Nitric oxide mediates hepatocyte injury

JIANG HUAI WANG, H. PAUL REDMOND, QIONG DI WU, AND DAVID BOUCHIER-HAYES
The Royal College of Surgeons in Ireland, Department of Surgery, Beaumont Hospital, Dublin 9, Ireland

Wang, Jia-Huai, H. Paul Redmond, Qiong Di Wu, and David Bouchier-Hayes. Nitric oxide mediates hepatocyte injury. Am. J. Physiol. 275 (Gastrointest. Liver Physiol. 38): G1117–G1126, 1998.—The degree of acute hepatic failure after severe trauma and sepsis is related to the extent of hepatocyte (HC) damage and cell death resulting from either necrosis or apoptosis. We have previously demonstrated that tumor necrosis factor-α (TNF-α) and lipopolysaccharide (LPS) can directly lead to HC necrosis, but not apoptosis. To date, the reactive oxygen intermediates (ROI) and nitric oxide (NO) have been shown to play a potential role in the induction of cell apoptosis. However, it is unknown whether ROI and NO are involved in HC cell death. Therefore, in this study we tested the hypothesis that NO and ROI exert different effects on HC cell death. TNF-α and LPS alone failed to induce HC apoptosis but when combined with antioxidants resulted in HC apoptosis and DNA fragmentation, which is correlated with apoptosis but when combined with antioxidants resulted in HC cell death. TNF-α and LPS induced HC apoptosis, whereas HO and L-NMMA. These results indicate that NO is involved in HC injury, primarily through the induction of HC apoptosis.

Materials and Methods

Reagents. The following reagents were used for the isolation and culture of rat HC and assessment of HC cell death. DMEM, RPMI 1640, HBSS without Ca2+ and Mg2+, fetal calf serum (FCS), penicillin, streptomycin sulfate, 0.05% trypsin-0.02% EDTA solution, glutamine, and insulin were purchased from Gibco BRL (Paisley, Scotland, UK). LPS (Escherichia coli O55:B5), SDS, sodium chloride, sodium phosphate, calcium chloride, sodium bicarbonate, potassium chloride, sodium nitrate, SNP, superoxide dismutase (SOD; 5,100 U/mg protein), catalase (CAT; 2,000 U/mg protein), glutathione (GSH), DMSO, N-acetyl cysteine (NAC), HEPES, EDTA, Tris, Triton X-100, glucose, propidium iodide (PI), and collagenase (type II) were purchased from Sigma (St. Louis, MO). Recombinant human TNF-α (2 × 107 U/mg) was obtained from Genzyme (Cambridge, MA). Nω-monomethyl-L-arginine (L-NMMA) was from ICN (Cleveland, OH). RNase A and d×174 DNA/HaeIII markers were obtained from Boehringer Mannheim Biochemica (Mannheim, Germany) and Promega (Madison, WI), respectively.

