

Review

Redox state and the potential role of antioxidant compounds in liver ischemia/reperfusion injury

Maria I. Korontzi^{1*}, Apostolos Papalois², Ilias Kouerinis¹, Andreas C. Lazaris³,
George Theodoropoulos¹ and George Zografos¹

¹First Department of Propaedeutic Surgery, Hippocraton Hospital, Athens Medical School, Athens, Greece.

²Experimental Research Center ELPEN, Athens, Greece.

³Department of pathology, Laiko Hospital, Athens Medical School, Athens, Greece.

Accepted 12 April, 2020

Hepatic ischemia/reperfusion (I/R) injury consists of a sequel of cellular and humoral events that finally leads to parenchymal and nonparenchymal cell death. It is of utmost importance as regards the outcome of liver transplantation and liver resections. There is ample evidence that the key role in the inflicted injury is ascribed to reactive oxygen species (ROS) generated mainly by Kupffer cells and neutrophils during reperfusion, with the participation of endothelial cells and hepatocytes. ROS can procure serious damage to cellular membranes and genomic material. The disaster culminates with the initiation of several inflammatory mediators. On the other hand, cells possess a very potent, enzymatic and non enzymatic antioxidant system capable to mitigate oxidant stress or scavenge ROS, thus preserving to some extent cellular redox state. When the imbalance between oxidant stress and antioxidant systems predominates, cell death ensues. The spectre of liver I/R injury therapeutic options includes a plethora of antioxidant agents, natural or synthetic, and extends to genetic modifications. The aim of this article is to review the current knowledge on the generation and mode of action of ROS and to give a further insight on the antioxidant compounds that comprise the therapeutic quiver of this complicated syndrome.

Key words: Reactive oxygen species, oxidant stress, liver ischemia reperfusion, redox state, inflammation, antioxidants.

INTRODUCTION

Ischemia-Reperfusion injury (I/R injury) is a complex process characterized by the anoxic cell injury as well as the generation of inflammatory mediators and reactive oxygen species (ROS), leading step by step to cell necrosis and apoptosis. It is of utmost importance as regards to graft viability after transplantations and the outcome of surgical procedures involving transient ischemic periods such as hepatectomy. Liver failure is a dramatic outcome of vascular occlusion during liver resection (Pringle maneuver) and transplantation. Graft non function after liver transplantation occurs in a rate of 2 to 6%, while graft dysfunction in 20 to 25% (Jassem et al., 2002).

Anoxic injury is the predominant event during ischemia

and mitochondria are the organelles first insulted by the O₂ depletion (Jassem et al., 2002). The decrease in mitochondrial energy production (oxidative phosphorylation, ATP) causes a dramatic fall in cell energy status, thus triggering a sequel of events such as disturbances of cellular ion homeostasis, activation of hydrolases and loss of mitochondrial and membrane barrier function. Intracellular concentration of Ca²⁺ increases, leading to activation of phospholipases and proteases and finally to mitochondrial membrane permeability transition (de Groot and Rauen, 2007). Ca²⁺ also activates the enzyme xanthine oxyreductase (XOD) which is one of the main sources of reactive oxygen species (ROS) production following reperfusion (Ishii et al., 1990). At a later stage, Na⁺ accumulates in the cytosol causing osmotic cellular swelling and degradation of plasma membrane. All this injury leads to necrotic cell death (de Groot and Rauen, 2007). Although the differences between warm and cold ischemia are vague, it is postulated that oxidative stress

*Corresponding author. E-mail: markorontzi@gmail.com. Tel: +306932662229.

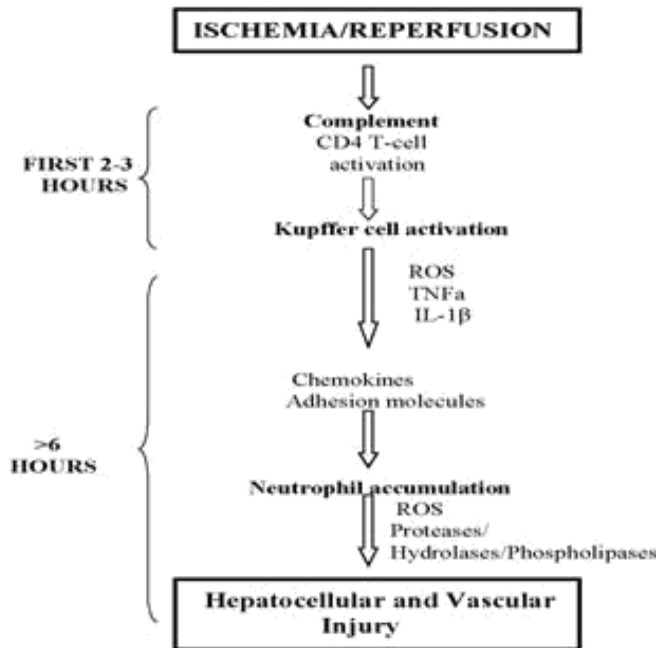


Figure 1. Pathophysiology of liver/ischemia reperfusion injury. During the early subacute phase of reperfusion which covers the first 2-3 h, the activation of Kupffer cells is the main feature. The factors responsible for this activation is hypoxia in combination with complement and CD4T-lymphocytes. During the late phase (> 6 h) of reperfusion, the inflammatory mediators augment cellular and vascular dysfunction by releasing neutrophils' chemoattractants and inducing adhesion molecules expression. Neutrophils' accumulation augments oxidative stress via ROS and protease release.

and mitochondrial dysfunction is greater after warm ischemia (Baumann et al., 1987). In warm ischemia, hepatocytes are mainly injured, whereas in cold ischemia the nonparenchymal cells (Kupffer cells, sinusoidal endothelial cells, Ito cells, biliary endothelium) are mainly insulted (Glatzounis et al., 2005).

Although ischemia is responsible for critical cellular damage, reperfusion represents the most destructive phase. Most researchers agree that reperfusion constitutes a biphasic process. During the early subacute phase of reperfusion which covers the first 2 - 3 h, the activation of Kupffer cells is the main feature (Jaeschke, 1991). The factors responsible for this activation are hypoxia in combination with complement and CD4T-lymphocytes (Jaeschke, 2003a). Kupffer cells are the most important source of ROS during this early period.

The xanthine oxidase system and mitochondria represent other sources of ROS early production (Parks and Granger, 1988). During the late phase of reperfusion (> 6 h), the inflammatory mediators augment cellular and vascular dysfunction by releasing neutrophils' chemoattractants and inducing adhesion molecules expression. Neutrophils' accumulation augments oxidative stress via ROS and protease release (Farmer et al., 2000) (Figure1).

ROS production and their role in liver I/R injury

Radicals are very reactive due to the unpaired electrons. The most important radicals in I/R injury are the superoxide anion ($O_2^{\cdot -}$), the hydroxyl radical (OH^{\cdot}) and the nitric oxide (NO). Some other intermediate species in the metabolism of O_2 and NO are also very reactive but they are not called radicals as they do not contain unpaired electrons. These intermediate species involved in I/R injury are hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), derivatives of O_2 metabolism (Glatzounis et al., 2005) and peroxynitrite ($ONOO^-$), a derivative of NO metabolism produced during reperfusion by the reaction of superoxide with NO (Cuzzocrea et al., 2001). The radicals together with the non-radical species are called Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS), depending on whether they are byproducts of O_2 or NO metabolism, respectively (Glatzounis et al., 2005). ROS and RNS can cause serious damage to a plethora of cellular components including proteins, enzymes, nucleic acids, DNA, RNA, cytoskeleton, cell membranes and lipids (Farmer et al., 2000). The major sources of ROS production include Kupffer cells during the initial reperfusion phase and polymorphonuclears (PMNs) during the late (> 6 h) reperfusion, with the contribution of endothelial cells and hepatocytes (Jaeschke, 1991). The xanthine/xanthine oxyreductase (xanthine/XOR) system, the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and the mitochondria have been speculated to play key roles in the I/R injury induced oxidant stress (Jaeschke, 2002a).

