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TOPIC HIGHLIGHT

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Role of nitric oxide in hepatic ischemia-reperfusion injury

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Abstract

Hepatic ischemia-reperfusion injury (IRI) occurs upon restoration of hepatic blood flow after a period of ischemia. Decreased endogenous nitric oxide (NO) production resulting in capillary luminal narrowing is central in the pathogenesis of IRI. Exogenous NO has emerged as a potential therapy for IRI based on its role in decreasing oxidative stress, cytokine release, leukocyte endothelial-adhesion and hepatic apoptosis. This review will highlight the influence of endogenous NO on hepatic IRI, role of inhaled NO in ameliorating IRI, modes of delivery, donor drugs and potential side effects of exogenous NO.

Key words: Nitric oxide; Liver; Ischemia-reperfusion injury; Drug delivery

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INTRODUCTION

Ischemia-reperfusion injury (IRI) is a series of multifaceted cellular events that takes place on the resumption of oxygen delivery after a period of hypoxia. This injury could be severe enough to lead to a significant morbidity and mortality.

The liver may be involved in IRI in procedures that are associated with sequential vascular impediment and restoration of blood flow; for example hepatic resections and orthotopic liver transplantation. During these procedures, unclamping of the vascular inflow to the liver after a temporary period of cross clamping results in major hepatocellular damage.

Nitric oxide (NO) has various protective effects on cells during IRI. NO has been demonstrated to inhibit oxidative stress, cytokine release, leukocyte endothelial adhesion and apoptosis[1]. On a cellular-signaling level, NO effects are mediated *via* redox-sensitive sites, and include: inhibition of protein kinase C, activation of tyrosine kinase, inactivation of nuclear factor (NF)-κB and activation of G proteins[2]. Previous studies have demonstrated that a reduction of NO during hepatic IRI, generally *via* a reduction in endothelial nitric oxide synthase activity, leads to liver injury[3]. Inhaled NO or NO donor drugs are novel treatments that have been used clinically to attenuate liver IRI[4]. This review will discuss the pathophysiology of liver involvement during IRI, and the clinical use of nitric oxide in ameliorating the impact of liver IRI.

BRIEF REVIEW OF THE PATHOPHYSIOLOGY OF IRI

The complex mechanisms of IRI have been revealed by advanced molecular biology[5] (Figure 1). During the isch-

