Review

Divergent roles of superoxide and nitric oxide in liver ischemia and reperfusion injury

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Liver ischemia and reperfusion-induced injury is a major clinical complication associated with hemorrhagic or endotoxin shock and thermal injury as well as liver transplantation and resectional surgery. Data obtained from several different studies suggest that an important initiating event in the pathophysiology of ischemia and reperfusion-induced tissue injury is enhanced production of superoxide concomitant with a decrease in the bioavailability of endothelial cell-derived nitric oxide. This review will summarize the evidence supporting the hypothesis that the redox imbalance induced by alterations in superoxide and nitric oxide generation creates a more oxidative environment within the different cells of the liver that enhances the nuclear transcription factor-κB-dependent expression of a variety of different cytokines and mediators that may promote as well as limit ischemia and reperfusion-induced hepatocellular injury. In addition, the evidence implicating endothelial cell nitric oxide synthase-dependent and -independent generation of nitric oxide as important regulatory pathways that act to limit ischemia and reperfusion-induced liver injury and inflammation is also presented.

***Key Words*: peroxynitrite, NF-κB, free radicals, cytokines, nitrite**

Liver ischemia and reperfusion (I/R)-induced injury is a major complication associated with hemorrhagic or endotoxin shock and thermal injury as well as liver transplantation and resectional surgery. The vast majority of studies performed in experimental animals have used *in situ* models of liver I/R suggesting that data obtained from these investigations are most relevant to pathophysiological situations or surgical manipulations involving "warm" (37°C) I/R such as hemorrhagic or endotoxin shock or resectional surgery. Indeed, the pathophysiological mechanisms responsible for warm I/R-induced liver injury may be significantly different than those that occur with the cold ischemia (4°C) associated with liver storage prior to transplantation.[1,2] Nevertheless, a great deal of mechanistic information has been derived from *in situ* I/R studies.[3] These studies demonstrate that reperfusion of ischemic tissue initiates a cascade of molecular and cellular events that culminate in the superoxide anion radical ($O_2^{\bullet-}$) dependent, nuclear transcription factor-κB (NF-κB)-mediated expression of both injurious and protective mediators in favor of the former (Fig. 1).

Post-ischemic liver injury is biphasic in nature consisting of an acute or early phase and a subacute or late phase.[4,5] The early phase of injury occurs in the absence of leukocyte infiltration and is thought to be initiated by a rapid alteration in the redox state of the tissue in favor of a more oxidative environment. The late phase of injury is dependent upon the production of several different cytokines and chemokines that promote the infiltration of large numbers polymorphonuclear neutrophils (PMNs) and lymphocytes into the liver interstitium via the up-regulation of endothelial cell adhesion molecules and formation of chemotactic gradients.[6,7] Interstitial PMNs become fully activated and release copius amounts of reactive oxygen species (ROS) together with extracellular matrix degrading enzymes such as collagenase and matrix metalloproteases.[7] The net result of this inflammatory infiltrate is an amplification of the acute injurious response resulting in extensive inflammatory tissue injury.

Over the past 10 years, an emerging body of experimental data suggest that endothelial cell nitric oxide synthase (eNOS)-derived NO may limit ROS- and PMN-mediated tissue injury thereby regulating the subsequent inflammatory response *in vivo*. Indeed, it is becoming clear that reperfusion of ischemic tissue induces a rapid reduction in the bio-availability of NO which is thought to represent an important initiating event in the pathophysiology of post-ischemic injury in a variety of different tissues including the liver, heart, kidney and gut.[8–10] This chapter presents evidence supporting the hypothesis that: a) I/R enhances the generation of the $O_2^{\bullet-}$ and b) the I/R-induced decrease in the bioavailability of NO is mediated directly or indireclty by the $O_2^{\bullet-}$-dependent decomposition of endogenous NO. In addition to reducing the steady state levels of this protective nitrogen oxide, post-ischemic overproduction of $O_2^{\bullet-}$ as well as other ROS would also alter the redox potential of the liver creating a more oxidative environment within the major cell types of liver including the hepatocytes, Kupffer cells (KCs) and sinusoidal endothelial cells (SECs; Fig. 1). Increasing the redox potential within these cells would activate NF-κB thereby promoting the expression of pro-inflammatory cytokines (e.g., TNF-α, IL-12, IL-1β) that may injure the tissue directly in the acute phase as well as induce chemokine and endothelial cell adhesion molecule expression in the later subacute phase. Activation of this transcription factor would also induce the expression of certain protective and anti-apopototic genes that could limit tissue injury following I/R. The evidence implicating endogenous eNOS-dependent and-independent generation of NO as important regulatory pathways that limit the early and late phases of I/R-induced liver injury are also presented in this review.

