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Controversial Role of Nitric Oxide in Hepatic Structural and Functional Injury

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Abstract

Nitric oxide (NO) may regulate hepatic metabolism directly by causing alterations in hepatocellular (hepatocyte and Kupffer cell) metabolism and function or indirectly as a result of its vasodilator properties. NO may be released from the hepatic vascular endothelium, platelets, nerve endings, mast cells, and Kupffer cells as a response to various stimuli such as endotoxemia, ischemia-reperfusion injury, and circulatory shock. It is synthesized by nitric oxide synthase (NOS), which has three distinguishable isoforms: NOS-1 (ncNOS), a constitutive isoform originally isolated from neuronal sources; NOS-2 (iNOS), an inducible isoform that may generate large quantities of NO and may be induced in a variety of cell types throughout the body by the action of inflammatory stimuli; and NOS-3 (ecNOS), a constitutive isoform originally located in endothelial cells. It is believed that Kupffer cells are the main source of NO during endotoxemic shock and that selective inhibition of this stimulation may have future beneficial therapeutic implications. NO may possess both cytoprotective and cytotoxic properties depending on the amount and the isoform of NOS by which it is produced. The mechanisms by which these properties are regulated are important in the maintenance of whole body homeostasis and remain to be elucidated.

Introduction

The function of any organ critically depends on an efficient blood supply that provides adequate tissue perfusion. This is particularly pertinent in a metabolically active organ such as the liver because any factors that alter sinusoidal perfusion will affect hepatic metabolism. The naturally occurring vasodilator nitric oxide (NO) exerts profound effects on hepatic vascular tone[1-2]. Surprisingly little is known regarding its direct actions on hepatic metabolism. The mechanisms by which NO can elicit changes in hepatic metabolism can be broadly divided into two areas by 1) exerting a direct effect on hepatic uptake, storage, detoxification, and clearance mechanisms[3] and 2) exerting an indirect effect either by induction of changes in hepatic vascular tone, which would ultimately affect these mechanisms or via modulation of the activity of other vasoactive substances such as prostaglandins[4]. Nitric oxide (NO) has been found to have a myriad of biological effects. After its initial discovery as playing an important role in the regulation of blood flow, it has been found to be involved in numerous physiological processes including neurotransmission, mediation of macrophage activity, memory formation, immunomodulation and apoptosis[5-7]. The inducible nitric oxide synthase (iNOS) is expressed by many cell types including macrophages, smooth muscle cells and hepatocytes[8]. Hepatocytes have been shown to express large levels of NO following exposure to endotoxins, such as bacterial lipopolysaccharide and/or cytokines, such as tumour necrosis factor-α (TNFα) and interleukin-1[9-10]. Increased levels of NO in hepatocytes have been reported to cause a decrease in protein synthesis, inhibit mitochondrial respiration and the activation of guanylate cyclase[11]. Increased levels of NO and iNOS have also been shown to be present also in hepatocyte suspensions following isolation, thereby suggesting that iNOS is induced in response to stresses occurring during the collagenase perfusion process[12-13]. One area in which NO is known to exert effects is cell death. NO can act as a prooxidant and thereby induce apoptotic cell death[14]. However, in other situations NO can function as a cellular antioxidant and protect cells from damage induced by reactive oxygen species (15). It is now well established that NO plays an important role in apoptosis and depending on unknown conditions NO has the ability to either induce or inhibit apoptosis (7). The liver performs a variety of important host defense and metabolic functions that include synthesis of acute phase proteins, gluconeogenesis, detoxification, and clearance of endogenous mediators, as well as secretion of proinflammatory cytokines[16]. Hepatic dysfunction after sepsis is a frequent event that is characterized by loss of synthetic function, hepatocellular necrosis, and release of inflammatory mediators such as tumor necrosis factor-α (TNF-α), interleukin (IL)-1, IL-6, prostaglandins, and nitric oxide (NO)[17-20]. The specific role of these various cytokines in the pathogenesis of hepatocellular dysfunction or necrosis after endotoxemia is still undefined. Several authors have shown that nonspecific inhibition of all three isoforms of NO synthase (NOS) during endotoxemia may augment hepatocellular injury[21-23]. NO donors have been shown to preserve hepatic perfusion during endotox-

emia and to prevent inflammatory changes in the microcirculation[24-25]. However, a growing body of evidence suggests that sustained production of NO resulting from upregulation of inducible NOS (iNOS) after lipopolysaccharide (LPS) challenge may cause hepatocellular injury, either directly[26], or indirectly, by forming reactive nitrogen intermediates[27]. Menezes et al.[28] recently demonstrated that a NO scavenger, NOX, decreased hepatocellular injury and improved survival after hemorrhagic shock. This review presents an overview of hepatic anatomy, hepatic metabolic processes, direct hepato-toxicity, synthesis of NO and direct and indirect effects of NO on hepatic metabolism.