Isolation of rat HC. Adult male pathogen-free Sprague-Dawley rats weighing 200–300 g (obtained from Charles River Breeding Laboratories, Kent, UK) were fasted overnight before experimentation and allowed water ad libitum. Rat HC were isolated by a modification of the techniques of Seglen (52) and Doolittle and Richter (19), and all of the procedures were performed under sterile conditions. Briefly, the rats were anesthetized with inhalation of halothane (May and Baker, Dagenham, UK), and then the portal vein was cut to expose the portal vein. An 18-gauge needle connected to a Masterflex perfusion pump (Cole-Parmer, Niles, IL) with a three-way stopcock was introduced into the liver through the portal vein, and the perfusate was connected to a Masterflex perfusion pump (Cole-Parmer, Niles, IL) with a three-way stopcock was introduced into the liver through the portal vein, and the perfusate was then perfused through the portal vein to perfuse the liver. The perfusate consisted of DMEM, RPMI 1640, HBSS without Ca2+ and Mg2+, fetal calf serum (FCS), penicillin, streptomycin sulfate, 0.05% trypsin-0.02% EDTA solution, glutamine, and insulin were purchased from Gibco BRL (Paisley, Scotland, UK). LPS (Escherichia coli O55:B5), SDS, sodium chloride, sodium phosphate, calcium chloride, sodium bicarbonate, potassium chloride, sodium nitrate, SNP, superoxide dismutase (SOD; 5,100 U/mg protein), catalase (CAT; 2,000 U/mg protein), glutathione (GSH), DMSO, N-acetyl cysteine (NAC), HEPES, EDTA, Tris, Triton X-100, glucose, propidium iodide (PI), and collagenase (type II) were purchased from Sigma (St. Louis, MO). Recombinant human TNF-α (2 × 107 U/mg) was obtained from Genzyme (Cambridge, MA). Nω-monomethyl-L-arginine (L-NMMA) was from ICN (Cleveland, OH). RNase A and d×174 DNA/HaeIII markers were obtained from Boehringer Mannheim Biochemica (Mannheim, Germany) and Promega (Madison, WI), respectively.
portal vein. While the inferior vena cava was being cut, the liver perfusion was begun at a constant speed of 30 ml/min with 500 ml of Ca²⁺-free HEPES buffer solution containing 160.8 mM sodium chloride, 3.15 mM potassium chloride, 0.7 mM sodium phosphate, 33 mM HEPES, and 5% glucose maintained at 37°C. This was immediately followed by perfusion of 300 ml Ca²⁺-free HEPES buffer solution supplemented with 0.025% collagenase type IV and 0.075% calcium chloride at a constant speed of 15 ml/min at 37°C. After the perfused liver was removed and dissociated, the cells, suspended in 80 ml of cold Ca²⁺-free HEPES buffer solution, were passed through a sterile 180-µm stainless steel sieve (Endecotts, London, UK) into a 125-ml beaker. Rat HC were separated from nonparenchymal cells by centrifugation at 50 g for 2 min, four times. HC pellets were resuspended in complete RPMI 1640 medium containing 10% FCS, penicillin (100 U/ml), streptomycin sulfate (100 µg/ml), 2 mM glutamine, 28 mM sodium bicarbonate, 8 mM HEPES, and 0.16 U/ml insulin. The remaining Kupffer cells were further eliminated by allowing Kupffer cells to adhere to 60-mm dishes for 1 h at 37°C in 5% CO₂ condition. This procedure yielded HC preparation with less than 5% Kupffer cell contamination as determined by esterase staining and with a viability greater than 80% according to the trypan blue exclusion. Before experimentation HC were cultured for 12 h at 37°C in humidified 5% CO₂ conditions, and culture medium was then replaced with fresh complete RPMI 1640.

Assessment of HC apoptosis. For determination of apoptosis by flow cytometry and DNA gel electrophoresis, HC were plated at 1 × 10⁶ cells per 40-mm dish (Nunc; GIBCO BRL) with different treatments at 37°C in humidified 5% CO₂ conditions. After 12- and 24-h incubation, HC were detached by using a low concentration of trypsin-EDTA at 0.025%-0.01% for detecting apoptosis. For detection of apoptosis by ELISA, HC were plated at 1 × 10⁵ cells/well in 24-well, flat-bottom plates (Falcon, Lincoln Park, NJ) and incubated with different treatments for 12 and 24 h at 37°C in humidified 5% CO₂ conditions.

Flow cytometry. HC apoptosis was assessed according to the percentage of cells with hypodiploid DNA, using the PI staining technique as previously described (44). Briefly, after centrifugation the cell pellets were gently resuspended in 1 ml of hypotonic fluorochrome solution (50 µg/ml PI, 3.4 mM sodium citrate, 1 mM Tris, 0.1 mM EDTA, 0.1% Triton X-100) and incubated in the dark at 4°C overnight before they were analyzed by a FACSscan flow cytometer (Becton Dickinson, Mountain View, CA). The forward scatter and side scatter of HC particles were simultaneously measured. The PI fluorescence of individual nuclei with an acquisition of fluorescence channel 2 (FL2) was plotted against forward scatter, and the data were registered on a logarithmic scale. The minimum number of 5,000 events was collected and analyzed on the software Lysis II. Apoptotic HC nuclei were distinguished by their hypodiploid DNA content from the diploid DNA content of normal HC nuclei. The forward threshold was raised to exclude HC debris from analysis. All measurements were performed under the same instrument settings.