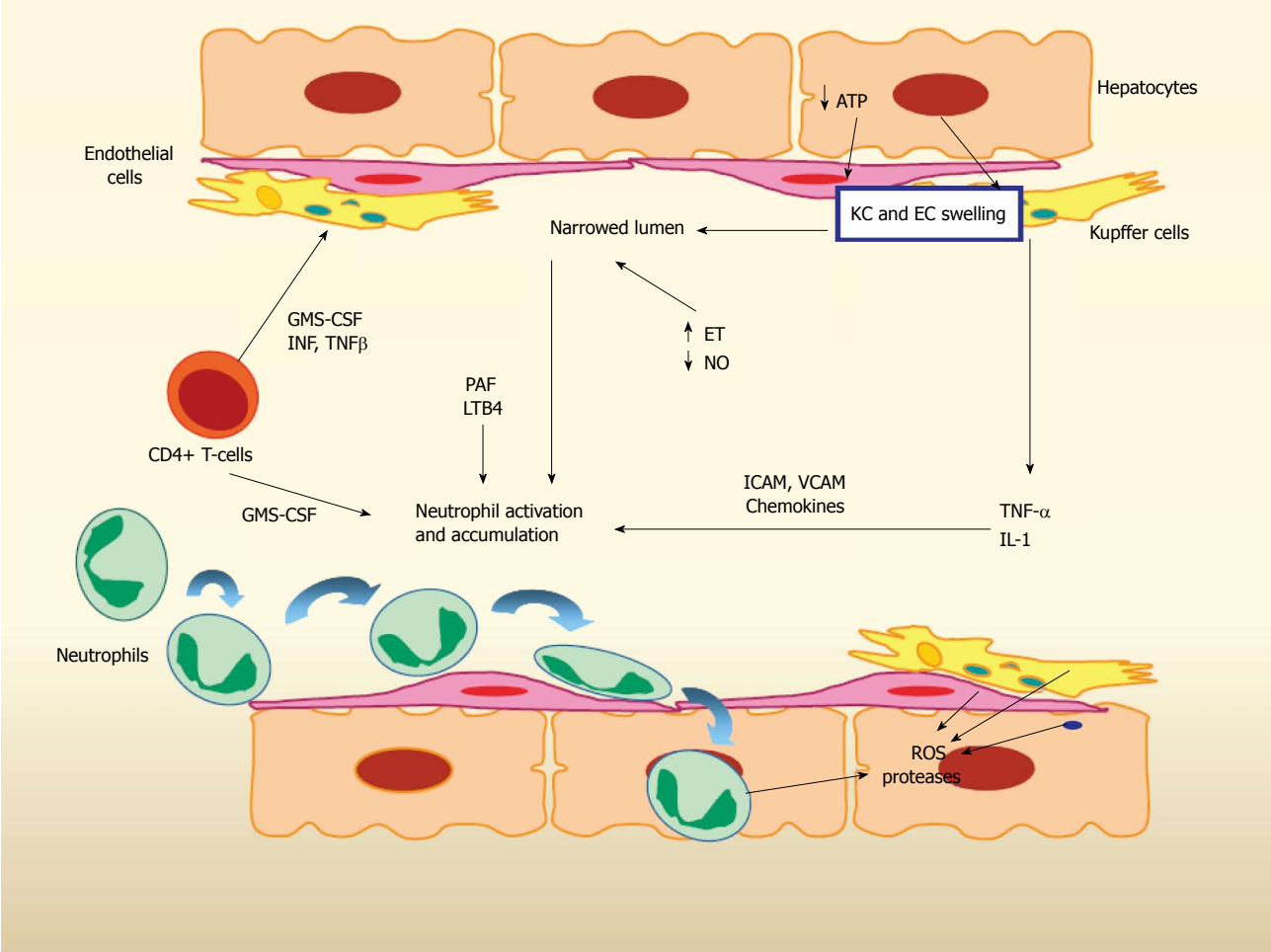

Figure 1 Multifaceted hepatic ischemia-reperfusion injury. Kupffer and endothelial cells produce cytokines and chemokines, recruiting neutrophils that further accentuate injury. EC: Endothelial cell; KC: Kupffer cell; ATP: Adenosine triphosphate; TNF: Tumor necrosis factor; IL: Interleukin; ICAM: Intercellular adhesion molecule; VCAM: Vascular adhesion molecule; PAF: Platelet activation factor; LTB4: Leukotriene B4; GMS-CSF: Granulocyte macrophage colony stimulating factor; INF: Interferon; ROS: Reactive oxygen species (Courtesy of Dr. Joan Rosello-Catafau, Barcelona, Spain).

emic phase, anaerobic metabolism ensues and produces an inadequate amount of high-energy phosphates which are fundamental to most cellular functions. Low levels of high-energy phosphates affect a myriad of cellular functions: homeostasis, signaling interactions, cellular proliferation and processing of the apoptotic death cycle. Adenosine triphosphate (ATP) depletion impairs sodium/potassium ATPase (Na^+/K^+-ATPase) function, resulting in an impairment of the efflux of sodium from the cell. Additionally, toxic metabolites, which are generated during ischemia, attract free water into ischemic cells and organelles leading to the formation of cellular edema[6]. If the ischemic insult lasts greater than 24 h, it is likely that ATP-synthase activity becomes irreversible after blood restoration, leading to cellular necrosis, apoptosis or neuroapoptosis[7]. Ischemia also causes an increased expression of adhesion molecules that leads to endothelial cell and neutrophil adhesion, resulting in vascular studding and occlusion[8]. Furthermore, disequilibrium between NO and endothelin (ET) induces vasoconstriction and subsequent microcirculatory failure even though blood circulation has been re-established[9]. Re-establishment of blood flow will serve to amplify inflammation with consequent injury that is highly variable but dependent on numerous variables including the extent of mediators produced (i.e. reactive oxygen species), the degree of endothelial and neutrophil adhesive responses and the degree of Kupffer cell activation.