Anatomy of the liver

The liver may be considered as a collection of numerous microscopic structural and functional units, the acini, whose effluents of blood and bile eventually drain into the main hepatic veins and common bile duct, respectively. Each functional unit, or acinus, comprises a cluster of cells that are arranged anatomically around the hepatic vasculature[29]. The hepatocellular architecture of the liver is highly complex partially because 1) the liver is composed of several cell types, 2) the ratio of different cell types may alter after hepatocellular injury owing to its high regenerative capacity, and 3) there are two afferent vasculatures, the hepatic artery (HA) and the portal vein (PV). Liver cells can be divided into the hepatic parenchymal cells (hepatocytes) and the nonparenchimal hepatic cells, which are further subdivided into 1) endothelial cells; 2) smooth muscle cells, which probably comprise the intrahepatic vascular resistance sites; 3) kupffer cells, the hepatic macrophages that are believed to be derived from monocytes and which make up to 90% of the total body tissue macrophage count[3], and 4) Ito cells, which are responsible for fat storage but may also play a role in regulation of sinusoidal resistance.

Hepatic metabolic processes

The numerous and diverse functions of the liver range from the synthesis of proteins and peptides and storage of carbohydrates to detoxification of drugs and gut metabolites and the inactivation of hormones. The liver is also responsible for the formation of bile, urea, the metabolism of fat, and also has important immune functions. An efficient blood supply is critical to many vital metabolic functions of the liver. In addition, many metabolic and pharmacologic reactions carried out in the liver are oxygen dependent and rely on adequate oxygenation and efficient gaseous exchange. Curiously, the direct influence of hepatic blood flow on regulation of hepatic metabolism has still to be fully elucidated[30].

Direct hepato-toxicity

The liver injury has been studied in some detail using the chlorinated hydrocarbon pesticide 'Dieldrin' as a model[31]. The first stage, that of enzyme induction, is characterized by an increase in liver weight, microsomal protein, smooth endoplasmic reticulum and cytochrome p450. During the second, steady state, the elevated levels are maintained and tolerance to otherwise fatal doses of 'Dieldrin' prevails. In the third stage, that of decompensation, elevation of liver weight, microsomal protein and p45O persists and the smooth endoplasmic reticulum appears abundant but the activity of the drug-handling enzymes decreases. This hypoactive, hypertrophic, smooth endoplasmic reticulum is accompanied by biochemical and morphological alterations in the mitochondria. This may develop before light microscopy shows changes in the hepatocytes and may reflect the border between adaptation and toxicity. All direct hepato-toxins interfere with protein synthesis. This is shown by an early fall in clotting factors such as prothrombin. The fatty change in the liver cell is probably related to failure of synthesis of carrier proteins. The hepatocellular necrosis is more difficult to explain and is not simply related to mitochondrial damage.

Hepatic cirrhosis

Cirrhosis is defined anatomically as widespread hepatic fibrosis with nodule formation. The responses of the liver to severe injury are strictly limited; the most important are collapse of hepatic lobules, formation of diffuse fibrous septa, and nodular regeneration of the hepatic parenchyma. When the liver cells become necrotic and disintegrate, the *reticulin framework collapses* with approximation of portal and central zones *(bridging)*. Some cells regenerate to form nodules of various sizes. These changes following lobular collapse are characteristic of severe virus hepatitis. New fibroblasts form round damaged liver cells and proliferated ductules. The fibrosis (collagen) progresses from a reversible to an irreversible state[32] where acellular permanent septa have developed in the portal zones and hepatic parenchyma. *Regenerative liver-cell nodules* distort the hepatic vascular tree; the flow of portal blood is impeded and portal hypertension results. Sinusoids persist at the periphery of regenerating nodules, shunting blood directly from the portal zones to the hepatic vein. Thus, portal blood is diverted past functioning liver tissue and this may lead to vascular insufficiency in the centre of the nodules and even to persistence of the cirrhosis after the initial causative injury has been controlled. Basement membranes form in the sinusoids which become capillarized so impeding metabolic exchange with the liver cells[33].

