Nitric oxide and chronic colitis

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MB GRISHAM, S AIKO. Nitric oxide and chronic colitis. Can J Gastroenterol 1996;10(3):199-202. Nitric oxide (NO) is thought to play an important role in modulating the inflammatory response by virtue of its ability to affect bloodflow, leukocyte function and cell viability. The objective of this study was to assess the role that NO may play in mediating the mucosal injury and inflammation in a model of chronic granulomatous colitis using two pharmacologically different inhibitors of nitric oxide synthase (NOS). Chronic granulomatous colitis with liver and spleen inflammation was induced in female Lewis rats via the subserosal (intramural) injection of peptidoglycan/polysaccharide (PG/PS) derived from group A streptococci. Chronic NOS inhibition by oral administration of N^G-nitro-L-arginine methyl ester (L-NAME) (15 µmol/kg/day) or amino-guanidine (AG) (15 µmol/kg/day) was found to attenuate the PG/PS-induced increases in macroscopic colonic inflammation scores and colonic myeloperoxidase activity. Only AG – not L-NAME – attenuated the PG/PS-induced increases in colon dry weight. Both L-NAME and AG significantly attenuated the PG/PS-induced increases in spleen weight whereas neither was effective at significantly attenuating the PG/PS-induced increases in liver weight. Although both L-NAME and AG inhibited NO production in vivo, as measured by decreases in plasma nitrite and nitrate levels, only AG produced significantly lower values (38±3 versus 83±8 µM, respectively, $P<0.05$). Finally, L-NAME, but not AG, administration significantly increased mean arterial pressure from 83 mmHg in colitic animals to 105 mmHg in the PG/PS+ L-NAME-treated animals ($P<0.05$). It is concluded that NO may play an important role in mediating some of the pathophysiology associated with this model of chronic granulomatous colitis.

Key Words: *Inflammation, Neutrophils, Oxygen radicals*

Oxyde nitrique et colite chronique

RÉSUMÉ : L'oxyde nitrique (ON) jouerait un rôle important dans la modulation de la réponse inflammatoire à cause de sa capacité d'affecter le débit sanguin, la fonction leucocytaire et la viabilité cellulaire. L'objectif de cette étude était d'évaluer le rôle de l'ON dans la l'atteinte et l'inflammation des muqueuses dans un modèle de colite granulomateuse chronique à l'aide de deux inhibiteurs pharmacologiquement différents de la synthétase de l'oxyde nitrique. La colite granulomateuse chronique accompagnée d'inflammation hépatique et splénique a été induite chez des rates Lewis par l'entremise d'une injection subséreuse (intramurale) de peptidoglycanes/polysaccharides (PG/PS) dérivés de streptocoques du groupe A. L'inhibition chronique de la synthétase de l'oxyde nitrique par l'administration orale de N^G-nitro-L-arginine méthyl ester (L-NAME) (15 µmol/kg/jour) ou d'amino-guanidine (AG) (15 µmol/kg/jour) s'est révélée apte à atténuer les augmentations des indices d'inflammation macroscopique du côlon et de l'activité de la myélo-peroxydase colonique induites par PG/PS. Seule l'AG et non pas le L-NAME a atténué les augmentations du poids sec du côlon induites par PG/PS. Le L-NAME et l'AG ont atténué significativement les augmentations du poids de rates induites par PG/PS alors que ni l'un ni l'autre n'a pu atténuer de façon significative les augmentations du poids hépatique induites par PG/PS. Comme en témoignaient les diminutions des taux plasmatiques de nitrite, seule l'AG a produit des taux significativement moindres (38±3 contre 83±8 µM, respectivement $P<0,05$). Finalement, le L-NAME, contrairement à l'AG, a significativement augmenté la tension artérielle moyenne, de 83 mmHg, chez les animaux atteints de colite à 105 mmHg chez les animaux traités par PG/PS+L-NAME ($P<0,05$). On en conclut que l'ON peut jouer un rôle important dans la physiopathologie associée à ce modèle de colite granulomateuse chronique.