ELISA kit. An ELISA kit (Boehringer Mannheim), which quantitatively detects cytosolic histone-associated DNA fragments, was used to assess apoptosis in adhered HC (38, 39). DNA fragments were detected according to the procedures described in the ELISA kit. Briefly, the cytosolic fraction (13,000 g supernatant) of HC was used as the antigen source with the use of the fluorescent probe 2′,7′-dichlorodihydrofluorescein diacetate (H₂DCFDA) (Molecular Probes, Eugene, OR) as described previously (23). Briefly, after experiments HC were washed twice and resuspended in 200 µl PBS at 5 × 10⁶ cells/ml. Cells were loaded with 20 µM H₂DCFDA and incu-
bated in a 37°C water bath for 10 min. The measurement of ROI generation was performed on a FACScan flow cytometer (Becton Dickinson) for detecting the log of the mean channel fluorescence intensity with an acquisition of fluorescence channel 1 (FL1). The minimum number of 5,000 events was collected and analyzed on the software Lysis II.

Peroxynitrite formation in HC was detected with the use of the fluorescent probe dihydrorhodamine-123 (Molecular Probes) as described previously (34, 64). Briefly, after experiments, 200 µl of HC suspensions (5 × 10⁵ cells/ml) were incubated with 20 µl of dihydrorhodamine-123 for 10 min at room temperature. The generation of peroxynitrite in HC was detected based on the generation of the fluorescent product (rhodamine) through the reaction of peroxynitrite with dihydrorhodamine-123, on a FACScan flow cytometer (Becton Dickinson) for detecting the log of the mean channel fluorescence intensity with an FL1. The minimum number of 5,000 events was collected and analyzed on the software Lysis II.

Statistical analysis. All data are means ± SD. Statistical analysis was performed using ANOVA. Differences were judged to be statistically significant when the P value was less than 0.05.

RESULTS

LPS and TNF-α, in the presence of antioxidants, result in HC apoptosis, which is abrogated by the NO synthase inhibitor L-NMMA. HC were treated with LPS and TNF-α in the presence or absence of antioxidants (SOD, CAT, DMSO, NAC, and GSH) for 12 and 24 h. There was no HC apoptosis found after 12 h incubation (data not shown). However, after 24 h incubation as shown in Fig. 1, A and C, TNF-α in combination with either SOD, DMSO, NAC, or GSH resulted in a significant increase in HC apoptosis (P < 0.05 vs. TNF-α alone), whereas TNF-α alone did not induce HC apoptosis. TNF-α plus CAT failed to induce HC apoptosis. Similar results were found when HC were treated with LPS alone and in combination with the antioxidants (Fig. 1, B and D). The addition of the NO synthase inhibitor L-NMMA significantly attenuated HC apoptosis induced by either TNF-α or LPS, in the presence of SOD, DMSO, and GSH (P < 0.05 vs. LPS or TNF-α plus the antioxidant) (Fig. 2). These findings were further confirmed by DNA gel electrophoresis. TNF-α in combination with either SOD, DMSO, NAC, or GSH induced a typical DNA “ladder” pattern, which indicates the occurrence of DNA fragmentation and thus apoptosis, whereas TNF-α alone failed to cause DNA fragmentation. L-NMMA prevented DNA fragmentation induced by TNF-α plus the antioxidant (Fig. 3). Antioxidants alone did not cause HC apoptosis (data not shown).

Antioxidants and L-NMMA attenuate HC damage induced by TNF-α. As shown in Fig. 4, HC damage directly induced by TNF-α was evident as elevations of both AST and LDH activities, represented as the percentage of total releasable enzyme after 12- and 24-h incubation. The addition of antioxidants, SOD, DMSO, and GSH significantly reduced TNF-α-mediated hepatocellular enzyme release (P < 0.05 vs. TNF-α alone).