PRINCIPAL PARTICIPANTS IN LIVER IRI

Sinusoidal endothelial cells

Injury to these cells is initiated during cold ischemia whereby Ca^{2+}-ATPase results in the accumulation of intracellular calcium[10]. Following this event, a series of actions occur making the endothelium more susceptible to platelet adhesion and reduced sinusoidal flow.

Kupffer cells

Kupffer cells are crucial in liver injury orchestration. Metabolic alterations of these cells occur during no-flow ischemia leading to the formation of reactive oxygen species during early reperfusion[11]. Additionally, at the onset of reperfusion, Kupffer cells undergo further activation by Toll-like receptor 4 signaling and/or by complement. Subsequently, Kupffer cells release pro-inflammatory cytokines such as TNF-α and interleukin-1 which them-

selves can perpetuate inflammatory injury by such means as leukocyte activation.

Hepatocytes

While major participants in the promotion of injury, during cold ischemia hepatocytes undergo intracellular bioenergetic perturbations that reduce ATP stores due to mitochondrial dysfunction and predispose these cells to injury during reperfusion[12].

Leukocytes and lymphocytes

As a result of IRI, cellular adhesion molecules (i.e. intercellular adhesion molecule-1 or ICAM-1, vascular adhesion molecule-1 or VCAM-1), selectins and integrins are activated and upregulated on the surface of endothelial cells, neutrophils and platelets. The activated neutrophils adhere to endothelial cells at the initial stages of reperfusion, and subsequently transmigrate across the endothelium where they continue to injury orchestration. The accumulation of activated neutrophils contributes to microcirculatory disturbances both locally and remotely. Activated neutrophils release reactive oxygen species, specifically superoxide radical (O_2^-•), proteases and various cytokines[13]. Monocytes and macrophages are also activated shortly following reperfusion[14]. Recent studies propose an important role for lymphocytes, especially $CD4^+$ T cells, in augmenting injury responses after IRI. However, lymphocytes may also play a protective role, but this is probably dependent on cell type and time course of injury[15].

Reactive oxygen species and reactive nitrogen species

During periods of ischemia, reactive oxygen species (ROS) and reactive nitrogen species (RNS) are generated which can promote intracellular damage. Due to electron transport chain alterations, mitochondrial dysfunction ensues leading to reductions in ATP production and with subsequent loss of inner membrane stability resulting in mitochondrial swelling and rupture. With the reintroduction of oxygen during reperfusion, ROS are produced due to reactions of oxygen introduced during reperfusion with xanthine oxidase. ROS serve to stimulate other cell lines including Kupffer cells to produce proinflammatory cytokines[16]. The major ROS are hydroxyl radical (OH•) and hydrogen peroxide (H_2O_2). Reactions of ROS such as O_2^-• with NO yield products such as peroxynitrite ($ONOO^-$), a RNS which can be an extremely aggressive oxidant.

Cytokines

Cytokines play a vital role in IRI, both by inducing and sustaining the inflammatory response, and by modulating IRI severity. Tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1) are the two cytokines most commonly implicated in liver IRI. TNF-α is a pleiotropic cytokine generated by various different cell types in response to inflammatory and immunomodulatory stimuli. TNF-α modulates leukocyte chemotaxis and activation, and induces ROS production in Kupffer cells[17]. Additionally, IL-1 is known to promote production of ROS, induce TNF-α synthesis by Kupffer cells and induce neutrophil recruitment[18].

Complement

The complement system also contributes significantly to IRI and is composed of approximately 30 soluble and membrane-bound proteins. This system can be stimulated in three pathways: (1) the antibody-dependent classical pathway; (2) the alternative pathway; or (3) the mannose-binding lectin pathway[19]. Complement, when activated, acts as a membrane-attacking complex that stimulates the production of proinflammatory cytokines and chemotactic agents. Furthermore, it can regulate adaptive immunity[20].