Alcohol and the liver

The association of alcoholism with cirrhosis of the liver was recognized by Matthew Baillie in 1793 and later by Addison[33]. The frequency of cirrhosis in alcoholics is undoubted. The susceptibility of the liver to the effects of alcohol is related to the fact that it is the only organ metabolizing it. Hepatic effects are the most important in terms of chronic invalidism and death. The effects consist of single cells showing lytic necrosis, the cytoplasm containing an irregular, frequently perinuclear, clump of highly refractile,

densely eosinophilic material, the alcoholic hyaline of Mallory. Polymorphs surround the necrosing liver cell. Kupffer cell proliferation is prominent. Cholestasis is noted if the patient is jaundiced. The portal zones show stellate fibrosis and infiltration with round cells. In severely malnourished alcoholics centrizonal fibrosis may proceed even to obliteration of hepatic venous radicles[33]. *Sclerosing central hyaline necrosis*. Fibrous septa, commencing in relation to cell necrosis ('creeping collagenosis') divide the liver up into small, regular, uniformly distributed nodules until a micronodular cirrhosis is produced. Fatty change bears a reciprocal relationship to fibrosis. The stromal collapse and cirrhosis seem to follow the necrosis rather than the fatty change[33].

Synthesis of nitric oxide

Only a broad outline of some of the most pertinent features of what is currently known relating to the biochemistry of NO is presented here. The reader will find extensive reviews in the literature[34-39]. NO is synthesized endogenously from L-arginine via the action of NOS, which possesses homology with the cytochrome P450-type enzymic group. Synthesis is achieved by NADPH-dependent oxidation of the guanidino nitrogen of L-arginine. It is believed that a more labile intermediary molecule, *N*-hydroxy L-arginine, is produced during this reaction and requires the oxidation of another molecule of NADPH before L-citrulline is produced with the liberation of NO[40] (Fig. 1).

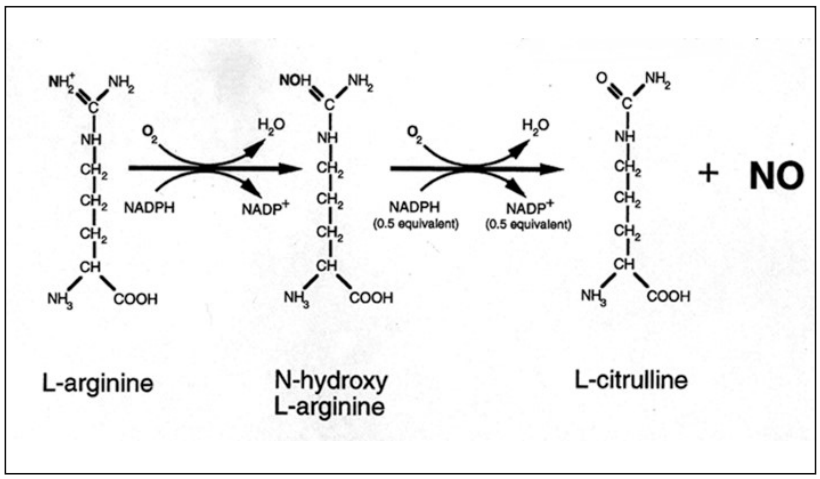

Fig. 1 The biosynthesis of nitric oxide (NO) from the catabolism of L-arginine to L-citrulline is a multistep process that involves the five electron oxidation of nitrogen. The process is catalyzed by a single enzyme, NOS, which makes the reaction unique and the underlying enzymology difficult to elucidate. NOS is a cytochrome P450 -type of enzyme and contains a hem complex at what is believed to be the active site for its reactivity. This reaction is largely based on analogies with other monoxygenase-type reactions that involve an initial two-electron oxidation that is aided by one molecule of NADPH to form the unstable intermediary N-hydroxy L-arginine. It is believed that this is rapidly catalyzed again by NOS, and 0.5 molecules of NADPH per reaction act as a cofactor for the addition of a second oxygen molecule to result in a three-electron transfer and the generation of L-citrulline and NO (Stamler and Feelisch 1996)[40].