Fig. 1. Induction of hepatocyte (HC) apoptosis after exposure to either tumor necrosis factor-α (TNF-α) (A and C) or lipopolysaccharide (LPS) (B and D) in the presence of antioxidants. HC apoptosis (A and B) and DNA fragments (C and D) were assessed as described in MATERIALS AND METHODS after incubation with either TNF-α (25 ng/ml) or LPS (0.1 μg/ml) in the presence or absence of the antioxidants superoxide dismutase (SOD; 400 U/ml), catalase (CAT; 2,000 U/ml), DMSO (0.5%), N-acetylcysteine (NAC; 7.5 mM), and glutathione (GSH; 7.5 mM) at 37°C in 5% CO₂ for 24 h. Data are means ± SD and are representative of 4 separate experiments. *Statistically significant compared with TNF-α alone or LPS alone, P < 0.05.
The combination of L-NMMA and the antioxidants resulted in further reductions in AST and LDH release (P<0.05 vs. TNF-α plus the antioxidant).

The combinations of antioxidants and L-NMMA were also found to protect against HC damage induced by LPS (data not shown).

The effect of LPS and TNF-α, with or without antioxidants, on ROI generation, NO production, and peroxynitrite formation in HC. Exposure of HC to either LPS or TNF-α for 6 h led to a significant increase in ROI generation in HC, whereas the antioxidants SOD, DMSO, or GSH significantly inhibited LPS- and TNF-α-mediated ROI formation (P<0.05 vs. LPS or TNF-α alone) (Fig. 5).

In contrast, TNF-α in combination with either SOD, DMSO, NAC, or GSH resulted in a significant increase in NO level (P<0.05 vs. TNF-α alone) (Fig. 6A), whereas TNF-α alone at different doses failed to augment NO production in HC (Table 1). Similar results were also found in HC treated with LPS alone (Table 1) or in combination with these antioxidants (Fig. 6B).

The oxidation of dihydrorhodamine-123 by peroxynitrite has been successfully utilized for the detection of peroxynitrite formation in vitro (34, 64) and in vivo (55). The method is sensitive and specific, since neither NO nor superoxide causes dihydrorhodamine-123 oxidation (34, 55, 64). As shown in Fig. 7, TNF-α significantly increased peroxynitrite formation in HC. The antioxidants SOD, DMSO, NAC, and GSH attenuated TNF-α-mediated peroxynitrite formation. Similar results were found in HC treated with LPS alone or in combination with antioxidants (data not shown).

Fig. 2. Attenuation of HC apoptosis by the nitric oxide (NO) synthase inhibitor Nω-monomethyl-L-arginine (L-NMMA). HC were treated with TNF-α (25 ng/ml), LPS (0.1 µg/ml), SOD (400 U/ml), DMSO (0.5%), GSH (7.5 mM), and L-NMMA (0.5 mM) in different combinations at 37°C in 5% CO2 for 24 h. HC apoptosis (A and B) and DNA fragments (C and D) were assessed as described in MATERIALS AND METHODS. Data are means ± SD and are representative of 4 separate experiments. Statistical significances were compared with TNF-α plus the antioxidant (SOD, DMSO, GSH) (A and C) or LPS plus the antioxidant (SOD, DMSO, GSH) (B and D). @P<0.05.

