We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

The Influences of Nitric Oxide on Liver Ischemia-Reperfusion Injury

Erik Stoltenberg, Alexander A. Vitin,
Bradley E. Hansen and John D. Lang, Jr.
*University of Washington, Seattle,
Department of Anesthesiology and Pain Medicine
USA*

1. Introduction

Ischemia-reperfusion injury (IRI) is a series of multifaceted cellular events that takes place on the resumption of oxygen delivery to the affected organ after a period of hypoxia. IRI occurs in the liver during procedures that are associated with vascular inflow obstruction followed by restoration of blood flow, particularly during orthotopic liver transplantation. IRI can result in major hepatocellular damage.

In the last decade, nitric oxide (NO) has been shown to have various protective effects on cells during IRI. NO was first described as endothelium derived relaxing factor (EDRF), and it was described as being released from vascular endothelium to induce smooth muscle vasorelaxation. Since that time, much more has been elucidated about the role of NO in biological systems. NO has been demonstrated to inhibit oxidative stress, cytokine release, leukocyte endothelial adhesion and apoptosis (Phillips, Toledo, Lopez-Neblina, Anaya-Prado, & Toledo-Pereyra, 2009). 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 NF- κ B and activation of G proteins (Y. M. Kim, de Vera, Watkins, & Billiar, 1997). Previous studies have demonstrated that a reduction of NO during hepatic IRI, generally via a reduction in endothelial nitric oxide synthase (eNOS) activity, leads to liver injury (Köken & Inal, 1999). Inhaled NO or NO donor drugs are novel treatments that have been used clinically to attenuate liver IRI (Zaky, Siriussawakul, Tostenrud, Pauldine, & J. Lang, 2009). This review will discuss the pathophysiology of liver involvement during IRI and the clinical use of NO and its sister compounds in ameliorating the impact of liver IRI.

2. An overview of hepatic ischemia reperfusion injury

As noted above, the pathophysiology of IRI is multifactorial and involves a multitude of oxidative and cellular mechanisms. Briefly, hepatic IRI can be described as a two phase process with early (acute) and late (sub-acute) injury (Fan, Zwacka, & Engelhardt, 1999; Zwacka, Zhang, Zhou, Halldorson, & Engelhardt, 1998b). The distinction is particularly important because potential therapeutic targets (i.e. methods of increasing NO in the hepatic

micro-environment) may have different effects on these two phases. Early injury is mediated by a rapid change in the biochemical redox state of the tissue to a more oxidative one. It occurs within 5 minutes, and is not associated with leukocyte infiltration. Following the acute state is an increase in endothelial cell adhesion molecules, chemokines and cytokines. These molecules then herald the late phase characterized by a significant infiltration of polymorphonuclear neutrophils, further release of a reactive oxygen species (ROS) and extensive inflammation and tissue injury.

NO plays a significant role in the acute phase of IRI, as this phase is associated with a rapid decrease in available NO. This decrease occurs either by depressed production by eNOS in sinusoidal endothelial cells (SECs), increased degradation by ROS, or both. The ROS implicated are chiefly $O_2^{\bullet-}$ (superoxide, see next paragraph), but also include hydrogen peroxide (H_2O_2). In the last few years, the implicated enzyme responsible for production of ROS has shifted from hepatocyte xanthine oxidase to NADPH oxidase in Kupffer cells or mitochondrial sources of ROS (Hines & Grisham, 2011).

The term “reactive oxygen species” in the context of hepatic IRI primarily refers to superoxide. Two studies that incorporated manganese superoxide dismutase (MnSOD) – an enzyme which degrades superoxide – into liver tissue showed attenuation of IRI (He et al., 2006; Zwacka et al., 1998a). Therefore superoxide itself seems important in IRI. The mechanism by which superoxide imparts its damage is somewhat unclear, but it is known that membrane lipid peroxidation is associated with oxidative damage. Perhaps more importantly, damage by superoxide to mitochondrial membrane proteins and therefore ATP generating capacity and may be a more important mechanism in IRI (Madresh & Hajnóczky, 2001; Moon et al., 2008).

3. Nitric oxide biochemistry

NO is a highly reactive molecule with other free radical species and possesses an extremely short half-life (Rubbo, Darley-Usmar, & Freeman, 1996). NO is produced endogenously or delivered exogenously where it can react with a variety of cellular targets resulting in vasorelaxation, enhanced neuronal transmission, reduced apoptosis, inhibition of neutrophil aggregation and adhesion, and modulation of vascular smooth muscle proliferation.

NO synthesis is dependent on the enzyme nitric oxide synthase (NOS). NOS catalyzes the net reaction:

(adapted from Alderton, Cooper, & Knowles, 2001)

This complex enzyme system generates NO from the terminal nitrogen atom of L-arginine in the presence of NADPH and dioxygen. NOS binds flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), heme, tetrahydrobiopterin (BH_4) and calmodulin from L-arginine and oxygen by a family of three NO synthases (NOS), all of which are expressed in a variety of cell types.