There is a growing body of experimental and clinical data suggesting that chronic inflammation of the colon is associated with enhanced production of nitric oxide (NO) (1-3). NO is thought to play an important role in modulating the inflammatory response via its ability to affect bloodflow and leukocyte function (4). Furthermore, this reactive nitrogen intermediate will rapidly and spontaneously interact with molecular oxygen or superoxide to yield potentially

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injurious oxidizing and nitrosating agents. Although the sources of this enhanced NO production in vivo have not been definitively identified, it is very probable that the phagocytic leukocytes (neutrophils, monocytes, macrophages) known to accumulate within the colonic interstitium are primary candidates (5). We have demonstrated that extravasated, but not circulating, neutrophils produce much larger amounts of NO via the up-regulation of both mRNA and inducible nitric oxide synthase (iNOS) enzymatic activity (6).

Intestinal inflammation induced in experimental animals or in human inflammatory bowel disease is associated with increases in at least one tissue-derived cytokine, such as tumour necrosis factor, interferon-gamma and interleukin-1-beta (7). Some of these cytokines are potent inducers of NO synthase in macrophages, neutrophils and endothelial cells (4). In addition, we and others have found that incubation of interleukin-1-beta, tumour necrosis factor and/or interferon with cultured rat intestinal epithelial cells for 24 h promotes the release of large quantities of NO_2^- and NO_3^- (8,9). Several investigators have demonstrated that NO produced from activated macrophages is capable of injuring microorganisms, tumour cells and some normal cells, such as hepatocytes, pancreatic islet cells and lymphocytes (10-14).

Because of the potential injurious and proinflammatory properties of NO produced by iNOS, we wanted to assess whether NO plays a role in mediating the mucosal injury and inflammation in a model of immunologically induced chronic granulomatous colitis. The objective of this study was to assess the role of NO or NO-derived metabolites as mediators of the mucosal injury and inflammation observed in a model of chronic granulomatous colitis in rats (2).

ANIMALS AND METHODS

Induction of colitis: Specific pathogen-free female Lewis rats (weighing between 150 and 175 g) were housed in wire mesh-bottomed cages and given free access to water and standard laboratory rat chow. Forty-four rats were randomized into four groups: control group (n=7), peptidoglycan/polysaccharide (PG/PS)-treated group (n=15), PG/PS + N^G-nitro-L-arginine methyl ester (L-NAME) group (n=15) and PG/PS + aminoguanidine (AG) group (n=7). The animals were anesthetized via inhalation of isofluorane and their descending colons were exposed by laparotomy using aseptic technique. Colitis was induced via nine to 10 intramural (subserosal) injections (50 to 60 μL/injection) of PG/PS (12.5 μg rhamnose/g body weight) into the distal colon (4 cm) using a 30 G needle (2). Control animals were treated identically using nine to 10 injections (50 to 60 μL/injection) of a sterile saline solution. The nitric oxide synthase (NOS) inhibitors L-NAME (15 μmol/kg/day) and AG (15 μmol/ kg/day) were administered to the colitic animals in their drinking water beginning three days before the induction of colitis and continuing for the entire three-week period.

Assuming that each drug freely equilibrates with the entire extracellular volume (30% of body weight), steady state concentrations of L-NAME and AG should approximate 50 μM each. This concentration of L-NAME produces near maximal inhibition of constitutive nitric oxide synthase (cNOS) as measured by its ability to cause substantial increases in MAP, but has little or no effect on phagocytic leukocyte (polymorphonuclear leukocytes, macrophage)-associated iNOS (15,16). Fifty micromolar AG, on the other hand, has been demonstrated to inhibit phagocyte iNOS completely, but has little or no effect on cNOS as judged by its lack of effect on MAP in vivo (17).

