NO modulates P-selectin and ICAM-1 mRNA expression and hemodynamic alterations in hepatic I/R

PEITAN LIU,1 BAOHUAN XU,1 CARL E. HOCK,1 ROBERT NAGELE,2 FRANK F. SUN,1 AND PATRICK Y-K WONG1

Departments of 1Cell Biology and 2Molecular Biology, University of Medicine and Dentistry of New Jersey, School of Osteopathic Medicine, Stratford, New Jersey 08084

Numerous studies have shown that nitric oxide (NO) promotes inflammation (6, 15, 34), whereas an equal number of studies demonstrates an anti-inflammatory role (21, 24, 25). These conflicting results may be due to concentration-dependent effects of NO, the variety of in vivo or in vitro experimental models, and/or differences in cell sensitivity to NO. The effects of NO on inflammation may also vary, depending on acute or chronic inflammatory states, the animal species used in experiments, and the specific organ(s) studied. For example, in acute inflammation certain vascular beds such as the pulmonary circulation may be subjected to excessive vasoconstriction, and direct inhalation of NO gas to vasodilate the pulmonary vasculature has been reported to be beneficial (29).

NO is known to modulate early steps in the inflammatory process, such as expression of adhesion molecules, leukocyte adhesion and migration, and secretion of inflammatory mediators (8, 17, 21). In addition, NO has important effects on the regulation of vascular smooth muscle tone (10). All of the above effects are believed to contribute to the role of NO in ischemia-reperfusion (I/R) tissue injury. The liver appears to be a particularly sensitive organ to NO-mediated vasodilation (12). In vivo administration of an NO donor caused limited hypotension, whereas almost completely blocking tumor necrosis factor-α-induced liver failure due to massive apoptosis. Organ-selective NO administration is a promising new approach to reduce cytotoxicity and improve perfusion to the organ.

On the other hand, detrimental effects of excessive production of NO have been demonstrated because high concentrations of NO are cytotoxic and contribute to cell injury in a variety of disease states, including acute lung injury (16), endotoxemia (24), and I/R injury (6, 19). Some of the cytotoxic effects of NO have now been attributed to generation of peroxynitrite, a potent oxidant and nitrating agent (27) formed by the reaction of NO with superoxide (2, 16, 19, 21). However, administration of NO synthase (NOS) inhibitors such as Nω-monomethyl-L-arginine (L-NMMA) or Nε-nitro-L-arginine methyl ester (L-NAME) increased mortality and decreased tissue perfusion (4, 14, 21, 24, 25) in models of endotoxemia and I/R. Thus the role of NO in acute inflammation is complex. Excessive production of NO can be cytotoxic and lead to the formation of the highly cytotoxic molecule peroxynitrite. On the other hand, inhibition of NO formation may lead to tissue hypoperfusion, increased neutrophil adhesion, and infiltration.

In our previous studies, I/R of the liver (22) and heart (19) induced overproduction of NO and the generation of peroxynitrite in ischemic organs. Inhibition of NOS by L-NAME reduced plasma and tissue NO concentration and the formation of peroxynitrite (21). However,
this decrease in NO and peroxynitrite generation also enhanced the liver tissue injury (21), suggesting that peroxynitrite may not be an important mediator in early reperfusion injury of the liver. Hepatic injury observed in our experiments with L-NAME was associated with enhanced neutrophil infiltration and superoxide production. In the present study we investigated the precise mechanism of the cytoprotective and anti-inflammatory role of endogenous and exogenous NO in acute inflammation induced by hepatic I/R (HI/R) in rats.

MATERIALS AND METHODS

Male Fischer rats 344 (280–340 g body wt) were purchased from Taconic Farm (Germantown, NY). Animals were given free access to food (Purina rodent chow J 001) and water. Experimental protocols followed the criteria of the National Institute of Health Guide for the Care and Use of Laboratory Animals and were approved by our Institutional Animal Care and Use Committee.

Materials. Alanine aminotransferase (ALT) activity was measured with a Sigma diagnostics kit. Nitrate reductase (from Aspergillus species), D-anisidine, β-NADPH, the reduced form (β-NADPH), L-NAME (hydrochloride), and sodium nitrite were purchased from Sigma Chemical (St. Louis, MO). S-nitroso-N-acetyl-penicillamine (SNAP) was purchased from Alexis (San Diego, CA). The RNA Stat-60 reagent, SuperScript II, Taq DNA polymerase, and GelMarker were purchased from GIBCO BRL (Gaithersburg, MD) and Tel-Test (Friendswood, TX), respectively.