THE INFLUENCE OF ENDOGENOUS NO ON LIVER IRI

Damage to the liver due to IRI is a culmination of inflammatory cross talk with the principal participants mentioned previously. IRI is the main cause of liver injury in response to vascular clamping during hepatic procedures such as hepatectomy and liver transplantation. This insult on the liver results in disturbances of the sinusoidal microcirculation and the generation of a variety of mediators such as ROS, cytokines, activation of chemokines and other cell signaling molecules previously mentioned.

Hepatic IRI can cause severe hepatocellular injury that contributes to morbidity and mortality after liver surgery. As briefly mentioned previously, reductions of NO during liver IRI occur and are associated with increased liver injury[3]. This is now appreciated to be due to decreases in NO steady state production resulting from low concentrations of endothelium-derived nitric oxide synthase (eNOS). This event coupled with NO inactivation due to reactions with abundant ROS, such as O_2^-•, results in reduced NO bioavailability. The consequences of this reduced bioavailability include, but are not exclusive to, increased oxidative stress, increased apoptosis, increased leukocyte adhesion, increased microcirculatory tone, and perturbed mitochondrial function. Interestingly, restoration of NO to more "physiologic" concentrations serves to diminish the liver ischemic injury *via* countering of the adverse actions mentioned previously. Studies have demonstrated findings that are consistent with the premise that eNOS is crucial for minimizing injury during liver IRI. For example, liver injury was demonstrated to be less in wild type mice compared to eNOS knockouts ($eNOS^{-/-}$)[21] (Figure 2), in addition to the findings that agents given to increase eNOS expression or donate NO afford greater liver IRI protection[22,23]. It is also well established that the NO concentrations during various inflammatory states are significantly increased by increasing expression of inducible nitric oxide synthase (iNOS). However, the influence of iNOS and its true contribution in conferring liver protection (or not) deserves additional studies. In a rat model of liver IRI, iNOS expression was significantly increased correlating with increases in iNOS RNA at 1 and 5 h[24]. This is consistent with other studies measuring iNOS expression in conditions of liver IR. In

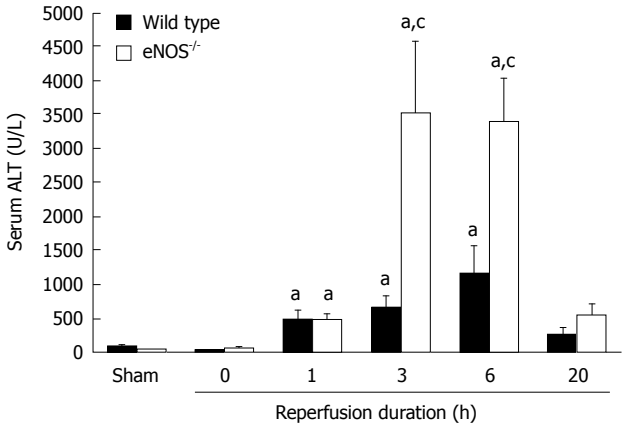

Figure 2 Increased liver injury as assessed by serum alanine aminotransferase in endothelium-derived nitric oxide synthase knockout mice compared with their wild type controls. $^{a}P < 0.05$ *vs* sham-operated controls. $^{c}P < 0.05$ *vs* time-matched wild type control. ALT: Alanine aminotransferase; eNOS: Endothelium-derived nitric oxide synthase (Courtesy of Dr. Ianes N. Hines, Chapel Hill, NC).

a porcine model of IRI, intraportal injection of the selective iNOS inhibitor, aminoguanidine, was demonstrated to decrease injury[25]. In an intriguing study, iNOS knockout mice (*iNOS*$^{-/-}$) exposed to warm liver IRI demonstrated a much greater magnitude of injury compared to wild type mice. Of notable interest was the finding that even though injury was greater in the iNOS knockout mice, little to no iNOS RNA was detectable in the wild type mice. It would appear that for now, the true influence of iNOS on liver injury during IR remains unclear.