Ischemia and Reperfusion Increases Reactive Oxygen Production, Decreases Nitric Oxide Bioavailability and Alters the Redox State of the Liver: *A recipe for disaster*

The early phase of hepatocellular I/R occurs within 1–6 h following reperfusion and is associated with Kupffer cell (KC) and possibly lymphocyte activation.[4–6,11] Data obtained from several different laboratories demonstrate that this acute, PMN-independent injury may be initiated by alterations in the redox

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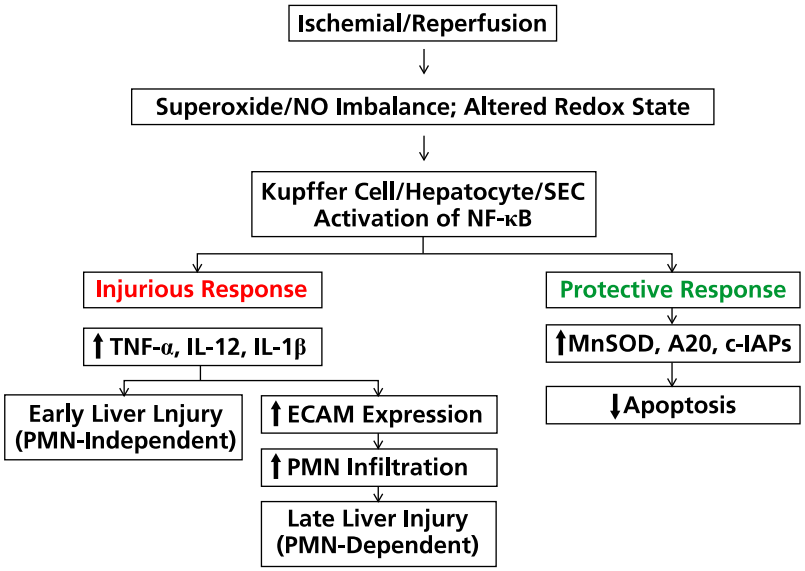

Fig. 1. NF-κB-dependent injurious and protective responses induced by liver ischemia and reperfusion. Ischemia and reperfusion of the liver induces an increase in the production of superoxide and other reactive oxygen species that may directly or indirectly decrease in the bioavailability of NO. This redox imbalance generates a more oxidative environment within the Kupffer cells, hepatocytes and sinusoidal endothelial cells that is thought to promote NF-κB-dependent expression of injurious and protective mediators.

state of the post-ischemic liver such that the redox potential becomes more positive thereby creating a more oxidative environment. Exactly how this happens has been the subject of active debate for more than a decade. We do know that one of the earliest events associated with I/R is a remarkable dysfunction of the SEC characterized by profound decreases in the steady state production of eNOS-derived NO.[8,12,13] This decrease in steady state levels of NO occurs very quickly (within 5 min of reperfusion) and appears to be due to decreased synthesis of NO, enhanced inactivation of NO by certain ROS or both. Co-incident with the decrease in NO production is the enhanced production of ROS such as $O_2^{\bullet-}$ and hydrogen peroxide (H_2O_2) during the first few minutes of reperfusion.[4,14–16] The cellular source(s) of these ROS are not known with certainty but several candidate enzymes/pathways have been suggested including hepatocyte xanthine oxidase and/or mitochondrial electron transport as well as KC- and SEC-associated NADPH oxidase.[4,14,15,17] Interest in xanthine oxidase has waned over the years in favor of mitochondrial sources of ROS and/or KC-associated NADPH oxidase.[7,15,18] It is well known that certain oxygen-or hemoprotein-derived free radicals such as $O_2^{\bullet-}$ and ferryl (Fe^{+4}) hemoproteins may interact with and decompose NO.[19–22] In addition, H_2O_2 can indirectly decrease steady state levels of NO via the peroxidase-catalyzed consumption of NO in different tissues.[23,24] Therefore, I/R-induced over-production of ROS not only reduces (or eliminates) the important vasoactive mediator and cytoprotective NO free radical, but it also induces the rapid oxidation of cellular GSH creating a more positive redox potential within the liver.[12,16,25,26] Indeed, a number of different studies, using pharmacologic interventions or genetic approaches have demonstrated that the protective effects of certain nonenzymatic or enzymatic antioxidants administered prior to inducing liver ischemia correlates with restoration of GSH levels within the tissue.[11,16,26]