Fig. 3. Gel electrophoresis for detection of HC DNA fragmentation. DNA gel electrophoresis was performed as described in MATERIALS AND METHODS. TNF-α (25 ng/ml) plus either SOD (400 U/ml), DMSO (0.5%), NAC (7.5 mM), or GSH (7.5 mM), but not TNF-α alone or TNF-α plus CAT (2,000 U/ml), induced the typical “ladder” pattern of DNA fragmentation. L-NMMA (0.5 mM) prevented TNF-α plus either SOD, DMSO, or GSH-mediated DNA fragmentation. Lanes 1–10 contained medium, TNF-α alone, TNF-α + SOD, TNF-α + CAT, TNF-α + DMSO, TNF-α + NAC, TNF-α + GSH, TNF-α + SOD + L-NMMA, TNF-α + DMSO + L-NMMA, and TNF-α + GSH + L-NMMA, respectively.
The NO donor SNP induces HC apoptosis and cell damage. The NO donor SNP was used in this study to further elucidate the effect of NO on HC injury. Exposure of HC to 0.5 mM SNP for 24 h led to HC apoptosis, as evidenced by flow cytometry and ELISA, whereas the antioxidants SOD, DMSO, and GSH augmented SNP-induced HC apoptosis and DNA fragmentation (P < 0.05 vs. SNP alone) (Fig. 8). In contrast, the addition of antioxidants significantly reduced SNP-mediated HC damage as represented by hepatocellular enzyme AST and LDH release (P < 0.05 vs. SNP alone) (Fig. 9).

DISCUSSION

The abnormal or excessive interactions of the proinflammatory mediators, such as exogenous LPS and endogenous TNF-α, with neutrophils and Kupffer cells are thought to play an important role in hepatic dysfunction. The extent of HC damage and cell death, presumably resulting from either necrosis or apoptosis, determines the degree of acute hepatic failure. Evidence from our laboratory has demonstrated that LPS and TNF-α, apart from their indirect effect on HC injury through neutrophil and Kupffer cell activation, can also directly result in HC damage and apoptosis (57). Furthermore, the induction of HC apoptosis has been shown to be responsible for the occurrence of hepatic injury and to precede hepatic failure in experimental murine shock models (39). However, the precise mechanisms involved in the induction of HC apoptosis during SIRS remain to be elucidated. The major finding in this in vitro study is that NO is involved in HC injury, primarily through its role in the induction of HC apoptosis.

Since the discovery that murine macrophages could produce NO when stimulated with interferon-γ (IFN-γ) and LPS (54) and the subsequent observation that
**L-arginine** serves as the substrate for macrophage NO production (29), a number of other cell types, including endothelial cells (46), neutrophils (49), vascular smooth muscle cells (12), myocardial cells (21), Kupffer cells (5), etc.

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**Fig. 6.** Augmentation of NO production in HC after treatment with either TNF-α (A) or LPS (B) in presence of antioxidants. NO production was measured after 24-h incubation with TNF-α (25 ng/ml), LPS (0.1 µg/ml), SOD (400 U/ml), CAT (2,000 U/ml), DMSO (0.5%), NAC (7.5 mM), and GSH (7.5 mM) at 37°C in 5% CO2 as described in MATERIALS AND METHODS. Data are means ± SD, representative of 4 separate experiments. Each experiment was carried out in triplicate. *Statistically significant difference vs. TNF-α alone or LPS alone (P < 0.05).

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**Table 1.** NO production in HC after treatment with TNF-α and LPS

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<th>Treatment</th>
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<th>P</th>
<th>72 h NO, µM</th>
<th>P</th>
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<td>Medium</td>
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<td>0.35</td>
<td>2.717 ± 0.806</td>
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<td>TNF-α, ng/ml</td>
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<td>25</td>
<td>3.233 ± 1.005</td>
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<tr>
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<td>0.237</td>
<td>4.478 ± 2.011</td>
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<td>LPS, µg/ml</td>
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<tr>
<td>0.1</td>
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<td>3.733 ± 1.365</td>
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<tr>
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<td>0.192</td>
<td>3.967 ± 1.206</td>
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**Fig. 7.** Attenuation of TNF-α-mediated peroxynitrite formation in HC by antioxidants. Peroxynitrite formation was detected after HC were treated with TNF-α (25 ng/ml), SOD (400 U/ml), CAT (2,000 U/ml), DMSO (0.5%), NAC (7.5 mM), or GSH (7.5 mM) for 12 h at 37°C in 5% CO2 as described in MATERIALS AND METHODS. Data are expressed as MCF/cell. Results are means ± SD, representative of 3 separate experiments. *Statistically significant difference vs. TNF-α alone (P < 0.05).

