Nitric Oxide
II. Nitric oxide protects in intestinal inflammation*

ALLAN M. LEFER AND DAVID J. LEFER
Department of Physiology, Jefferson Medical College, Thomas Jefferson University, Philadelphia, Pennsylvania 19107; and Department of Physiology, Louisiana State University Medical Center, Shreveport, Louisiana 71130

Lefer, Allan M., and David J. Lefer: Nitric Oxide. II. Nitric oxide protects in intestinal inflammation. Am. J. Physiol. 276 (Gastrointest. Liver Physiol. 39): G572–G575, 1999.—This article examines the evidence for nitric oxide (NO) as a protective agent in splanchnic ischemia-reperfusion and other forms of acute intestinal inflammation. Four major points emerge from this body of data. First, acute intestinal inflammation results in an early (i.e., <5 min) and severe decrease in endothelium-derived NO. Thus the early trigger event in this condition is a functional loss of NO. Second, administration of exogenous NO, NO donors, or NO precursorsameliorates splanchnic ischemia-reperfusion and other forms of acute intestinal inflammation (i.e., splanchnic trauma). These beneficial effects occur at physiological levels of NO when given early in the course of the inflammatory state. Third, blockade of nitric oxide synthase (NOS) or gene deletion of NOS exacerbates intestinal inflammation. Fourth, there are a variety of signaling mechanisms that may mediate the protective effect of NO.

synthase blockers; nitric oxide donors; nitric oxide synthase gene-targeted mice; L-arginine

THE PURPOSE OF THIS THEMES article is to provide a brief overview of the protective effects of nitric oxide (NO) in intestinal inflammation. Four questions will be addressed in this brief review: 1) What happens to endogenous NO concentrations in acute intestinal inflammatory states? 2) What does exogenous NO do during acute intestinal inflammatory states? 3) What effects do inhibition or ablation of nitric oxide synthase (NOS) have in acute intestinal inflammation? and 4) What are the cellular mechanisms of the effects of NO in acute intestinal inflammation?

NO AND ENDOThelial Dysfunction IN INTESTINAL INFLAMmATION

It has been known for many years that a variety of acute shocklike states (e.g., hemorrhage-reinfusion, soft tissue trauma, and mesenteric ischemia-reperfusion) all produce a profound endothelial dysfunction characterized by a marked decrease in endothelium-derived nitric oxide (EDNO) (20). This loss of EDNO occurs both in basal release of NO as well as in agonist-mediated pulsed NO release (20). One of the primary sites of this endothelial dysfunction is the superior mesenteric artery (SMA) (4, 20). Moreover, the time course of this SMA endothelial dysfunction has been characterized in soft tissue trauma (24) and in mesenteric ischemia-reperfusion (4) (13). In both cases, this endothelial dysfunction occurred within 5 min of the injury (24) or the reperfusion of the ischemic bowel (13).

The magnitude of this loss of EDNO was in the range of 40–60% at 5 min and gradually worsened over time to 75–85% losses at 2 h (12, 23).

In both trauma and SAO/R, the loss of EDNO led to an increase in adherence of neutrophils to the mesenteric endothelium by 20 min postinjury or reperfusion (13, 24), and this resulted in an infiltration of neutrophils into the intestine by 30–60 min (13, 24), signifying a well-developed state of acute intestinal inflammation. This enhanced neutrophil adherence is due largely to endothelial P-selectin upregulation in the traumatized (2) and ischemic-reperfused (6) intestine.

Although the precise mechanism for the reduced EDNO is not clear, the best available data suggest that the NO loss is largely due to the rapid release of superoxide radicals on reintroduction of oxygen following hypoxia or ischemia (11). Superoxide radicals are known to inactivate or quench NO. Recent experimental evidence (4) also suggests that neutrophils can contribute to the loss of EDNO following SMA occlusion and reperfusion. The consequences of this inflammatory cascade, 1) loss of EDNO, 2) upregulation of P-selectin on the endothelium, 3) increased adherence of neutrophils to the intestinal endothelium, and 4) infiltration of polymorphonuclear neutrophils into the inflamed intestine, are tissue injury and fluid loss (19) leading to hypotension and shock (13, 24). Thus loss of EDNO acts as the trigger mechanism (i.e., the endothelial trigger), and this event becomes aggravated by the involvement of neutrophils (i.e., the leukocyte amplification phase). In this sequence of pathophysiologic events, the key early step in acute intestinal inflammation is an abrupt decrease in local production-release of NO, which is sustained for at least several hours.

