Nitric Oxide
V. Therapeutic potential of nitric oxide donors and inhibitors

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Muscará, Marcelo N., and John L. Wallace. Nitric Oxide. V. Therapeutic potential of nitric oxide donors and inhibitors. Am. J. Physiol. 276 (Gastrointest. Liver Physiol. 39): G1313–G1316, 1999.—Nitric oxide is a crucial mediator of gastrointestinal mucosal defense, but, paradoxically, it also contributes to mucosal injury in several situations. Inhibitors of nitric oxide synthesis and compounds that release nitric oxide have been useful pharmacological tools for evaluating the role of nitric oxide in gastrointestinal physiology and pathophysiology. Newer inhibitors with selectivity for one of the isoforms of nitric oxide synthase are even more powerful tools and may have utility as therapeutic agents. Also, agents that can scavenge nitric oxide or peroxynitrite are promising as drugs to prevent nitric oxide-associated tissue injury. Compounds that release nitric oxide in small amounts over a prolonged period of time may also be very useful for prevention of gastrointestinal injury associated with shock and with the use of drugs that have ulcerogenic effects. Indeed, the coupling of a nitric oxide-releasing moiety to nonsteroidal anti-inflammatory drugs has proven to be a valid means of substantially reducing the gastrointestinal toxicity of these drugs without decreasing their efficacy.

inflammation; ulcer; inflammatory bowel disease; ischemia-reperfusion

An enduring theme in nitric oxide (NO) research is the paradoxical roles played by this molecule: in so many tissues and in so many circumstances, NO mediates both normal tissue function and tissue injury. This is certainly the case in the gastrointestinal tract, where NO is a critical mediator of mucosal defense and repair, but NO also plays a role as a contributor to tissue injury in a number of digestive diseases. As a result of this dual role, the development of drugs that will modulate the synthesis or actions of NO has been extremely challenging. Attempting to inhibit the overproduction of NO at a site of mucosal inflammation has often led to impairment of mucosal defense and thus an exacerbation of tissue injury. In this themes article, we review some of the strategies that have been employed to modulate levels of NO within gastrointestinal tissues and the potential utility of such approaches for treatment of digestive diseases.

Two major types of gastrointestinal disorders are characterized by an involvement of NO: motility disorders and inflammatory/ulcerative diseases. NO is a key mediator of smooth muscle relaxation mediated by nonadrenergic, noncholinergic nerves and is therefore extremely important in regulating transit of chyme, sphincter tone, and so forth. NO donors or inhibitors could be useful in modulating smooth muscle tone in the gut just as they are used clinically for modulating vascular smooth muscle tone. NO produced in large amounts [via the inducible isoform of nitric oxide synthase (iNOS)] has been suggested to be important in mediating tissue injury associated with gastroduodenal ulceration and inflammatory bowel disease, as well as the gastrointestinal tissue injury that accompanies endotoxic shock and ischemia-reperfusion (14). It is the potential use of NO donors and inhibitors as they pertain to gastrointestinal inflammation and ulceration that is the major focus of this themes article.

NO DONORS

Because of the limited utility of authentic NO gas in many experimental systems and the short half-life of NO in vivo, chemicals that have the capacity to release NO (with or without a requirement for enzymatic metabolism) have been widely employed as pharmacological tools to investigate the role of NO in gastrointestinal physiology and pathophysiology. Of course, several NO donors have been used in clinical settings for decades (e.g., nitroglycerin, nitroprusside), although these are rarely used for treatment of digestive disorders. The growth of interest in the physiology of NO since the mid-1980s led to the development of a variety of new NO donors that offered several advantages over what had previously been available, such as compounds that spontaneously release NO in solution (e.g., S-nitrosopenadilamine), compounds that can release NO over a prolonged period of time at predictable rates (e.g., NCX-4016), and compounds that selectively release NO in certain tissues (18). It is these newer compounds that represent useful therapeutic agents for the treatment of digestive disorders.
One of the potential applications of NO donors is as protective agents in situations in which the gastrointestinal mucosa is exposed to potentially noxious substances or in which mucosal defense is impaired. Experimental evidences to support such applications include the observations that NO donors reduce the severity of gastric mucosal damage induced by ethanol (11) and the gastric and small intestinal injury associated with ischemia-reperfusion (16). These effects may be related to actions of NO on several targets, including prevention of leukocyte-endothelial adherence, maintenance of mucosal blood flow, and stimulation of mucus secretion (22).