A number of other endogenous NO-mediated mechanisms thought to confer protection have been published. For example, NO has been shown to inhibit caspase proteases *via S*-nitrosylation, thereby inhibiting apoptosis[26]. This appears to be somewhat concentration-dependent. Low physiological concentrations of NO may inhibit apoptosis. In contrast, higher concentrations may lead to the formation of toxic products such as $ONOO^{-}$ or other ROS which lead to cell necrosis and apoptosis[27]. Other published mechanisms of NO-mediated protection include inhibition of NF-κB[28], reversible inhibition of mitochondrial complex I, and decreased mitochondrial calcium accumulation[29]. As to be expected, controversy exists concerning "if" and "how" NO exerts cellular protection. For instance, in a study by Jaeschke *et al*[11], administration of a NO synthase inhibitor did not attenuate or accentuate liver injury during the initial reperfusion period. Inhibition of NO was observed not to influence neutrophil migration to the injured sites. While this contradicts a number of other studies, based on their findings, the authors concluded that NO availability was unlikely to be involved in the post-ischemic oxidant stress and reperfusion injury[30]. Nevertheless, the majority of published literature has demonstrated the beneficial effects of NO during liver IRI. These conflicting results might be explained by the fact that the mechanism of NO-mediated protection varies depending on cell type, quantities supplied, laboratory methods applied, timing and duration of NO exposure.

While iNOS was shown to be protective against hepatic IRI in some studies, it was shown to be deleterious in others. In a rat model of hepatic IRI, Takamatsu *et al*[31] observed increased hepatic expression of iNOS mRNA, ALT, and plasma iNOS at 3, 12, and 24 h after hepatic reperfusion. Concomitantly, there was evidence of histologic damage and nitrotyrosine formation in the liver sampled post-reperfusion. These changes were absent in the control group given the selective iNOS inhibitor, ONO-1714. The authors concluded that peroxynitrite may be involved in iNOS-mediated hepatic injury following IR[31].

In another model of hepatic IR in rats, Wang *et al*[32] observed an increase in iNOS protein and mRNA expression on the first day following hepatic reperfusion. Higher levels of iNOS correlated with evidence of increased hepatic injury in the form of elevated serum levels of ALT and AST. Administration of the non-selective nitric oxide synthase (NOS) inhibitor, L-NAME, significantly increased AST and ALT, while administration of the selective iNOS inhibitor, AE-ITU, significantly decreased AST and ALT levels, respectively[32]. The authors postulated that the deleterious effects of L-NAME were due to inhibition of eNOS, while the protective effects of AE-ITU were due to inhibition of injury-provoking iNOS. In a rat model of hepatic IR and small-for-size living-related liver transplantation, Jiang *et al*[33] observed increased iNOS mRNA and protein expression post-reperfusion from a warm ischemic insult with peak expression at 3 h post-reperfusion. This was accompanied by significant increases in concentrations of AST, ALT, malondialdehyde (MDA) and histologic evidence of damage compared to controls. The authors postulated that iNOS-induced hepatic damage was *via* significant production of ROS[33]. We summarize some key studies investigating endogenous NO and NOS in hepatic IRI in Table 1[3,21,25,31-37].

THE USE OF EXOGENOUS NO ADMINISTRATION IN ATTENUATING HEPATIC IRI

Inhaled nitric oxide

Inhaled NO was approved by the US Food and Drug Administration in December of 1999 for the treatment of persistent hypertension of the newborn. Over the last decade, the primary advantage of inhaled nitric oxide (iNO) was seen to be its ability to selectively decrease pulmonary vascular resistance with minimal effects on systemic blood pressure; however, there is currently much interest in exploring its other benefits, including its antioxidant properties and its cytoprotective abilities[4]. In many animal studies, iNO decreased infarct size and left ventricular dysfunction after IRI, increased coronary artery patency after thrombosis, increased blood flow in brain, kidney and peripheral vasculature, decreased leukocyte adhesion in bowel during ischemia-reperfusion, and decreased platelet aggregation[38]. Date *et al*[39] reported the use of iNO in 15 out of 32 patients who suffered from immediate severe allograft dysfunction, with iNO administered at 20 to 60 ppm. The mortality was significantly lower in the

Table 1 Effect of endogenous nitric oxide and nitric oxide synthase on liver ischemia-reperfusion injury