It has been confirmed that one molecule of NO is produced for each molecule of citrulline and that ultimately the process involves a multistep, five-electron oxidation of nitrogen[41]. In the liver at least, L-citrulline may be converted back to L-arginine via the transamination of the α-amino nitrogen of aspartic acid via the urea cycle. L-arginine is also the natural substrate for arginase, which catalyzes the irreversible conversion of arginine to ornithine to remove ammonia from the body by the production of urea. It remains unclear whether the Km for NOS is greater than that for arginase or whether this may alter according to prevailing physiologic conditions.

Isoforms of nitric oxide synthase

The isoforms of NOS have been further subdivided and now fall into three basic categories: 1) NOS-1 (ncNOS), a neuronal and constitutive isoform; 2) NOS-2 (iNOS), the inducible form that is distributed ubiquitously in the body after appropriate induction or stimulation; and 3) NOS-3 endothelial and constitutive NOS (eNOS), the constitutive form that is located in endothelial cells. eNOS and ncNOS are calmodulin-dependent for activity and are dimeric complexes, and iNOS function does not depend on calmodulin binding and activation, as this is prebound to the molecule and exists as trimeric and tetrameric complexes[35]. The basic categories are based on their localization, their dependency for Ca^{2+}/calmodulin, and their molecular identity. The basic structural subdivision between iNOS and the two constitutive isoforms of NOS (eNOS) exists because of the unbound state of the calmodulin receptor on eNOS. This is tightly bound on iNOS (NOS-2), which, therefore, does not depend on calcium-calmodulin binding for activity. Activation of iNOS is currently believed to depend on exposure to immunologic or inflammatory stimuli such as bacterial endotoxins, LPS, IL-1, and tumor necrosis factor (TNF). It has been reported[42] that an inducible NOS isoform that can be stimulated by Ca^{2+}/calmodulin has also been isolated in the rat liver.

Direct and indirect effects of nitric oxide on hepatic metabolism

Much of the literature available concerning the direct effects of NO on hepatic metabolism involves the characterization of various isoforms of NOS that have been identified. The degree of activation of NO cannot be directly measured in terms of receptor binding activity and is indirectly measured by the activity and expression of NO synthase. Much interest has centered on the role of Kupffer cell function on hepatocyte, and Kupffer cell metabolism, as they are both capable of synthesizing NO. Kupffer cells act as hepatic macrophages at fixed anatomic locations and, like circulating macrophages, are phagocytic and are able to ingest substances ranging from exogenously delivered particulate matter such as carbon colloid to microorganisms and bacterial toxins[43]. It has been proposed[44] that the ratio of Kupffer cell to hepatocyte number may be important in the role of Kupffer

cell function and NO production in hepatocyte cytotoxicity. The ratio is higher (that is, Kupffer cells increase) after endotoxin or immunostimulant injection[44]. Both hepatocytes and Kupffer cells are capable of synthesizing NO under normal and diseased conditions, but Kupffer cells may also synthesize other superoxide radicals, proteins, eicosanoids, TNF and IL-1[3]. On exposure to inflammatory stimuli such as LPS and γ-interferon, Kupffer cells have been shown to release TNF and IL-1[45]. It is believed[46] that the release of TNF and particularly IL-1[45] from Kupffer cells may then stimulate the synthesis of iNOS in hepatocytes. The simultaneous release of several cytokines could act synergistically to induce hepatocyte iNOS[45-46]. The complex actions of Kupffer cell inflammatory mediators, which include TNF, IL-1, IL-6, and NO, are largely undetermined in vivo, and whether they exert cytotoxic or cytoprotective actions remains unclear (Fig. 2).

Hepatic metabolism critically depends on adequate delivery of blood to the liver, and total hepatic blood flow may extract up to 25% from the cardiac output. It is now known that NO may modulate PV and HA vascular tone and may, therefore, alter hepatic metabolism indirectly by inducing changes in hepatic blood flow. This is in contrast to its direct cytotoxic or cytoprotective actions on hepatocyte and Kupffer cell metabolism discussed earlier.