Superoxide is the major ROS involved in post-ischemic liver injury. There is a large body of work suggesting that the major ROS responsible for post-ischemic hepatocellular injury is $O_2^{\bullet-}$. A variety of different studies have demonstrated that adenoviral transfection of the liver with manganese superoxide dismutase (MnSOD)[14,16] as well as exogenous administration of long-lived SODs attenuate I/R-induced injury suggesting that $O_2^{\bullet-}$

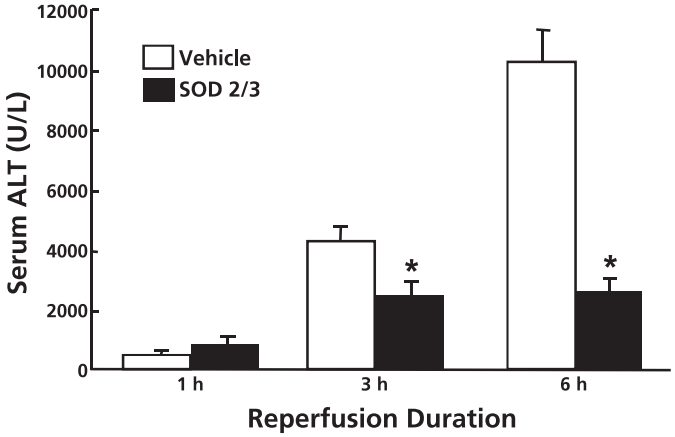

Fig. 2. Effect of SOD2/3 on ischemia and reperfusion-induced liver injury. Mice were treated with the fusion protein SOD2/3 (1,000 U/kg, iv) or vehicle 15 min prior to being subjected to 90 min of ischemia and 6 h of reperfusion. *: $p<0.05$ vs vehicle-treated mice controls. $n\geq6$ animals per group. Reproduced from (3) with permission.

per se rather than secondary oxidants is directly or indirectly responsible for I/R-induced tissue damage (Fig. 2).[3,15,27–29] A number of cellular sources of $O_2^{\bullet-}$ have been proposed to account for post-ischemic tissue injury in the absence of an inflammatory infiltrate. The most likely candidate is KC-associated NADPH oxidase although SEC NADPH oxidase may play an as yet-to-be discovered role. This multimeric $O_2^{\bullet-}$-producing enzymatic complex has been well-characterized in phagocytic leukocytes and Kupffer cells. Several studies have determined that KC-derived NADPH oxidase is critically involved in promoting post ischemic liver injury.[15,30,31] Exactly how cell-derived $O_2^{\bullet-}$ promotes liver injury is not known but emerging data suggest that multiple mechanisms are likely involved. Several studies had originally suggested that $O_2^{\bullet-}$ dependent lipid peroxidation represented an important pathway for I/R-induced damage; however other investigations cautioned that oxidative degradation

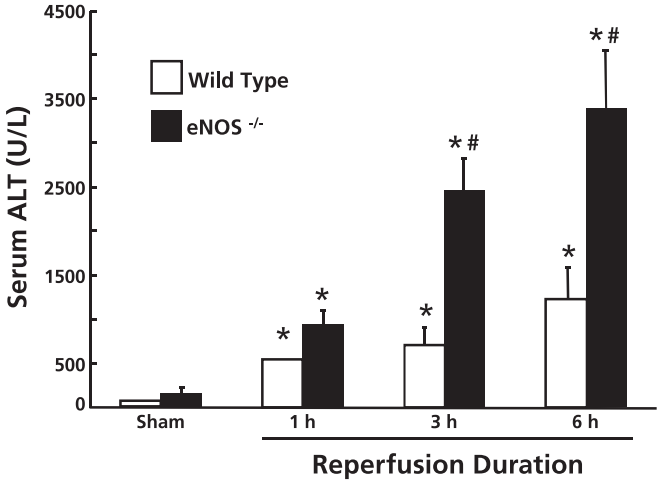

Fig. 3. Effect of eNOS deficiency on ischemia and reperfusion liver injury. eNOS deficient ($eNOS^{-/-}$) or wild type mice were subjected to 45 min of ischemia and 6 h of reperfusion. *: $p<0.05$ vs sham-operated mice; #: $p<0.05$ vs time matched wild type muse. $n\geq 6$ animals per group. Data reproduced from (3) with permission.

of membrane lipids may, in reality, be a consequence rather than a cause of I/R-induced liver injury.[32] More recent studies suggest that $O_2^{\bullet-}$ may directly or indirectly mediate cell and tissue injury by rapidly interacting with and damaging mitochondrial membrane *proteins* leading to the loss of inner membrane potential and ATP generating capacity.[18,33]