Three distinct isoforms are known: 1) Neuronal NOS (NOS I), is produced in central and peripheral nerves and is pivotal in neuronal transmission and cell-to-cell communication

within the central nervous system. 2) Inducible NOS (NOS II is induced by an inflammatory stimulus such as a microbe (Parul Tripathi, Prashant Tripathi, Kashyap, & Singh, 2007). Unlike the other types of NOS (I and III), NOS II is not constitutive and is independent of calcium regulation. While NOS II is expressed by immune cells such as neutrophils and macrophages, it is also present in other cell lines including hepatocytes. Endothelial NOS (NOS III), is constitutively expressed by endothelial cells and is critical for the regulation of vascular function, more specifically vasorelaxation.

(Modified, with permission from <http://www.wiley.com/college/boyer>)

Fig. 1. Mechanisms of smooth muscle relaxation. NO diffuses across the muscle cell membrane and binds to guanylyl cyclase. Guanylyl cyclase catalyzes the synthesis of cyclic GMP from GTP. cGMP activates a cGMP-dependent protein kinase which stimulates the uptake of calcium by the endoplasmic reticulum of the muscle cell. The reduced levels of cytoplasmic calcium cause the muscle cell to relax. As a consequence of muscle cell relaxation, vasodilation occurs. PKG – protein kinase G

The generation of NO leads to several actions that promote smooth muscle relaxation. First, activation of guanylate cyclase raises the level of intracellular cGMP which in turn inhibits the entry of calcium into the cell thereby inducing smooth muscle relaxation. Second,

activation of K⁺ channels leads to cellular hyperpolarization and relaxation. Finally, stimulation of cGMP-dependent protein kinase activates of myosin light chain phosphatase leading to dephosphorylation of myosin light chains resulting in smooth muscle relaxation. NOSs are related but encoded by distinct genes. Classically, the ability of NO to elicit vasorelaxation is due to its ability to increase intracellular levels of cyclic guanosine monophosphate (cGMP) through the activation of soluble guanylate cyclase (sGC). cGMP-dependent protein kinases in turn decrease the sensitivity of myosin to calcium-induced contraction and lower intracellular calcium by activation of calcium-sensitive potassium channels and inhibits the release of calcium from the sacroplasmic reticulum. Mechanisms of smooth muscle relaxation are shown on Figure 1.

4. Brief review of the pathophysiology of IRI

Fig. 2. Multifaceted mechanism are involved in the causation of hepatic ischemia-reperfusion injury (IRI). Kupffer and endothelial cells produce cytokines and chemokines, recruiting neutrophils that further accentuate injury. EC, endothelial cell. NO (nitric oxide) is decreased as a result of IRI allowing for decreased perfusion and exaggerated injury (Massip-Salcedo, Roselló-Catafau, Prieto, Avila, & Peralta, 2007). KC, Kupffer cell. ATP, adenosine triphosphate. TNF, tumor necrosis factor. IL, interleukin. ICAM, intercellular adhesion molecule. PAF, platelet activation factor. LTB4, leukotrien B4. GMS-CSF, granulocyte macrophage colony stimulating factor. INF, interferon. ROS, reactive oxygen species. (Slightly modified with permission of Dr. Joan Rosello-Catafau, Barcelona, Spain)

During the ischemic 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 affects a myriad of cellular functions: homeostasis, signaling interactions, cellular proliferation and processing of the apoptotic death cycle. Adenosine triphosphate (ATP) depletion impairs the 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 (Jennings, Shen, Hill, Ganote, & Herdson, 1978). If the ischemic insult lasts greater than 24 hours, it is likely that ATP-synthase activity becomes irreversible after blood restoration, leading to cellular necrosis, apoptosis or necroapoptosis (Sammur et al., 2000). Ischemia also causes an increased expression of adhesion molecules that leads to endothelial cell and neutrophil adhesion resulting in vascular studding and occlusion (Yadav et al., 1998). Furthermore, disequilibrium between NO and endothelin (ET) induces vasoconstriction and subsequent microcirculatory failure even though blood circulation has been re-established (Montalvo-Jave, Escalante-Tattersfield, Ortega-Salgado, Piña, & Geller, 2008). Reestablishment of blood flow will serve to amplify inflammation with consequent injury that is highly variable but dependent on numerous variables that include 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.

5. Principal participants in liver IRI

5.1 Sinusoidal endothelial cells (SEC)

Injury to these cells is initiated during cold ischemia whereby Ca^{2+} -ATPase results in the accumulation of intracellular calcium (Bigelow & Thomas, 1987). Following this event, a series of actions occur making the endothelium more susceptible to platelet adhesion and reduced sinusoidal flow.

5.2 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 (Jaeschke, Bautista, Spolarics, & Spitzer, 1991). 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 $\text{TNF-}\alpha$ and Interleukin-1 which themselves can perpetuate inflammatory injury by leukocyte activation.

While major participants in the promotion of injury, during cold ischemia they undergo intracellular energetic bioenergetic perturbations that reduce ATP stores due to mitochondrial dysfunction and predispose these cells to injury during reperfusion (Kamiiike et al., 1988).