When tissue becomes ischemic, ATP is progressively degraded to other adenine nucleotides, nucleosides and finally to purine catabolites. Once cellular membranes are damaged due to free radicals attack during ischemia, they become leaky to constituents of adenine nucleotides. The adenine nucleotides are converted to nucleosides in the interstitial space, and then they are taken up by interstitial cells and catabolised to hypoxanthine (Kobayashi et al., 1991). XOD exists in two forms, xanthine dehydrogenase (XDH) and xanthine oxidase (XO) which coexist *in vivo*. These enzymes catalyse two consecutive reactions: The conversion of hypoxanthine to xanthine and xanthine to uric acid (Parks and Granger, 1986). Under normal physiological conditions the dehydrogenase predominates. During ischemia XDH is transformed to XO by proteolytic cleavage or by oxidation of sulphidyl residues (Hammerman et al., 1999). Plasma XO increases after hepatic occlusion-reperfusion (Weinbroum et al., 1995). During reperfusion, once oxygen is reintroduced, XDH and XO produce hydrogen peroxide (H_2O_2) and superoxide radicals ($O_2^{\cdot -}$). $O_2^{\cdot -}$ produced by XDH are inhibited by NAD, whereas $O_2^{\cdot -}$ generated by XO are not inhibited and induce oxidant stress (Parks and Granger, 1986) (Figure 2).

An alternative system of $O_2^{\cdot -}$ generation is NADPH oxidase. It is a membrane bound FAD-containing

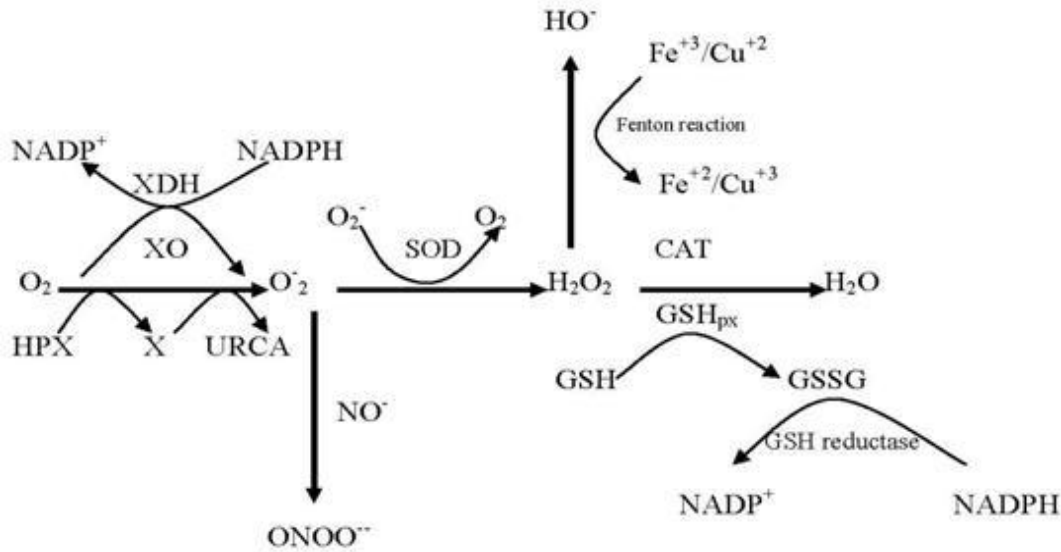


Figure 2. ROS production and their role in liver I/R injury XDH transfers electrons to the oxidised form of NAD (nicotinamide adenine dinucleotide), while XO transfers electrons to molecular oxygen. During reperfusion, once oxygen is reintroduced, both enzymes produce hydrogen peroxide (H_2O_2) and superoxide radicals (O_2^-). O_2^- produced by XDH are inhibited by NAD, whereas O_2^- generated by XO are not inhibited and induce oxidant stress. Superoxide anions can be converted to H_2O_2 and H_2O with the mediation of SOD (superoxide dismutase) and CAT (catalase). Moreover, highly toxic hydroxyl radicals (OH^\bullet) can be released by the reaction of H_2O_2 with reduced transition metals such as Fe^{+3}/Cu^{+1} (Fenton reaction). H_2O_2 can either enter the Fenton reaction to produce the highly reactive hydroxyl radicals or be reduced to water by CAT and glutathione peroxidase (GSHpx) which catalyses the conversion of glutathione (GSH) to oxidized glutathione disulfide (GSSG). GSSG can then be retransformed to GSH and oxidized NADP (NADPH) by the enzyme glutathione reductase.

flavoprotein which transfers electrons from NADPH to molecular oxygen, resulting in the production of superoxide anions (O_2^-). Superoxide anions can be converted to H_2O_2 and H_2O with the mediation of superoxide dismutase (SOD) and catalase (CAT). Moreover, highly toxic hydroxyl radicals (OH^\bullet) can be released by the reaction of H_2O_2 with reduced transition metals such as Fe^{+3}/Cu^{+1} (Fenton reaction) (Todo et al., 1996; Cesaratto et al., 2004) (Figure 2).

The role of mitochondria as a major source of ROS production after liver ischemia/reperfusion has been postulated and confirmed in animal trials and human liver transplantations (Honda et al., 2005). Normally, most of O_2 is reduced to water to produce energy (ATP) by the process of oxidative phosphorylation in the inner mitochondrial membrane. Only 2 - 4% of O_2 is converted to superoxide anion (O_2^-) due to electron transport chain leakage. As already mentioned, superoxide anion is converted to H_2O_2 and H_2O by SOD. Further, H_2O_2 can either enter the Fenton reaction to produce the highly reactive hydroxyl radicals or be reduced to water by CAT and glutathione peroxidase (GSHpx) which catalyses the conversion of glutathione (GSH) to oxidized glutathione disulfide (GSSG). GSSG can then be retransformed to GSH and oxidized NADP (NADPH) by the enzyme glutathione reductase (Jassem, 2002; Cesaratto et al.,

2004). In the ischemic phase, the complexes of the mitochondrial respiratory chain (mainly cytochrome c) are damaged, antioxidants like GSH and SOD are reduced, matrix Ca^{2+} is increased. All these changes result in significant electron leakage upon reperfusion and consequently in an enhanced ROS generation from mitochondria that leads to the formation of mitochondrial membrane permeability transition pores and finally to the membrane degradation, ATP depletion and killing of the cell (Jassem et al., 2002; Honda et al., 2005; de Groot and Rauen., 2007) (Figure 2).

Cell membrane transition permeability together with NADPH oxidation and GSH decrease, culminate ROS formation by mitochondria and trigger the release of apoptotic factors into the cytosol such as cyt c and apoptosis inducible factor, finally killing the cell by apoptosis (Liu et al., 1996; Susin et al., 1999). In fact, there is a great debate among authors regarding the predominant mode of cell death (apoptosis or necrosis) in liver I/R injury with some authors emphasizing apoptotic cell death (Helling et al., 1999; Kohli et al., 1999; Selzner et al., 2002; Gao et al., 1998) and others supporting necrotic cell death (Ikebe et al., 2000; Rentsch et al., 2001; Jaeschke, 2002b; Jaeschke, 2003b). In necrosis, groups of contiguous cells die by bleb formation and swelling as a result of ATP depletion, whereas in

apoptosis programmed death ligand-death receptor interactions lead to cytochrome c release, caspase (cystein proteases) activation and death of separated individual cells by shrinkage (Lemasters et al., 1981; Lemasters et al., 1983). Recently, Malhi have yielded an insight in the existing controversy, postulating that apoptosis or necrosis represent alternative outcomes of the same pathways leading to cell death that are both generated by ROS induced mitochondrial permeability transition (Mahli et al., 2006). Previously, Huet had attested that depending on the duration of ischemia early sinusoidal endothelial cell necrosis is followed by later hepatocyte apoptosis (Huet et al., 2004).