Experimental protocol. The experimental protocol for partial no-flow hepatic ischemia and measurement of associated hemodynamic variables has been previously described (11, 20). Briefly, under pentobarbital anesthesia (60 mg/kg ip) the trachea was cannulated (PE-240) to maintain a patent airway. Polyethylene catheters (PE-50) filled with heparinized 0.9% NaCl (10 U heparin/1 ml saline) were inserted into the left femoral artery (PE-50) and left femoral vein (PE-90) for measurement of mean arterial blood pressure (MABP) and drug or vehicle infusion, respectively. A PE-90 catheter was inserted into the right external jugular vein for the measurement of central venous pressure (CVP) by connection to a blood pressure transducer (Statham P23 AC, Hatoey, PR). The blood pressure transducers were connected to a Grass model 7D polygraph (Quincy, MA). The catheter inserted into the external jugular vein was also used for bolus saline injection (i.e., 200 µl) for the determination of cardiac output (CO). A 1.5-Fr thermistor probe (Columbus Instruments) was advanced into the right common carotid artery to the arch of the aorta. The position of the carotid thermistor probe was adjusted to ensure that a change in temperature of at least 0.3°C was recorded at the aortic arch when 200 µl of room temperature normal saline was injected into the right arium. The PE-50 catheter inserted into the left femoral artery was connected to a blood pressure transducer (Gould Statham P23 1D), and the blood pressure transducer and thermistor were connected to a Cardiomax II CO computer (Columbus Instruments) for measurement of MABP, CO, stroke volume (SV), and heart rate (HR).

Twenty minutes after all surgical procedures were completed the baseline MABP, CO, SV, HR, and CVP were recorded. A laparotomy was performed, and the artery supplying the left lateral and median lobes of the liver, which comprise ~75% of the total liver weight, was occluded with an atraumatic Glover bulldog clamp for 30 min. The remaining caudal three lobes retained an intact portal and arterial blood supply, as well as venous outflow, thereby preventing the development of intestinal venous hypertension. Reperfusion was initiated by removal of the clamp, the animal received 1 ml of the sterile saline intraperitoneally, and the wound was closed with 4–0 silk and wound staples. In some rats L-NAME (10 mg/kg iv through the penile vein) was given 10 min before the start of reperfusion (I/R-L-NAME), and control rats were given saline (I/R). Another group of rats was given SNAP (25 µmol/kg iv) 10 min before reperfusion, followed by 20 µmol·kg⁻¹·h⁻¹ in 1 ml saline infused for 4 h. Animals in the sham control group were subjected to the same surgical operation without occlusion of the artery supplying the left and lateral lobes of liver. The hemodynamic parameters (MABP, CO, SV, HR, and CVP) were recorded at the beginning of ischemia and at different time points during reperfusion. Blood samples (500 µl) were obtained at 4 h of reperfusion for determination of ALT activities, nitrite-nitrate concentration (as an index of NO production). Biopsies of the ischemic lobes of the liver were taken after 4 h of reperfusion for extraction of total RNA and measurement of superoxide generation. Samples of liver tissue were saved in 4% neutral-buffered paraformaldehyde for subsequent histological or immunohistochemical study.

ALT activity. Plasma ALT activities were measured with the Sigma test kit, COUG 159-UV, and expressed as internal units per liter.

Nitrite-nitrate assay. Nitrite was measured using a Nitric Oxide Analyzer (NOA; model 270B, Sievers Instruments, Denver, CO) (22). The NOA measures NO in biological fluids by a modified gas-stripping technique with high sensitivity (~10 pmol/ml of solution). Pieces of ischemic and nonischemic lobes of liver were homogenized in physiological saline (10% wet wt tissue suspension) on ice. This suspension was centrifuged at 10,000 g, 4°C for 10 min. Then 100 µl of tissue supernatant were incubated in the presence of nitrate reductase (0.05 U/ml) and NADPH (0.1 mM) at 37°C for 15 min to convert all nitrate to nitrite. A sample (20 µl) was then injected into the purge vessel of the NOA, which contained 2 ml of 1% sodium iodide in acetic acid to convert the nitrite to NO gas. A stream of nitrogen was passed through the purge vessel under vacuum to eliminate oxygen. The amount of nitrite was calculated from a standard curve of sodium nitrite (0–400 pmol; r = 0.99). Blood samples were centrifuged at 1,000 g for 5 min to obtain plasma. Plasma samples (100 µl) were incubated with nitrate reductase and NADPH for 15 min before injection into the NOA.