Another mechanism that has been proposed to account for $O_2^{\bullet-}$ dependent injury to the postischemic liver is through the generation of even more potent oxidants via its interaction with NO. This interaction is known to produce the potent cytotoxic oxidizing and nitrating specie peroxynitrite ($ONOO^-$) and its conjugate acid peroxynitrous acid (ONOOH).

$$O_2^{\bullet-} + NO \rightarrow ONOO^- + H^+ \longleftrightarrow ONOOH$$

Although an attractive hypothesis, the data *do not* support a role for this pathway. Indeed, we have found that I/R-induced liver injury in eNOS-deficient ($eNOS^{-/-}$) mice or wild type mice pretreated with the NOS inhibitor N(G)-nitro-L-arginine methyl ester (L-NAME) exacerbates liver injury suggesting that NO is protective in nature (Fig. 3).[34] In addition to its direct biochemical effects, $O_2^{\bullet-}$ may mediate hepatocellular damage indirectly via the up-regulation (or down-regulation) of certain redox-sensitive genes known to be important in cell proliferation, apoptosis and the inflammatory response.[10,35] In addition to the above mentioned mechanisms, there is good evidence to suggest that I/R-induced $O_2^{\bullet-}$ production may mediate hepatocellular injury and inflammation by creating a more oxidative environment within the different cells of the liver thereby activating redox-sensitive transcription factors such as NF-κB.[4,14,36–39]

Ischemia and reperfusion activates NF-κB: a link between ROS generation and hepatocellular injury? Studies from several different laboratories have shown that I/R-induces the rapid up regulation of several different cytokines (TNF-α, IL-1β, IL-2, IL-6, IL-8, IL-12), endothelial cell adhesion molecules (ICAM-1, VCAM-1, E-selectin and mucosal addressin cell adhesion molecule-1), CXC chemokines (macrophage inflammatory protein-2, keritinocyte-derived chemokine, and epithelial neutrophil-activating peptide) as well as nitric oxide synthase-2 (iNOS) and cyclooxygenase-2 (COX2).[39] The expression of the majority of these genes is regulated by the transcription factor NF-κB and thus there is great interest in understanding how the molecular events associated with I/R-mediated activation of NF-κB and the subsequent induction and roles of the different cytokines, chemokines, adhesion molecules and enzymes (Fig. 4). Indeed, it is now well-appreciated that the expression of TNF-α, IL-1β and IL-12 are important for mediating post-ischemic liver injury.[15,39,40]

A large body of experimental data suggest $O_2^{\bullet-}$ and/or H_2O_2 may act as downstream signaling and/or costimulatory molecules required for the activation of NF-κB.[41] Although an attractive hypothesis that continues to receive substantial attention in the literature, there is in fact little data directly demonstrating that ROS mediate NF-κB activation *in vivo*.[42,43] It may be that ROS function to activate NF-κB indirectly by promoting the expression of certain cytokines and/or mediators (e.g., TNF-α and IL-1β) which can in turn activate this transcription factor. Indeed, it has been demonstrated that a major mediator of post-ischemic liver injury is TNF-α.[15,44] The mechanisms by which TNF-α promotes hepatocellular injury are not entirely clear but are most likely multifactorial. Previous studies have suggested that much of the post-ischemic liver injury may be a result of TNF-α-induced endothelial cell and hepatocyte apoptosis.[45] It is known that TNF-α will induce hepatocyte injury via the ROS-dependent activation of numerous cell signaling pathways resulting in apoptosis.[38] However, Jaeschke *et al.* found, using well-defined histopathological criteria, that virtually all liver injury occurred by "osmotic necrosis" with very little injury occurring by way of apoptosis.[45] Nevertheless, it should be remembered that cells that begin to die by apoptosis may also undergo necrosis if intracellular ATP is low or absent as would be the case during ischemia.