Kupffer cells and neutrophils can induce oxidant cellular damage both by the direct effect of ROS on cellular components and indirectly by proteases release, since ROS inhibit anti-protease in the vicinity around neutrophils (Weiss, 1990). PMNs mainly generate hyperoxide anions ($O_2^{\cdot-}$) through NADPH oxidase (Jaeschke, 2006). These oxygen radicals can then be converted to H_2O_2 by SOD. Further conversion of H_2O_2 by Fenton reaction is prevented due to lactoferrin which keeps iron ions at low concentration (Jaeschke, 1991). Alternatively, myeloperoxidase (MPO) released from neutrophils azurophilic granules can catalyze the conversion of H_2O_2 to HOCl with the subsequent formation of toxic chloramines (El-Benna et al., 2005). Endothelial cells are abundant to xanthine oxidase since this enzyme is attached to glycosaminoglycans of the endothelial cell surface (Tan et al., 1993). Also, hepatocytes are a potential source of XO (Jaeschke, 1991). In addition, the presence of NADPH oxidase has been recently confirmed in hepatocytes (Ozaki et al., 2000) and in endothelial cells (Li and Shah, 2001).

Intracellular and extracellular ROS, RNS and particularly $O_2^{\cdot-}$ coordinate inflammatory mediators through the activation of transcriptional factors such as Nuclear Factor κ B (NF κ B) and AP-1 (Fan et al., 2003; Glatzounis et al., 2005). NF κ B triggers the expression of many inflammatory cytokines namely: Tumor Necrosis Factor- α (TNF- α), Interleukin-1 (IL-1), Interferon- γ (INF- γ), chemokines (CXC) such as MIP-1, IL-8, Kupffer cells (Lentsch et al., 1999; Jaeschke., 2003b) and adhesion molecules such as Intracellular Adhesion Molecules (ICAM) (Jaeschke., 2003b), P- and E-selectin, Vascular Cell Adhesion Molecules (VCAM) (Ghosh et al., 1998). NF κ B resides in cytoplasm and its activation depends on I κ B family inhibitory proteins. When I κ B proteins are bound to NF κ B, NF κ B is inactivated. In contrast, during oxidative stress, I κ B proteins are phosphorylated and degraded, leaving NF κ B free to translocate to the nucleus and bind to DNA, where it induces the transcription of the above mentioned inflammatory genes with subsequent neutrophil accumulation and tissue damage (Ghosh et al., 1998; Lentsch et al., 2000). It is also noteworthy that some data indicates a protective anti-apoptotic role of NF κ B. NF κ B activation is also related to liver regeneration after transplantation. Thus, NF κ B may play a dual

role behaving both in a pro-apoptotic, cytotoxic or anti-apoptotic, cytoprotective manner depending on the type of the cells, or on the triggering of other signals or transcription factors such as AP-1 (Thanos and Maniatis, 1995; Baichwal and Baeuerle, 1997; Fan et al., 1999). Llacuna et al., support the notion that complete NF κ B suppression may be detrimental inhibiting the expression of survival genes, while a partial NF κ B suppression may attenuate the up-regulation of inflammatory mediators and activate protective genes (Llacuna et al., 2000).

Recently, a special interest is given to Toll-like receptors (TLRs) which are expressed mostly on antigen presenting cells, recognize pathogen associated molecular patterns (PAMP) in different organisms and induce a proinflammatory response mediated by neutrophils, macrophages and complement. They are also expressed on cells of the innate immune system like basophils, eosinophils, neutrophils and mast cells. Except of PAMP, TLRs also recognize endogenous ligands released from damaged cells. These endogenous ligands include stress proteins, heat shock proteins (HSP), extracellular matrix components, fibrinogen, fibrin, hyaluronan fragments, heparin sulfate. TLR4 is related to inflammatory process. Disruption of TLR4 pathway was shown to reduce hepatocellular injury in TLR4 mutant mice (Foley and Chari, 2007; Zhai et al., 2008).

AP-1 is another transcriptional factor implicated in ROS mediated liver I/R injury. Like NF κ B, Ap-1 is activated by oxidant stress and pro-inflammatory cytokines (Wisdom, 1999). Ap-1 involvement in I/R injury is ambivalent; Ap-1 mediates both apoptosis and regeneration, since the proteins most involved in AP-1 activation, c-jun and junD, demonstrate opposing roles: C-jun promotes cell growth while junD mitigates cell proliferation (Fan et al., 1999). It is postulated that ROS can also enhance the expression of cytoprotective genes such as HSP and particularly heme oxygenase-1 (HO-1) through the activation of AP-1 (Jaeschke, 2003b). In conclusion, the role of NF κ B and AP-1 in liver I/R injury is not yet very well delineated. Existing data is ambivalent and more insight is needed on the parameters influencing the expression and mode of action of these genes.

Another mechanism of ROS mediated tissue damage is elaborated through the action of proteases released by neutrophils and Kupffer cells (Jaeschke, 1991). Normally, proteases are inactivated by anti-proteases. During oxidant stress, ROS abrogate anti-proteases leaving the proteases undisturbed to cause intracellular oxidant stress, proteolysis and cell death of hepatocytes (Jaeschke et al., 1999). In the presence of ROS, proteases are allowed to procure significant parenchymal damage in proximal to neutrophil or phagocyte environment. Remote organs are protected from proteases since they are inactivated by antiproteases as soon as they escape from the ROS range of action (Weiss, 1990).

In addition, ROS can directly inflict serious cellular damage through lipid peroxidation (LPO), protein oxidation and DNA damage. The result is membrane damage,

protein and nucleic acid modification, enzyme inactivation and DNA strand breaks (Ernster, 1988). DNA can be damaged directly by ROS or indirectly via lipid peroxidation products (Garcea et al., 2006). Jaeschke reviewed that the extent of lipid peroxidation during reperfusion is moderate and can not be responsible for the severe tissue damage (Jaeschke, 1991; Jaeschke, 2003b). A possible explanation comes from the toxic cascades mediated by LPO products. These cascades promote the synthesis of many products involved in the continuation of inflammatory response such as leukotrienes, thromboxane (Ernster, 1988; Jaeschke, 2003b).

ANTIOXIDANT THERAPEUTIC

COMPOUNDS *In vivo* synthesized

compounds *Thiol donor molecules*

The therapeutic administration of several *in vivo* synthesized molecules has given promising results in attenuating liver I/R injury. Exogenous administration of GSH during the post- ischemic phase alleviated liver warm I/R injury in rats (Schauer et al., 2004). The dose of GSH seems to be decisive since protection from liver I/R injury was achieved in a dose between 0.1 - 2.0 mM (Glatzounis et al., 2005). The main problem with exogenous GSH administration is the large size of the molecule and its difficulty to enter the cell. GSH intracellular buildup needs cysteine provision from outside. N-acetylcysteine (NAC) is a synthetic thiol-containing compound which is deacetylated to cysteine and acts not only as a GSH precursor but also as a direct free radical scavenger (Jin et al., 2007). Kupffer cells use cysteine as a free radical scavenger and furthermore NAC can directly inhibit ROS generation from monocytes (Fusai et al., 2005). Recent *in vivo* trials showed significant reduction of warm liver I/R injury in rabbits with continuous NAC administration prior and/or during reperfusion with diminished ROS and RNS production (Fusai et al., 2005). However, there is data demonstrating neutral or even negative influence of NAC in liver I/R (Glatzounis et al., 2005).

Another GSH precursor is S-Adenosyl-L-methionine (SAM). SAM is a methyl donor molecule, present in all cells. It is a precursor molecule to the transsulfuration pathway which leads to GSH production (Bottiglieri, 2002). Pretreatment with SAM reduced sequential cold and warm I/R injury in experimental liver transplantation in rats (Dunne et al., 1997) and diminished hepatocellular and mitochondrial oxidative stress in warm I/R injury of steatotic rat livers (Kaneshiro et al., 1998).

Lipoic acid is, like GSH and NAC, a thiol donor molecule. Lipoic acid is synthesized *de novo* in mammalian cells (Glantzounis et al., 2006). Lipoic acid has been tested for its antioxidant efficacy in animal experiments and proved to improve liver function and

histological parameters in clinical trials of hepatic resection (Dulundu et al., 2007).