Perhaps more significant from a clinical perspective is the ability of NO donors to accelerate the healing of preexisting ulcers in the gastrointestinal tract. Gastric ulcers in rats were shown to be healed at a greatly accelerated rate when the rats were treated daily with NO donors (4). An NO donor (glyceryl trinitrate) has also been successfully employed in a clinical setting to accelerate the healing of anal fissures associated with Crohn's disease (10).

NO-releasing anti-inflammatory drugs. Nonsteroidal anti-inflammatory drugs (NSAIDs) represent one of the most widely used classes of therapeutic agents, but their tendency to cause ulceration and bleeding in the gastrointestinal tract is a major problem (23). Studies performed in the early 1990s identified neutrophil adherence to the vascular endothelium of the splanchic circulation as one of the earliest events following NSAID administration to laboratory animals (23). Prevention of this neutrophil adherence, and of the reduced mucosal blood flow that occurred following NSAID administration, resulted in prevention of gastric ulceration. NO was among the substances that could prevent the NSAID-associated vascular events and the mucosal injury. It was therefore proposed that addition of an NO-releasing moiety to standard NSAIDs would result in the formation of anti-inflammatory drugs with markedly reduced ulcerogenic properties (25). These so-called "NO-NSAIDs" have been extensively characterized in several species, including humans, and have been shown to have comparable or superior anti-inflammatory and analgesic activity to standard NSAIDs while sparing the gastrointestinal tract and kidney of injury (24, 25). Interestingly, NO-NSAIDs have been shown to accelerate the healing of preexisting gastric ulcers (4) and to accelerate the recovery of renal function and structure in rats subjected to five-sixths renal ablation (6). Unlike the parent drugs from which they are derived, NO-NSAIDs do not cause a reduction in gastric mucosal blood flow and do not evoke leukocyte adherence to the vascular endothelium in mesenteric venules (24, 25). The compounds release small amounts of NO (insufficient to alter systemic arterial blood pressure in normotensive rats) over a prolonged period of time (up to 12 h after oral administration).

The success of the NO-NSAID approach has led to this strategy being applied to other classes of drugs that could benefit from the addition of an NO-releasing moiety, such as glucocorticoids, N-bisphosphonates and 5-aminosalicylic acid. The latter is one of the most commonly used drugs in the treatment of inflammatory bowel disease. In an experimental colitis model in the rat, an NO-releasing derivative of 5-aminosalicylic acid was recently shown to have markedly enhanced anti-inflammatory activity compared with the parent drug (unpublished observations).

NO INHIBITORS

Most of the animal models of human diseases in which the occurrence of large amounts of iNOS-derived NO has been observed make use of "NO inhibitors" to study the involvement of NO overproduction as either the cause or the consequence of the particular disease. Even before the chemical identification of endothelium-derived relaxing factor as being NO, hemoglobin and superoxide anion (O$_2^-$) were known to block the vasodilator effects of this labile factor. Because l-arginine was identified as the naturally occurring substrate for NO synthesis, several l-arginine-derived analogs (e.g., N-monomethyl-l-arginine and nitro-l-arginine methyl ester) were synthesized and characterized as competitive nitric oxide synthase (NOS) inhibitors. These early inhibitors shared a common feature of nonselectivity with respect to the source of NO (i.e., isoform of NOS).

Selective NOS inhibitors. Many NOS inhibitors considered as "isoform selective" are now available, mainly exhibiting their selectivity for the neuronal isoform of NOS (nNOS; e.g., 7-nitroindazole and analogs) or iNOS [e.g., aminoguanidine, l-N$^6$-(1-iminoethyl)lysine, S-alkylated isothiourea derivatives]. A comprehensive review of iNOS inhibitors has recently been published (20). It is toward iNOS that most efforts to find a selective inhibitor are being aimed, largely because of the importance of NO derived from iNOS in mediating the cardiovascular disturbances that characterize septic shock. These agents are also being tested in the context of intestinal inflammation, such as in experimental models of colitis. Earlier studies using nonselective NOS inhibitors produced, perhaps not surprisingly, discrepant results. Some studies demonstrated that NOS inhibitors reduced the severity of experimental colitis, whereas others demonstrated exacerbation of colitis (see Refs. 9 and 14 for reviews of this controversy). As outlined above, such discrepant results are almost certainly due to the dual ability of NO to mediate tissue injury and tissue defense. Even more recent studies that utilized more selective iNOS inhibitors and iNOS-deficient mice have failed to produce convincing and consistent evidence for a role of iNOS-derived NO in colitis (13, 17, 21).