EXOGENOUS NO ADMINISTERED IN INTESTINAL INFLAMmATION

The first study in which NO was administered in an intestinal inflammatory state was conducted in 1990 by
Aoki et al. (1). These investigators intravascularly infused low concentrations of authentic NO gas dissolved in physiological saline and compared it to acidified NaNO₂, a nonorganic NO donor. Both NO and acidified NaNO₂ stabilized mean arterial blood pressure in cats subjected to SAO/R, significantly prolonged survival, and markedly reduced lysosomal disruption, plasma proteolysis, and the formation of myocardial depressant factor from the ischemic pancreas (1). Recently, this concept was extended by Fox-Robichaud et al. (9) who employed inhaled NO in cats that were subjected to mesenteric ischemia-reperfusion. These investigators found that inhalation of NO markedly attenuated leukocyte rolling, adherence, and transmigration in the ischemic-reperfused intestine but not in the normal intestine (9). These two studies provide strong support for the concept that NO preserves parenchymal and vascular cell function in acute intestinal inflammation produced by ischemia-reperfusion.

Not only does authentic NO gas protect in SAO/R, but a variety of organic NO donors also protect (5, 10, 16, 17). These include C87–3754, a sydnonmine class NO donor (5), S-nitroso-N-acetylpenicillamine (10), the NONOate spermine-NO (16), and sodium nitroprusside (16, 17). These NO donors protected a variety of important functions, including attenuation of leukocyte-endothelium interaction, prevention of microvascular fluid and protein leakage, prevention of P-selectin expression, prevention of hypotension, and attenuation of endothelial dysfunction. This is consistent with the finding that superior mesenteric venous nitrate/nitrate levels were reduced in SAO/R (16). NO donors also attenuated platelet-leukocyte aggregation under these conditions (16, 19). In addition to NO and organic and inorganic NO donors, other NO-generating agents also protect in intestinal inflammation. These agents include low doses of peroxynitrite (i.e., 400–800 nM) and the NO precursor, L-arginine (12). In the case of peroxynitrite, which decomposes to NO and superoxide, the NO is carried as S-nitrosothiols by plasma proteins and glutathione. The liberated NO can protect against leukocyte-endothelium interactions in the mesenteric microvasculature (22). Recently, infusions of the amino acid L-arginine have been shown to prolong survival, prevent neutrophil infiltration into the intestine, and preserve mesenteric endothelial function in rats subjected to traumatic shock due to blunt splanchic soft tissue injury (12). The L-arginine appeared to work by enhancing EDNO release from the vascular endothelium.

Thus there is abundant evidence that replacement of NO can protect and preserve intestinal function in the face of severely lethal forms of acute intestinal inflammation. This replacement therapy can take the form of (1) authentic NO given either intravascularly in solution or by inhalation of low levels of NO gas, (2) inorganic NO releasing compounds (e.g., acidified NaNO₂), (3) organic NO donors (e.g., sydnonimines, sodium nitroprusside, cysteine analogs, NONOates, and so forth), (4) low concentrations of peroxynitrite, and (5) the precursor of NO (i.e., L-arginine). The variety and number of these positive studies comprise strong evidence that physiological levels of NO given early in the inflammatory state are a potent and highly effective means of counteracting the fulminating nature of acute intestinal inflammation.

**EFFECTS OF NOS BLOCKADE OR GENE DISRUPTION**

If NO levels are decreased early in the course of intestinal inflammation and replacement of NO protects against the acute effects of this inflammation, then one wonders what would happen if the enzymes responsible for NO biosynthesis (e.g., NOS) are either missing or blocked. The first clear demonstration that NOS enzymatic inhibition is deleterious to the intestine is the now classic study of Kubes et al. (18) who showed that NOS inhibition enhances leukocyte adherence to the mesenteric vascular endothelium in the cat, which could be blocked by a monoclonal antibody directed against the β₂-integrins. This was a seminal finding that pointed toward a lack of NO as being important in the early phases of acute intestinal inflammation. This finding was extended by Davenpeck et al. (7) who found that NOS inhibition enhanced leukocyte-endothelium interactions in the rat. This latter study clearly linked inhibition of NO to the first step in leukocyte-endothelium interaction (i.e., leukocyte rolling). Moreover, the leukocyte rolling induced by nitro-L-arginine methyl ester (L-NAME) was shown by immunohistochemistry to be largely due to upregulation of P-selectin on the mesenteric endothelial cell surface, and a P-selectin-neutralizing antibody blocked both rolling and adherence of leukocytes (7). This effect may be linked to the action of NO on endothelial cell guanylate cyclase activity (7). L-NAME was also shown to enhance microvascular leakage of albumin (16) and result in significant alterations in fluid dynamics in the inflamed intestine.