SOD and CAT derivatives

The use of exogenous SOD and CAT has been tested in various liver I/R experimental models with controversial results (Tanaka et al., 1990; Lardot et al., 1996). The main difficulties in the use of the native products are large size which restrains intracellular delivery, short half-life, antigenicity and expense (Cuzzocrea et al., 2001). To circumvent those difficulties, SOD and CAT derivatives have been manufactured (Yabe et al., 1999; Fujita et al., 1992). Conjugation of SOD with carbohydrates has been tested, namely carboxymethyl (SOD-CMD) and diethyliminoethyl (SOD-DEAED) dextrans, galactosylated (Gal-SOD) and mannosylated (Man-SOD) derivatives. Galactosylation and mannosylation of SOD result in enhanced pharmacokinetic properties targeting drug to parenchymal and non- parenchymal cells respectively and prolonging its half-life. Moreover, conjugation of SOD with polyethylene glycol (PEG-SOD) increases SOD half-life and intracellular permeability and allows SOD to resist to proteolysis as seen from I/R experiments in rats (Fujita et al., 1992).

Similar modifications of CAT molecule have been introduced in order to improve its biodistribution and hepatic clearance uptake. In fact, mannosylated CAT (Man-CAT) and succinylated CAT (Suc-CAT) are preferably delivered to non-parenchymal cells, galactosylated CAT (Gal-CAT) to parenchymal cells and polyethylene glycol conjugated CAT (PEG -CAT) was retained in plasma for a longer period. The hepatic clearance uptake of all three carbohydrate-conjugated molecules was much greater than this of native CAT. Suc-CAT and Man-CAT proved to be the more potent than PEG-CAT and Gal-CAT in attenuating liver I/R injury in mice (Yabe et al., 1999).

Another novel therapeutic approach is metalloporphyrins. Metalloporphyrins are stable catalytic antioxidants. Manganese-based metalloporphyrin complexes (MnP) demonstrate SOD and CAT activity, scavenging $O_2^{\cdot -}$, HO^{\cdot} , $ONOO^-$ and lipid peroxy radicals. The SOD activity of MnP is based on the dismutation reaction with $O_2^{\cdot -}$ by reduction of Mn(III) to the Mn(II) oxidation state, while the CAT activity is ascribed to their conjugated ring system that can undergo reversible oxidations (Cuzzocrea et al., 2001). MnP complexes have been proved effective in decreasing tissue damage, lipid peroxidation and protein nitration in isolated liver I/R injury of a mouse model (Wu et al., 2007).

Peptides and hormones

The three isoforms of heme oxygenase (HO), HO^{\cdot} or HSP

32, HO²⁻, and HO³⁻ catalyses the conversion of the pro-oxidant molecule heme into biliverdin and bilirubin, Fe²⁺ free iron which is incorporated in ferritin and carbon monoxide (CO). Biliverdin and bilirubin are potent peroxyl radical scavengers and inhibit lipid peroxidation. The sequestration of Fe²⁺ by ferritin reduces its oxidative capacity and makes it less likely to accumulate in membranes while CO induces vasodilation, inhibits mRNA expression of adhesion molecules and platelet aggregation. In conditions of inflammation and oxidant stress HO⁻ is mainly expressed, rather than HO²⁻ and HO³⁻ which are expressed constitutively. HO⁻ and products of HO degradation have been shown to attenuate liver I/R injury in rat models (Foley and Chari, 2007; Zhang et al., 2007; Vardanian et al., 2008).

Another potent antioxidant in mammalian tissues is reduced coenzyme Q₁₀ (CoQ₁₀H₂). Exogenous administration of CoQ₁₀H₂ suppressed lipid peroxidation by scavenging peroxyl radicals (Wu et al., 2007).

Melatonin is a hormone produced by the pineal gland. It is confirmed that melatonin is a potent ROS and RNS scavenger (Reiter et al., 2003). Melatonin can increase the activity of antioxidant enzymes such as GSHpx and SOD (Reiter et al., 2003), preserve the mitochondrial function by sustaining mitochondrial redox status and increasing the activity of complexes I and IV of the respiratory chain (Rodríguez-Reynoso et al., 2001). Melatonin has been proved to reduce liver warm I/R injury in several animal models and in humans' liver resections (Sewerynek et al., 1996; Glatzounis et al., 2005; Zhang et al., 2006). In addition, melatonin was found to ameliorate other clinical conditions related with significant oxidant stress in humans (Baykara et al., 2009).

Carnosine (-alanyl-L-histidine) is the first neuropeptide ever described and acts as an active scavenger of superoxide, hydroxyl radicals and singlet oxygen molecule. The administration of melatonin and carnosine together had better results compared to the sole administration of each agent in liver ischemia /reperfusion of rat models (Baykara et al., 2009).

Atrial Natriuretic Peptide (ANP) is also a hormone believed to play a cytoprotective role in rats liver I/R injury probably by reinforcing cells defense against damage induced by ROS. This cytoprotection is mediated via cGMP formation (Bilzer et al., 1999).

Dietary compounds

Some dietary compounds have been recognized as effective in attenuating lipid peroxidation and liver damage after I/R. α -Tocopherol (vitamin E derivative) is a potent antioxidant of lipid bilayers of the cellular membranes (Giakoustidis et al., 2006) capable to maintain lipid peroxidation at low levels in warm I/R injury of rat livers (Taha et al., 2004). Donor treatment with α -tocopherol before harvesting reduced parenchymal and

non-parenchymal I/R induced liver cell damage in a rat model (Gondolesi et al., 2002). Moreover, α -Tocopherol is capable to abate liver I/R from a biochemical and an histological point of view, possibly by scavenging lipid peroxyl radicals production (Taha et al., 2004). There is evidence that α -Tocopherol can also inhibit pro-inflammatory cytokine production (Vitaglione et al 2004).

Pentoxifylline was found almost equally effective to vitamin E, but the concomitant administration of vitamin E and pentoxifylline obtained even better results regarding the lipid peroxidation and histological parameters (Vardareli et al., 1998).

D-allose, a rare sugar has also been documented to moderately attenuate liver I/R injury mainly by decreasing neutrophil infiltration and ROS production (Hossain et al., 2004).

Synthetic compounds

Allopurinol is a XO inhibitor. Oral administration of allopurinol during the pre-ischemic phase in pigs managed to increase glutathione levels and decrease lipid peroxides production during warm I/R injury (Durmu et al., 1994), whereas no amelioration of oxidation parameters, morbidity or mortality was found in a human clinical trial of partial hepatectomy (Vriens et al., 2002). Jaeschke reviewed that although small doses of allopurinol are adequate to inhibit XO, only high and very high doses of allopurinol exerted a protective effect in liver I/R injury *in vivo*. This fact may be regarded as a hint that other mechanisms outside XO inhibition are responsible for the protective effect of allopurinol in liver I/R injury (Jaeschke, 1991).

Steroids and non-steroid anti-inflammatory drugs (NSAID) have been reported to inhibit superoxide production and moreover NSAID can impede ROS production from arachidonic acid by inhibiting cyclooxygenase (Kirimlioglu et al., 2006).

Cyclooxygenase (COX) is a key enzyme in the arachidonic acid cascade which induces the formation of prostaglandin E₂ (PGE₂). COX has two isoenzymes: COX-1 and COX-2. COX-2 is more commonly found in immune and inflammatory cells. Celecoxib, a selective COX-2 inhibitor reduces liver damage after I/R injury (Zhang et al., 2007).

Lazaroids are a new class of 21-aminosteroids lacking glucocorticoid and mineralocorticoid action. They act as potent antioxidants inhibiting iron dependent lipid peroxidation. Lazaroids are reported to be 10,000 times more potent than methylprednisolone in inhibiting lipid peroxidation (Braugher et al., 1987). Moreover, they abrogate cytokine generation, arachidonic acid release, neutrophil and Kupffer cell activation, adhesion molecule expression (Todo et al., 1996). They have also been reported to possess a vitamin E-like, a superoxide scavenging and an iron chelating activity (Hall and Travis, 1988). They have

been considered to be cytoprotective in various experimental models of injuries (Todo, 1996; Kuwaki et al., 2000).