Superoxide assay. Superoxide anion production in ischemic and nonischemic lobes of liver was measured using the method of Cherry et al. (3). Briefly, tissue samples (60–170 mg) were incubated in Krebs-bicarbonate buffer (pH 7.4), consisting of (in mM) 118 NaCl, 4.7 KCl, 1.5 CaCl₂, 25 NaH₂CO₃, 1.1 MgSO₄, 1.2 K₂HPO₄, and 5.6 glucose. Tissues were gassed with 95% O₂–5% CO₂ for 30 min and placed in plastic scintillation vials containing 0.25 mM lucigenin in 1 ml of Krebs-bicarbonate buffer containing Hepes (pH 7.4). The chemiluminescence elicited by superoxide in the presence of lucigenin was measured using a Mark 5303 scintillation counter (Analytic, Elk Grove Village, IL). After 3 min of dark adaptation, vials containing only the cocktail (blanks) were counted three times for 6 s each time. Tissue samples were subsequently added to vials, allowed 3 min of dark adaptation, and counted twice (6 s each time).

Histology. Ischemic and nonischemic lobes of liver were fixed by immersion in 10% Formalin solution. Tissues were dehydrated in ethanol and embedded in paraffin. Sections (5 µm) were cut using a microtome, attached to glass slides with poly-L-lysine and stained with hematoxylin and eosin for light
microscopic examination. Polymorphonuclear neutrophils (PMN) were counted in 50 high-power fields (HPF) at ×40 magnification.

RT-PCR amplification of mRNA. The liver tissues from rats of different experimental groups were snap frozen in liquid nitrogen and stored at −70°C until analysis. Total cellular RNA was isolated by homogenizing tissues with a polytron homogenizer in RNA Stat-60 reagent (Tel-Test). Total RNA was extracted by chloroform and then centrifuged at 12,000 g for 15 min at 4°C. The RNA was precipitated by isopropanol, and the pellet dissolved in diethyl pyrocarbonate water (Sigma). Total RNA concentration was determined by spectrophotometric analysis at 260 nm wavelength, and 4 μg of total RNA was reverse transcribed into cDNA in a 30-μl reaction mixture containing SuperScript II (GIBCO BRL) and oligo(dT) 12–18 primers. The cDNA was amplified using specific primers with a Perkin-Elmer DNA Thermal Cycler 480. The amplification mixture contained 1 μl of 10 μM forward primer, 1 μl of 10 μM reverse primer, 5 μl of 10 × buffer, 1.5 μl of 15 μM MgCl₂, 5 μl of the reverse transcribed cDNA samples, and 1 μl of Taq polymerase. Primers were designed from the published mRNA sequences using the Oligo Primer Detection Program. The cDNA was amplified after determining the optimal number of cycles. The mixture was first incubated for 5 min at 94°C and then cycled 29 times at 94°C for 45 s, 60°C for 60 s, and extended at 72°C for 90 s. After amplification, the sample (10 μl) was separated on a 2% agarose gel containing 0.3 μg/ml (0.003%) of ethidium bromide, and bands were visualized and photographed using ultraviolet transillumination. The size of each PCR product was determined by comparing to the standard DNA size marker. Semiquantification of gene expression was performed using the Image Master VDS program (Pharmacia Biotech). The designed primer sequences are shown as sense primer: P-selectin, 5′-TGT ATC CAG CCT CTT GGG CAT TCC-3′; intercellular adhesion molecule 1 (ICAM-1), 5′-ACA GAC ACT AGA GGA-3′; and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), 5′-GTT GAA GGT CGG TGT CAA CGG ATG-3′ and antisense primer: P-selectin 5′-TGG CAG AGG AAG TGT TAC ACC-3′; ICAM-1, 5′-TGT AGC GTC CAT ATT TAG CCA GG-3′; and GAPDH, 5′-GAT GCC AAA GGT GTG ATG GAT GAC C-3′.

Statistical analysis. Data were analyzed using one-way ANOVA through the Sigma Stat program (Jandel Scientific). Differences between groups were then determined using the Newman-Keuls test. If test of normality failed, Dunn's test of ANOVA by ranks was used. Groups were deemed to be significantly different from one another when P < 0.05.