Quantitative indexes of colonic injury and inflammation: Macroscopically visible injury and inflammation, colonic myeloperoxidase (MPO) activity and colon dry weight were quantified and used as indexes of colonic inflammation, granulocyte infiltration and interstitial fibrosis, respectively (2). Plasma nitrate and nitrite levels were used as indirect indexes of NO metabolism in vivo and were quantified by converting nitrate to nitrite using *Escherichia coli* nitrate reductase and then measuring total nitrite using the Griess reagent (10).

RESULTS

Subserosal (intramural) injection of PG/PS into the distal colon results in an acute and spontaneously reactivating chronic colitis characterized by leukocyte infiltration, colonic granulomas, adhesions and bowel wall thickening at three weeks post-PG/PS injection (2). Chronic NOS inhibition by L-NAME or AG attenuated the PG/PS-induced increases in macroscopic colonic inflammation scores (2.5±0.2 and 3.0±0.4 versus 4.4±0.4, respectively, $P<0.05$) and colonic MPO activity (1.7±0.2 and 1.6±0.1 versus 6.1±0.8 units/cm, respectively, $P<0.01$). Only AG (not L-NAME) attenuated the PG/PS-induced increases in colon dry weight (0.0135±0.002 versus 0.022±0.002 g dry weight/ cm, $P<0.05$). Both L-NAME and AG significantly attenuated the PG/PS-induced increases in spleen weight (3.8±0.7 and 2.8±0.2 versus 6.6±2 mg/g body weight, respectively, $P<0.05$), whereas neither drug was effective at significantly attenuating the PG/PS-induced increases in liver weight. Although both L-NAME and AG inhibited NO production in vivo, as measured by decreases in plasma nitrite and nitrate levels, only AG produced significantly lower values (38±3 versus 83±8 μM, respectively, $P<0.05$). Finally, L-NAME, but not AG, administration significantly increased MAP, from 83 mmHg in colitic animals to 105 mmHg in the PG/PS+L-NAME-treated animals ($P<0.05$)

DISCUSSION

We have found that intramural injection of PG/PS induces acute and chronic granulomatous inflammation of the distal colon (2). This model, compared with other models of colitis induced by the intrarectal administration of noxious organic acids and/or solvents, produces a chronic colonic inflammation via one injection of a nontoxic biopolymer. Although injections of the bacterial cell wall polymer were made into the distal colon, the animals developed systemic

inflammation, including arthritis and hepatic and splenic granulomas. Certain inflammatory mediators, cytokines and bacterial products (such as lipopolysaccharides and PG/PS) activate monocytes, macrophages and neutrophils (4). This activation may be expressed in a variety of ways, including synthesis and release of certain cytokines and proinflammatory mediators (eg, platelet-activating factor, leukotriene B4), secretion of protease and release of reactive oxygen metabolites (eg, superoxide, hydrogen peroxide) (4). L-arginine-dependent production of NO is induced in phagocytic leukocytes by these mediators (4). Macrophages and neutrophils contain a calcium-independent NO synthase that is transcriptionally activated by lipopolysaccharide and interferon-gamma (4).

Because histological inspection of colonic and hepatic tissue revealed the presence of acute and chronic inflammatory cells such as neutrophils, macrophages and monocytes in inflamed intestine and the liver three to four weeks after PG/PS injection, we quantified the plasma levels of nitrite and nitrate as an index of NO synthase regulation in vivo (2). We found that intramural administration of PG/PS enhanced plasma levels of these nitrogen oxides by fourfold at three weeks, suggesting that this bacterial polymer may induce NO synthase in at least one population of cells in vivo. These data suggest that hepatic and colonic macrophages and neutrophils may be major sources of these nitrogen oxides in the chronically inflamed animal. This suggestion is supported by our observations that elicited rat peritoneal macrophages and neutrophils produce relatively large amounts of nitrite in an L-arginine dependent manner when cultured with PG/PS at concentrations calculated to be achieved in vivo (2). It should be emphasized that although the major source of nitrite and nitrate in vivo in response to inflammatory mediators is assumed to be macrophages (and possibly neutrophils) it is possible that other cells such as endothelial cells, smooth muscle cells, fibroblasts, hepatocytes and/or mast cells may contribute to the overall production of nitrogen oxides in vivo (4).