Role of nitric oxide in cirrhosis

Cirrhotic patients exhibit a hyperdynamic splanchnic circulation that has been attributed to increased peripheral release of NO[48]. Initial reports[49] demonstrated that non-specific inhibition of NO activity by methylene blue, which inhibits cGMP, in a patient with decompensated alcoholic cirrhosis, reversed the severe hypotension associated with this disease. Perfused mesenteric arteries from portal-hypertensive and cirrhotic rats are less responsive to the vasoconstrictors noradrenaline, vasopressin, potassium chloride, and methoxamine compared with normal rats, and this was partly attributed to increased release of NO[50-51]. In addition, inhibition of NO synthesis in portal vein partially ligated rats did not alter the reduced basal perfusion pressure compared with controls, although the responsiveness to vasoconstrictors was restored[52]. Others[53] showed in a similar model of portal hypertension that L-NMMA restored the reduced systemic blood pressure to control values, although portal vascular resistance remained unchanged. A recent study[54] has reported, however, that the hyporesponsiveness to methoxamine in isolated perfused mesenteric vascular bed preparations from rats with carbon tetrachloride-induced cirrhosis could only be partly restored to normal using L-NAME. It may also be likely that the

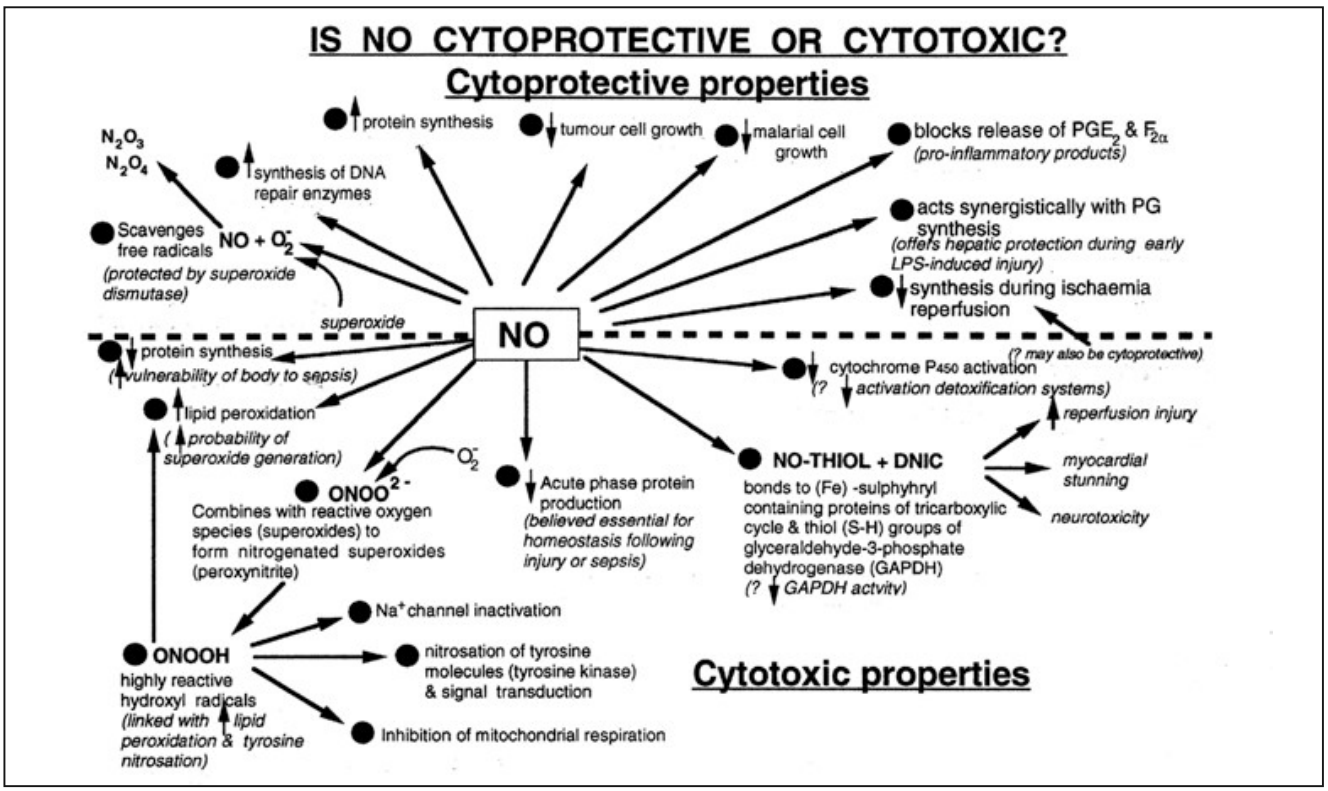

Fig. 2 The potential for nitric oxide (NO) to exert cytotoxic or cytoprotective actions appears to be related to its ability to interact with other molecules in the immediate environment and also to the quantity of NO produced. This is probably because its half-life is only 5 s. NO is itself a superoxide molecule, but it may also form NO_2 O_3 and N_2O_4 in the presence of O_2^- and may be considered as cytoprotective in this context. In addition, it has been suggested[47] that these two molecules may be of physiologic relevance in their own right. The cytotoxic properties of NO have been associated with the ability of NO to react with superoxide radicals to form nitrogenated superoxides such as peroxynitrite ($ONOO^{2-}$). This forms the highly reactive hydroxyl radical ONOOH, which has been shown to increase lipid peroxidation and increase the probability of further superoxide generation. The ability of NO to react with Fe-nitrosyl and thiol-containing ligands to form nitrosyl iron cystein (DNIC) and nitrosothiol ligands (NO-THIOL) is believed to be related to NO-induced reduction of GAPDH activity[38].

changes in vascular tone reported during portal hypertension may vary according to anatomic location; for example, isolated aortic rings from carbon tetrachloride-induced cirrhotic rats had impaired responsiveness to angiotensin II (not found in the hepatic microvasculature), and this was attributed to increased NO synthesis[55]. Other studies[52] conducted in isolated segments of mesenteric arteries from portal hypertensive rats did not show a reduction in vascular reactivity to vasoconstrictors, in fact an increased maximum responsiveness to vasoconstrictors was demonstrated which was absent in the aortae of these rats. This increased maximum responsiveness could not be produced in mesenteric arteries from control rats in the presence of L-NMMA, and therefore it was concluded that the increased responsiveness to vasoconstrictors in portal hypertensive rats is not due to a diminished responsiveness of the smooth muscle possibly via decreased release of NO. L-NMMA, moreover did not alter arterial cGMP concentrations and reduced portal tributary blood flow and vascular resistance by similar proportions in control and cirrhotic rats and consequently reduced the portal hypertension[52]. Because the reduction in NO synthesis was identical in both groups, it is possible that NO synthesis may not be involved in cirrhosis[56].

Discussion

NO exerts many physiologic actions, the complexities of which have only begun to be addressed. It was originally difficult to believe that such a short-lived and simple molecule could exert profound effects and be of such physiologic significance. Perhaps, it is those characteristics that render the molecule so physiologically versatile. In addition, the ability of NO to interact with a host of other substances, such as prostaglandins and oxygen-derived free radicals, and to be the messenger in signal transduction mechanisms for molecules such as ATP and acetylcholine, implies a highly complex chemistry and a diverse range of physiologic and pathophysiologic actions[30]. Isolation of rat hepatocytes by the two-step collagenase method resulted in the formation of large amounts of nitrites. This increase in nitrites was inhibited by the NOS inhibitor L-NAME, suggesting that this increase in extracellular nitrite formation results from the activation of NOS and subsequent formation of NO. This is in agreement with previous findings demonstrating both an increase in nitrites[57] and NOS mRNA[58] following hepatocyte isolation. The fundamental reason for the activation of NOS is apparently related to the presence of collagenase during the liver perfusion. This was demonstrated by Wang et al.