5.3 Leukocytes and lymphocytes

As a result of IRI, cellular adhesion molecules (ie, intracellular 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 the endothelium where they continue to orchestrate tissue injury. The accumulation of activated neutrophils contributes to microcirculatory disturbances both locally and remotely. Activated neutrophils release reactive oxygen species, specifically superoxide radical ($O_2^{\bullet-}$), proteases and various cytokines (Teoh & Farrell, 2003). Monocytes and macrophages are also activated shortly following reperfusion (Ysebaert et al., 2000). 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 (Ysebaert et al., 2000).

5.4 Reactive oxygen species (ROS) and reactive nitrogen species (RNS)

During periods of ischemia, ROS and 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 and possible xanthine oxidase (or mitochondrial sources of ROS). ROS serve to stimulate other cell lines including Kupffer cells to produce proinflammatory cytokines (Diesen & Kuo, 2011). The major ROS are hydroxyl radical ($OH^{\bullet}$) and hydrogen peroxide (H_2O_2). Reactions of ROS such as $O_2^{\bullet-}$ with NO yield products such as peroxynitrite ($ONOO^-$), a RNS which can be an extremely aggressive oxidant.

5.5 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 (Colletti et al., 1996). Additionally, IL-1 is known to promote production of ROS, induce TNF- α synthesis by Kupffer cells and induce neutrophil recruitment (Kato, Gabay, Okaya, & Lentsch, 2002).

5.6 Complement

The complement system also contributes significantly to IRI and is composed of approximately thirty 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 (Qin & Gao, 2006). When activated, complement acts as a membrane-attacking complex that stimulates the production of proinflammatory cytokines and chemotactic agents. Furthermore, it can regulate adaptive immunity (Boros & Bromberg, 2006).

6. 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. Injury due to ischemia and reperfusion 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 reactive oxygen species, 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 (Köken & Inal, 1999). This is now appreciated to be due to decreases in NO steady-state production resulting from low concentrations of eNOS. This event coupled with NO inactivation due to reactions with abundant ROS, such as $O_2^{\bullet-}$, 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 with of NO to more “physiologic” concentrations serves to diminish the liver ischemia injury via countering the adverse actions mentioned previously. Other 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 less in wild type mice compared to eNOS knockouts (eNOS $-/-$), in addition to the findings that agents given to increase eNOS expression or donate NO afford greater liver IRI protection (Duranski et al., 2006; Katsumi, Nishikawa, Yamashita, & Hashida, 2008). It is also well established that the NO concentrations during various inflammatory states are significantly increased by increased expression of inducible nitric oxide synthase or iNOS. However, the influence of iNOS and its true contribution in conferring liver protection deserves additional studies. In a rat model of liver IRI, iNOS expression was significantly increased as per increases in iNOS RNA at 1 and 5 hrs (Hur et al., 1999). This is consistent with other studies measuring iNOS expression of conditions of liver IRI. In a porcine model of IRI, intraportal injection of the selective iNOS inhibitor, aminoguanidine was demonstrated to decrease injury (M Isobe et al., 2000). In an intriguing study, NOS knockout mice (iNOS $-/-$) exposed to warm liver IRI demonstrated a much greater magnitude of injury compared to wild type mice. Interestingly, 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 for now, the true influence of iNOS’s influence 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 (Maejima, Adachi, Morikawa, Ito, & Mitsuaki Isobe, 2005). 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 reactive nitrogen species such as $ONOO^{\bullet-}$ or reactive oxygen species which lead to cell necrosis and apoptosis (P. K. Kim, Zamora, Petrosko, & Billiar, 2001). Other published mechanisms of NO-mediated protection include inhibition of nuclear factor kappa B (Marshall, Hess, & Stamler, 2004), reversible inhibition of mitochondrial complex I, and decreased mitochondrial calcium accumulation (Burwell & Brookes, 2008). As to be expected, controversy exists concerning “if” and “how” NO exerts cellular protection. For instance, in a study by Jaeschke *et al* (Jaeschke et al., 1991), 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 (Jaeschke, Schini, & Farhood, 1992). 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. Here, we summarize some key studies studying endogenous NO and NOS in hepatic IRI Table 1.

Species	Experimental Methods	Ischemic Time	NO or NOS Effects	Reference
Pigs	Aminoguanidine, 5 min before ischemia	120 min	NO derived from iNOS, antioxidant	(M Isobe et al., 2000)
Dogs	FK 409, 30 min before ischemia and 15 min before and to 45 min after reperfusion	60 min	NO, improve hepatic microcirculation	(Aiba et al., 2001)
Rats	L-arginine, 7 days before IRI	60 min	NO, antioxidant	(Chattopadhyay et al., 2008)
Rats	L-NAME 60 min before ischemia	30 min	NO, antioxidant	(Köken & Inal, 1999)
Mouse	- Gadolinium chloride 24h before ischemia - L-nitroarginine (L-NAME) methyl ester 15 min prior to ischemia.	45 min	NO derived from eNOS, antioxidant, suppresses Kupffer cell function, regulated basal hepatic blood flow, but not affects blood flow after reperfusion, attenuated neutrophils infiltration.	(Hines et al., 2005)
Rats	- L-arginine or Sodium nitroprusside or L-Name prior to ischemia	60 min	NO, improve peripheral liver blood flow after reperfusion, cytoprotective	(Nilsson, Delbro, Wallin, & Friman, 2001)
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 min	NO, antioxidant, antiproinflammatory cytokines, improves microcirculation by the cGMP pathway, Inhibit neutrophils infiltration and platelet aggregation.	(Cottart et al., 2003)

