATP is available from glycolysis and still-intact mitochondria, Cyt c activate downstream caspases and other executioner enzymes of apoptosis. When MPT onset is abrupt and involves most mitochondria, ATP becomes profoundly depleted, which blocks caspase activation. Instead, ATP depletion culminates with plasma membrane rupture and the onset of necrotic cell death (Jaeschke & Lemasters, 2003). Hence, the new term "necroptosis" has been coined to describe a process that begins with a common death signal and which culminates in either cell lysis

(necrotic cell death) or programmed cellular resorption (apoptosis), depending on factors such as the decline of cellular ATP levels (see Fig. 2).

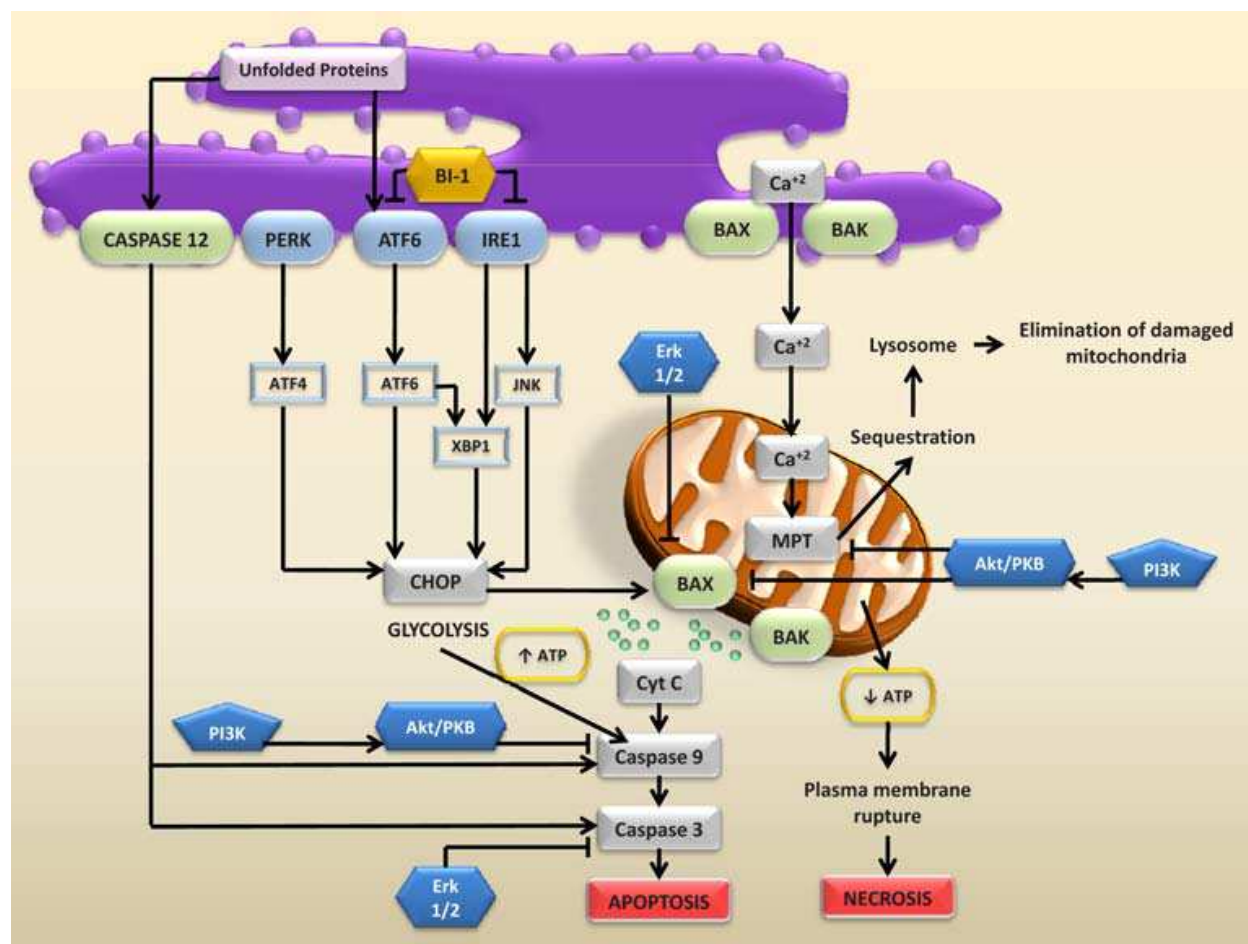


Fig. 2. Scheme of possible cell death pathway in hepatic I/R. (Alfany et al., 2009; Ben Mosbah et al., 2010; Casillas et al., 2006; Fernández et al., 2004; Ghobrial et al., 2005; Jaeschke & Lemasters, 2003; Malhi et al., 2006; Massip-Salcedo et al., 2007; Selzner et al., 2000; Yin, 2000)

#### 4. Steatosis in hepatic ischemia-reperfusion

Several hypotheses have been suggested to explain the decreased tolerance of steatotic liver to I/R injury compared with non-steatotic livers. The impairment of the microcirculation is considered a major event of reperfusion injury in steatotic livers (Ijaz et al., 2003). A reduction in hepatic microcirculation has been observed in human fatty donor livers and in experimental models of hepatic steatosis (Ijaz et al., 2003; Seifalian et al., 1999). An imbalance between vasoconstrictors (e.g., ET1) and vasodilators (e.g., NO) negatively affect the hepatic microcirculation (Massip-Salcedo et al., 2007; Peralta et al., 2000a). In addition, fatty accumulation in the cytoplasm of hepatocytes is associated with an increase in cell volume that reduces the size of the hepatic sinusoid space by 50% compared with a normal liver and may result in partial or complete obstruction of the hepatic sinusoid space (Ijaz et al., 2003; Seifalian et al., 1999). Using Doppler flowmetry, Seifalian et al., 1999 demonstrated reduced sinusoidal perfusion in fatty human liver donors compared with healthy livers.

Analogous studies in rabbits with diet-induced steatosis confirmed that this reduction in perfusion correlated with the severity of fat accumulation in hepatocytes. The reductions in sinusoidal perfusion appear to arise initially from the effects of enlarged hepatic parenchymal cells, swollen with accumulated lipid, which widen the parenchymal cell plates and narrow and distort the lumens of sinusoids. Other investigators have shown that as a result of the structural alterations around them, the sinusoids become inefficient conduits of blood with resulting impairment of tissue perfusion, evidenced by the significant reductions in the numbers of perfused sinusoids per microscopic field (Teoh et al., 2010).

Hepatocyte damage appears remarkably higher in steatotic livers than in non-steatotic livers (Casillas et al., 2006; Selzner et al., 2000). Several evidences indicate that an increased sensitivity of fatty hepatocytes to the injurious effects of ROS could explain the poor tolerance of steatotic livers to I/R (Koneru et al., 2005; Soltys et al., 2001). It has been postulated that steatotic livers are more susceptible than nonsteatotic livers to lipid peroxidation because of either their lower antioxidant defenses or their greater production of ROS or both (Fernández et al., 2004). Mitochondrial ROS generation dramatically increases during reperfusion and mitochondrial structures are exposed to the attack of the ROS generated both outside and inside these organelles leading eventually to the dysfunction of important mitochondrial processes including those responsible for the ATP synthesis. In ROS generation systems, the inhibition of XOD with allopurinol effectively protected against the greater liver and lung damage in transplantation of steatotic livers (Fernández et al., 2004). Higher levels of IL-1 $\beta$  and lower IL-10 levels were observed in steatotic livers compared with non-steatotic livers after I/R. This imbalance between pro- and anti-inflammatory ILs was responsible for the vulnerability of steatotic livers to I/R (Serafin et al., 2004). Previous studies from our group indicated less glutathione (GSH) and SOD levels in steatotic livers than in non-steatotic livers as consequence of hepatic I/R (Fernández et al., 2004; Serafin et al., 2002).