Species	Experimental methods	Ischemic time (min)	NO or NOS effects	Ref.
Pigs	Aminoguanidine, 5 min before ischemia	120	NO derived from iNOS, antioxidant	[25]
Dogs	FK 409, 30 min before ischemia and15 min before and 45 min after reperfusion	60	NO, improves hepatic microcirculation	[34]
Rats	L-arginine, 7 d before IRI	60	NO, antioxidant	[35]
Rats	L-NAME 60 min before ischemia	30	NO, antioxidant	[3]
Mouse	Gadolinium chloride 24 h before ischemia L-NAME methyl ester 15 min prior to ischemia	45	NO derived from eNOS, antioxidant, suppresses Kupffer cell function, regulated basal hepatic blood flow, but did not affect blood flow after reperfusion, attenuated neutrophils infiltration	[21]
Rats	L-arginine or Sodium nitroprusside or L-Name prior to ischemia	60	NO, improves peripheral liver blood flow after reperfusion, cytoprotective	[36]
Male rats	Arginine or L-NAME or 8-bromo guanosine 3′ 5′-cyclic monophosphate or rat atrial natriuretic peptide (ANP 1-28) 30 min before ischemia	45	NO, antioxidant, antiproinflammatory cytokines, improves microcirculation by the cGMP pathway, inhibits neutrophil infiltration and platelet aggregation	[37]
Male rats	IRI group: had partial clamping of portal vein and hepatic artery ONO-1714 group: as above plus ONO-1714 just prior to reperfusion and 6 h thereafter Control group: sham operation	90	iNOS expression peaked at 3 h and diminished at 24 h post reperfusion in IRI and ONO-1714 groups ONO-1714 significantly inhibited plasma nitrates at 24 h post reperfusion ONO-1714 significantly inhibited plasma ALT at 12 h post reperfusion, together with inhibiting histological damage and peroxynitrate expression in liver	[31]
Male rats	Microvessel clamping of portal vein and left hepatic artery L-NAME and AE-ITU given to each of 6 rats exposed to microvessel clamping (time unknown)	60	L-NAME worsened, elevated levels of ALT/AST in IRI groups AE-ITU mildly and significantly decreased levels of AST	[32]
Male rats	Portal vein, hepatic artery and bile ducts clamped by microvessel clamp followed by reperfusion	60	Significant elevation of AST/ALT, MDA/SOD in IRI and small-for-size liver transplantation groups	[33]

NO: Nitric oxide; NOS: Nitric oxide synthase; iNOS: Inducible nitric oxide synthase; eNOS: Endothelium-derived nitric oxide synthase; cGMP: Cyclic guanosine monophosphate; L-NAME: L-nitroarginine; ANP: Atrial natriuretic peptide; IRI: Ischemia-reperfusion injury; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; AE-ITU: Aminoethyl-isothiourea; MDA: Malondialdehyde; SOD: Superoxide dismutase.

iNO group (7% and 24%, respectively). The gross benefits reported were that iNO improved oxygenation, decreased pulmonary artery pressure, shortened the period of postoperative mechanical ventilation, and reduced airway complications and mortality[39]. Likewise, a recent retrospective study also presented a picture of improvement of overall respiratory functions. The authors encouraged the administration of iNO for the prevention and treatment of early graft failure in lung transplant recipients[40]. Varadarajan *et al*[41] were the first group to study the relationship between NO metabolism and IRI in human liver transplantation[41]. From their study, they concluded that reduced bioavailability of eNOS contributed to IRI one hour after portal reperfusion. On the other hand, iNOS did not contribute to early IRI after human liver transplantation. Clinical and mechanistic reports on therapeutic use of iNO demonstrated action well beyond vascular relaxation, subsequently inactivated by oxy- or deoxyhemoglobin in the red blood cells. iNO has various positive effects on extrapulmonary systems. However, how iNO mediates these extrapulmonary effects remains unclear. Evidence supporting stable forms of iNO is probably strongest for *S*-nitrosothiols (SNOs) and nitrite[38]. In a prospective, blinded, placebo-controlled study, 80 ppm of iNO was administered to patients undergoing orthotropic liver transplantation[42]. Many advantages were reported in the iNO group, including reduced platelet transfusion, an improvement in the rate at which liver function was restored post-transplantation, and a decrease in the length of hospital stay. Most interesting was the finding of an approximate 75% reduction of hepatocellular apoptosis in patients treated with iNO[42] (Figure 3). Possible biochemical intermediates of iNO include plasma and red blood cell nitrate, nitrite, SNOs, C- or N-nitrosamines and red blood cell ferrous nitrosylhemoglobin. In this study, a detailed analysis indicated that the most likely candidate transducer of iNO in liver IRI was nitrite.