Table 1. Effect of endogenous NO and NOS on liver IRI

7. Efficacy of exogenous NO, nitrite anion and NO donor administration in attenuating hepatic IRI

7.1 Inhaled nitric oxide (iNO)

Inhaled NO was approved by the U.S. Food and Drug Administration in December 1999, for the treatment of Persistent Hypertension of the newborn. Over the last decade, the primary advantage of 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 (Zaky et al., 2009). In many animal studies, iNO decreased infarct size and left ventricular dysfunction after ischemia-reperfusion injury, 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 (McMahon & Doctor, 2006). Date *et al* reported the use of iNO in 15 out of 32 patients who suffered from immediate severe allograft dysfunction with iNO at 20 to 60 ppm. The mortality was significantly lower in the iNO group (7% and 24%, respectively). The gross benefits reported were that iNO improves oxygenation, decreases pulmonary artery pressure, shortens the period of postoperative mechanical ventilation, and reduces airway complications and mortality (Date et al., 1996). Likewise, a recent retrospective study also presented an 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 (Yerebakan, Ugurlucan, Bayraktar, Bethea, & Conte, 2009). Varadarajan *et al* were the first group to study the relationship between NO metabolism and IRI in human liver transplantation (Varadarajan et al., 2004). 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 demonstrating well beyond vascular relaxation, subsequently inactivated by oxy-ordeoxyhemoglobin in the red blood cells. iNO has various positive effects on extrapulmonary systems. However, how iNO mediates extrapulmonary effects remains unclear. Evidence supporting of stable forms of iNO is probably strongest for S-nitrosothiol (SNOs) and nitrite (McMahon & Doctor, 2006). In a prospective, blinded, placebo-controlled study, iNO at 80 ppm was administered to patients undergoing orthotopic liver transplantation (J. D. Lang et al., 2007). 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 on, and a decrease in the length of hospital stay. Most interesting was the finding of an approximated 75% reduction of hepatocellular apoptosis in patients treated with iNO (J. D. Lang et al., 2007). Possible biochemical intermediates of iNO including plasma and red blood cell nitrate, nitrite, S-nitrosothiols, 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 on liver IRI was nitrite.

7.2 iNO delivery systems

An iNO delivery system should allow for constant and accurate measurements of NO and nitrogen dioxide [NO₂] concentration in inspired gas as well as minimize the contact time between oxygen and NO in order to decrease the feasibility of producing high NO₂

concentrations. The measurement of iNO and NO₂ 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 (Mupanemunda & Edwards, 1995). 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 (Macrae et al., 2004). 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 (Ambalavanan, St John, Carlo, Bulger, & Philips, 2002). Finally, the exhausted gas should be scavenged by passing it through carbon and filters, soda lime or activated charcoal (Ambalavanan, El-Ferzli, Roane, Johnson, & Carlo, 2009).

7.3 Potential toxicities during inhalation

In the presence of high concentrations of O₂, NO oxidizes to nitrogen dioxide (NO₂). NO₂ reacts with the alveolar lining fluid to form nitric acid. NO dissolves in the alveolar lining fluid reacts with O₂⁻ yielding OONO₂⁻ then decomposes into a hydroxyl anion (Pryor & Squadrito, 1995). Nitration of tyrosine residues of proteins is used as a marker of oxidative stress (Ischiropoulos, 1998). The rate at which NO is oxidized to NO₂ 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/8 hr/24 hour interval as the upper safe limit of human exposure (Fullerton & McIntyre, 1996). In order to protect against NO₂ toxicity, iNO should be given with the least possible O₂ concentration. Inhaled NO and NO₂ 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.

7.4 Nitrite

The simple molecule nitrite had been thought to be just an index of NO production for decades (Köken & Inal, 1999). Recently, a number of evidence suggests that nitrite is a pro-mediator of NO homeostasis. Administration of nitrite at near physiological concentrations (<5 µg) leads to vasodilatation in animals and human studies (Fullerton & McIntyre, 1996). Gladwin *et al* 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 (Shiva & Gladwin, 2009). 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 P₄₅₀ and xanthine oxidoreductase), and components of the mitochondrial electron transport chain (Zaky et al., 2009). Since nitrite can be converted back to NO during hypoxia nitrite therefore is expected to be utilized during ischemia reperfusion injury. Furthermore, nitrite shows more potential benefits than NO in terms of safety and ease of administration. In other words, nitrite concentrations administered need only a small dose in order to increase plasma and tissue nitrite level several-fold. Routes of administration are oral, intravenous injection or infusion, intraperitoneal, nebulizer or topical (Duranski et al., 2005). Nitrite has now been

demonstrated to have cytoprotective effects in animal models of ischemia reperfusion in organs. Duranski *et al* evaluated the effects of nitrite therapy in vivo murine models of hepatic and myocardial ischemia reperfusion injury and showed that nitrite was associated with cytoprotective effects. In the setting, nitrite reduced cardiac infarct size by 67% and limited elevations of liver enzymes in a dose-dependent manner. They also demonstrated that nitrite was reduced to NO regardless of eNOS and heme oxygenase-1 enzyme activities (Duranski et al., 2005). The exact mechanisms of how nitrite protects against the 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 prevents cytochrome-C release (Shiva & Gladwin, 2009).