Recently, a new approach has been employed to investigate the role of NO synthesis in intestinal inflammation. This approach utilizes specific gene-targeted mice. Thus selective gene deletion has been accomplished for all three isoforms of NOS, the endothelial constitutive NOS (ecNOS), neuronal NOS (nNOS), and the inducible NOS (iNOS). The results obtained with these mice relevant to intestinal inflammation are rather interesting. Hickey et al. (14) showed that iNOS knockout mice subjected to endotoxemia have enhanced leukocyte-endothelium interactions (i.e., increased leukocyte rolling and adherence in hepatic postsinusoidal venules). Many more leukocytes were recruited in iNOS-deficient mice, suggesting that upregulated iNOS in wild-type mice and the subsequent elevation in NO is a homeostatic adjustment serving to minimize leukocyte recruitment into inflamed tissue. More recently, ecNOS and nNOS knockout mice have been studied in terms of their microvascular responses to thrombin stimulation (D. J. Lefer et al., unpublished observations). Both ecNOS and nNOS knockout mice showed marked increases in both leukocyte rolling and adherence in thrombin-stimulated mouse peri-intesti-
nal venules. These increased leukocyte-endothelium interactions were on the order of four- to sevenfold increases and are due in part to upregulation of P-selectin. These changes in the ecNOS knockout mice are even more significant, since they experienced an increased mean arterial blood pressure and thus increased microvascular shear forces.

The major conclusion one must reach from all of these data is that NO acts as a homeostatic regulatory molecule designed to attenuate leukocyte-endothelium interaction and thus attenuate local inflammation. In the absence of NOS (e.g., NOS gene deletion) or in the case of pharmacological inhibition of NOS (e.g., NOS inhibitors), there is a marked enhancement of leukocyte-endothelium interaction and the inflammatory process is exacerbated. These results are consistent with the salutary effects of NO in acute intestinal inflammation.

MECHANISMS OF THE ANTI-INTESTINAL-INFLAMMATORY EFFECTS OF NO

Although it is clear that NO exerts significant anti-inflammatory effects in the intestinal microvasculature and epithelium, the precise mechanism of these effects is not fully elucidated. At present, there are several pathways that appear to be involved in potential effects of NO on leukocyte-endothelium interaction.

The first potential mechanism of action of NO is its interaction with the superoxide radical. As a consequence of this interaction, superoxide is inactivated or quenched. This is because superoxide radicals exert important inflammatory-induced leukocyte-endothelial interactions (26) that can be abrogated by superoxide dismutase (7, 26). Superoxide radicals can lead to the formation of hydrogen peroxide, which is known to induce P-selectin expression. In cultured human endothelial cells, L-NAME markedly upregulated P-selectin mRNA and protein (3), which led to marked increases in leukocyte adherence to endothelial monolayers. Moreover, this upregulation of P-selectin could be reversed by coincubation with the NO donor, SPM-5185 (3). That P-selectin is crucial to the leukocyte-endothelium interaction in ischemia-reperfusion is clearly shown in wild-type mice subjected to hepatic ischemia-reperfusion (15) in contrast to P-selectin knockout mice in which the leukocyte rolling was totally absent.

Thus one key event in this process is the mechanism of upregulation of P-selectin by ischemia-reperfusion and the means whereby NO suppresses this process.
One of the major messengers in the upregulation of P-selectin, and other endothelial cell adhesion molecules, is nuclear transcription factor-κB (NF-κB) (8). NF-κB is a transcription factor that translocates to the cell nucleus on activation by oxidants, whereupon it acts as a signal for increased transcription of P-selectin (2, 8). Recently, NF-κB upregulation (i.e., binding of a P-selectin-specific NF-κB element to nuclear extracts) was found in the organs of rats subjected to traumatic shock (2). The highest degree of activation occurred in the intestine (2). This increase in P-selectin mRNA was consistent with Western blot analysis of increased p52 homodimers of NF-κB in trauma (2). Very recently, the mechanism of this enhanced P-selectin mRNA appears to relate to IκB-α promoter activity (25), since NO donors reduced endothelial cell adhesion molecule expression by up to 70% via induction of IκB-α promoter activity. IκB-α is the inhibitory subunit of NF-κB and thus inhibits expression of NF-κB, effectively diminishing P-selectin and other adhesion molecule (e.g., intercellular adhesion molecule-1) expression on endothelial cells.

Another potential mechanism of the anti-inflammatory effect of NO is via inhibition of protein kinase C (PKC) (23). This may represent another pathway whereby NO blocks endothelium-leukocyte interaction.

Figure 1 summarizes some of the potential mechanisms of NO inhibition of leukocyte-endothelium interaction in intestinal inflammation. Thus NO inhibits P-selectin expression and transcription via activation of soluble guanylate cyclase, inhibition of NF-κB, and finally by inhibition of PKC activity. The bottom line is that NO functionally inhibits leukocyte-endothelium interaction as well as epithelial permeability, two key events in the early phase of intestinal inflammation.

This work was supported by National Institute of General Medical Sciences Research Grant GM-45434.

Address for reprint requests: A. M. Lefer, Dept. of Physiology, Jefferson Medical College, 1020 Locust St., Philadelphia, PA 19107.

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