RESULTS

Plasma ALT activity. Partial hepatic no-flow ischemia for 30 min induced significant cell injury in the liver during the first 4 h of reperfusion, as indicated by the greater than 26-fold increase of ALT activity in the plasma of the I/R group. Administration of L-NAME (10 mg/kg) to rats with I/R exacerbated the liver injury as evidenced by a 64 and 69% increase in ALT activity at 4 h of reperfusion compared with I/R and I/R-SNAP rats, respectively (Fig. 1). It should be emphasized that liver injury was not enhanced by administration of SNAP to rats with I/R because there was no significant difference of ALT activity between I/R alone and I/R-SNAP rats.

Plasma nitrite-nitrate. Plasma nitrite-nitrate levels were increased 4.5-fold in rats with HI/R compared with shams. Administration of L-NAME to rats with I/R decreased plasma levels of nitrite-nitrate. Nitrite-nitrate levels in the I/R-SNAP group of rats were markedly elevated compared with sham control (7.5-fold increase), I/R (1.6-fold increase), and I/R-L-NAME (15.2-fold increase) animals, respectively (Fig. 2).

Superoxide generation. Figure 3 illustrates the superoxide generation in the various treatment groups. At 4 h of reperfusion, superoxide generation in the ischemic lobes of rats with L-NAME was significantly increased by 223 and 164% compared with sham-operated and I/R-SNAP animals, respectively. There was no significant difference between I/R and I/R-L-NAME groups, although there was a 44% increase in the I/R-L-NAME group compared with I/R rats.

Neutrophil quantitation. Neutrophil infiltration and accumulation in the sinusoids of ischemic lobes of the liver increased from 28 ± 3 PMN/50 HPF in sham control animals to 337 ± 25 PMN/50 HPF in animals subjected to HI/R. Treatment with L-NAME increased PMN accumulation in ischemic lobes of livers (549 ± 73...
PMN/50 HPF, P < 0.05, compared with sham control and I/R, respectively). PMN accumulation in the ischemic lobes of rats with I/R-SNAP was markedly decreased (294 ± 29 PMN/50 HPF) compared with rats with I/R-L-NAME, but was not significantly different from rats with I/R (Fig. 4).

RT-PCR of adhesion molecules. The mechanism of enhancement of neutrophil migration in the liver was studied at the molecular level by using the RT-PCR. P-selectin and ICAM-1 gene expression were normalized with GAPDH gene expression, which is considered a housekeeping gene. The results of gene expression were semiquantified with the Image Master VDS program (Pharmacia Biotech) (Fig. 5, A and B). I/R significantly induced gene expression of P-selectin and ICAM-1 compared with control group. Moreover, administration of L-NAME to rats with I/R dramatically enhanced gene expression of P-selectin and ICAM-1 compared with rats with I/R and I/R-SNAP, respectively. Administration of SNAP to rats with I/R attenuated P-selectin and ICAM-1 gene expression compared with that of rats with I/R; however, this decrease was not significant (Fig. 5, A and B).

Hemodynamic parameters. The initial MABP values, systemic vascular resistance index (SVRI), cardiac index (CI), and stroke volume index (SVI) for the I/R, I/R-L-NAME, sham-L-NAME and I/R-SNAP experimental groups were not significantly different.

MABP. L-NAME can significantly increase MABP because values of MABP in rats subjected to I/R-L-NAME or sham control-L-NAME were rapidly and markedly increased compared with that of rats with I/R or I/R-SNAP, respectively. This elevation was maintained during the 4-h reperfusion period. Values of MABP in the I/R-SNAP group were slightly decreased at the concentration of SNAP used in this experiment; however, this decrease was not significant compared
with rats with I/R (Fig. 6). MABP values were not
significantly different between I/R-L-NAME and sham
control-L-NAME groups.

CI and SVI. Importantly, there was a significant
decrease in CI (Fig. 7A) and SVI (Fig. 7B) after
administration of L-NAME to rats subjected to I/R or
sham control compared with rats with I/R and I/R-
SNAP, respectively. This decrease in CI and SVI con-
tinued for the entire 4 h of reperfusion. Interestingly,
values of CI and SVI in rats with I/R-SNAP at 2, 3, and
4 h of reperfusion were significantly increased com-
pared with rats with I/R (Fig. 7A and B). The CI and
SVI values were not significantly different between
I/R-L-NAME and sham control-L-NAME groups.