It is clear that I/R-induced activation of NF-κB within KCs and SECs induces an injurious/pro-inflammatory response via the upregulation of numerous cytokines, enzymes and chemokines (Fig. 1).[38,39] However, the function of NF-κB in hepatocytes appears to be more complicated (Fig. 1). Emerging evidence suggests that NF-κB activation may be to protect a variety of cells from the injurious effects of certain pro-inflammatory cytokines such as TNF-α.[38,39] It is well known that pre-treatment of cultured cells with non-lethal concentrations of TNF-α protects these cells from a subsequent challenge by lethal amounts of this same cytokine and that this protective response is dependent upon NF-κB activation.[38,39] In addition, IKKα has been shown to be required for embryonic development of skin and limbs whereas IKKβ appears essential for normal liver development with massive liver apoptosis, injury and embryonic lethality occurring in IKKβ-deficient mice.[38,39] Similar effects have been observed in mice that genetically lack the p65 (relA) component of NF-κB. Taken together, these studies suggest that NF-κB activation is essential for normal liver development and that inhibition of NF-κB nuclear translocation promotes liver injury via massive apoptosis. Studies by Brenner and coworkers have confirmed that over-expression of liver IκB-α in mice inhibited NF-κB activation resulting in massive hepatocyte injury and apoptosis following partial hepatectomy in these animals.[38,39,46] The mechanisms by which NF-κB activation protects the liver are only now becoming understood. It is known that NF-κB activation induces several "anti-apoptotic" genes including MnSOD, a zinc finger protein termed A20, cellular inhibitors of apoptosis-2 (c-IAP-2), and Bcl-2 family members such as A1. Most of these genes (i.e. MnSOD, A20 and A1) are also known to inhibit NF-κB activation and thus may control the expression of certain pro-inflammatory genes (Fig. 1).

Despite the recent body of experimental data demonstrating that NF-κB may limit hepatocyte injury, there is also an equally convincing body of data demonstrating that I/R-induced, TNF-α-mediated liver injury is mediated by NF-κB activation. For example, it has been shown that much of the post-ischemic liver injury is abrogated in TNF-α-deficient mice.[47] These are important studies as they demonstrate that I/R-induced liver injury is

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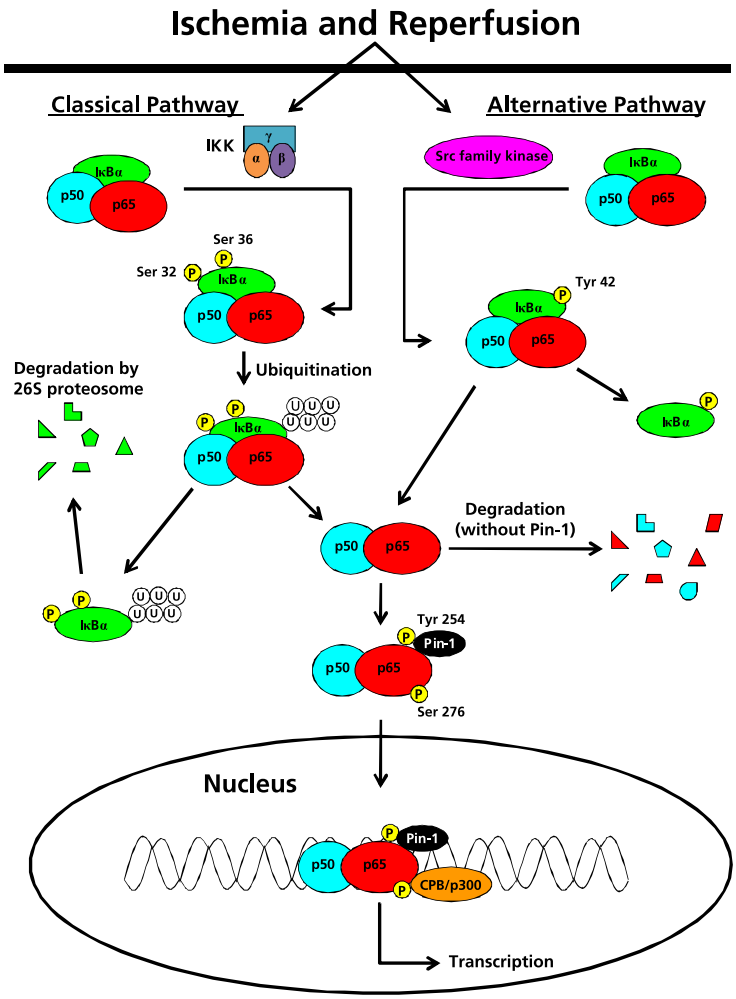

Fig. 4. Classical and Alternative Pathways for ischemia and reperfusion-induced activation of NF-κB. Derived from (39).