Tetrahydrobiopterin (THB) is a common cofactor for all of the NOS isoforms. However, its role in the NO synthesis mechanism depends on the type of NOS isoform. For example, it contributes to the stabilization of nNOS and iNOS isoform dimerization, helps to prevent NOS inhibition by NO through a redox mechanism involving the prosthetic heme Fe atom, modulates the relative production of NO and O$_2^-$ by endothelial NOS, and helps to protect endothelial cells by scavenging...
ing $\text{H}_2\text{O}_2$. At present, attempts are being made to identify THB antagonists that are able to effectively block iNOS activity without significant effects on the other isoforms.

Peroxynitrite “scavenging.” NO reacts with $\text{O}_2$ in alkaline solutions to render the peroxynitrite anion ($\text{ONOO}^-; \text{PN}$). This reaction can also occur in biological systems (1). The formation of PN has been reported as a secondary and parallel event in several pathological situations in which large amounts of NO derived from iNOS have been identified. With the discovery of PN, previous work on the involvement of iNOS-derived NO in pathological processes had to be revised, and the reaction of PN formation from NO and $\text{O}_2$

$$\text{NO} + \text{O}_2 \rightarrow \text{ONOO}^-$$

was (and is) seen as the “good” → the “bad” → the “ugly.”

The identification of proteins containing 3-nitrotyrosine residues is the most widely employed strategy to detect the formation of PN in biological systems (2) given the highly reactive and unstable nature of this anion under physiological conditions (half-life of 1.9 s).

As outlined above, NO derived from iNOS has been suggested to play a role in the tissue injury associated with experimental colitis and human inflammatory bowel disease. PN has also been implicated as a mediator of tissue injury in these disorders, through demonstration of increased formation (i.e., nitrotyrosine staining) in inflamed ileum and colon from guinea pigs (15) and humans (8), respectively. It is important to bear in mind that nitrotyrosine staining may occur independently of PN formation (3). On the other hand, mercaptoprotothylguanidine has recently been shown to exert beneficial effects in hapten-induced colitis in the rat, and the effects of this agent were attributed to its dual ability to inhibit iNOS and quench PN (26). It is noteworthy that Sandoval et al. (19) have demonstrated that 5-aminoalicylic acid, one of the most widely used drugs for treating inflammatory bowel disease, is a very potent scavenger of PN. It is possible that this activity contributes significantly to the beneficial effects of this drug in the treatment of inflammatory bowel disease.

Endogenous, unrelated compounds such as uric acid, porphyrins, and vitamins C and E have been shown to offer effective protection against the PN toxicity in different biological systems. Kamisaki et al. (7) have recently found that rat spleen homogenates are able to reverse the PN-induced nitration of protein tyrosine residues and that this “nitrotyrosine denitrase” activity is enhanced in endotoxin-treated animals. The search for the expression of this (or a similar) activity in other tissues and the knowledge of their mechanisms of induction will be of fundamental importance for a rational pharmacological manipulation of PN-induced toxicity in an iNOS-independent way.

NO scavengers. The high reactivity of NO with diverse chemical groups (such as reduced thiols, primary amines, and transition metals) is the main basis for the design of drugs to be used as NO scavengers.

Ruthenium compounds (mainly as coordination complexes) have been proposed as efficient NO scavengers in animal models of sepsis. Fricker et al. (5) have shown encouraging results regarding the recovery of the hemodynamic functions in the rat after the administration of one such ruthenium compound (JM-1226) 20 h after challenge with endotoxin. 2-Phenyl-4,4,5,5-tetramethylimidazolin-1-oxyl-3-oxide (PTIO) is another well-known NO scavenger widely used in several animal models that are characterized by the participation of iNOS-derived NO (12). To our knowledge, this compound has not been assessed in models of gastrointestinal inflammation.

**SUMMARY**

NO represents a very attractive therapeutic target with respect to several gastrointestinal disorders. There is considerable evidence that iNOS is strongly expressed in several inflammatory conditions in the digestive system and that NO derived from this isofom contributes to tissue injury. However, there is also evidence to the contrary. The development of iNOS inhibitors that are truly selective in vivo will help to finally sort out the contribution of this isoform to gastrointestinal damage and their utility as therapeutic agents. Scavengers of NO and of PN are also very promising tools for determining the roles of these substances in various digestive disorders, and there is the strong possibility that these compounds will be used clinically. In addition to the approaches to reduce NO formation or scavenge NO and PN, compounds that release small amounts of NO (insufficient to cause hypotension or motility disorders) over a prolonged period of time have great promise. NO-NSAIDs are an example of such compounds that are now being developed by a number of pharmaceutical companies for treatment of inflammatory conditions and thrombotic disorders. NO donors may be useful for preventing gastrointestinal injury associated with septic and hemorrhagic shock. The ability of NO to accelerate ulcer healing is another therapeutic avenue that is presently being pursued by several groups.

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