SVRI. SVRI was calculated as (MABP – CVP)/CI,
and the results are illustrated in Fig. 8. After adminis-
tration of L-NAME to rats with I/R or sham control,
values of SVRI were rapidly and significantly in-
creased, parallel with the change in MABP. This ele-
vation of SVRI was maintained for the 4-h period of
reperfusion compared with I/R and I/R-SNAP, respec-
tively. There was a slight decrease in SVRI in rats with
I/R-SNAP compared with rats with I/R; however, this
attenuation was not significant (Fig. 8). The values of
SVRI were not significantly different between I/R-L-
NAME and sham control-L-NAME groups. There were
no statistically significant differences in HR among the
four experimental groups (data not shown).

DISCUSSION

Recent reports indicate that NO is a major mediator
of vasorelaxation and hypotension and that inhibition
of NOS may provide a novel approach in the treatment
of septic shock (6, 26, 34). It has also been reported
that NOS inhibitors have potentially deleterious effects,
including their tendency to enhance PMN infiltration
and platelet aggregation (17, 21, 28) and to decrease
CO and tissue perfusion (14), thereby increasing the
mortality rate (4). The preservation of blood flow to
individual organs to maintain adequate tissue nutri-
tion and oxygen supply must be balanced with the
maintenance-elevation of blood pressure by using vaso-
constrictors. MABP, CO, and systemic vascular resis-

discussion

Recent reports indicate that NO is a major mediator
of vasorelaxation and hypotension and that inhibition
of NOS may provide a novel approach in the treatment
of septic shock (6, 26, 34). It has also been reported
that NOS inhibitors have potentially deleterious effects,
including their tendency to enhance PMN infiltration
and platelet aggregation (17, 21, 28) and to decrease
CO and tissue perfusion (14), thereby increasing the
mortality rate (4). The preservation of blood flow to
individual organs to maintain adequate tissue nutri-
tion and oxygen supply must be balanced with the
maintenance-elevation of blood pressure by using vaso-
constrictors. MABP, CO, and systemic vascular resis-

Fig. 6. Time course of changes in mean arterial blood pressure
(MABP) in 4 experimental groups. Data represent means ± SE of 5
(I/R and sham + L-NAME) and 6 (I/R + L-NAME and I/R + SNAP)
animals/group. *P < 0.05 compared with I/R. #P < 0.05 compared
with I/R + SNAP animals.

Fig. 7. Time course of changes in cardiac index (A) and stroke volume
index (B) in 4 experimental groups. Data represent means ± SE of 5
(I/R and sham + L-NAME) and 6 (I/R + L-NAME and I/R + SNAP)
animals/group. BW, body weight. *P < 0.05 compared with I/R. #P <
0.05 compared with I/R + SNAP animals. & P < 0.05 compared with
I/R group.

Fig. 8. Time course of changes in systemic vascular resistance index
in 4 experimental groups. Data represent means ± SE of 5 (I/R and
sham + L-NAME) and 6 (I/R + L-NAME and I/R + SNAP) animals/
group. *P < 0.05 compared with I/R. #P < 0.05 compared with I/R +
SNAP animals.
tance (SVR) are important factors in determining the adequacy of organ perfusion. MABP is determined by the balance between neural and humoral constrictors and dilators. Any therapeutic approach that shifts this balance by increasing vasoconstrictor(s) or decreasing vasodilator(s) could cause enhanced constriction of the blood vessels, an increase in vascular resistance, and a subsequent decrease in tissue perfusion.

SNAP and nitroglycerin have been shown to be equipotent vasodilators in the dog, and SNAP has continuous hemodynamic effects throughout 10 h of infusion in rats (32). The mechanism of NO release from SNAP is unknown but it is presumed to be through spontaneous homolytic cleavage (32). Ichimori et al. (13) observed that the concentration of NO generated by 10 µM SNAP in biological fluids is similar to that produced by endothelial cells stimulated with 1 µM acetylcholine. Our preliminary data indicated that injection of SNAP at a dose of 45 µmol/kg intravenously to rats with I/R induced a rapid and significant decrease in MABP. The data presented in this study showed that a slow injection (in 5 min) of SNAP at a dose of 25 µmol/kg intravenously followed by 20 µmol·kg\(^{-1}\)·h\(^{-1}\) in 1.0 ml of saline infused over 4 h of reperfusion, led to a limited decrease in MABP, which was not statistically significant compared with rats with I/R alone (Fig. 6). In contrast, after administration of L-NAME to rats with I/R, MABP was 1.95-fold higher than I/R-SNAP and 1.61-fold higher than the I/R group at the end of 4 h of reperfusion (Fig. 6). Similarly, administration of L-NAME to rats with I/R produced a 3.65-fold increase in SVRI compared with I/R-SNAP and a 2.46-fold increase compared with I/R rats (Fig. 8).