attenuated in the complete absence of NF-κB activation ie in the absence of both the injurious/inflammatory and the protective responses. It may be that the balance between injurious vs protective genes upregulated during I/R as well as the cellular location of NF-κB activation dictates whether NF-κB activation will promote injury or protection. A more direct approach to the role of hepatocyte NF-κB in the pathophysiology of post-ischemic tissue injury has been provided by investigators who showed that I/R-induced injury was attenuated in mice with hepatocyte-specific ablation of IKKβ.[48] They found that the protective effect was associated with decreased expression of hepatocyte-derived TNF-α.[48] In apparent contradiction to these results, these same investigators reported that I/R-induced liver injury in mice with a hepatocyte-specific deletion of IKKγ (NEMO) was dramatically increased compared to wild type mice suggesting a protective effect of hepatocyte NF-κB.[49] In an attempt to reconcile these seemingly contradictory results, the authors suggested that hepatocyte-specific deletion of IKKβ may possess some residual NF-κB activity whereas IKKγ (NEMO)-deficient hepatocytes have no detectable NF-κB activity and thus no protective response to limit liver damage. Taken together, these data suggest that further work is needed to define the role of hepatocyte NF-κB.

Role of Leukocytes in I/R Injury

The early phase of I/R injury is a PMN-independent process in which KCs are activated, ROS are generated by these resident phagocytes and by dysfunctional mitochondria in KCs, SECs and hepatocytes and proinflammatory cytokines (TNF-α, IL-12, IL-1β) are synthesized. This initial response induces the upregulation of additional inflammatory mediators including activated complement factors (e.g., C5a), platelet activating factor and different CXC chemokines, such as interleukin-8 (IL-8), macrophage inflammatory protein-2 (MIP-2), keratinocyte-derived chemokine, and cytokine-induced neutrophil chemoattractant (CINC-1).[7] These inflammatory mediators promote the enhanced expression of CD11b/CD18 which prime PMNs and promote their extravasation from the sinusoids and post-sinusoidal venules into the tissue thereby initiating the late or subacute phase of post-ischemic tissue injury. This inflammatory response is characterized by the activation of PMNs resulting in the production of large amounts of ROS and release of a variety of different proteases (gelatinase, collagenase). The net result of this acute inflammatory response is oxidant-mediated mitochondrial dysfunction as well as degradation of the interstitial matrix and damage to the parenchymal cells.[7] In addition, TLR4-mediated production of cytokines and inflammatory mediators may represent an additional important pathway for the amplification of the inflammatory response. Although it is well-recognized that PMNs play an important role in the pathophysiology of liver I/R injury, other leukocytes may also be involved in this innate immune response. For example, it has been reported that CD4+ T-cells

play an important role in recruiting PMNs into the post-ischemic liver and that CD4+ T-cell-deficiency remarkably attenuated both PMN infiltration and tissue injury following I/R.[50] However, more recent studies have found that CD4+ T-cell-deficient mice responded to liver I/R with an exacerbation in liver injury despite the fact that these mice displayed fewer PMNs infiltrating the post-ischemic liver.[6] The mechanisms responsible for these seemingly contradictory results are not clear at the present time but may relate to the suppressive or regulatory nature of the infiltrating CD4+ T-cells in the later study.

Suppression of I/R-Induced Liver Damage by Endogenous or Exogenous Nitric Oxide

It is becoming increasingly appreciated that I/R results in rapid endothelial dysfunction in a number of different tissues that is characterized by a marked decrease in steady-state production of endothelial cell-derived NO.[8,12,51,52] The decrease in NO bioavailability occurs within the first few minutes following reperfusion and appears to be due to decreased synthesis of NO, enhanced inactivation of NO by $O_2^{\bullet-}$ (or other ROS) or both. Because endogenous NO is known to interact with and rapidly decompose certain ROS such as $O_2^{\bullet-}$, $HO^{\bullet}$, and ferryl hemoproteins, decreased steady state production of NO would in all likelihood increase the redox potential of hepatocytes, KCs and/or SECs in favor of a more oxidative environment.[26,52] Indeed, we have demonstrated that many of the pathophysiological characteristics induced by I/R (i.e. oxidative stress, leukocyte adhesion, enhanced vascular permeability) may be recapitulated in normal tissue by administration of certain NOS inhibitors[12,51,52] suggesting that eNOS-derived NO may act as an endogenous anti-inflammatory mediator in the microcirculation.