7.5 NO donor drugs

Since nitric oxide is not considered to be an ideal gas for the treatment of ischemia reperfusion injury, 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 drugs 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 (Yang, Chen, Kong, Xu, & Lou, 2007). On the other hand, the mechanism of NO release from sodium nitroprusside is more complex as demonstrated by

Model	Drugs	Outcomes	Reference
Canine liver IRI	FK-409,	Promote hepatic tissue blood flow, decrease serum Endothelin-1, cytoprotection	(Aiba et al., 2001)
Isolated hepatocytes	S-nitroso-N-acetylpenicillamine (SNAP)	- drug induced the expression of heat shock protein 70 mRNA and protein resulting in cytoprotection from TNF α	(Y. M. Kim et al., 1997)
Murine liver IRI	Sodium nitroprusside	- Promote hepatic tissue blood flow after reperfusion - cytoprotection	(Nilsson et al., 2001)
Murine liver IRI	PEG-poly SNO-BSA, a sustained release of NO	- Decreased neutrophils accumulation - Prevented the excessive production of iNOS	(Katsumi et al., 2008)
Murine liver IRI	Macromolecule S-nitrosothiols	Prevented hepatocellular injury	(Katsumi et al., 2009)

Table 2. Nitric oxide donors

Yang et al in a murine model of hepatic IRI. Sodium nitroprusside is thought to down-regulate the mRNA expression of several enzymes related to hepatic injury (Katsumi et al., 2008). Lastly, enhanced eNOS activation affords hepatoprotection during IRI and serves as yet another potential treatment option. Interestingly, liver preservation solutions supplemented with the agents trimetazidine (TMZ), 5-amino-4-imidazole carboxamide riboside (AICAR) or activated protein C (APC) have demonstrated allograft protection during conditions of cold ischemia (Katsumi, Nishikawa, Yasui, Yamashita, & Hashida, 2009). Below, we summarize other novel NO donor drugs in Table 2.

8. 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 as hepatic resections and orthotopic liver transplantations. Relative NO deficiency is central in the pathogenesis of this injury. Replacing NO per se either by inhalation, nitrate anion or via donor drugs represents a novel means in ameliorating IRI. Further randomized controlled drugs are needed to evaluate this therapy in patients undergoing operative procedures causing IRI.

9. References

- Aiba, M., Takeyoshi, I., Ohwada, S., Kawashima, Y., Iwanami, K., Sunose, Y., Yamada, T., et al. (2001). Novel nitric oxide donor (FK409) ameliorates liver damage during extended liver resection with warm ischemia in dogs *Journal of the American College of Surgeons*, 193(3), 264–271.
- Alderton, W. K., Cooper, C. E., & Knowles, R. G. (2001). Nitric oxide synthases: structure, function and inhibition *The Biochemical journal*, 357(Pt 3), 593–615.
- Ambalavanan, N., El-Ferzli, G. T., Roane, C., Johnson, R., & Carlo, W. A. (2009). Nitric oxide administration using an oxygen hood: a pilot trial *PloS one*, 4(2), e4312. doi:10.1371/journal.pone.0004312
- Ambalavanan, N., St John, E., Carlo, W. A., Bulger, A., & Philips, J. B. (2002). Feasibility of nitric oxide administration by oxygen hood in neonatal pulmonary hypertension *Journal of perinatology : official journal of the California Perinatal Association*, 22(1), 50–56. doi:10.1038/sj.jp.7210652
- Bigelow, D. J., & Thomas, D. D. (1987). Rotational dynamics of lipid and the Ca-ATPase in sarcoplasmic reticulum. The molecular basis of activation by diethyl ether *The Journal of biological chemistry*, 262(28), 13449–13456.
- Boros, P., & Bromberg, J. S. (2006). New cellular and molecular immune pathways in ischemia/reperfusion injury *American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons*, 6(4), 652–658. doi:10.1111/j.1600-6143.2005.01228.x
- Burwell, L. S., & Brookes, P. S. (2008). Mitochondria as a target for the cardioprotective effects of nitric oxide in ischemia-reperfusion injury *Antioxidants & redox signaling*, 10(3), 579–599. doi:10.1089/ars.2007.1845
- Chattopadhyay, P., Verma, N., Verma, A., Kamboj, T., Khan, N. A., & Wahi, A. K. (2008). L-arginine protects from pringle manoeuvre of ischemia-reperfusion induced liver injury *Biological & pharmaceutical bulletin*, 31(5), 890–892.