More importantly, at the end of 4 h of reperfusion CI values in the I/R-L-NAME group were only 52% of CI values in the I/R-SNAP group and 67% in the I/R group (Fig. 7A), respectively. The values of SVI after administration of L-NAME to rats with I/R were 51% of SVI values in the I/R-SNAP group and 67% in the I/R group at the end of 4 h of reperfusion (Fig. 7B). The values of MABP, CI, SVI, and SVRI were not significantly different between I/R-L-NAME and sham-L-NAME groups. This pattern of L-NAME effects is similar to the effects of norepinephrine, which increases SVRI and decreases CI and SVI, resulting in a decrease in tissue perfusion and an increase in MABP. There was no evidence of SNAP-induced cytotoxicity at the concentration used in this study as determined by enzymatic examination of ALT activity at 4 h of reperfusion (Fig. 1).

L-NAME, a nonselective inhibitor for NOS, produces generalized vasoconstriction in vascular beds, e.g., arteries, arterioles, and veins. Inhibition of NOS with the competitive inhibitor L-NMMA resulted in a significant increase in blood pressure and SVR and a concomitant decrease in CI and SVI (10). Therefore, this increase in MABP occurs at the expense of tissue blood flow. Our results are consistent with the data reported by Henderson et al. (10) that the NOS inhibitor (i.e., L-NMMA) caused a statistically significant decrease in CO, oxygen delivery, and regional blood flow compared with the control group in a septic model, despite increased blood pressure and SVR (10). Similarly, treatment with NOS inhibitors in an animal model of septic shock successfully increased SVR but also increased mortality rate (4).

In the normal resting state, organ blood flow is principally determined by CO, vascular tone, MABP, and organ-specific regulation of the microvasculature. Systemic arterial pressure determines the driving force for blood flow through each vascular bed. The balance of the major neural and humoral constrictors (catecholamine, PGF\(_2\)\(_a\)) and dilators (PGF\(_2\), NO) in each vascular bed serves to regulate organ blood flow. Administration of L-NAME may decrease organ blood flow by one or more of the following mechanisms: 1) increased afterload; 2) decreased venous return; 3) decreased cardiac pump function (i.e., negative inotropy, subsequent occurrence of coronary ischemia due to vasoconstriction or adhesion of leukocytes and platelets); 4) facilitation of norepinephrine release (31); and 5) reduction in the vasodilatory effects of NO in the microcirculation. The MABP and SVR correlate with survival in patients with septic shock (9). The decrease in SVRI by NO occurs via at least two independent mechanisms: 1) stimulation of guanylyl cyclase in smooth muscle cells leading to the generation of cGMP and vascular relaxation and 2) reduction in norepinephrine release from sympathetic nerves in the rat heart (31).

Besides the effects on vascular tone, NO has many anti-inflammatory properties, including reduction of platelet aggregation, limitation of vascular smooth muscle cell proliferation, inhibition of leukocyte adhesion to endothelium, prevention of monocyte chemotaxis, and contribution to antimicrobial defense mechanisms (8, 17, 21). It has been reported that different NO donors inhibit interleukin-\(1\alpha\)-stimulated vascular cell adhesion molecule 1 (VCAM-1) expression in a concentration-dependent manner by 35–55%. This inhibition was paralleled by reduced monocyte adhesion to endothelial monolayers in nonstatic assays (5). NO also decreased the endothelial expression of other leukocyte adhesion molecules (E-selectin, ICAM-1) and secretable cytokine interleukin-6 and -8 (35). These results are consistent with our data that endogenous NO inhibits P-selectin and ICAM-1 gene expression in acute inflammation induced by H\(_2\)/R.

Our results agree with the hypothesis that endogenous production of NO induced by inflammatory stimuli is sufficient in limiting P-selectin and ICAM-1 gene expression. Administration of L-NAME induced a significant augmentation of P-selectin and ICAM-1 gene expression and PMN accumulation in the ischemic lobes (Figs. 4 and 5, A and B). At sites of inflammation the increase in NO production by endothelium, macrophages, and vascular smooth muscle cells is the essential compensatory response for maintaining organ blood flow and inhibiting PMN adhesion and platelet aggregation. P-selectin is important in promoting rolling and adherence of leukocytes to the endothelium. These effects on leukocyte rolling act to capture or tether leukocytes and bring them into contact with the endothelium, where they can undergo firm adhesion, a
process regulated by other adhesion molecules, i.e., ICAM-1 and VCAM-1. Moreover, P-selectin is also involved in cell signaling, inflammation, and oxygen-derivered free radical injury (23). In our studies, H1/R did not induce E-selectin and VCAM-1 mRNA expression in I/R and I/R-SNAP groups of rats at 4 h of reperfusion. This may be due to the fact that E-selectin and VCAM-1 play a critical role in a later phase of reperfusion. Interestingly, there was E-selectin and VCAM-1 mRNA expression in the ischemic lobes of rats with I/R-L-NAME (data not shown), which suggests that inhibition of NO synthesis by administration of L-NAME may be able to accelerate E-selectin and VCAM-1 gene expression.