Cyclosporine and azathioprine, the known immunosuppressive drugs, can diminish lipid peroxidation and ameliorate warm liver I/R injury in rats (Konuko lu et al., 1998). In comparison to cyclosporine monotherapy, best results were obtained by the combination of cyclosporine with ibuprofen, a non-steroid anti-inflammatory agent (Konuko lu et al., 1998). Another immunosuppressant, tacrolimus or FK506, has been documented to possess apart from an anti-inflammatory effect, a free radical inhibitory property as well, as estimated by the reduction in antioxidant enzymes levels in rats (Garcia-Criado et al., 1997).

Trimetazine (1-(2,3,4-trimethoxybenzyl)-piperazine dihydrochloride) is a cellular anti-ischemic drug proved to exert a cytoprotective role in liver ischemia reperfusion injury. It was found to protect isolated rat mitochondria from endogenous and exogenous ROS by restoring GSHpx activity (Elimadi et al., 1988). Metal chelation has also been implicated in its mode of action (Glatzounis et al., 2005).

Two iron chelators TPEN (N,N,N,N-tetrakis-[2-pyridylmethyl]-ethylenediamine) and deferoxamine can protect hepatic tissue after warm liver I/R. TPEN attenuated the apoptotic I/R insult in isolated rat liver (Hochhauser et al., 2005). Caraceni et al. experimented in perfused isolated rat hepatocytes and concluded that deferoxamine at low concentrations exhibits an iron chelating action and inhibits lipid peroxidation without preventing O_2^- generation whereas in high concentrations acts as a non specific ROS scavenger (Caraceni et al., 1995). Colletti et al. reports another mechanism of action of deferoxamine namely the reduction of TNF release. In addition, Colletti postulates that deferoxamine, apart from its iron chelating property, can reduce neutrophil infiltration and neutrophil induced ROS generation since neutrophil recruitment and activation is depended on TNF release (Colletti et al., 1994). Another novel iron chelator desferrioxochelin 772SM (D-Exo) significantly reduced lipid peroxidation in I/R rat liver model when combined with a P-selectin antagonist (Amersi et al., 2001).

Edaravone is another potent free-radical scavenger that beyond its clinical use in the acute phase of the myocardial infarction has been used in hepatic I/R injury with positive results on oxidative stress (Taniguchi et al., 2007).

Bucilamine is another synthetic thiol donor molecule which suppressed lipid peroxide levels and preserved GSH level in *ex vivo* and *in vivo* cold I/R injury experiments in rats (Amersi et al., 2002).

Antioxidant gene therapy

Another recently developed treatment approach to I/R injury is gene therapy that is targeting of specific cells and

organs with vectors carrying a certain gene. This vector can be viral or non-viral. Due to the low level production of late viral proteins and the immunogenicity of these proteins (Chia et al., 1998), there are efforts to substitute viral vectors with non-viral vectors such as poly lipid nanoparticles (He et al., 2006), ribozymes, synthetic oligodeoxynucleotides, short interfering RNA (siRNA), and vector-expressing short hairpin RNA (shRNA). Short interfering RNA is double-stranded RNA that is incorporated into an RNA-induced silencing complex and serves as a guide for silencing their corresponding target mRNAs based on complimentary base pairing (Foley and Chari, 2007). Lysosomes and DNA-complexes are some other non-viral vectors (Glatzounis et al., 2005). Second generation adenoviral vectors which contain additional deletions of early transcriptional units has also been developed (Colletti et al., 1994).

He et al. (2006) used poly lipid nanoparticles to deliver the human extracellular dismutase (EC-SOD) or CAT gene via the portal vein one day prior to liver I/R in a mice model and noticed significant I/R injury attenuation attributed to the elevated antioxidant enzyme activity as the result of the gene therapy. Zwacka et al. (1998) delivered mitochondrial SOD (Mn-SOD) gene using an adenoviral vector in mouse liver I/R model and reported amelioration of I/R injury by inactivation of NFkB and AP-1 transcription factors. The role of Cu/ZnSOD in oxidant injury is more ambivalent since some researchers report aggravation of lung I/R injury in rats with adenoviral induced overexpression of Cu/ZnSOD (Danel et al., 1998), while others postulate abrogation of lipid peroxidation and liver function parameters in a cholestatic model of liver injury in rats (Prosser et al., 2006). It is however noteworthy that in rodents, gene delivery is preferentially targeted to liver and this may be a plausible explanation to this discord.

CONCLUSION

Hepatic I/R injury is a multifactorial process tightly related to cellular redox state. It has been clearly delineated that ROS play a pivotal role in hepatic I/R injury while other bioactive substances such as cytokines, chemokines are also very important. A plethora of antioxidant agents have been tested *in vitro* and *in vivo* with promising results. Nonetheless, only a few have been corroborated in human trials. In line with experimental and clinical conclusions, combination therapy of two or more therapeutic compounds can further improve liver resections and transplantations' outcome. Antioxidant gene therapy is another modality recently added to our therapeutic arsenal, but as for transplantations, it encounters an ethical hurdle regarding the genetic pretreatment of a living donor.

Abbreviations: I/R injury, Ischemia-reperfusion injury; ROS, reactive oxygen species; RNS, reactive nitrogen species; XOR,

xanthine oxyreductase; **NADPH**, nicotinamide adenine dinucleotide phosphate; **PMNs**, polymorphonuclears; **XDH**, xanthine dehydrogenase, **XO**, xanthine oxidase; **SOD**, superoxide dismutase; **CAT**, catalase; **GSH_{bx}**, glutathione peroxidase; **GSH**, glutathione; **GSSG**, oxidized glutathione disulfide; **MPO**, myeloperoxidase; **CXC**, chemokines; **NFκB**, nuclear factor κB; **TNF-α**, tumor necrosis factor-α; **IL-1**, interleukin-1; **INF-γ**, interferon-γ; **TLR**, toll-like receptors; **PAMP**, pathogen associated molecular patterns; **ICAM**, intracellular adhesion molecules; **VCAM**, vascular cell Adhesion molecules; **HO-1**, heme oxygenase-1; **LPO**, lipid peroxidation; **NAC**, N-acetylcysteine; **SAM**, S-Adenosyl-L-methionine; **MnP**, manganese-based metalloporphyrin complexes; **HSP**, heat shock proteins; **CO**, carbon monoxide; **ANP**, atrial natriuretic peptide; **NSAID**, non-steroid anti-inflammatory drugs; **COX**, cyclooxygenase; **PGE₂**, Prostaglandin E₂.