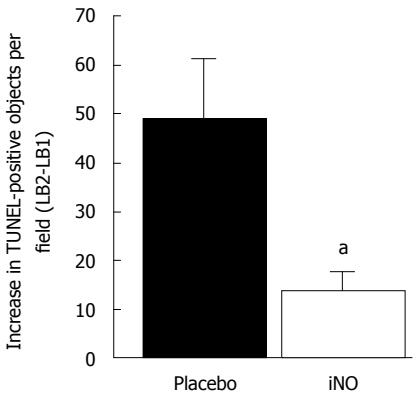

Figure 3 Decreased apoptosis indicated by TUNEL staining in patients treated with inducible nitric oxide compared to controls (Courtesy of John D. Lang, MD, Seattle, WA). $^{a}P < 0.05$. iNO: Inducible nitric oxide.

iNO delivery systems

An iNO delivery system should allow for constant and accurate measurements of NO and nitrogen dioxide (NO_2)

Table 2 Nitric oxide donors

Model	Drugs	Outcomes	Ref.
Canine liver IRI	FK-409	Promoted hepatic tissue blood flow, decreased serum endothelin-1, cytoprotection	[34]
Isolated hepatocytes	S-nitroso-N-acetylpenicillamine	Drug induced the expression of heat shock protein 70 mRNA and protein resulting in cytoprotection from TNFα	[2]
Murine liver IRI	Sodium nitroprusside	Promotes hepatic tissue blood flow after reperfusion-cytoprotection	[36]
Murine liver IRI	PEG-poly SNO-BSA, a sustained release of NO	Decreased neutrophil accumulation, prevented the excessive production of iNOS	[54]
Murine liver IRI	Macromolecule S-nitrosothiols	Prevented hepatocellular injury	[55]

NO: Nitric oxide; SNO: S-nitrosothiol; iNOS: Inducible nitric oxide synthase; IRI: Ischemia-reperfusion injury; TNF: Tumor necrosis factor.

concentration in inspired gas, as well as minimization of the contact time between oxygen and NO, in order to decrease the feasibility of producing high NO_2 concentrations. The measurement of iNO and NO_2 concentrations can be undertaken using chemiluminescence or electrochemical devices. There are some drawbacks of chemiluminescence devices such as cost, the need for a relatively high sample volume, noise and maintenance difficulties[43]. However, an electrochemical analyzer is relatively insensitive, and these measurements may be affected by pressure, humidity, temperature and the presence of other gases in the environment[44]. The delivery system should display the pressure of iNO in the cylinder and should have a backup power supply to avoid sudden discontinuation of iNO. Inhaled NO is usually supplied in nitrogen at various concentrations. The gas mixture concentration should be sampled downstream of the input port just proximal to the patient manifold. iNO also can be administered *via* nasal cannula, oxygen mask and oxygen hood[45]. Finally, the exhausted gas should be scavenged by passing it through carbon and filters, soda lime or activated charcoal[46].

POTENTIAL TOXICITIES DURING INHALATION

In the presence of high concentrations of O_2, NO oxidizes to nitrogen dioxide (NO_2). NO_2 reacts with the alveolar lining fluid to form nitric acid. NO dissolved in the alveolar lining fluid reacts with O_2^- yielding $OONO^-$, then decomposes into a hydroxyl anion[47]. Nitration of tyrosine residues of proteins is used as a marker of oxidative stress[48]. The rate at which NO is oxidized to NO_2 depends on the square of NO concentration and fractional concentration of oxygen to which it is exposed. The Occupational and Health Administration recommend 5 ppm exposure to NO per 8 h per 24-h-interval as the upper safe limit of human exposure[49]. In order to protect against NO_2 toxicity, iNO should be given with the least possible O_2 concentration. Inhaled NO and NO_2 concentrations should be monitored, exhaled gases should be scavenged, and a soda lime canister should be placed in the inspiratory limb of the breathing circuit.