The regulation of adhesion molecule gene expression is complex and occurs at multiple levels ranging from gene transcription to posttranslational protein modifications. Nuclear factor-κB (NF-κB) is a transcription factor that upregulates the expression of many inflammatory mediators. The gene expression of P-selectin and ICAM-1 requires activation of the transcription factor NF-κB (1). Electrophoretic mobility shift assays indicated that NO suppresses VCAM-1 gene transcription in part by inhibiting NF-κB (18). It is proposed that NO downregulates adhesion molecule gene transcription, in part, by inhibiting NF-κB. Because the activation of NF-κB occurs in part via the action of reactive oxygen species, NO may inhibit NF-κB by scavenging and inactivating superoxide anion, via the formation of peroxynitrite (30).

It will be important to test the profile of action of various NOS inhibitors to determine whether they have beneficial effects in I/R, in addition to their reported vasopressor effects. More specific NOS inhibitors are required to determine whether selective inducible NOS inhibition is beneficial (7) in selected groups of patients. It remains to be determined whether the elevation of NO levels during I/R is a beneficial or deleterious response to acute inflammation (33). The answer to this question may be dependent on the time following reperfusion (i.e., early vs. late, acute vs. chronic). Caution must be exercised regarding the use of NOS inhibitors in patients with acute or chronic inflammation until both the physiological and pathophysiological profiles of NO have been fully defined in these inflammatory states.

Our results support the hypothesis that in the early acute phase of inflammation induced by I/R, the compensatory response of increased NO production occurs together with increased CO, decreased SVR, reduced PMN adhesion and migration, and attenuated superoxide generation compared with NOS inhibition with L-NAME. These effects are key responses to inflammatory injury, and they may be more important in determining the extent of organ injury than the cytotoxic potential of NO and/or peroxynitrite. Exogenous NO (i.e., SNAP) further improved CI and SVI but did not further attenuate P-selectin and ICAM-1 gene expression and PMN accumulation at the dose used in the present study.

This work was supported by Grant-in-Aid NJ-97-GS-16 from the American Heart Association, New Jersey Affiliate, to P. Liu. Address for reprint requests: P. Y-K. Wong, Dept. of Cell Biology, UMDNJ-School of Osteopathic Medicine, 2 Medical Center Dr., Stratford, NJ 08084.

Received 19 May 1998; accepted in final form 21 August 1998.

REFERENCES

2. Beckman, J. S., M. Carson, C. D. Smith, and H. W. Koppe- lansky. H2O2 generation increased in I/R and I/R-SNAP groups of rats at 4 h of reperfusion. This may be due to the fact that E-selectin and VCAM-1 play a critical role in a later phase of reperfusion. Interestingly, there was E-selectin and VCAM-1 mRNA expression in the ischemic lobes of rats with I/R-L-NAME (data not shown), which suggests that inhibition of NO synthesis by administration of L-NAME may be able to accelerate E-selectin and VCAM-1 gene expression.

The regulation of adhesion molecule gene expression is complex and occurs at multiple levels ranging from gene transcription to posttranslational protein modifications. Nuclear factor-κB (NF-κB) is a transcription factor that upregulates the expression of many inflammatory mediators. The gene expression of P-selectin and ICAM-1 requires activation of the transcription factor NF-κB (1). Electrophoretic mobility shift assays indicated that NO suppresses VCAM-1 gene transcription in part by inhibiting NF-κB (18). It is proposed that NO downregulates adhesion molecule gene transcription, in part, by inhibiting NF-κB. Because the activation of NF-κB occurs in part via the action of reactive oxygen species, NO may inhibit NF-κB by scavenging and inactivating superoxide anion, via the formation of peroxynitrite (30).