REFERENCES

- Amersi F, Dulkanchainun T, Nelson SK, Farmer DG, Kato H, Zaky J, Melinek J, Shaw GD, Kupiec-Weglinski JW, Horwitz LD, Horwitz MA, Busuttill RW (2001). "A novel iron chelator in combination with a P-selectin antagonist prevents ischemia/reperfusion injury in a rat liver model." *Transplantation* 71: 112-118.
- Amersi F, Nelson SK, Shen XD, Kato H, Melinek J, Kupiec-Weglinski JW, Horwitz LD, Busuttill RW, Horwitz MA (2002). "Bucillamine, a thiol antioxidant, prevents transplantation-associated reperfusion injury." *PNAS* 99: 8915-8920.
- Baichwal VR, Baeuerle P (1997). "Activate NF-kappa B or die?" *Curr. Biol.* 7: R94-R96.
- Baumann M, Bender E, Stommer G, Gross G, Brand K (1987). "Effects of warm and cold ischemia on mitochondrial functions in brain, liver and kidney." *Mol. Cell Biochem.* 87: 137-145.
- Baykara B, Tekmena I, Pekcetina C, Ulukus C, Tuncel P, Sagol O, Ormen M, Ozogul C (2009). "The protective effects of carnosine and melatonin in ischemia-reperfusion injury in the rat liver." *Acta Histochem.* 111: 42-51.
- Bilzer M, Jaeschke H, Vollmar AM, Paumgartner G, Gerbes AL (1999). "Prevention of Kupffer cell-induced oxidant injury in rat liver by atrial natriuretic peptide." *Am. J. Physiol. Gastrointest Liver Physiol.* 276: G108-116.
- Bottiglieri T (2002). "S-Adenosyl-L-methionine (SAME): from the bench to the bedside—molecular basis of a pleiotropic molecule." *Am. J. Clin. Nutr.* 76(suppl): 115: 1S-7S.
- Braugher JM, Pregenzer J, Chase RL, Duncan LA, Jacobsen EJ, McCall JM (1987). "Novel 21-amino steroids as potent inhibitors of iron-dependent lipid peroxidation." *J. Biol. Chem.* 262.
- Caraceni P, Thiel D, Borle AB (1995). "Dual effect of deferoxamine on free radical formation and reoxygenation injury in isolated hepatocytes." *Am. J. Gastrointest Liver Physiol.* 269: G132-G137.
- Cesaratto L, Vascotto C, Calligaris S, Tell G (2004). "The importance of redox state in liver damage." *Ann. Hepatol.* 3: 86-92.
- Chia SH, Geller D, Kibbe MR, Watkins SC, Fung JJ, Starzl TE, Murase N (1998). "Adenovirus mediated gene transfer to liver grafts: An improved method to maximize infectivity." *Transplantation* 66: 1545-1551.
- Colletti LM, Remick D, Campbell DA (1994). "Desferal attenuates TNF release following hepatic ischemia reperfusion." *J. Surg. Res.* 57: 447-453.
- Cuzzocrea S, Riley D, Caputi AP, Salvemini D (2001). "Antioxidant therapy: A new pharmacological approach in shock, inflammation, and Ischemia/Reperfusion injury." *Pharmacol. Rev.* 53: 135-159.
- Danel C, Erzurum S, Prayssac P, Eissa NT, Crystal RG, Herve P, Baudet B, Mazmanian M, Lemarchand P (1998). "Gene therapy for oxidant injury-related diseases: adenovirus mediated transfer of superoxide dismutase and catalase cDNAs protects against hyperoxia but not against ischemia/reperfusion lung injury." *Hum. Gene Ther.* 9: 1487-1496.
- de Groot H, Rauen U (2007). "Ischemia-Reperfusion Injury: Processes in Pathogenetic Networks: A Review." *Transplant Proc.* 39: 481-487.
- Dulundu E, Ozel Y, Topaloglu U, Sehirlir O, Ercan F, Gedik N, Sener G (2007). "Alpha lipoic acid protects against hepatic ischemia reperfusion in rats" *Pharmacol.* 79: 163-170.
- Dunne JB, Piratvisuth T, Williams R, Tredger JM (1997). "Treatment of experimental ischemia / reperfusion injury with S-adenosylmethionine: Evidence that donor pre-treatment complements other regimens." *Transplantation* 63: 500-506.
- Durmu O, Aricioglu A, Guven T, Ouz M, Yel M, Trkzkan N (1994). "The effect of allopurinol on the liver ultrastructure, reduced glutathione and lipid peroxide levels during liver ischemia in pigs." *Gen. Pharmac.* 25: 781-786.
- El-Benna J, Dang P, Gougerot-Pocidallo MA, Elbim C (2005). "Phagocyte NADPH oxidase: a multicomponent enzyme essential for host defenses." *Arch Immunol. Ther. Exp. (Warsz)* 53: 199-206.
- Elimadi A, Settaf A, Morin D, Sapena R, Lamchouri F, Cherrah Y, Tillement JP (1998). "Trimetazidine counteracts the hepatic injury associated with ischemia-reperfusion by preserving mitochondrial function." *JPET* 286: 23-28.
- Ernster L (1988). "Biochemistry of reoxygenation injury." *Crit. Care Med.* 16: 947-953.
- Fan C, Li Q, Ross D, Engelhardt JF (2003). "Tyrosine phosphorylation of IB activates NFB through a redox-regulated and c-Src-dependent mechanism following hypoxia/reoxygenation." *J. Biol. Chem.* 278: 2072-2080.
- Fan C, Zwacka R, Engelhardt JF (1999). "Therapeutic approaches for ischemia/reperfusion injury in the liver." *J. Mol. Med.* 77: 577-596.
- Farmer DG, Amersi F, Kupiec-Weglinski J, Busuttill R (2000). "Current status of ischemia and reperfusion injury in the liver." *Transpl. Rev.* 14: 106-126.
- Foley DP, Chari R (2007). "Ischemia-reperfusion injury in transplantation: novel mechanisms and protective strategies." *Transplant Rev.* 21: 43-53.
- Fujita T, Furitsu H, Nishikawa M, Takakura Y, Sezaki H, Hashida M (1992). "Therapeutic effects of superoxide dismutase derivatives modified with mono- or polysaccharides on hepatic injury induced by ischemia/reperfusion." *Biochem. Biophys. Res. Commun.* 189: 191-196.
- Fusai G, Glatzounis G, Hafez T, Yang W, Quaglia A, Sheth H, Kanoria S, Parkes H, Seifalian A, Davidson BR (2005). "N-Acetylcysteine ameliorates the late phase of liver ischaemia/reperfusion injury in the rabbit with hepatic steatosis." *Clin. Sci.* 109: 465-473.
- Gao W, Bentley R, Madden JF, Clavien PA (1998). "Apoptosis of sinusoidal endothelial cells is a critical mechanism of preservation injury in rat liver transplantation." *Hepatology* 27: 1652-1660.
- Garcea G, Gescher A, Steward W, Dennison A, Bery D (2006). "Oxidative stress in humans following the Pringle manoeuvre." *Hepatobiliary Pancreat Dis. Int.* 5: 210-214.
- Garcia-Criado FJ, Palma-Vargas J, Valdunciel-Garcia JJ, Toledo AH, Misawa K, Gomez-Alonso A, Toledo-Pereyra LH (1997). "Tacrolimus (FK506) down-regulates free radical tissue levels, serum cytokines, and neutrophil infiltration after severe liver ischemia." *Transplantation* 64: 594-598.
- Ghosh S, May M, Kopp EB (1998). "NF-κB and Rel proteins: evolutionarily conserved mediators of immune responses." *Annu. Rev. Immunol.* 16: 225-260.
- Giakoustidis D, Papageorgiou G, Iliadis S, Giakoustidis A, Kostopoulou E, Kontos N, Botsoglou E, Tsantilas D, Papanikolaou V, Takoudas D (2006). "The Protective Effect of α-Tocopherol and GdCl₃ Against Hepatic Ischemia/Reperfusion Injury." *Surg. Today* 36: 450-456.
- Glantzounis GR, Yang W, Koti RS, Mikhailidis DP, Seifalian AM, Davidson BR (2006). "The role of thiols in liver ischemia-reperfusion injury." *Curr. Pharm. Des.* 12: 2891-901.
- Glatzounis GK, Salacinski H, Yang W, Davidson BR, Seifalian AM (2005). "The Contemporary Role of Antioxidant Therapy in Attenuating Liver Ischemia-Reperfusion Injury: A Review." *Liver Transpl.* 11: 1031-1047.
- Gondolesi GE, Lausada N, Schinella G, Vidal, MS, Luna GC, Toledo J, de Buschiazzo PM, Raimondi JC (2002). "Reduction of Ischemia-Reperfusion Injury in Parenchymal and Nonparenchymal Liver Cells by Donor Treatment With DL-α-Tocopherol Prior to Organ Harvest." *Transplant Proc.* 34: 1086-1091.