The use of certain NOS inhibitors to evaluate the role of NO *in vivo* has proven problematic. It is known for example that L-NAME and L-NMMA are nonspecific inhibitors of both eNOS and iNOS as well as potent vasoconstrictors *in vivo*.[53] Thus, the non-selective nature of these NOS inhibitors coupled to the fact that their exacerbatory effects may be due to their vasoconstrictor effects in a model of hypoperfusion makes their usefulness less than ideal to probe the role for NO in liver I/R. The potentially protective role of endogenous NO in liver I/R injury is supported by studies demonstrating enhanced hepatocellular injury in post-ischemic animals rendered deficient in eNOS (Fig. 3).[34,54] In addition, investigators have demonstrated that NO donors or over-expression of liver eNOS protects mice from liver I/R-induced injury.[55–57] Although eNOS has been shown by several groups of investigators to play a critical protective role in liver I/R injury, the role of iNOS is less clear. Studies from our laboratory suggest that I/R-induced liver injury is enhanced in iNOS-deficient mice however interpretation is complicated by the fact that we could not demonstrate upregulation of iNOS message in post-ischemic livers of wild type mice suggesting that the source of iNOS is not in the liver (e.g., in the intestine) or that a small population of cells such as KCs or SECs upregulate iNOS which we are unable to detect in whole liver preparations.[58,59]

The mechanisms by which endogenous NO protects the liver against the injurious effects of I/R are now beginning to be defined (Fig. 5). NO is known to interact with and decompose $O_2^{\bullet-}$ or other reactive radicals or oxidants thereby limiting the formation of $O_2^{\bullet-}$-derived oxidants and preventing the downstream oxidant-induced signaling pathways.[25] If this antioxidant/free radical scavenging activity of NO is a major protective mechanism, then one would predict that administration of a long lived SOD such as the fusion protein SOD2/3 to *eNOS-deficient* mice would attenuate I/R-induced injury to the liver. In fact, we found that although SOD2/3 markedly attenuates post-ischemic injury in wild type mice, it does not attenuate liver injury in eNOS-deficient animals.[34] These data suggested that the protective effect of

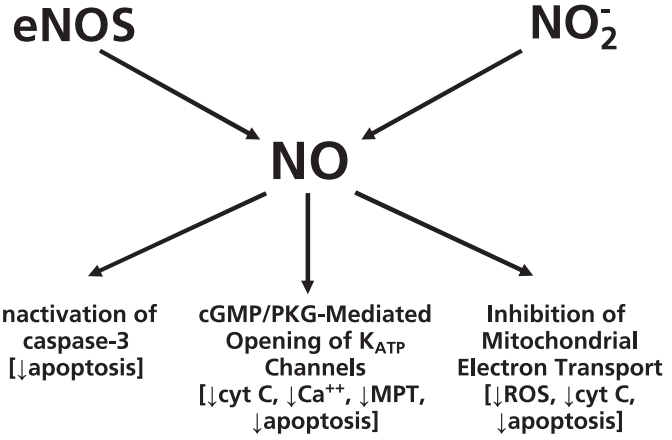

Fig. 5. Proposed cytoprotective mechanisms for eNOS- or nitrite (NO_2^-)-derived nitric oxide (NO). NO derived from eNOS or NO_2^- may protect tissue subjected to ischemia and reperfusion by: a) Inactivation of caspase-3 via the NO-dependent S-nitrosation of the protein; b) cGMP/protein kinase G (cGMP/PKG)-mediated opening of mitochondrial ATP-dependent potassium channels (K_{ATP}) which reduces the loss of cytochrome C, decreases calcium accumulation within the mitochondria, prevent the opening of the mitochondrial permeablility transition (MPT) pore and decrease apoptosis; c) Inhibition of mitochondrial electron transport via the direct or indirect (S-nitrosation) inhibition of Complex I (and possible Complex IV) resulting in decreased reactive oxygen specie (ROS) generation, reduced cytochrome C release and decreased apoptosis.

SOD2/3 in wild type mice is due to its ability to increase the bioavailability of NO by preventing the $O_2^{\bullet-}$ mediated inactivation of NO. We then return to the question: How does NO limit I/R-induced tissue damage? There is data suggesting that NO inhibits NF-κB activation at different levels. For example, DeCaterina and coworkers provide evidence that NO enhances the denovo synthesis and/or stabilization of the natural inhibitor IκB-α.[60] In addition, NO-mediated S-nitrosation of a specific cysteine on the p50 and/or p65 subunits of NF-κB results in inhibition of binding of this heterodimer to it consensus sequences upstream of the different pro-inflammatory genes.[61–63] Another possible mechanism may be that NO-dependent activation of soluble guanylyl cyclase (sGC) with the subsequent production of the vasorelaxant cGMP may protect against reperfusion injury by enhancing blood flow thereby limiting the degree of ischemia to the liver. It has also been proposed that NO-mediated activation of protein kinase G via the sGC/cGMP pathway opens mitochondrial K_{ATP} channels thereby reducing calcium accumulation within the mitochondria and preventing the loss of cytochrome c from the mitochondrial intermembranal space.[9] Alternatively or in addition to, NO may reversibly inhibit mitochondrial respiration via interaction with complex I and/or cytochrome c oxidase. This would inhibit apoptosis, maintain small but significant amounts of oxygen during ischemia and allow for a more controlled resumption of respiration following reperfusion.[9] Similar observations have been made with NO-dependent S-nitrosation of caspase-3 resulting in inactivation of this enzyme and inhibition of apoposis.[9] This would minimize free radical-mediated damage to the mitochondrial membrane and preserve cellular function. NO may attenuate the later stages of post-ischemic tissue damage by inhibiting platelet/leukocyte-endothelial cell interactions.[12,51,64]