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**Fig. 8.** Induction of HC apoptosis (A) and DNA fragments (B) after exposure to SNP. Isolated HC were incubated either with SNP alone (0.5 mM) or in combination with SOD (400 U/ml), DMSO (0.5%), and GSH (7.5 mM) at 37°C in 5% CO2 for 24 h. HC apoptosis and DNA fragments were assessed as described in MATERIALS AND METHODS. Data are means ± SD, representative of 4 separate experiments. *P < 0.05 vs. medium; @P < 0.05 vs. SNP alone.
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Fig. 9. Induction of HC damage by SNP as represented by hepatocellular enzyme release. AST (A) and LDH (B) activities, represented as % of total releasable enzyme, were measured as described in MATERIALS AND METHODS after incubation with SNP, either alone (0.5 mM) or in combination with SOD (400 U/ml), DMSO (0.5%), or GSH (7.5 mM) at 37°C in 5% CO2 for 12 h. Data are means ± SE from 3 separate experiments. Statistical significances were compared with medium as control (*P < 0.05) or with SNP alone (@P < 0.05).

and HC (15, 22), have been shown to produce NO. It has been suggested that different cell types may vary not only in the timing and quantity of NO production but also in the signals that stimulate the target cell to produce NO (5, 15, 22, 46, 48). In rat HC, Curran et al. (15, 16) and Geller et al. (22) have shown that single cytokine but rather combinations of TNF-α, LPS, IFN-γ, and interleukin-1β are required for the induction of HC NO production in vitro, which correlates with the expression of inducible NO synthase in HC (16, 22). In contrast, other investigators have found that exposure of rat HC to either TNF-α alone (45) or LPS alone (48) can induce a significant increase in NO production and that endotoxin-induced NO production is partially mediated by HC-derived TNF-α (48). In the present study, we demonstrate that TNF-α and LPS alone failed to induce NO production in HC. However, when TNF-α or LPS was combined with different antioxidants such as SOD, DMSO, NAC, or GSH, NO production was significantly augmented. The possible mechanism for this phenomenon is that ROI, particularly superoxide anion, and NO can react rapidly to form peroxynitrite anion through the near-diffusion-limited reaction, which reduces the level of biologically active NO (8, 20). Therefore, it is possible that TNF-α or LPS-mediated ROI generation lowered the level of NO, possibly through peroxynitrite formation, as both of these oxygen species can be produced in HC by TNF-α or LPS. Moreover, antioxidants scavenging ROI inhibited peroxynitrite formation and thus increased the NO level in TNF-α or LPS-stimulated HC cultures. This finding is supported by the observation that oxygen radical scavengers were found to elevate the NO level in interleukin-1-treated chondrocyte cultures (8).

There are two distinct mechanisms involved in eukaryotic cell death, apoptosis and necrosis, which can be distinguished by morphological and biochemical criteria (13, 51). Apoptosis, characterized by cell shrinkage, nuclear condensation, and DNA fragmentation, is a form of programmed cell death that is genetically controlled and regulated by signal transduction-coupled events. Apoptosis plays an important role in controlled deletion of cells during metamorphosis, differentiation, and normal cell turnover. However, recent accumulating evidence has expanded the definition of apoptosis, and it is now recognized that apoptosis is not always physiological (32). In endothelial cells, the induction of apoptosis has been implicated in the development of increased vascular permeability as a consequence of the loss of barrier function, which may lead to significant organ dysfunction (1, 11, 58, 59). In different murine models of acute inflammatory liver failure, hepatic damage induced in d-galactosamine-sensitized mice by endotoxin infection was found to be initiated by processes typical of HC apoptosis (39).