- Hall ED, Travis M (1988). "Inhibition of arachidonic acid induced vasogenic brain edema by the non-glucocorticoid 21-aminosteroid U74006F" *Brain Res.* 451.
- Hammerman C, Goldschmidt D, Caplan MS, Kaplan M, Schimmel MS, Eidelman AI, Branski D, Hochman A (1999). "Amelioration of Ischemia-Reperfusion Injury in Rat Intestine by Pentoxifylline-Mediated Inhibition of Xanthine Oxidase." *J. Pediatr. Gastroenterol. Nutr.* 29: 69-74.
- He SQ, Zhang Y, Venugopal SK, Dicus CV, Perez RV, Ramsamooj R, Nantz MH, Zern MA, Wu J (2006). "Delivery of Antioxidative Enzyme Genes Protects Against Ischemia/Reperfusion-Induced Liver Injury in Mice." *Liver Transpl.* 12: 1869-1879.
- Helling TS, EC, Helling TS, Jr, Chang CC, Hodges MC, Dhar A, VanWay C (1999). "Hepatic Apoptotic Activity Following Transient Normothermic Inflow Occlusion and Reperfusion in the Swine Model." *J. Surg. Res.* 86: 70-78.
- Hochhauser E, Ben-Ari Z, Pappo O, Chepurko Y, Vidne BA (2005). "TPEN attenuates hepatic apoptotic ischemia/ reperfusion injury and remote early cardiac dysfunction." *Apoptosis* 10: 53-62.
- Honda H, Korge P, Weiss J (2005). "Mitochondria and Ischemia/reperfusion injury." *Ann. N Y Acad. Sci.* 1047: 248-58.
- Hossain MA, Izuishi K, Tokuda M, Izumori K, Maeta H (2004). "D-Allose has a strong suppressive effect against ischemia/reperfusion injury: a comparative study with allopurinol and superoxide dismutase." *J. Hepatobiliary Pancreat. Surg.* 11: 181-189.
- Huet PM, Nagaoka MR, Desbiens G, Tarrab E, Brault A, Bralet MP, Bilodeau M (2004). "Sinusoidal endothelial cell and hepatocyte death following cold ischemia—warm reperfusion of the rat liver." *Hepatology* 39: 1110-1119
- Ikebe N, Akaike T, Miyamoto Y, Hayashida K, Yoshitake J, Ogawa M, Maeda H (2000). "Protective Effect of S-Nitrosylated 1-Protease Inhibitor on Hepatic Ischemia-Reperfusion Injury." *J. Pharmacol. Exp. Ther.* 295: 904-911.
- Ishii K, Suita S, Sumimoto H (1990). "Effect of verapamil on conversion of xanthine dehydrogenase to oxidase in ischemic rat liver." *Res. Exp. Med. (Berl)* 190: 389-399.
- Jaeschke H (1991). "Reactive oxygen and ischemia/reperfusion injury of the liver." *Chem. Biol. Interact.* 79: 115-136.
- Jaeschke H (2002a). "Xanthine oxidase-induced oxidant stress during hepatic ischemia-reperfusion: Are we coming full circle after 20 years?" *Hepatology* 36: 761-763.
- Jaeschke H (2002b). "Reperfusion Injury after Warm Ischemia or Cold Storage of the Liver: Role of Apoptotic Cell Death" *Transplantation Proceedings* 34: 2656-2658.
- Jaeschke H (2003a). "Molecular mechanisms of hepatic ischemia-reperfusion injury and preconditioning." *Am. J. Physiol. Gastrointest. Liver Physiol.* 284: G15-G26.
- Jaeschke H (2003b). "Role of reactive oxygen species in hepatic ischemia-reperfusion injury and preconditioning." *J. Invest. Surg.* 16: 127-140.
- Jaeschke H (2006). "Mechanisms of neutrophil-induced liver cell injury during hepatic ischemia-reperfusion and other acute inflammatory conditions." *Am. J. Physiol. Gastrointest. Liver Physiol.* 290: G1083-G1088.
- Jaeschke H, Ho Y-S, Fisher MA, Lawson JA, Farhood A (1999). "Glutathione peroxidase deficient mice are more susceptible to neutrophil-mediated hepatic parenchymal cell injury during endotoxemia: Importance of an intracellular oxidant stress." *Hepatology* 29: 443-450.
- Jassem W, Fuggle, Rela M, Koo DDH, Heaton ND (2002). "The role of mitochondria in ischemia/reperfusion injury." *Transplantation* 73: 493-499.
- Jin X, Wang L, Wu HS, Zhang L, Wang CY, Tian Y, Zhang JH (2007). "N-acetylcysteine inhibits activation of toll-like receptor 2 and 4 gene expression in the liver and lung after partial hepatic ischemia-reperfusion injury in mice." *Hepatobiliary Pancreat. Dis. Int.* 6: 284-289.
- Kaneshiro Y, Nakano H, Kumada K, Boudjema K, Kitamura N, Shimura H, Barama A, Kigawa G, Tatsuno M, Fujiwara Y, Baek Y, Sasaki J, Nagasaki H, Yamaguchi M (1998). "Augmentation of mitochondrial reduced glutathione by S-adenosyl-L-methionine administration in ischemia-reperfusion injury of the rat steatotic liver induced by choline-methionine-deficient diet" *Eur. Surg. Res.* 30: 34-42.
- Kirimlioglu V, Kirimlioglu H, Yilmaz S, Piskin T, Tekerekoglu S, Bayindir Y (2006). "Effect of steroid on mitochondrial oxidative stress enzymes, intestinal microflora, and bacterial translocation in rats subjected to temporary liver inflow occlusion" *Transplant Proc.* 38: 378-381.
- Kobayashi H, Nonami T, Kurokawa T, Sugiyama S, Ozawa T, Takagi H. (1991). "Mechanism and preservation of ischemia-reperfusion-induced liver injury in rats." *J. Surg. Res.* 51: 240-244.
- Kohli V, Selzner M, Madden JF, Bentley RC, Clavien PA (1999). "Endothelial cell and hepatocytes deaths occur by apoptosis after ischemia-reperfusion injury in the rat." *Liver Transpl.* 67: 1099-1105.
- Konuko lu D, Tasç I, Çetinkale O (1998). "Effects of cyclosporin A and ibuprofen on liver ischemia-reperfusion injury in the rat." *Clin. Chim. Acta* 275: 1-8.
- Kuwaki K, Komatsu K, Sohma H, Abe T (2000). "Improvement of Ischaemia-reperfusion njury by lazarold U74389G in rat lung transplantation model." *Scand. Cardiovasc. J.* 34: 209-212.
- Lardot C, Broeckaert F, Lison D, Buchet JP and Lauwerys R (1996). "Exogenous catalase may potentiate oxidant-mediated lung injury in the female Sprague-Dawley rat." *J. Toxicol. Environ. Health* 47: 509-522.
- Lemasters JJ, Ji S, Thurman RG (1981). "Centrilobular injury following hypoxia in isolated, perfused rat liver." *Sci.* 213: 661-663.
- Lemasters JJ, Stenkowski C, Ji S, Thurman RG (1983). "Cell surface changes and enzyme release during hypoxia and reoxygenation in the isolated, perfused rat liver." *J. Cell Biol.* 97: 778-786.
- Lentsch AB, Kato A, Yoshidome H, McMasters KM, Edwards MJ (2000). "Inflammatory mechanisms and therapeutic strategies for warm hepatic ischemia/reperfusion injury." *Hepatology.* 32: 169-173.
- Lentsch AB, Yoshidome H, Warner RL, Ward PA, Edwards MJ (1999). "Secretory leukocyte protease inhibitor in mice regulates local and remote organ inflammatory injury induced by hepatic ischemia/reperfusion." *Gastroenterol.* 117: 953-961.
- Li JM, Shah AM (2001). "Differential NADPH- versus NADH-dependent superoxide production by phagocyte-type endothelial cell NADPH oxidase." *Cardiovasc. Res.* 52: 477-486.
- Liu X, Kim C, Yang J, Jemmerson R, Wang X (1996). "Induction of apoptotic program in cell-free extract; requirement for dATP and cytochrome c." *Cell* 86: 147-157.
- Llacuna L, Mari M, Lluís JM, Garcia-Ruiz C, Fernandez-Checa JC, Morales A (2000). "Reactive Oxygen Species Mediate Liver Injury Through Parenchymal Nuclear Factor- κ B Inactivation in Prolonged Ischemia/Reperfusion." *AJP* 174: 1776-1785.
- Mahli H, Gores G, Lemasters JJ (2006). "Apoptosis and Necrosis in the Liver: A Tale of Two Deaths?" *Hepatology.* 43: S31-S44.
- Ozaki M, Deshpande SS, Angkeow P, Bellan J, Lowenstein CJ, Dinauer MC, Goldschmidt-Clermont PJ, Irani K (2000). "Inhibition of the Rac1 GTPase protects against nonlethal ischemia/reperfusion-induced necrosis and apoptosis *in vivo*." *FASEB J* 14: 418-429.
- Parks DA, Granger D (1986). "Xanthine oxidase: Biochemistry, distribution and physiology." *Acta Physiol. Scand.* 548(Suppl) : 87-99.
- Parks DA, Granger D (1988). "Ischemia-reperfusion injury: a radical view." *Hepatology.* 8: 680-682.
- Prosser CC, Yen R, Wu J (2006). "Molecular therapy for hepatic injury and fibrosis: Where are we?" *World J. Gastroenterol.* 12: 509-515.
- Reiter RJ, Tan D, Mayo JC, Sainz RM, Leon J, Czarnocki Z (2003). "Melatonin as an antioxidant: biochemical mechanisms and pathophysiological implications in humans." *Acta Biochim. Pol.* 50: 1129-1146.
- Rentsch M, Beham A, Iesalnieks I, Mirwald T, Anthuber M, Jauch KW (2001). "Impact of prolonged cold ischemia and reperfusion on apoptosis, activation of caspase 3, and expression of bax after liver transplantation in the rat." *Transplant Proc.* 33: 850-851.
- Rodríguez-Reynoso S, Leal C, Portilla E, Olivares N, Mu iz J (2001). "Effect of Exogenous Melatonin on Hepatic Energetic Status during Ischemia/Reperfusion: Possible Role of Tumor Necrosis Factor- α and Nitric Oxide." *J. Surg. Res.* 100: 141-149.
- Schauer RJ, Gerbes A, Vonier D, Meissner H, Michl P, Leiderer R, Schildberg F, Messmer K, Bilzer M (2004). "Glutathione Protects the Rat Liver Against Reperfusion Injury After Prolonged Warm Ischemia." *Ann. Surg.* 239: 220-231.
- Selzner M, Rudiger H, Selzner N, Thomas DW, Sindram D, Clavien PA.