[58], who showed that hepatocytes isolated without the use of collagenase had negligible levels of iNOS mRNA. There are a number of changes that occur during the hepatocyte isolation process that may have the potential to induce NOS. Initially, perfusion with ethyleneglycolbis(b-aminoethylether)-N,N,N,N-tetraacetic acid and subsequent calcium addition results in sheer stress and structural changes to the architecture of hepatocytes in the liver[59]. These alterations, and changes in calcium fluxes, may affect the induction of NOS. As endotoxin is a well established inducer of NOS in hepatocytes[9], a problem associated with collagenase perfusion may be the high level of endotoxin that can be present in the collagenase. Isolation of hepatocytes in the presence of an endotoxin neutralising agent results in a decrease in subsequent nitrite formation[13]. Finally, oxidative stress during isolation of hepatocytes also contributes to the induction of NOS, and it has been reported that antioxidants present in the perfusion buffers result in a decrease in subsequent nitrite formation[13]. It is likely therefore that there is more than one main cause for the induction of NOS during hepatocyte isolation, the induction of NOS by the cell probably represents the response of it to a range of stresses imposed during the entire procedure. Although NO levels were increased in hepatocyte monolayers, this increase had apparently no effect on basal levels of ATP or GSH which was demonstrated by the fact that L-NAME had no effect on these parameters. Measurement of cellular ATP and GSH give an insight into the mechanism of cell death since both play an important role in apoptosis. The redox status of a cell plays an important role in determining the mode of cell death, apoptosis or necrosis[60]. GSH is one of the cell's major antioxidant defences and therefore any alterations in GSH will affect the cell redox state. In accordance, Fernandes and Cotter[61] demonstrated that a decrease in cellular GSH switches the mode of cell death from apoptosis to necrosis, since GSH is thought to play a role in the activation of caspases during apoptosis[62]. Measurement of cellular ATP gives an indication of mitochondrial viability. Mitochondria are known to play a pivotal role in the initiation and control of apoptosis[63]. ATP is required for apoptosis since many of the events occurring during apoptosis, for example DNA fragmentation and vesicle formation, are energy-dependent processes. Therefore, a decrease in cellular ATP levels has been shown to change the mode of cell death from apoptosis to necrosis[64]. Therefore, any decreases in ATP or GSH will reflect the fact that cell death is occurring primarily by necrosis and not apoptosis. The increase in hepatocyte NO levels had no effect on basal apoptosis, as observed by the lack of effect of L-NAME on basal ATP and GSH levels and on basal levels of the activating downstream effector caspase, caspase-3[65]. Basal levels of hepatocyte apoptosis are low therefore any potential effect of L-NAME may have been too small to detect. NO is a well established inhibitor of apoptosis; however, the precise mechanism of action for this inhibition is not completely understood. One possible mechanism for the inhibitory effects NO has on apoptosis is the interaction between NO and heat shock proteins (HSPs)[66]. NO is also known to have an additional inhibitory effect on apoptosis by directly affecting the activity of the proteases responsible for apoptosis, caspases. NO has been shown to inhibit apoptosis by directly or indirectly inhibiting caspase-3 activation via a cGMP-dependent mechanism, and additionally by directly inhibiting caspase-3 activity via protein S-nitrosylation[67]. In summary, the hepatocyte monolayers produce large amounts of nitrite following isolation as a result of NOS activation and subsequent production of NO. This increase in NO was shown to have no effect on basal levels of hepatocyte apoptosis but an inhibitory effect on apoptosis induced. Further research is required to determine the full extent of the effects of NO production in hepatocyte monolayers. Some authors