It is well-known that steatotic livers synthesise less ATP than non-steatotic livers during post-ischemic reperfusion (Caraceni et al., 2005). Fatty degeneration induces a series of ultra-structural and biochemical alterations in both human and animal mitochondria. The lower ATP and adenine nucleotide content observed in steatotic livers preserved in UW solution could be caused by mitochondrial damage (Ben Mosbah et al., 2006; Caraceni et al., 2005; Massip-Salcedo et al., 2007). Caraceni et al., 2004 reported that alterations in oxidative phosphorylation during preservation is greatly enhanced by fatty infiltration resulting from damage to respiratory chain complex I and F<sub>0</sub>F<sub>1</sub>-ATP synthase. Others studies have discovered that in steatotic livers under conditions of either warm ischemia or transplantation, the content of mitochondrial uncoupling protein-2 (UCP-2) is four to five times higher than in non-steatotic livers (Chavin et al., 2004; Wan et al., 2008). This finding was associated with reduced ability to synthesize ATP upon reperfusion (Chavin et al., 2004). If cold storage time exceeds 10-12 h, complications in biliary structures occur in more than 25% of liver transplant recipients (Kukan & Haddad, 2001). Several factors, including poor recovery after ATP depletion appear to contribute to bile duct cell damage after liver transplantation. Furthermore, isolated rat bile duct epithelial cells are noticeably sensitive to oxidative stress, possibly because their cellular stores of reduced glutathione are seven times lower than those of hepatocytes (Noack et al., 1993). Taking these observations into account, bile production failure in steatotic livers could be explained, at least partially, by the lower



ATP and increased oxidative stress presented by this type of liver compared with non-steatotic liver.

Toll-like receptor 4 (TLR4) has been implicated as a mediator of steatotic liver damage after I/R (Ellett et al., 2009). The loss of TLR4 in steatotic livers from TLR4-knockout HFD animals reduces pro-inflammatory cytokines and liver injury and improves survival (Ellett et al., 2009). Although TLR4 signaling is relevant in hepatic I/R injury, there is some controversy over which of the pathways [(myeloid differentiation factor 88 (MyD88)-dependent) or Toll/IL-1 receptor domain-containing adaptor inducing interferon- $\beta$  (TRIF/IRF-3 signalling pathway)] is activated in hepatic I/R (Kang et al., 2011). Neutrophils have been involved in the increased vulnerability of steatotic livers to I/R injury, especially in alcoholic steatotic livers. However, neutrophils do not account for the differentially greater injury in the non-alcoholic steatotic liver during the early or late hours of reperfusion. Similarly, the role of TNF- $\alpha$  in the vulnerability of steatotic livers to I/R injury may be dependent on the type of steatosis (Serafin et al., 2002). These observations could be of clinical interest because pharmacological strategies that could be effective in alcoholic fatty livers by reducing the neutrophil infiltration and or TNF- $\alpha$  action may not be sufficient to reduce the hepatic I/R injury in non-alcoholic fatty livers.

Cell death can occur by either necrosis or apoptosis and intracellular ATP level appear to play a role as a putative apoptosis/necrosis switch: when ATP depletion is severe, necrosis ensues before the activation of the energy-requiring apoptotic pathway (Casillas et al., 2006; Massip-Salcedo et al., 2007) (See Fig. 2). In steatotic liver graft undergoing 6 h of cold ischemia, necrosis was the predominant cell death whereas no apoptosis signs were found (Alfany et al., 2009; Fernández et al., 2004). Since apoptosis is an energy-requiring process, the impaired maintenance of ATP levels observed after reperfusion in steatotic livers submitted to long periods of cold ischemia may be linked with a failure to induce apoptosis. Thus, it is not surprising that data reported previously indicate that necrosis rather than apoptosis is the predominant process by which steatotic livers undergo cell death (Alfany et al., 2009; Fernández et al., 2004; Selzner et al., 2000).

Previous studies from our group have indicated that steatotic livers differed from non-steatotic livers in their response to UPR and ER stress. Steatotic livers showed a reduced ability to respond to ER stress as the activation of two UPR arms, IRE1 and PERK, was weaker in the presence of steatosis. (Ben Mosbah et al., 2010). Different hypotheses, including decreased ATP production and dysfunction of regulators of apoptosis, such as Bcl-2, Bcl-xL and Bax have been proposed to explain the failure of apoptosis in steatotic livers. The results on ER stress in steatotic livers undergoing I/R may throw some light on this question. Reduced proapoptotic factors related to ER stress such as caspase 12, C/EBP-homologous protein (CHOP) and Jun N-terminal kinase (JNK) were observed in steatotic livers under conditions of I/R compared with non-steatotic livers. This may be related to the reduced activation of the two UPR arms, inositol-requiring enzyme-1 (IRE1) and PERK, which are responsible for caspase 9 and 12 activation, JNK activation and CHOP induction (Ben Mosbah et al., 2010) (see Fig. 2). We believe that the damaged ER and mitochondria are intimately linked and that mitochondrial cell death and ER-induced cell death cannot be separated in hepatic I/R. Thus, caspase activation and Cyt *c* release from mitochondria consequently to hepatic I/R (Ben Mosbah et al., 2010) can be attributed to ischemic disturbance or damage to the ER. Given these results in steatotic livers under warm

ischemia conditions, it is therefore tempting to speculate that increased ER stress may be involved in the vulnerability of steatotic liver grafts to I/R injury associated with transplantation and in the sensitivity of other marginal grafts to I/R injury, such as liver grafts from aging donors. Indeed, aging donors have an increased incidence of steatosis, which may favor cold preservation injury (Busuttil & Tanaka, 2003; Massip-Salcedo et al., 2007). Alterations in the activation of inflammatory transcription factors and expression of cytoprotective proteins, increased intracellular oxidants and decreased mitochondrial function and protein misfolding accumulation, and aggregation also characterize many age-related diseases (Massip-Salcedo et al., 2007; Pallet et al., 2009).

## **5. Strategies designed in past years to prevent hepatic I/R injury**

Despite improvements in pharmacological treatments, preservation solutions and gene therapy aimed at reducing hepatic I/R injury, the results to date have not been conclusive. Figure 3 shows some of the therapeutic strategies developed to prevent I/R injury in LT. Possible reasons for the failure of these strategies in clinical applications are now discussed.

### **5.1 Pharmacological treatment**

Numerous experimental studies have focused on inhibiting the harmful effects of I/R-associated inflammatory response. In this respect, drugs such as chloroquine and chlorpromazine have been administered in order to prevent mitochondrial dysfunction and loss of liver cell phospholipids during hepatic ischemia. Antioxidant therapy using either tocopherol, GSH ester, or allopurinol has been applied in an attempt to inhibit ROS effects in reperfusion, and anti-TNF antiserum pre-treatment has also been employed to block the damaging effects of this cytokine. Therapies with dopamine or ATP-MgCl<sub>2</sub> have been administered to reduce hepatic I/R injury-related microcirculatory disorders. Drugs such as adenosine, NO donors, L-arginine, and anti-ICAM-1 and anti-P-selectin antibodies have been used to inhibit neutrophil accumulation. However, none of these treatments has managed to prevent hepatic I/R injury. The possible side effects of the some drugs may frequently limit their use in human LT (Casillas et al., 2006). For example, idiosyncratic liver injury in humans is documented for chlorpromazine, pernicious systemic effects have been described for nitric oxide (NO) donors, allopurinol therapy can cause haematological changes and gadolinium can induce coagulation disorders (Casillas et al., 2006).

Hepatic failures have been observed after administration of these two thiazolidinediones (TZDs) and some case reports of acute hepatotoxicity attributed to rosiglitazone have been published, including one death (Reynaert et al., 2005). The toxicity of TZDs is thought to be mainly metabolic idiosyncratic, although in some cases possible immunological mechanism has been implicated (Reynaert et al., 2005). High dose resveratrol was found to be a pro-oxidant with aggravation of liver injury; and experiments are in progress to devise a pharmaceutical form appropriate for clinical use (Hassan et al., 2008). The development of therapeutic strategies that utilize the protective effect of Heme oxygenase-1 (HO-1) induction is hampered by the fact that most pharmacological inducers of this enzyme perturb organ function by themselves and that gene therapy for up-regulation of HO-1 has potential negative side effects, which currently preclude its clinical application under these conditions (Schmidt, 2010) (see Fig. 3).