It will be important to test the profile of action of various NOS inhibitors to determine whether they have beneficial effects in I/R, in addition to their reported vasopressor effects. More specific NOS inhibitors are required to determine whether selective inducible NOS inhibition is beneficial (7) in selected groups of patients. It remains to be determined whether the elevation of NO levels during I/R is a beneficial or deleterious response to acute inflammation (33). The answer to this question may be dependent on the time following reperfusion (i.e., early vs. late, acute vs. chronic). Caution must be exercised regarding the use of NOS inhibitors in patients with acute or chronic inflammation until both the physiological and pathophysiological profiles of NO have been fully defined in these inflammatory states.

Our results support the hypothesis that in the early acute phase of inflammation induced by I/R, the compensatory response of increased NO production occurs together with increased CO, decreased SVR, reduced PMN adhesion and migration, and attenuated superoxide generation compared with NOS inhibition with L-NAME. These effects are key responses to inflammatory injury, and they may be more important in determining the extent of organ injury than the cytotoxic potential of NO and/or peroxynitrite. Exogenous NO (i.e., SNAP) further improved CI and SVI but did not further attenuate P-selectin and ICAM-1 gene expression and PMN accumulation at the dose used in the present study.

This work was supported by Grant-in-Aid NJ-97-GS-16 from the American Heart Association, New Jersey Affiliate, to P. Liu. Address for reprint requests: P. Y-K. Wong, Dept. of Cell Biology, UMDNJ-School of Osteopathic Medicine, 2 Medical Center Dr., Stratford, NJ 08084.

Received 19 May 1998; accepted in final form 21 August 1998.

REFERENCES

2. Beckman, J. S., M. Carson, C. D. Smith, and H. W. Koppe- lansky. H2O2 generation increased in I/R and I/R-SNAP groups of rats at 4 h of reperfusion. This may be due to the fact that E-selectin and VCAM-1 play a critical role in a later phase of reperfusion. Interestingly, there was E-selectin and VCAM-1 mRNA expression in the ischemic lobes of rats with I/R-L-NAME (data not shown), which suggests that inhibition of NO synthesis by administration of L-NAME may be able to accelerate E-selectin and VCAM-1 gene expression.

The regulation of adhesion molecule gene expression is complex and occurs at multiple levels ranging from gene transcription to posttranslational protein modifications. Nuclear factor-κB (NF-κB) is a transcription factor that upregulates the expression of many inflammatory mediators. The gene expression of P-selectin and ICAM-1 requires activation of the transcription factor NF-κB (1). Electrophoretic mobility shift assays indicated that NO suppresses VCAM-1 gene transcription in part by inhibiting NF-κB (18). It is proposed that NO downregulates adhesion molecule gene transcription, in part, by inhibiting NF-κB. Because the activation of NF-κB occurs in part via the action of reactive oxygen species, NO may inhibit NF-κB by scavenging and inactivating superoxide anion, via the formation of peroxynitrite (30).

It will be important to test the profile of action of various NOS inhibitors to determine whether they have beneficial effects in I/R, in addition to their reported vasopressor effects. More specific NOS inhibitors are required to determine whether selective inducible NOS inhibition is beneficial (7) in selected groups of patients. It remains to be determined whether the elevation of NO levels during I/R is a beneficial or deleterious response to acute inflammation (33). The answer to this question may be dependent on the time following reperfusion (i.e., early vs. late, acute vs. chronic). Caution must be exercised regarding the use of NOS inhibitors in patients with acute or chronic inflammation until both the physiological and pathophysiological profiles of NO have been fully defined in these inflammatory states.

Our results support the hypothesis that in the early acute phase of inflammation induced by I/R, the compensatory response of increased NO production occurs together with increased CO, decreased SVR, reduced PMN adhesion and migration, and attenuated superoxide generation compared with NOS inhibition with L-NAME. These effects are key responses to inflammatory injury, and they may be more important in determining the extent of organ injury than the cytotoxic potential of NO and/or peroxynitrite. Exogenous NO (i.e., SNAP) further improved CI and SVI but did not further attenuate P-selectin and ICAM-1 gene expression and PMN accumulation at the dose used in the present study.

This work was supported by Grant-in-Aid NJ-97-GS-16 from the American Heart Association, New Jersey Affiliate, to P. Liu. Address for reprint requests: P. Y-K. Wong, Dept. of Cell Biology, UMDNJ-School of Osteopathic Medicine, 2 Medical Center Dr., Stratford, NJ 08084.

Received 19 May 1998; accepted in final form 21 August 1998.