demonstrated that NO mediates hepatocellular injury after endotoxic shock[26]. Inhibition of eNOS leads to decreased hepatic perfusion and increased hepatocellular injury in a model of hemorrhagic shock[68]. NOS-2 (iNOS) is calcium independent and may be induced in large quantities by inflammatory stimuli, including LPS[69]. Small quantities of NO derived from eNOS may exert a protective role in the liver by *1)* preserving hepatic arterial and portal blood flow[25], *2)* preventing infiammation in the hepatic microcirculation[24], or *3)* inhibiting reactive oxygen intermediate release[70]. However, excess NO produced in infiammation may be deleterious. Although there have been conflicting reports regarding the role of NO in hepatocellular damage, Nadler et al. [26] demonstrated that NO, or its reactive nitrogen intermediates, may promote liver injury after endotoxemia, ischemia/reperfusion, or hemorrhagic shock[71]. Mustafa et al.[71] used platelet-activating factor receptor antagonists to inhibit NO formation and prevent hepatic injury in LPS-challenged livers and in Kupffer cell culture. The hepatocellular injury attributed to NO may be due to its direct cytotoxicity or its diffusion-limited reaction with superoxide to produce the toxic nitrogen metabolite peroxynitrite[72]. Ma et al.[73] pretreated mice with endotoxin to induce hepatic NO production before ischemia/reperfusion, which resulted in increased hepatocellular injury, implicating peroxynitrite as a causative agent. The mechanism of NO-mediated hepatocellular injury also remains somewhat controversial. Early reports suggested that LPS-induced hepatic dysfunction was primarily due to necrosis rather than apoptosis[74]. However, Redmond et al.[75] used LPS in conjunction with antioxidants to induce hepatocellular apoptosis. Inhibition of NO production reduced both hepatocyte necrosis and apoptosis in this model. Wang et al.[14] confirmed these results by illustrating that the NO donor, SNAP, could induce hepatocellular apoptosis. However, in the presence of reactive oxygen intermediates, NO led to hepatic necrosis. Nadler et al.[26] showed that LPS challenge induced hepatic injury via necrosis rather than apoptosis.

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