Several recent studies have reported that L-NAME is a much more selective inhibitor for cNOS whereas AG is much more selective for iNOS (15-17). A dose of 15 μmol/kg/day of either L-NAME or AG produces steady state concentrations of 50 μM each, assuming that each drug freely equilibrates with the entire extracellular volume (30% of body weight). As previously stated, this concentration of L-NAME produces near maximal inhibition of cNOS as measured by its ability to cause substantial increases in MAP, but has little or no effect on phagocytic leukocyte-associated iNOS (15,16). To reiterate, a steady state concentration of 50 μM AG, on the other hand, has been demonstrated to inhibit phagocyte iNOS completely, but has little or no effect on cNOS as judged by its lack of effect on MAP in vivo (17).

In this study we found that chronic NOS inhibition using 15 μmol/kg/day of either L-NAME or AG attenuated colonic injury and inflammation as judged by similar reductions in macroscopically visible lesions and colonic MPO activity. Histological inspection of the colons revealed similar anti-inflammatory activities of L-NAME and AG based on the lack of leukocyte infiltration and maintenance of epithelial and crypt integrity.

Interestingly, only AG (not L-NAME) significantly attenuated the PG/PS-induced increases in the dry weight of the colon and plasma levels of nitrate and nitrite, suggesting differences in the ability of L-NAME and AG to inhibit protein (collagen) deposition in the inflamed colon and NOS inhibition in vivo. The precise mechanisms for these differences are unknown; however, it has been demonstrated that L-NAME, at the concentration calculated to be present in vivo in our model of colitis, is much more effective than AG at inhibiting cNOS. Indeed, L-NAME but not AG was found to increase MAP by 30 mmHg.

These data are consistent with the idea that the enhanced levels of nitrate and nitrite observed during chronic gut, liver and spleen inflammation result from up-regulation of iNOS. Because cNOS is regulated by the small and transient increases in intracellular calcium, this isoenzyme produces only small amounts of NO for short periods whereas iNOS produces much larger amounts during times of chronic inflammation. Thus, the overall contribution of systemic NO made by cNOS is expected to be minimal. In fact, one can predict that selective inhibition of cNOS by L-NAME is expected to increase MAP but has little effect on circulating levels of nitrate and nitrite. Indeed, this is exactly what happens. Selective inhibition of iNOS by AG, on the other hand, is expected to produce no effect on blood pressure but to inhibit systemic nitrate and nitrite levels substantially. Again, this is observed. The mechanisms responsible for L-NAME- or AG-dependent inhibition of leukocyte accumulation in the PG/PS-treated colons are not clear. As in other models of inflammation-induced vascular injury, it can be argued that inhibition of cNOS by L-NAME would reduce bloodflow to the colon and thereby deliver circulating leukocytes to the tissue (18). This explanation is unlikely in our model because AG appears not to alter cNOS but to inhibit leukocyte accumulation.

Enhanced and sustained production of NO may alter the normal physiology of the colon and possibly the liver and spleen. NO, also known as endothelial-derived relaxing factor, is a potent vasodilator that will increase tissue bloodflow quite dramatically (4). Clinical and experimental studies demonstrate that the bloodflow of the inflamed colon increases quite dramatically during active episodes of gut inflammation. Furthermore, NO is known to injure (reversibly or irreversibly) parenchymal and leukocytic cells such as hepatocytes, pancreatic islet cells and lymphocytes (12-14). This may be an important mechanism by which PG/PS mediates injury and fibrosis to the gut, liver and spleen in our model. More recent data suggest that enhanced production of NO has the potential to induce mutagenic and possibly carcinogenic alterations in the gut epithelium via the formation of potent N-nitrosating agents derived from the spontaneous decomposition of NO in oxygenated solutions (19).

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