There is ample evidence demonstrating that eNOS-derived NO as well as exogenous NO-releasing compounds attenuate I/R-induced liver injury.[13,34,55,57,65] More recent studies demonstrate that animals and humans are capable of producing substantial quantities of NO via eNOS-*independent* pathways that utilize nitrogen oxides that are produced from the decomposition of NO

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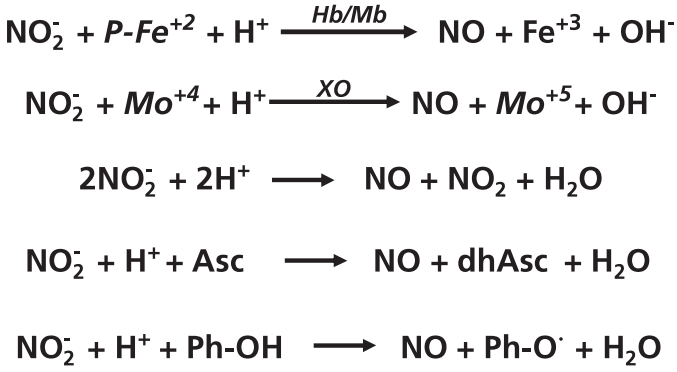

Fig. 6. Proposed mechanisms for the conversion of nitrite (NO_2^-) to nitric oxide (NO). P-Fe^{+2} represents ferrous hemoglobin (Hb) or myoglobin (Mb). Mo^{+4} represents molybdenum at the active site of xanthine oxidase (XO); Asc and Ph-OH represent ascorbic acid and an aromatic phenolic compound, respectively. Figure derived from reference (66).

or are ingested in the diet. Like any important signaling molecule, the generation and lifetime of NO is very short-lived *in vivo*. Indeed, NO may be rapidly inactivated by a variety of different mechanisms.[66] A physiologically-relevant set of pathways for the inactivation of NO are mediated by metal-catalyzed oxidation reactions. The copper-containing protein ceruloplasmin has been shown to rapidly oxidize NO to NO_2^- under physiological conditions. In addition to ceruloplasmin, ferrous dioxygenated hemoglobin (Hb-$Fe^{+2}O_2$; oxyhemoglobin) or myoblobin rapidly decomposes NO to yield *nitrate*.

Historically, it has been assumed that blood and tissue nitrate and nitrite simply reflect inert endproducts of NO metabolism and food contaminants. However, it is now recognized that these oxidized metabolites of nitrogen can be metabolized *in vivo* to regenerate NO (Fig. 6).[66] Because the rate of NO generation from nitrite is linearly dependent on reductions in tissue pO2 and pH, nitrite may be metabolized to NO in ischemic tissue (via deoxyHb or Mb) and exert NO-dependent protective effects site-specifically. Indeed, the tissue-specific reduction of nitrite to generate cytoprotective NO has important clinical significance in that treatment of different ischemic disorders with nitrite would reduce the untoward cardiovascular effects of systemic delivery of inhaled NO or intravenous administration of S-nitrosothiols. A growing number of studies demonstrate that nitrite is cytoprotective in different models of tissue I/R including the liver, heart, kidney and brain (reviewed in Ref. 9). The clinical usefulness of nitrite is currently being investigated as a potential therapeutic agent to treat ischemic disorders.

Concluding Remarks

There is now overwhelming evidence supporting the concept that I/R-induced tissue damage is associated with the $O_2^{\cdot -}$-dependent decrease in steady-state levels of NO. Furthermore, accumulating evidence suggests that eNOS-dependent as well as eNOS-independent generation of NO acts to limit post-ischemic tissue injury. The mechanisms by which this nitrogen oxide protects the post-ischemic liver remain to be defined and are the subject of active investigation. Therapeutic uses of NO or nitrite are currently underway in several different laboratories to treat a variety of ischemic disorders.

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