- Colletti, L. M., Kunkel, S. L., Walz, A., Burdick, M. D., Kunkel, R. G., Wilke, C. A., & Strieter, R. M. (1996). The role of cytokine networks in the local liver injury following hepatic ischemia/reperfusion in the rat *Hepatology (Baltimore, Md.)*, 23(3), 506–514. doi:10.1002/hep.510230315
- Cottart, C. H., Nivet-Antoine, V., Do, L., Al-Massarani, G., Descamps, G., Xavier-Galen, F., & Clot, J.-P. (2003). Hepatic cytoprotection by nitric oxide and the cGMP pathway after ischaemia-reperfusion in the rat *Nitric oxide : biology and chemistry / official journal of the Nitric Oxide Society*, 9(2), 57–63.
- Date, H., Triantafillou, A. N., Trulock, E. P., Pohl, M. S., Cooper, J. D., & Patterson, G. A. (1996). Inhaled nitric oxide reduces human lung allograft dysfunction *The Journal of thoracic and cardiovascular surgery*, 111(5), 913–919.
- Diesen, D. L., & Kuo, P. C. (2011). Nitric oxide and redox regulation in the liver: part II. Redox biology in pathologic hepatocytes and implications for intervention *The Journal of surgical research*, 167(1), 96–112. doi:10.1016/j.jss.2009.10.006
- Duranski, M. R., Greer, J. J. M., Dejam, A., Jaganmohan, S., Hogg, N., Langston, W., Patel, R. P., et al. (2005). Cytoprotective effects of nitrite during in vivo ischemia-reperfusion of the heart and liver *The Journal of clinical investigation*, 115(5), 1232–1240. doi:10.1172/JCI22493
- Duranski, M. R., Elrod, J. W., Calvert, J. W., Bryan, N. S., Feelisch, M., & Lefer, D. J. (2006). Genetic overexpression of eNOS attenuates hepatic ischemia-reperfusion injury *American journal of physiology. Heart and circulatory physiology*, 291(6), H2980–6. doi:10.1152/ajpheart.01173.2005
- Fan, C., Zwacka, R. M., & Engelhardt, J. F. (1999). Therapeutic approaches for ischemia/reperfusion injury in the liver *Journal of molecular medicine (Berlin, Germany)*, 77(8), 577–592.
- Fullerton, D. A., & McIntyre, R. C. (1996). Inhaled nitric oxide: therapeutic applications in cardiothoracic surgery *The Annals of thoracic surgery*, 61(6), 1856–1864. doi:10.1016/0003-4975(96)00046-X
- He, S.-Q., Zhang, Y.-H., Venugopal, S. K., Dicus, C. W., Perez, R. V., Ramsamooj, R., Nantz, M. H., et al. (2006). Delivery of antioxidative enzyme genes protects against ischemia/reperfusion-induced liver injury in mice *Liver Transplantation*, 12(12), 1869–1879. doi:10.1002/lt.21001
- Hines, I. N., Harada, H., Flores, S., Gao, B., McCord, J. M., & Grisham, M. B. (2005). Endothelial nitric oxide synthase protects the post-ischemic liver: potential interactions with superoxide *Biomedicine & pharmacotherapy = Biomédecine & pharmacothérapie*, 59(4), 183–189. doi:10.1016/j.biopha.2005.03.011
- Hines, I. N., & Grisham, M. B. (2011). Divergent roles of superoxide and nitric oxide in liver ischemia and reperfusion injury. *Journal of clinical biochemistry and nutrition*, 48(1), 50–56. doi:10.3164/jcbrn.11-016FR
- Hur, G. M., Ryu, Y. S., Yun, H. Y., Jeon, B. H., Kim, Y. M., Seok, J. H., & Lee, J. H. (1999). Hepatic ischemia/reperfusion in rats induces iNOS gene transcription by activation of NF-kappaB *Biochemical and biophysical research communications*, 261(3), 917–922. doi:10.1006/bbrc.1999.1143
- Ischiropoulos, H. (1998). Biological tyrosine nitration: a pathophysiological function of nitric oxide and reactive oxygen species *Archives of Biochemistry and Biophysics*, 356(1), 1–11. doi:10.1006/abbi.1998.0755