Among the inducers of cell apoptosis and the mechanisms that permit the cell to die in this stereotypical fashion, both ROI and NO have been implicated. In the present study, stimulation with TNF-α and LPS failed to induce HC apoptosis. However, attenuation of ROI with antioxidants such as SOD, DMSO, NAC, or GSH resulted in the induction of apoptosis by either TNF-α or LPS, which correlated with an elevation in NO production, indicating the involvement of endogenous NO in HC apoptosis. This notion was further confirmed in experiments in which the addition of the NO synthase inhibitor L-NMMA prevented apoptosis and DNA fragmentation in TNF-α- or LPS-treated HC in the presence of antioxidants. NO also appeared to cause HC damage, as demonstrated by evidence that L-NMMA resulted in further reductions in TNF-α-mediated hepatocellular AST and LDH release. To further confirm these novel findings, we therefore used the NO donor SNP to examine the effect of exogenous NO on HC cell death. We found that SNP at 0.5 mM was responsible for HC apoptosis and hepatocellular enzyme release. The augmentation of SNP-induced HC apoptosis and DNA fragmentation by antioxidants indicates that NO is a primary inducer of HC apoptosis, as peroxynitrite formation through ROI and NO is blocked by antioxidants. On the other hand, the reduction of SNP-induced hepatocellular enzyme release by antioxidants
indicates that HC damage is possibly mediated by NO through peroxynitrite formation.

NO has been implicated in a variety of diverse cellular functions and biological responses. Although much of the interest in NO has focused on its role as a signaling or effector molecule in the cardiovascular, nervous, and immune systems (18, 28, 35), whether NO exerts a detrimental or protective effect on SIRS is still controversial. Consistent with our findings, Billiar et al. (5), Curran et al. (15), and Stadler et al. (53) have shown that NO biosynthesis in HC in vitro is associated with the suppression of hepatic protein synthesis and the inhibition of mitochondrial function. Other investigators also reported that addition of the NO synthase inhibitor L-NMMA to HC was responsible for reversal of endotoxin-induced inhibition of cell growth, protein synthesis, and mitochondrial function (36, 37). Furthermore, NO exerts cytotoxic properties by participating in Kupffer cell and neutrophil-mediated HC injury (6, 42). Interestingly, in vivo studies suggest that NO may protect against hepatic damage during endotoxemia (7, 26, 27). In contrast, data from Laskin et al. (37) and Ma et al. (40) indicate that NO may contribute to hepatic injury with structural alterations after acute endotoxemia or hepatic ischemia-reperfusion in rats. In addition, to the role of NO in hepatic dysfunction during SIRS, an expanding body of literature has revealed that overproduction of NO may be involved in the pathophysiology of endotoxemic and septic shock, as well as ischemia-reperfusion injury, which is associated with tissue injury and eventual organ failure (30, 33, 49, 56).

ROI, a series of potent oxidants, have been shown to be responsible for hepatic damage either through a direct effect or through effector cell-mediated HC injury (4, 17, 60). More recently, ROI have been implicated as potential mediators in the induction of apoptosis in different cell types (1, 11, 59, 61). In the present study, we found that exposure of HC to either TNF-α or LPS led to ROI generation with an elevation in hepatocellular enzyme release. This result is consistent with other observations in which LPS or TNF-α incubated with HC induced increased superoxide anion release (60), diminished intracellular antioxidant levels (2, 47, 60), and accumulated lipid peroxidation (47, 60). However, in this study TNF-α or LPS-mediated ROI generation did not induce HC apoptosis, and ROI seemed to lower the level of biologically active NO through the pathway of peroxynitrite formation.

In summary, we present two novel findings. 1) NO is the potential inducer of HC apoptosis. Endogenously derived NO generated by the proinflammatory mediators TNF-α or LPS in the presence of antioxidants or exogenous NO supplied by the NO donor SNP induces typical apoptotic changes in HC. Pharmacological inhibition of NO production by the NO synthase inhibitor L-NMMA ameliorates HC apoptosis. 2) TNF-α- or LPS-stimulated ROI generation is responsible for HC damage and appears to prevent endogenously produced NO from inducing apoptosis, possibly through peroxynitrite formation. Further studies are required to elucidate the possible mechanisms by which NO leads to HC apoptosis.

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Present address and address for reprint requests: J. H. Wang, Surgical Research Laboratory, Clinical Sciences Bldg., Cork Univ. Hospital, Wilton, Cork, Ireland.

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