Nitrite

The simple molecule nitrite had been thought to be just an index of NO production for decades[3]. Recently, a number of lines of evidence suggest that nitrite is a pro-mediator of NO homeostasis[50]. Administration of nitrite at near physiological concentrations (< 5 μg) leads to vasodilatation in animal and human studies[46]. Shiva *et al*[51] observed that nitrite was metabolized across the peripheral circulation. In addition, nitrite caused an increase in peripheral forearm blood flow when 80 ppm iNO was administered[51]. Under distinct conditions such as hypoxia and acidosis, nitrite can be reduced to NO by a number of deoxyhemeproteins (hemoglobin, myoglobin, neuroglobin and cytoglobin), enzymes (cytochrome P_{450} and xanthine oxidoreductase), and components of the mitochondrial electron transport chain[4]. Since nitrite can be converted back to NO during hypoxia, nitrite therefore is expected to be utilized during IRI. Furthermore, nitrite shows more potential benefits than NO in terms of safety and ease of administration. In other words, nitrite concentrations administered need only to be a small dose in order to increase plasma and tissue nitrite levels several folds. Routes of administration are oral, intravenous injection or infusion, intraperitoneal, *via* nebulizer or topical[52]. Nitrite has now been demonstrated to have cytoprotective effects in animal models of ischemia-reperfusion in organs. Duranski *et al*[52] evaluated the effects of nitrite therapy in *in vivo* murine models of hepatic and myocardial IRI, and showed that nitrite was associated with cytoprotective effects. In that setting, nitrite reduced cardiac infarct size by 67% and limited elevations of liver enzymes in a dose-dependent manner. These workers also demonstrated that nitrite was reduced to NO regardless of eNOS and heme oxygenase-1 enzyme activities[52]. The exact mechanisms as to how nitrite protects against this particular condition are being explored, but it appears that the benefit is mediated through the modulation of mitochondrial function by involving the posttranslational *S*-nitrosation of complex I to attenuate reperfusion oxygen radical generation and prevent cytochrome-C release[51].

NO donor drugs

Since nitric oxide is not considered to be an ideal gas for the treatment of IRI, NO donor drugs are now being explored as an alternative to the parent compound. Novel drugs have been developed and used for the delivery of NO in order to compensate for the very short half-

life of NO *in vivo*. However, there are only two types of NO donor drugs that are currently used clinically: organic nitrates and sodium nitroprusside. Organic nitrates are the most commonly used NO donor drug treatment for coronary artery disease and congestive heart failure because the drugs produce clear clinical responses through their vasodilatory effects. Preparations of drugs include slow release oral forms, ointments, transdermal patches, nebulizers and traditional intravenous forms. The main limitation of organic nitrates is the induction of drug tolerance with prolonged continuous use. NO release from nitroglycerin is likely *via* the enzyme, mitochondrial aldehyde dehydrogenase[53]. The mechanism of NO release from sodium nitroprusside, on the other hand, is more complex, as demonstrated by Yang *et al*[53] in a murine model of hepatic IRI. Sodium nitroprusside is thought to down-regulate the mRNA expression of several enzymes related to hepatic injury[54]. We summarize other novel NO donor drugs in Table 2[2,34,36,54,55].

CONCLUSION

Ischemia-reperfusion injury is a well-defined threat to the liver during periods of interruption and restoration of oxygen delivery, as occurs in certain procedures such as hepatic resections and orthotopic liver transplantations. Relative NO deficiency seems central in the pathogenesis of this injury. Replacing NO *per se* either by inhalation, nitrate anion or *via* donor drugs represents a novel means for ameliorating IRI. Further randomized controlled trials are needed to evaluate this therapy in patients undergoing operative procedures causing IRI.

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