- Isobe, M., Katsuramaki, T., Kimura, H., Nagayama, M., Matsuno, T., Yagihashi, A., & Hirata, K. (2000). Role of inducible nitric oxide synthase on hepatic ischemia and reperfusion injury *Transplantation proceedings*, 32(7), 1650–1652.
- Jaeschke, H., Bautista, A. P., Spolarics, Z., & Spitzer, J. J. (1991). Superoxide generation by Kupffer cells and priming of neutrophils during reperfusion after hepatic ischemia *Free radical research communications*, 15(5), 277–284.
- Jaeschke, H., Schini, V. B., & Farhood, A. (1992). Role of nitric oxide in the oxidant stress during ischemia/reperfusion injury of the liver *Life sciences*, 50(23), 1797–1804.
- Jennings, R. B., Shen, A. C., Hill, M. L., Ganote, C. E., & Herdson, P. B. (1978). Mitochondrial matrix densities in myocardial ischemia and autolysis *Experimental and molecular pathology*, 29(1), 55–65.
- Kamiike, W., Burdelski, M., Steinhoff, G., Ringe, B., Lauchart, W., & Pichlmayr, R. (1988). Adenine nucleotide metabolism and its relation to organ viability in human liver transplantation *Transplantation*, 45(1), 138–143.
- Kato, A., Gabay, C., Okaya, T., & Lentsch, A. B. (2002). Specific role of interleukin-1 in hepatic neutrophil recruitment after ischemia/reperfusion *The American journal of pathology*, 161(5), 1797–1803. doi:10.1016/S0002-9440(10)64456-2
- Katsumi, H., Nishikawa, M., Yamashita, F., & Hashida, M. (2008). Prevention of hepatic ischemia/reperfusion injury by prolonged delivery of nitric oxide to the circulating blood in mice *Transplantation*, 85(2), 264–269. doi:10.1097/TP.0b013e31815e902b
- Katsumi, H., Nishikawa, M., Yasui, H., Yamashita, F., & Hashida, M. (2009). Prevention of ischemia/reperfusion injury by hepatic targeting of nitric oxide in mice *Journal of controlled release : official journal of the Controlled Release Society*, 140(1), 12–17. doi:10.1016/j.jconrel.2009.07.013
- Kim, P. K., Zamora, R., Petrosko, P., & Billiar, T. R. (2001). The regulatory role of nitric oxide in apoptosis *International immunopharmacology*, 1(8), 1421–1441.
- Kim, Y. M., de Vera, M. E., Watkins, S. C., & Billiar, T. R. (1997). Nitric oxide protects cultured rat hepatocytes from tumor necrosis factor- α -induced apoptosis by inducing heat shock protein 70 expression *The Journal of biological chemistry*, 272(2), 1402–1411.
- Köken, T., & Inal, M. (1999). The effect of nitric oxide on ischemia-reperfusion injury in rat liver *Clinica chimica acta; international journal of clinical chemistry*, 288(1-2), 55–62.
- Lang, J. D., Teng, X., Chumley, P., Crawford, J. H., Isbell, T. S., Chacko, B. K., Liu, Y., et al. (2007). Inhaled NO accelerates restoration of liver function in adults following orthotopic liver transplantation. *The Journal of clinical investigation*, 117(9), 2583–2591. doi:10.1172/JCI31892
- Macrae, D. J., Field, D., Mercier, J.-C., Møller, J., Stiris, T., Biban, P., Cornick, P., et al. (2004). Inhaled nitric oxide therapy in neonates and children: reaching a European consensus In *Intensive care medicine* (Vol. 30, pp. 372–380). Presented at the Intensive care medicine. doi:10.1007/s00134-003-2122-3
- Madesh, M., & Hajnóczy, G. (2001). VDAC-dependent permeabilization of the outer mitochondrial membrane by superoxide induces rapid and massive cytochrome c release *The Journal of cell biology*, 155(6), 1003–1015. doi:10.1083/jcb.200105057
- Maejima, Y., Adachi, S., Morikawa, K., Ito, H., & Isobe, Mitsuaki. (2005). Nitric oxide inhibits myocardial apoptosis by preventing caspase-3 activity via S-nitrosylation *Journal of molecular and cellular cardiology*, 38(1), 163–174. doi:10.1016/j.yjmcc.2004.10.012

- Marshall, H. E., Hess, D. T., & Stamler, J. S. (2004). S-nitrosylation: physiological regulation of NF-kappaB *Proceedings of the National Academy of Sciences of the United States of America*, 101(24), 8841–8842. doi:10.1073/pnas.0403034101
- Massip-Salcedo, M., Roselló-Catafau, J., Prieto, J., Avila, M. A., & Peralta, C. (2007). The response of the hepatocyte to ischemia *Liver international : official journal of the International Association for the Study of the Liver*, 27(1), 6–16. doi:10.1111/j.1478-3231.2006.01390.x
- McMahon, T. J., & Doctor, A. (2006). Extrapulmonary effects of inhaled nitric oxide: role of reversible S-nitrosylation of erythrocytic hemoglobin *Proceedings of the American Thoracic Society*, 3(2), 153–160. doi:10.1513/pats.200507-066BG
- Montalvo-Jave, E. E., Escalante-Tattersfield, T., Ortega-Salgado, J. A., Piña, E., & Geller, D. A. (2008). Factors in the pathophysiology of the liver ischemia-reperfusion injury *The Journal of surgical research*, 147(1), 153–159. doi:10.1016/j.jss.2007.06.015
- Moon, K.-H., Hood, B. L., Mukhopadhyay, P., Rajesh, M., Abdelmegeed, M. A., Kwon, Y.-I., Conrads, T. P., et al. (2008). Oxidative inactivation of key mitochondrial proteins leads to dysfunction and injury in hepatic ischemia reperfusion *Gastroenterology*, 135(4), 1344–1357. doi:10.1053/j.gastro.2008.06.048
- Mupanemunda, R. H., & Edwards, A. D. (1995). Treatment of newborn infants with inhaled nitric oxide *Archives of disease in childhood. Fetal and neonatal edition*, 72(2), F131–4.
- Nilsson, B., Delbro, D., Wallin, M., & Friman, S. (2001). Protective effect of nitric oxide and prostaglandin E(2) in ischemia/reperfusion injury of the liver *Transplantation proceedings*, 33(4), 2518–2520.
- Phillips, L., Toledo, A. H., Lopez-Neblina, F., Anaya-Prado, R., & Toledo-Pereyra, L. H. (2009). Nitric oxide mechanism of protection in ischemia and reperfusion injury *Journal of investigative surgery : the official journal of the Academy of Surgical Research*, 22(1), 46–55. doi:10.1080/08941930802709470
- Pryor, W. A., & Squadrito, G. L. (1995). The chemistry of peroxynitrite: a product from the reaction of nitric oxide with superoxide *The American journal of physiology*, 268(5 Pt 1), L699–722.
- Qin, X., & Gao, B. (2006). The complement system in liver diseases *Cellular & molecular immunology*, 3(5), 333–340.
- Rubbo, H., Darley-USmar, V., & Freeman, B. A. (1996). Nitric oxide regulation of tissue free radical injury *Chemical research in toxicology*, 9(5), 809–820. doi:10.1021/tx960037q
- Sammur, I. A., Burton, K., Balogun, E., Sarathchandra, P., Brooks, K. J., Bates, T. E., & Green, C. J. (2000). Time-dependent impairment of mitochondrial function after storage and transplantation of rabbit kidneys *Transplantation*, 69(7), 1265–1275.
- Shiva, S., & Gladwin, M. T. (2009). Nitrite mediates cytoprotection after ischemia/reperfusion by modulating mitochondrial function *Basic research in cardiology*, 104(2), 113–119. doi:10.1007/s00395-009-0009-3
- Teoh, N. C., & Farrell, G. C. (2003). Hepatic ischemia reperfusion injury: pathogenic mechanisms and basis for hepatoprotection *Journal of gastroenterology and hepatology*, 18(8), 891–902.
- Tripathi, Parul, Tripathi, Prashant, Kashyap, L., & Singh, V. (2007). The role of nitric oxide in inflammatory reactions *FEMS immunology and medical microbiology*, 51(3), 443–452. doi:10.1111/j.1574-695X.2007.00329.x