- (2002). "Transgenic mice over expressing human Bcl-2 are resistant to hepatic ischemia and reperfusion." *J. Hepatol.* 36: 218-225.
- Sewerynek E, Reiter R, Melchiorri D, Ortiz GG, Lewinski A (1996). "Oxidative damage in the liver induced by ischemia-reperfusion: protection by melatonin." *Hepatogastroenterol.* 43: 898-905.
- Susin SA, Lorenzo H, Zamzami N, Marzo I, Snow BE (1999). "Molecular characterization of mitochondrial apoptosis-inducing factor." *Nature* 397: 441-446.
- Taha MO, Carvalho CA, Souza HS, Fagundes DJ, Simoes MJ, Novo NF, Caricati-Neto A (2004). "Effects of Alpha-Tocopherol on Ischemia/Reperfusion Injury in Rat Liver: Biochemical and Histological Evaluation." *Transplant Proc.* 36: 276-282.
- Tan S, Yokohama Y, Dickens E, Cash TG, Freeman BA, Parks DA (1993). "Xanthine oxidase activity in the circulation of rats following hemorrhagic shock." *Free Rad. Biol. Med.* 15: 407-414.
- Tanaka J, Malchesky PS, Omokawa S, Goldcamp JB, Harasaki H, Vogt DP, Broughan TA, Nosé Y (1990). "Effects of prostaglandin I₂, superoxide dismutase, and catalase on ischemia-reperfusion injury in liver transplantation." *ASAIO Trans* 36(3): M600-M603.
- Taniguchi M, Uchinami M, Doi K, Yoshida M, Sasaki H, Tamagawa K, Horiuchi T, Tanaka K (2007). "Edaravone Reduces Ischemia-Reperfusion Injury Mediators in Rat Liver." *J. Surg. Res.* 137: 69-74.
- Thanos D, Maniatis T (1995). "Identification of the rel family members required for virus induction of the human beta interferon gene." *Mol. Cell Biol.* 15: 152-164.
- Todo S, Hamada N, Zhu Y, Zhang S, Subbotin V, Nemoto A, Takeyoshi I, Starzl TE (1996). "Lazaroid U-74389G for 48 h canine liver preservation." *Transplantation* 61: 189-194.
- Vardanian AJ, Busuttil R, Kupiec-Weglinski JW (2008). "Molecular Mediators of Liver Ischemia and Reperfusion Injury: A Brief Review." *Mol. Med.* 14: 337-345.
- Vardareli E, Saricam T, Koken T, Degirmenci I, Aral E, Erenoglu E (1998). "The effect of alpha-tocopherol and pentoxifylline on ischemia-reperfusion." *Hepatogastroenterol.* 45: 1505-1508.
- Vitaglione P, Morisco F, Caporaso N, Fogliano V (2004). "Critical Reviews in Food Science and Nutrition." *Crit. Rev. Food Sci. Nutr.* 44: 575-586.
- Vriens MR, Marinelli A, Harinck HI, Zwinderman KH, van de Velde CJ (2002;). "The role of allopurinol in human liver ischemia/reperfusion injury: a prospective randomized clinical trial." *Hepatogastroenterol.* 49: 1069-1073.
- Weinbroum A, Nielsen VG, Tan S, Gelman S, Matalon S, Skinner KA, Bradley E Jr, Parks DA (1995). "Liver ischemia-reperfusion increases pulmonary permeability in rat: role of circulating xanthine-oxidase." *Am. J. Physiol. Gastrointest. Liver Physiol.* 268: G988-G996.
- Weiss SJ (1990). "Tissue destruction by neutrophils." *N. Engl. J. Med.* 320: 365-376.
- Wisdom R (1999). "AP-1: One switch for many signals." *Exp. Cell Res.* 253: 180-185.
- Wu TJ, Khoo N, Zhoo F, Day BJ, Parks DA (2007). "Decreased hepatic ischemia-reperfusion injury by manganese-porphyrin complexes." *Free Radic. Res.* 41: 127-134.
- Yabe Y, Nishikawa M, Tamada A, Takakura Y, Hashida M (1999). "Targeted delivery and improved therapeutic potential of catalase by chemical modification: combination with superoxide dismutase derivatives." *J. Pharmacol. Exp. Ther.* 289: 1176-1184.
- Zhai Y, Qiao B, Shen XD, Gao F, Busuttil RW, Cheng G, Platt JL, Volk HD, Kupiec-Weglinski JW (2008). "Evidence for the Pivotal Role of Endogenous Toll-Like Receptor 4 Ligands in Liver Ischemia and Reperfusion Injury." *Transplantation* 85: 1016-1022.
- Zhang W, Li J, Zhou Y (2006). "Melatonin abates liver ischemia/reperfusion injury by improving the balance between nitric oxide and endothelin." *Hepatobiliary Pancreat. Dis. Int.* 5: 574-579.
- Zhang W, Wang M, Xie HY, Zhou L, Meng XQ, Shi J, Zheng S (2007). "Role of Reactive Oxygen Species in Mediating Hepatic Ischemia-Reperfusion Injury and Its Therapeutic Applications in Liver Transplantation." *Transplant Proc.* 39: 1332-1337.
- Zwacka RM, Zhou W, Zhang Y, Darby CJ, Dudus L, Halldorson J, Oberley L, Engelhardt JF (1998). "Redox gene therapy for ischemia/reperfusion injury of the liver reduces AP1 and NF-kappaB activation" *Nat. Med.* 4: 698-704.