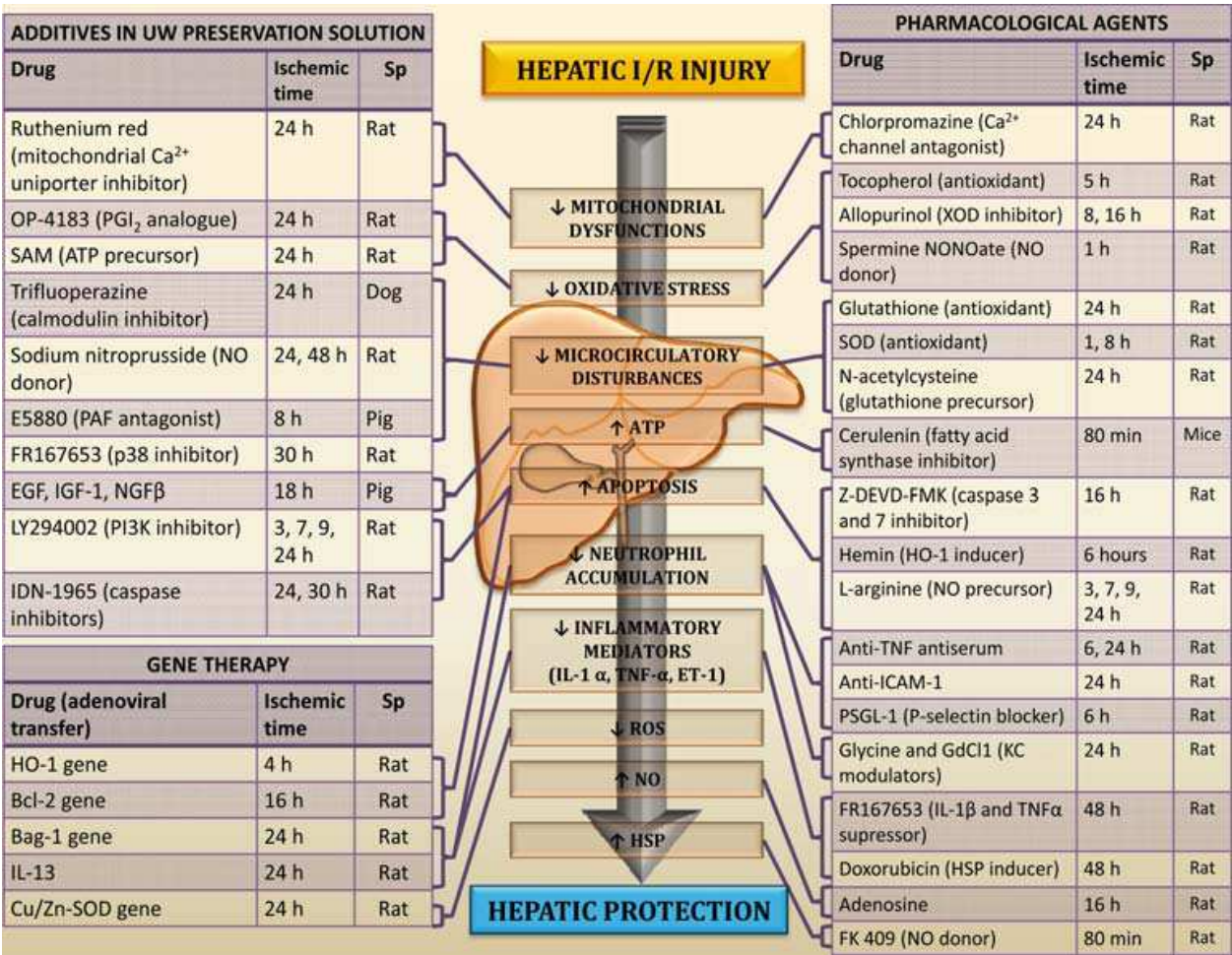


Fig. 3. Strategies used to prevent hepatic I/R injury. (Sp, species). (Carini et al., 2004; Carrasco et al., 2005; Casillas et al., 2006; Chavin et al., 2004; Cheng et al., 2003; Esfandiari et al., 2007; Fan et al., 1999; Hassan et al., 2008; Massip-Salcedo et al., 2006; Nakano et al., 2007; Natori et al., 1999; Peralta et al., 2001a, 2001b; Polyak et al., 2000; Reynaert et al., 2005; Schmidt, 2010; Selzner et al., 2000, 2003; Teoh et al., 2003; Vajdova et al., 2002; Yoshinari et al., 2001)

The difficulty of blocking the inflammation related to this process must be taken into account because, among other factors, many mediators and cell types are involved in this kind of inflammatory response. Pharmacological treatment-derived difficulties must also be considered. In this regard, superoxide dismutase (SOD) and glutathione show inadequate delivery to intracellular sites of ROS action (Polyak et al., 2000). The administration of anti-TNF antibodies does not effectively protect against hepatic I/R injury, and this finding has been related to the failure of complete TNF-α neutralization locally (Peralta et al., 2001b). Additionally, special attention should be given to drugs that suppress TNF-α, because its potential dual effects (Teoh et al., 2003). Small changes in the dose of NO donors produce totally opposite effects (Peralta et al., 2001a). Although this also occurs in non-steatotic livers, modulating I/R injury in steatotic livers poses a greater problem. Until now, data about the effectiveness of the administration of antioxidants on the deleterious effects of ROS in steatotic livers was controversial. Some studies in obese Zucker rats, a well-characterized model of nutritionally induced obesity, indicated that the administration of



tocopherol, which possesses antioxidant properties, improved tolerance to warm ischemia. However, other experimental studies in steatotic livers, induced by a choline-methionine-deficient diet, show that the administration of GSH precursors, such as N-acetylcysteine, could help to restore hepatocellular integrity in the steatotic liver but without scavenging free radical. In addition, both dietary high fat and alcohol exposure produced SOD/catalase-insensitive ROS that may be involved in the mechanism of failure of steatotic livers after orthotopic LT (Casillas et al., 2006; Massip-Salcedo et al., 2007; Serafin et al., 2002; Soltys et al., 2001).

Differences in the action mechanisms between steatotic and non-steatotic livers mean that therapies which are effective in non-steatotic livers may prove useless in the presence of steatosis, and the effective drug dose may differ between the two liver types. Findings such as these must be taken into consideration when applying pharmacological strategies in the same way to steatotic and non-steatotic livers, because the effects may be very different. Apoptosis was the predominant form of hepatocyte death in the ischemic non-steatotic liver, whereas the steatotic livers developed massive necrosis after an ischemic insult. Thus, caspase inhibition, a highly protective strategy in non-steatotic livers, had no effect on hepatocyte injury in steatotic livers (Selzner et al., 2000). For instance, whereas in an LT experimental model a NO donor reduced oxidative stress in non-steatotic livers, the same dose increased vulnerability of steatotic grafts to I/R injury (Carrasco et al., 2005). The injurious effects of exogenous NO donors on hepatic injury and oxidative stress in steatotic grafts could be explained by peroxynitrite generation caused by ROS overproduction (Carrasco et al., 2005). HO-1 activators such as cobalt (III) protoporphyrin IX, might protect both liver types against warm I/R injury. However, a lower dose of HO-1 activator was required to protect steatotic livers effectively, as steatotic livers undergoing I/R showed higher HO-1 levels than nonsteatotic livers (Massip-Salcedo et al., 2006). Furthermore, there may be drugs that would only be effective in steatotic livers. In the context of LT, steatotic donors have been reported to show a higher content of mitochondrial uncoupling protein-2 (UCP-2) and a reduced ability to synthesize ATP upon reperfusion, thus leading to increased mortality following I/R (Cheng et al., 2003). Studies by Chavin *et al* have discovered that in ob/ob mice (approximately 70%-80% of liver lipid content) expression of UCP-2 is four to five times higher than in normal liver tissues (Chavin et al., 1999; Wan et al., 2008). Hence, compounds such as cerulenin that reduce UCP-2 expression in steatotic livers, offer protection as a result of increased availability of ATP prior to I /R (Chavin et al., 2004). However, this strategy may be ineffective in non-steatotic livers because the latter do not show an overexpression of UCP-2 (Chavin et al., 1999). Similar results have been obtained with carnitine administration (Tolba et al., 2003; Yonezawa et al., 2005).

All the aforementioned results point up the fact that the different mechanisms of cell death in steatotic vs. non-steatotic livers as well the differences in the mechanisms involved in hepatic I/R injury in terms of the type of steatosis could explain the difficulties in effectively preventing steatotic livers from I/R injury. Further investigations are required to optimize some treatments because long-term therapy appears to be necessary to exert the desired effects. For example, the pre-treatment times for rosiglitazone was between 6 to 12 weeks (Nakano et al., 2007); and, S-adenosylmethionine (SAM) between 14 and 17 weeks (Esfandiari et al., 2007). Similarly, long-term IL-6 treatment (10 days) reduced hepatic steatosis and markedly prevents I/R-induced liver injury in ob/ob mice and mice fed high-



fat diets (Hong et al., 2004). However, there are obvious difficulties concerning the feasibility of long-term drug administration in some I/R processes, in particular, liver transplantation from cadaveric donors, because this is an emergency procedure in which there is very little time to pre-treat the donor with drugs.

## 5.2 Preservation solutions

Since its introduction by Belzer et al. in the late eighties, the University of Wisconsin (UW) solution has become the standard solution for the preservation of most organs in transplantation. The inclusion of some components in the UW solution has been both advocated and criticised. For instance, adenosine has been added to UW solution as a substrate for the regeneration of adenine nucleotides. However, simplified variants of UW solution in which adenosine was omitted were shown to have similar or even higher protective potential during cold liver storage. The colloid hydroxyethyl starch (HES) included in UW preservation solution prevents interstitial edema but produces extended and accelerated aggregation of erythrocytes that may result in stasis of blood and incomplete washout of donor organs before transplantation. Another limitation of the UW solution is that some of its constituent compounds (allopurinol, lactobionate) do not offer very good protection because they are not present at a suitable concentration and encounter problems in reaching their site of action. Indeed, studies in humans have suggested that the allopurinol in the UW preservation solution was unable to prevent the subsequent XDH/XOD-derived superoxide radical production during reperfusion (Casillas et al., 2006; Pesonen et al., 1998).

A variety of ingredients such as stable protacyclin (PGI<sub>2</sub>) analogue OP-4183, p38 mitogen-activated protein kinase (MAPK) inhibitor FR167653, NO donor sodium nitroprusside, platelet-activating factor (PAF) antagonist E5880, calmodulin inhibitors, Ca<sup>2+</sup> channel blockers such as nisoldipine, trophic factors, caspase or calpain inhibitors, S-adenosylmethionine (SAM), insuline, or fructose-1,6-biphosphate (FBP) were introduced into UW preservation solution, with promising results (Casillas et al., 2006). However, none of these modifications to UW solution composition have found their way into routine clinical practice. For instance, studies aimed at enrichment of UW solution with caspase inhibitors showed that this prevents sinusoidal endothelial cells apoptosis (Vajdova et al., 2002), but it has also been demonstrated that such inhibitors have little effect on necrosis, and this could mean no protection in the steatotic liver where the predominant form of cell death is necrosis (Selzner, 2003). Along this line, addition of precursors for ATP resynthesis such as SAM only resulted in a poor initial ATP recovery during liver reperfusion (Vajdova et al., 2002) (see Fig. 3). Insulin and FBP were recommended and added to UW preservation solution with the aim of stimulating glycolysis and modulating KC activity, respectively. However, further studies showed that these modifications in UW solution may exacerbate graft ischemic injury and decrease the graft survival rate in rat LT.

The failure of UW solution enrichments could be related either to factors intrinsic to the drugs themselves (i.e. toxic side-effects, lack of specificity, etc.) or disagreement in their mechanisms of modulation. For instance, LY294002 was added to UW in order to maintain calcium homeostasis through the inhibition of phosphatidylinositol-3-OH kinase (PI3K) activity (see Fig. 3). Despite LY294002 reduces apoptosis in the grafts, the beneficial effects of the survival pathway activated by PI3K were also suppressed (Carini et al., 2004).

Additives to UW solution might further improve survival rate and graft viability if their concentration could be increased, but this is not always possible. For example, the solubility of FR167653 in UW solution was found to be limited. In addition, these additives are rinsed from the liver graft before implantation, so they should have prolonged action (Yoshinari et al., 2001). For instance, addition of precursors for ATP re-synthesis, such as S-adenosylmethionine, only resulted in a poor ATP recovery during reperfusion, since they can be rescued only partially after liver flush before implantation (Vajdova et al., 2002). Another limitation is that suitable concentrations of additives, such as caspase inhibitor IDN-1965, can be achieved only with prolonged storage of the organ in the presence of the inhibitor (Natori et al., 1999). However, this exacerbates the cold ischemic injury.

Numerous studies have reported equivalent patient and graft survival for deceased donor liver allografts preserved with UW and HTK solutions (Steawart et al., 2009). The reduced viscosity of HTK as compared to UW has been hypothesized to be protective against the development of biliary complications. However, the impact of HTK versus UW preservation on biliary complications remains unclear, as some centers report equivalent, increased or reduced rates of biliary complication with HTK preservation of deceased donor liver allografts (Feng et al., 2007; Steawart et al., 2009).

Clinical studies indicated that HTK preservation was associated with higher odds of early graft loss as compared to UW preservation with a more pronounced effect on allograft with cold ischemia time over 8 h, donor after cardiac death allografts and donors over 70 years (Steawart et al., 2009). As previously reported, HTK is not so efficient for longer periods of cold ischemia causing a higher incidence of delayed graft function (Olschewski et al., 2008; Straatsburg et al., 2002).

### 5.3 Gene therapy

Advances in molecular biology provide new opportunities to reduce liver I/R injury by using gene therapy. To suppress the ROS burst, SOD and catalase have been transfected by either adenovirus, liposomes or polyethyleneglycol (Fan et al., 1999; Selzner, 2003). To inhibit apoptosis, overexpression of Bag-1 and Bcl-2, mainly by using adenovirus, has been tested (Selzner, 2003) (see Fig. 3). To limit neutrophil recruitment and activation, reduction in ICAM-1 expression was obtained by using liposomes. Cytoprotective strategies based on expression of genes such as HO-1, anti-inflammatory cytokine IL-13 and interleukin-1 receptor antagonist (IL-1Ra) have been developed employing adenoviral or liposome vector (Casillas et al., 2006). Attempts have also been made to modulate the NF $\kappa$ B effect through adenoviral transfection of a mutant inhibitor of kappaB-alpha (IkB $\alpha$ ), which would inhibit NF $\kappa$ B and ameliorate the hepatic inflammatory response to I/R (Fan et al., 1999; Casillas et al., 2006) (see Fig. 3). However, there are a number of problems inherent in gene therapy, for example, vector toxicity, difficulties in increasing transfection efficiencies and protein expression at the appropriate time and site, and the problem of obtaining adequate mutants (in the case of NF $\kappa$ B) due to controversy about NF $\kappa$ B activation (Chaisson et al., 2002; Somia & Verma, 2000). Although non-viral vectors (such as naked DNA and liposomes) are likely to present fewer toxic or immunological problems, they suffer from inefficient gene transfer (Somia & Verma, 2000). In addition, LT is an emergency procedure in most cases, which leaves very little time to pre-treat the donor with genetic approaches.

## 6. Directions for the future

New potential strategies that could be promissory in LT are now discussed. The present review will now centre on emerging protective strategies such as enrichments of UW solution and pharmacological treatments with favourable results in I/R injury but that up to now have not been tested in clinical LT. Moreover, we will discuss ischemic preconditioning taking into account the novel clinical reports that suggest the effectiveness of this surgical procedure in LT.

### 6.1 Pharmacological treatments and preservation solutions

#### 6.1.1 Trimetazidine and AICAR

Trimetazidine (TMZ), which has been used as an anti-ischemic drug in the heart for over 35 years (Ikizler et al., 2003) reduced liver injury and improved liver regeneration and survival rate in partial hepatectomy under vascular occlusion (Casillas et al., 2006). TMZ has been used as an additive in UW solution to protect steatotic livers exposed to prolonged cold ischemia in an *ex vivo* model of hepatic ischemia (Ben Mosbah et al., 2006). This could be of interest since irreversible injury has been reported in liver grafts preserved in UW after prolonged cold ischemic periods (between 16 h to 24 h) (Ben Mosbah et al., 2006). Studies examining the underlying protective mechanisms of TMZ suggest that mitochondria, energy metabolism, oxidative stress and microcirculation might be important targets through which TMZ exerts its cytoprotective effect (Ben Mosbah et al., 2006; Ikizler et al., 2003). Interestingly, these mechanisms are responsible for the vulnerability of steatotic livers to I/R. Similarly to the benefits of TMZ, the addition of AMPK activators to UW solutions such as 5-amino-4-imidazole carboxamide riboside (AICAR), protected steatotic livers against their vulnerability to I/R. TMZ, by means of AMPK, increased NO, thus protecting steatotic livers against their vulnerability to I/R injury (Ben Mosbah et al., 2006, 2007; Carrasco et al., 2005). Taking these observations into account, TMZ and AICAR may constitute new additives to UW solution in steatotic liver preservation, whereas a combination of both seems unnecessary.

#### 6.1.2 Modulators of renin-angiotensin system

Previous researches have observed an important role for the renin-angiotensin system (RAS), known for its regulation of blood pressure and fluid homeostasis, in both I/R injury and liver regeneration after partial hepatectomy (Ramalho et al., 2002; 2009). Furthermore, angiotensin-converting enzyme (ACE) inhibitors (captopril and enalapril) and angiotensin II (Ang-II) type 1 receptor blockers (losartan and candesartan) reduced inflammatory response associated with I/R injury (Araya et al., 2002). In addition, ACE inhibitors (lisinopril, captopril and enalaprilat) promoted liver regeneration after partial hepatectomy (Ramalho et al., 2002). Candesartan, a potent and long-lasting Ang-II type 1 receptor antagonist, up-regulated the hepatocyte growth factor (HGF), the most potent mitogen for mature hepatocytes (Araya et al., 2002). Steatotic livers against I/R. In conditions of partial hepatectomy under I/R, Angiotensin receptors (AT1R and AT2R) antagonists for steatotic livers improved regeneration in the remnant liver. AT1R antagonist, through NO inhibition, protected steatotic livers against oxidative stress and damage. The combination of AT1R and AT2R antagonists in steatotic livers showed stronger liver regeneration than either

antagonist used separately and also provided the same protection against damage as that afforded by AT1R antagonist alone. These results could be of clinical interest in liver surgery (Ramalho et al., 2009). BK seems to be a key mediator in the benefits of all the blockers of Ang II activity (ACE inhibitors, AT1R antagonists, and AT2R antagonists) in steatotic livers undergoing I/R (Casillas et al., 2008). In liver transplantation, Ang II is an appropriate therapeutic target only in non-steatotic livers. It was observed an upregulation of ACE2 in steatotic liver grafts, which was associated with decreased Ang II and high Ang-(1-7) levels. Ang-(1-7) receptor antagonist reduced necrotic cell death and increased survival in recipients transplanted with steatotic liver grafts. These results indicate a novel target for therapeutic interventions in liver transplantation within the RAS cascade, based on Ang- (1-7), which could be specific for this type of liver (Alfany et al., 2009). Further studies will be required to elucidate whether these strategies based on regulating RAS can be useful in hepatic I/R injury. ACE inhibitors are widely used in clinical practice. However, hepatotoxicity and cholestatic liver diseases have been reported under ACE inhibition (Casillas et al., 2008). Previous studies have indicated that losartan is as effective as captopril in its cardiovascular effects but has fewer adverse effects (Zhu et al., 2000). Thus, AT1R antagonists may be a safer protective pharmacologic strategy than ACE inhibitors for hepatic I/R injury.

### **6.1.3 Modulators of activating pro-survival kinase cascades, PI3K-Akt and Erk 1/2 pathway**

Trophic factors such as insulin-like growth factor (IGF), EGF, cardiotrophin-1 and fibroblast growth factor (FGF) have been shown to protect against I/R injury through the activation of phosphatidylinositol-3-OH kinase (PI3K)-Akt and p42/p44 extra-cellular signal-regulated kinases (Erk 1/2). This pathway has been implicated in cellular survival, through recruitment of anti-apoptotic protection pathways. PI3K-Akt has been shown to increase NO, inhibit opening of the MPT pore, and activate protein kinase C (PKC) and mitochondrial Raf-1, which has been shown to phosphorylate and inactivate the pro-apoptotic factor, Bad. Activation of either the PI3K-Akt or the Erk 1/2 pathway inhibits the conformational change in Bax required for its translocation to the mitochondria. Moreover Erk 1/2 kinase activation has been shown to inhibit apoptosis, by inhibiting caspase 3 activation and Akt activation can suppress the mitochondrial apoptotic death pathway by inactivating caspase 9. Interestingly, PI3K-Akt is a cell signalling mechanism also involved in the benefits of liver ischemic preconditioning in isolated hepatocytes. The modulation of therapeutic targets such as the anti-apoptotic pro-survival PI3K-Akt and Erk 1/2 kinase cascades could open new perspectives for limiting I/R injury associated with LT (Casillas et al., 2006).

Cardiotrophin-1 (CT-1) and alpha-lipoic acid (LA) could be promising drugs against I/R injury associated with LT because their benefits on pro-survival kinase cascades. The pretreatment of isolated hepatocytes with the pro-apoptotic mediator transforming growth factor-beta stimulates CT-1 production. In addition, pretreatment with CT-1 protects rats against fulminant liver failure after subtotal hepatectomy. This protective effect was associated with reduced caspase-3 activity and activation of Erk1/2 and PI3K/Akt pathways (Bustos et al., 2003). Recent research points to the potential of preconditioning with LA for hepatic IRI, which is mediated via the PI3K/Akt pathway. However, neither



Bad nor eNOS phosphorylation was increased after LA pretreatment, suggesting a new mechanism by which LA exerts antinecrotic but not antiapoptotic action during hepatic I/R (Muller et al., 2003). This could be of special interest to protect steatotic liver grafts, given that necrosis rather than apoptosis is the predominant type of cell death in such cases.

The results, based on isolated perfused liver, indicated that the addition of EGF and IGF-I (separately or in combination) to UW reduced hepatic injury and improved function in both liver types. A combination of EGF and IGF-I resulted in hepatic injury and function parameters in both liver types similar to those obtained by EGF and IGF-I separately. EGF increased IGF-I, and both additives up-regulated AKT in both liver types. This was associated with glycogen synthase kinase-3 $\beta$  (GSK3 $\beta$ ) inhibition in non-steatotic livers and peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ ) over-expression in steatotic livers. The benefits of EGF and IGF-I as additives in UW solution were also clearly seen in the LT model, because the presence of EGF and IGF-I (separately or in combination) in UW solution reduced hepatic injury and improved survival in recipients who underwent transplantation with steatotic and nonsteatotic liver grafts. Thus, EGF and IGF-I may constitute new additives to UW solution in steatotic and nonsteatotic liver preservation, whereas a combination of both seems unnecessary (Zaouali et al., 2010).

## 6.2 Antiapoptotic strategies

An interesting research in hepatic warm ischemia by Bailly-Maitre et al. has pointed to BAX inhibitor-1 (BI-1) as a regulator of the endoplasmic reticulum (ER) stress-mediated apoptosis pathway (Bailly et al., 2006). The results could lead to new strategies for reducing I/R injury associated with LT. Some mechanisms of ER stress-mediated apoptosis are briefly described below. During liver ischemia, hypoxia-induced ATP deficiency promotes the release of Ca<sup>2+</sup> from ER to cytosol. The depletion of ER Ca<sup>2+</sup> stores triggers downstream ER stress pathways that induce apoptosis. The pro-apoptotic Bcl-2 family members BAX and BAK, localized to the ER, also induce emptying of ER Ca<sup>2+</sup> pools concomitantly with Ca<sup>2+</sup> translocation into the mitochondria (Breckenridge et al., 2003). In addition, I/R initiates protein misfolding in the ER, which can activate a highly conserved unfolded protein response (UPR) signal transduction pathway. The UPR is characterized by coordinated activation of three ER transmembrane proteins, IRE1, PKR-like ER kinase (PERK) and activating transcription factor (ATF)-6. If the damage is so severe that homeostasis cannot be restored, ER stress signal transduction pathways ultimately initiate apoptosis (Oyadomari & Mori, 2004; Xu et al., 2005). The study by Bailly-Maitre indicated that the ER membrane protein BI-1 protects against apoptosis induced by ER stress. Compared to wild-type BI-1 mice, BI-1 knockout mice subjected to hepatic ischemia/reperfusion exhibited greater elevation in caspase-9 activity, more activation of IRE1, ATF6 and JNK, and greater increases in expression of CHOP and spliced X-box binding protein 1 (XBP-1) (Bailly et al., 2006). Thus, strategies aimed at modulating BI-1 as well as other component of ER stress-mediated apoptosis could protect not only against ER stress but also against the mitochondrial-dependent apoptosis pathway. In liver, the small molecule chemical chaperones, 4-PBA and Tauroursodeoxycholic acid (TUDCA) protect against I/R-induced ER stress-mediated cell death in non-steatotic livers undergoing ischemic conditions (Falasca et al., 2001; Vilatoba et al., 2005). 4-PBA reduced inflammatory response, apoptosis and mortality in non-steatotic livers undergoing total hepatic ischemia (Vilatoba et al., 2005). The addition of TUDCA to UW preservation solution protected non-steatotic livers, specifically sinusoidal lining cells and hepatocytes

against cold ischemia injury (Falasca et al., 2001). Recent studies indicated that PBA, and especially TUDCA, reduced inflammation, apoptosis and necrosis, and improved liver regeneration in both steatotic and non-steatotic livers in partial hepatectomy under vascular occlusion. Both compounds, especially TUDCA, protected both liver types against ER damage, as they reduced the activation of two of the three pathways of UPR (namely inositol-requiring enzyme and PKR-like ER kinase) and their target molecules caspase 12, c-Jun N-terminal kinase and C/EBP homologous protein-10. Only TUDCA, possibly mediated by extracellular signal-regulated kinase upregulation, inactivated glycogen synthase kinase-3 $\beta$ . This in turn, inactivated mitochondrial voltage-dependent anion channel, reduced Cyt c release from the mitochondria and caspase 9 activation and protected both liver types against mitochondrial damage (Ben Mosbah et al., 2010). Also, strategies aimed at modulating component of ER stress-mediated cell death could protect not only against ER stress but also against the mitochondrial-dependent apoptosis pathway. A recent study indicated that TUDCA reduced ER stress in steatotic liver transplantation. Further studies will be required to elucidate whether these chemical chaperones such as 4-PBA and TUDCA could be considered as useful strategies in clinical LT. They have been used for clinical treatment of urea cycle disorders, cholestatic liver diseases and cirrhosis (Ben Mosbah et al., 2010). Results of clinical trials have shown that 4-PBA has few side effects and is safe for patients since it is well tolerated at high dose for long periods of time (Özcan et al., 2006). TUDCA is a derivate of an endogenous bile acid, and it has been safely used as a hepatoprotective agent in humans with cholestatic liver diseases (Falasca et al., 2001).

Recently, autophagy has been described to be activated in stress conditions to ensure cell survival by limiting necrosis or apoptosis *in vivo*. Autophagy is a catabolic pathway triggered following various stress conditions, such as starvation or transient hypoxia, and aimed to restore adequate intracellular ATP and aminoacids levels and to eliminate damaged organelles (Degli et al., 2011). Autophagy has been shown to retard cell death by suppressing ER stress. Thus, the possibility that activation of autophagy may be involved in ER stress attenuation in steatotic livers, and that the modulation of autophagy and ER stress can have beneficial effects in clinical LT should not be discarded.

### 6.3 Omega-3 PUFAs

Manipulation of the chemical composition of hepatic lipids may evolve as a useful strategy to expand the donor pool and improve the outcome after LT. Macrosteatotic livers disclosed an abnormal omega-6: omega-3 PUFA ratio that correlates with a microcirculatory defect that enhanced reperfusion injury (El-Badry et al., 2007). Therefore, normalization of the  $\Omega$ -6: $\Omega$ -3 FA ratio appears to be crucial for protection of the steatotic liver from reperfusion injury. Preoperative dietary omega-3 PUFAs protect macrosteatotic livers against reperfusion injury and might represent a valuable method to expand the live liver donor pool (El-Badry et al., 2007). Clavien *et al.*, treated three live liver donors with moderate degrees of steatosis by oral administration of X-3 FAs. All donors showed a significant reduction of hepatic fatty infiltration within one month. Subsequently, LT was carried out for three candidates with uneventful outcomes for both donors and recipients. A very promising option to prevent post-transplant complications appears to be the use of a pretreatment with X- 3 FAs. However, the approach is only feasible in living donation since requires oral administration of X-3 FAs before organ procurement (McCormack et al., 2011).

Due to large inconsistencies in the qualitative and quantitative measurement of fat deposits in the liver, new techniques of assessment of steatosis are needed. Computerized programs have been developed to more objectively quantitate hepatic steatosis by determining the area occupied by lipid droplets in a given field of a liver section (El-Badry et al., 2009). However, these quantitative methods provide information only on the total amount of fat, omitting any data on the chemical composition of hepatic lipids. Therefore, novel and objective tools, such as measurement of the X-6 and X-3 FAs and prostanoid levels in liver biopsy samples, may help prediction of the magnitude of reperfusion injury (McCormack et al., 2011).

#### **6.4 Adipocytokines derived from liver and/or adipose tissue**

To date, adipose tissue has been considered the major site for endogenous adiponectin production, although there are other potential sources, including the liver (Massip-Salcedo et al., 2008; Neumeier et al., 2006). A recent study indicated that steatotic livers can generate adiponectin as a consequence of I/R (Massip-Salcedo et al., 2008). The role of adiponectin in hepatic I/R injury remains unclear. Adiponectin silent small interfering RNA (siRNA) treatment decreased oxidative stress and hepatic injury in steatotic livers. PPAR- $\alpha$  agonists as well as ischemic preconditioning (PC), through PPAR- $\alpha$ , inhibited mitogen-activated protein kinase expression following I/R. This in turn inhibited the accumulation of adiponectin in steatotic livers and reduced its negative effects on oxidative stress and hepatic injury (Massip-Salcedo et al., 2008). However, another study by Man et al., 2006 in small fatty grafts, adiponectin treatment exerted anti-inflammatory effects that down-regulated TNF- $\alpha$  mRNA and vasoregulatory effects that improved the microcirculation. Adiponectin anti-inflammatory effects also include the activation of cell survival signaling via the phosphorylation of Akt and the stimulation of NO production. Additionally, the studies by Man et al., 2006 showed the anti-obesity and proliferative properties of adiponectin in small fatty transplants. Thus, on the basis of the different results reported to date in hepatic I/R, it is difficult to discern whether we should aim to inhibit adiponectin, or administer adiponectin to protect steatotic livers against cold ischemia associated with transplantation.

Levels of adiponectin are reduced in obese subjects (Bugianesi et al., 2005; Targher et al., 2006; Weyer et al., 2001) and in experimental models of fatty livers, irrespective of the type of steatosis (induced by diet or alcohol) (Rogers et al., 2008; Xu et al., 2003). Indeed, in a cohort of 68 obese individuals, serum levels of adiponectin significantly predicted hepatic steatosis and hepatic damage (Schäffler et al., 2005; Targher et al., 2004). Research aimed at identifying prognostic factors in LT are both necessary and relevant. Further investigations will be required to elucidate whether measurements of adiponectin in serum, a non-invasive tool, might predict the severity of steatosis and liver damage and contribute to the identification of steatotic liver donors with a high risk for transplantation. The decision to implant or reject a steatotic liver is difficult due to the risk of impaired graft function or even failure after implantation. How much fat, and what types of fat, represent a significant risks for primary non-function of the graft remain under debate. The assessment of donor liver fat is a difficult task for the transplant team due to large inconsistencies in the qualitative and quantitative measurement of fat deposits in the liver (El-Badry et al., 2009; McCormack et al., 2011).

Retinol binding protein 4 (RBP4) is an adipokine synthesized by the liver, whose known function is to transport retinol in circulation. However, the role of RBP4 in the liver is largely unknown. A recent study indicated that steatotic liver grafts were found to be more vulnerable to the down-regulation of RBP4 and the over-expression of PPAR $\gamma$ . RBP4 treatment (through AMP-activated protein kinase (AMPK) induction) reduced PPAR $\gamma$  over-expression, thus protecting steatotic liver grafts against I/R injury associated with transplantation. In terms of clinical application, therapies based on RBP4 treatment and PPAR $\gamma$  antagonists might open new avenues for steatotic LT and improve the initial conditions of donor livers with low steatosis that are available for transplantation. (Casillas et al., 2011).

### 6.5 Surgical strategies

The response of hepatocyte to ischemia never ceases to be surprising. In fact, contrary to what might be expected, the induction of consecutive periods of ischemia to the liver does not provoke an additive effect in terms of the hepatocyte lesion. Murry et al. have reported that ischemic PC based on a brief period of ischemia followed by a short interval of reperfusion prior to a prolonged ischemic stress protects against I/R injury (Murry et al., 1986). The molecular basis for PC consists of a sequence of events: in response to the triggers of PC, a signal must be rapidly generated which is then transduced into an intracellular message leading to the amplification of the effector mechanism of protection (Cutrin et al., 2002; Serafin et al., 2004b). As in the pathophysiology of hepatic I/R, in the modulation of hepatic injury induced by IP there is a complex interaction between different cell types.

The present review is focused on some of the proposed mechanisms leading to the development of hepatocyte resistance to I/R injury following hepatic PC (see Fig. 4). Vasoactive substances such as adenosine, NO, bradykinin, etc, have been considered as the major players in triggering preconditioning (Cutrin et al., 2002). In addition to the extracellular mediators, PC involves activation of intracellular messengers such as PKC, AMPK, p38 MAPK, Ik kinase; signal transducer and activator of transcription-3 (STAT3) and transcription factors including NF $\kappa$ B and heat shock transcription factor 1 (HSF1) (Carini & Albano, 2003; Selzner, 2003) (see Fig. 4). The downstream consequences of these pathways could be cytoprotective by abrogation of cell death pathways, stimulating antioxidant and other cellular protective mechanisms including MnSOD and heat shock proteins (HSPs), and by initiating entry into the cell cycle (Cutrin et al., 2002; Selzner, 2003). The benefits of PC on energy metabolism, inflammatory mediators including ROS and TNF, mitochondrial dysfunction, KC activation, and microcirculatory disorders associated with I/R injury have also been described (Casillas et al., 2006; Massip-Salcedo et al., 2007). PC via AMPK activation, reduced the ATP depletion thus attenuating the accumulation of glycolytic intermediates and lactate production during hepatic sustained ischemia (Peralta et al., 2000b). The benefits of PC on oxidative stress could be explained by the induction of antioxidants, such as SOD and HSPs as well as by its effect on XDH/XOD (Carini & Albano, 2003; Casillas et al., 2006; Massip-Salcedo et al., 2007). PC reduced the accumulation of xanthine during ischemia and prevented the conversion of XDH to XOD, thus preventing the deleterious effect of this ROS generating system on liver (Fernández et al., 2002; Serafin et al., 2004b) (see Fig. 4). It is possible that NF $\kappa$ B and p38 MAPK-regulated transcription factors (ATF-2 and MEF2C) might be responsible for inducing the expression of protective



genes, including SOD. HSPs induced by PC might contribute to improve membrane potential and respiratory control in hepatic mitochondria, allowing a faster recovery of ATP on reoxygenation (Carini & Albano, 2003; Massip-Salcedo et al., 2007). The modulation of inflammatory response by hepatic PC has been also reported in different experimental models of warm and cold hepatic ischemia. PC reduces neutrophil accumulation, the generation of different cytokines and interleukins including TNF and IL-1 (Casillas et al., 2006; Cutrin et al., 2002; Massip-Salcedo et al., 2007). The benefits of PC were also observed on hepatic microcirculation by inhibiting the effects of different vasoconstrictor mediators such as ETs, thus ameliorating sinusoidal perfusion and microvascular dysfunction (Peralta et al., 1996; Peralta et al., 1999a). The benefits of PC regulating Ang II and adipocytokines such as adiponectin and RBP4 have been also reported in hepatic I/R. PC, through PPAR $\alpha$  inhibits adiponectin accumulation in steatotic livers and adiponectin-worsening effects on oxidative stress and hepatic injury in hepatic resections (Massip-Salcedo et al., 2008). In liver transplantation PC, which increases RBP4 levels, reduced PPAR $\gamma$  levels and hepatic injury in steatotic livers (Casillas et al., 2011). As ER stress activates an adaptive response to injury, modulating ER stress before transplantation by PC could improve the grafted organ viability (see Fig. 4). Along these lines, it has been proposed that induction of ER chaperones, particularly of BiP, underlies the phenomenon of PC in the heart, in which exposure to a transient episode of brief ischemia provides subsequent protection from a

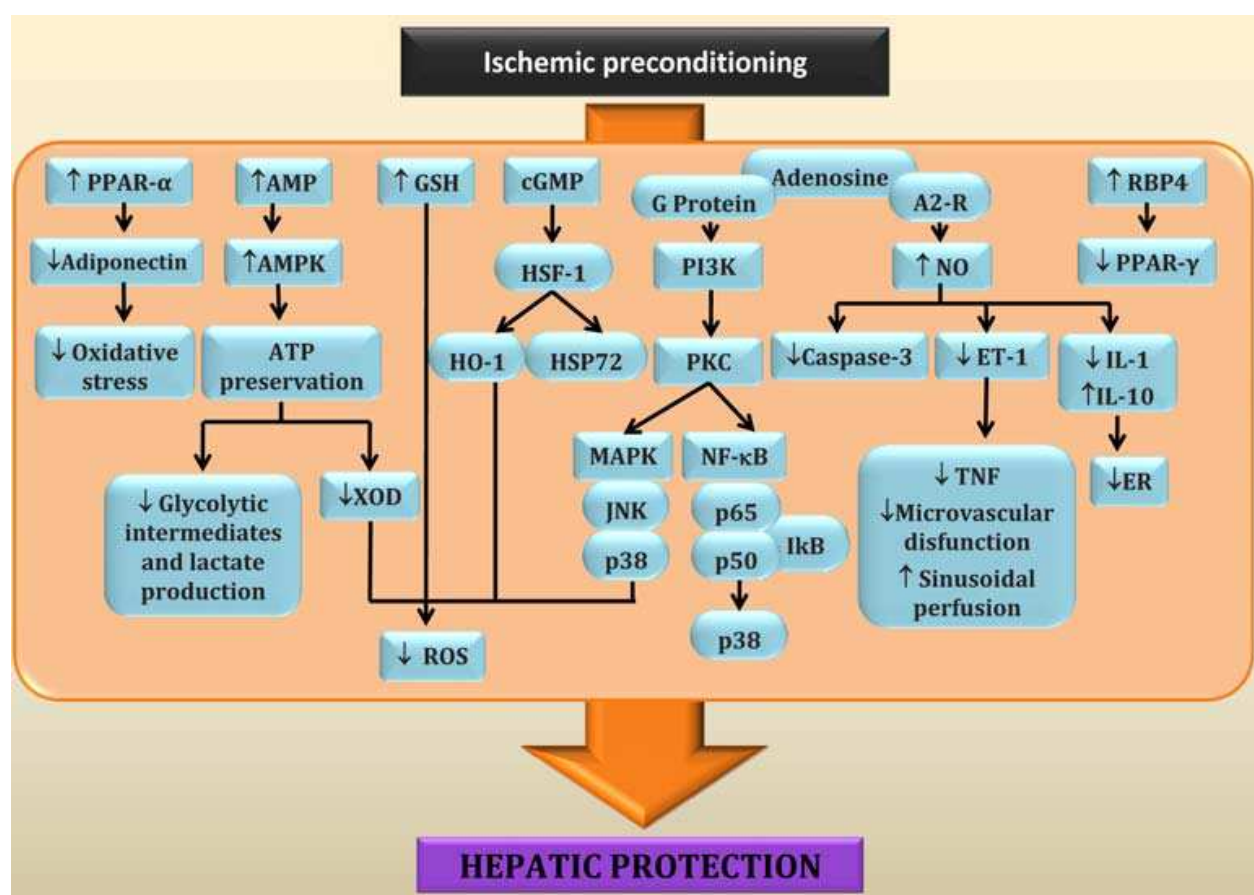


Fig. 4. Molecular basis of the ischemic preconditioning protection. (Carini & Albano, 2003; Casillas et al., 2006; Cutrin et al., 2002; Fernández et al., 2002; Massip-Salcedo et al., 2007, 2008; Peralta et al., 1996, 1999a, 2000b; Selzner, 2003; Serafin et al., 2004b)

sustained ischemic challenge (Kim et al., 2008). It is tempting to speculate that PC activates the UPR, particularly the adaptive and pro-survival aspects of ER stress (Pallet et al., 2009).

Since the effectiveness of PC was first described, numerous efforts have been made to find strategies capable of mimicking its beneficial effects. One of these strategies is known as heat shock preconditioning, in which the organ or the whole body is temporarily exposed to hyperthermia prior to hepatic ischemia. Chemical preconditioning with either doxorubicine, atrial natriuretic peptide or oxidants decreases hepatic injury in several experimental models of I/R. However, their possible clinical application seems limited owing to difficulties in implementing them in clinical practice, toxicity problems and the side-effects that have been identified (Casillas et al., 2006; Massip-Salcedo et al., 2007; Peralta et al., 1999a).

The benefits of PC observed in experimental models of hepatic warm and cold ischemia created the need for human trials of PC. To date, PC has been successfully applied in human liver resections in both steatotic and non-steatotic livers. The effectiveness of PC in hepatic surgery was first reported by Clavien et al., 2003, but unfortunately, in this study, it proved ineffective in elderly patients. It is well known that the impact of cold ischemia on organ function becomes even more significant as the age of the donor increases (Busuttil & Tanaka, 2003). Recent research indicates that melatonin prevents oxidative stress and inflammatory response in hepatocytes from elderly rats and this could improve the viability of liver grafts from elderly donors and increase the effectiveness of PC (Castillo et al., 2005).

Prevention of post-hepatectomy liver insufficiency by PC, particularly in patients with cirrhotic or steatotic livers has also been demonstrated (Nuzzo et al., 2004). A clinical study by Koneru and colleagues showed no effects of PC on cadaveric donor livers compared with controls. However, the study consisted of clamping the hepatic vessels for a period of 5 min, and as the authors concluded, that may be insufficient to obtain a beneficial effect from PC (Koneru et al., 2005). Another clinical study carried out by Azoulay and colleagues using the model of cadaveric whole liver transplantation showed that PC based on 10 min of ischemia was associated with better tolerance to ischemia. However, this was at the price of decreased early function (Azoulay et al., 2005). Beginning this year, Jassem and colleagues concluded that 10 min of preconditioning was effective to protect cadaveric donor allografts from cold ischemia, reduced inflammatory response and resulted in better graft function (Jassem et al., 2006). Further randomised clinical studies are necessary to confirm whether PC is appropriate for LT in clinical practice. The potential applications of PC in human LT are numerous. PC also has the potential to increase the number of organs suitable for LT since it can improve the outcome for marginal grafts that would not otherwise have been transplanted. Its benefits to reduce the vulnerability of steatotic grafts to I/R injury have also been reported in different experimental studies of LT (Carrasco et al., 2005; Fernández et al., 2004). Interestingly, the effectiveness of PC in clinical practice in major liver hepatectomy opens up new possibilities in living donor liver transplantation, since the ischemia period is similar in both surgical procedures. Moreover, PC increases liver regeneration, the most critical aspect to be considered in living donor liver transplantation (Franco et al., 2004). Again, PC may also have a role in the transplantation of small grafts whose pathophysiology overlaps with I/R injury. In fact, a study published by Barrier et al., 2005 has shown the benefits of PC in transplantation from living human liver donors. PC is easy to apply, inexpensive and does not require the use of drugs with potential side effects.

One disadvantage of PC is that it requires a period of pre-ischemic manipulation for organ protection.

## 7. Conclusion and perspectives

The hope of finding new surgical and pharmacological therapeutic applications provides a strong impetus to identify the mechanisms responsible for the failure of fatty livers. We must continue conducting researches attempting to improve the outcomes of LT using fatty liver grafts. Before a complete definition of a successful therapeutic strategy based on regulating hepatic I/R injury is stated, several additional points need to be addressed. The effects of the new potential protective strategies (TMZ, AICAR, RAS modulators, PI3K and ERK1/2 modulators, anti-apoptotic strategies, omega-3 PUFA, adiponectin, RBP4 and PC) on the pathways involved in the inflammatory process and lipid metabolism have only just been mapped. The success of these protective strategies might depend on the surgical procedure. Moreover, the response of different type of liver to these treatments might differ and involve different signal transduction pathways that are at present marginally understood. Whether the above-mentioned approaches can be translated into as viable options in the clinical practice remain unknown, but further researches are required to optimize their use (e.g. dose, pharmacokinetics...etc). Such approaches have the potential to increase the number of organs suitable for transplantation, since they may improve the outcomes of marginal grafts that would not otherwise have been used.

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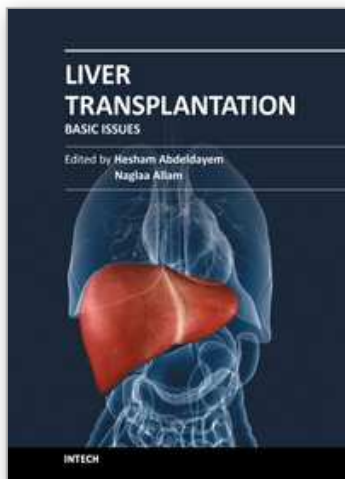
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This book covers a wide spectrum of topics including history of liver transplantation, ischemia-reperfusion injury, immunology of liver transplantation, viral hepatitis and liver transplantation, other indications for liver transplantation, prognostic factors and perioperative period. The authors of the chapters are experts in their respective fields. They are proponents covering different aspects of liver transplantation and come from many centers across the world. The interdisciplinary approach and the authority of the contributors resulted in a valuable reference to anyone interested in developing a global view in liver transplantation including medical students, residents, fellows, nurses, and practicing physicians and surgeons as well as researchers in the field of liver transplantation.

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