- Varadarajan, R., Golden-Mason, L., Young, L., McLoughlin, P., Nolan, N., McEntee, G., Traynor, O., et al. (2004). Nitric oxide in early ischaemia reperfusion injury during human orthotopic liver transplantation. *Transplantation*, 78(2), 250–256.
- Yadav, S. S., Howell, D. N., Gao, W., Steeber, D. A., Harland, R. C., & Clavien, P. A. (1998). L-selectin and ICAM-1 mediate reperfusion injury and neutrophil adhesion in the warm ischemic mouse liver *The American journal of physiology*, 275(6 Pt 1), G1341–52.
- Yang, S.-L., Chen, L.-J., Kong, Y., Xu, D., & Lou, Y.-J. (2007). Sodium nitroprusside regulates mRNA expressions of LTC₄ synthesis enzymes in hepatic ischemia/reperfusion injury rats via NF-kappaB signaling pathway *Pharmacology*, 80(1), 11–20. doi:10.1159/000102595
- Yerebakan, C., Ugurlucan, M., Bayraktar, S., Bethea, B. T., & Conte, J. V. (2009). Effects of inhaled nitric oxide following lung transplantation *Journal of cardiac surgery*, 24(3), 269–274. doi:10.1111/j.1540-8191.2009.00833.x
- Ysebaert, D. K., De Greef, K. E., Vercauteren, S. R., Ghielli, M., Verpooten, G. A., Eyskens, E. J., & De Broe, M. E. (2000). Identification and kinetics of leukocytes after severe ischaemia/reperfusion renal injury *Nephrology, dialysis, transplantation: official publication of the European Dialysis and Transplant Association - European Renal Association*, 15(10), 1562–1574.
- Zaky, A., Siriussawakul, A., Tostenrud, R., Pauldine, R., & Lang, J. (2009). Clinical Update on Therapeutic Use of Nitric Oxide. *Contemporary Critical Care*, 7(6), 1–12.
- Zwacka, R. M., Zhou, W., Zhang, Y., Darby, C. J., Dudus, L., Halldorson, J., Oberley, L., et al. (1998a). Redox gene therapy for ischemia/reperfusion injury of the liver reduces AP1 and NF-kappaB activation *Nature medicine*, 4(6), 698–704.
- Zwacka, R. M., Zhang, Y., Zhou, W., Halldorson, J., & Engelhardt, J. F. (1998b). Ischemia/reperfusion injury in the liver of BALB/c mice activates AP-1 and nuclear factor kappaB independently of IkappaB degradation *Hepatology (Baltimore, Md.)*, 28(4), 1022–1030. doi:10.1002/hep.510280417

IntechOpen

Liver Transplantation - Basic Issues

Edited by Prof. Hesham Abdeldayem

ISBN 978-953-51-0016-4

Hard cover, 418 pages

Publisher InTech

Published online 15, February, 2012

Published in print edition February, 2012

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

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Erik Stoltenberg, Alexander A. Vitin, Bradley E. Hansen and John D. Lang, Jr. (2012). The Influences of Nitric Oxide on Liver Ischemia-Reperfusion Injury, Liver Transplantation - Basic Issues, Prof. Hesham Abdeldayem (Ed.), ISBN: 978-953-51-0016-4, InTech, Available from: <http://www.intechopen.com/books/liver-transplantation-basic-issues/the-influences-of-nitric-oxide-on-liver-ischemia-reperfusion-injury>

INTECH
open science | open minds

InTech Europe

University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](https://creativecommons.org/licenses/by/3.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen