Protective role of heme oxygenase-1 on trinitrobenzene sulfonic acid-induced colitis in rats


Departments of Pharmacology and Medicine, Faculty of Medicine, The University of Hong Kong, Hong Kong, People’s Republic of China

Received 7 September 2000; accepted in final form 27 March 2001

Wang, W. P., X. Guo, M. W. L. Koo, B. C. Y. Wong, S. K. Lam, Y. N. Ye, and C. H. Cho. Protective role of heme oxygenase-1 on trinitrobenzene sulfonic acid-induced colitis in rats. Am J Physiol Gastrointest Liver Physiol 281: G586–G594, 2001.—Preliminary studies showed that the inducible form of heme oxygenase (HO-1) was induced and played a protective role in the process of inflammation. The present study investigated the possible role of HO-1 in 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis in rats. We measured HO-1 activity in TNBS-induced colitis in rats and analyzed the severity of colitis along with altered HO activity by assessing lesion area and myeloperoxidase activity. HO-1 mRNA and protein expressions were determined at different time points after TNBS induction. Free radical production and inducible nitric oxide synthase (iNOS), which participate in oxidative injury, were also assayed. HO activity and HO-1 gene expression increased markedly after TNBS induction. Administration with tin mesoporphyrin (SnMP), a HO inhibitor, potentiated the colonic damage and to study whether HO-1 induction could protect reactive oxygen species; inducible nitric oxide synthase

IN HEME METABOLISM, heme oxygenase (HO) is an initial and rate-limiting enzyme. It oxidatively degrades heme into carbon monoxide (CO), iron, and biliverdin, which is subsequently converted to bilirubin by biliverdin reductase (40). Two distinct isoforms of HO have been identified (17, 19, 41). These isoforms are different gene products and vary in their tissue expression as well as in their molecular properties (17, 19, 41). The HO-2 isoform is constitutive and physiologically expressed. In contrast, the HO-1 isoform is ubiquitously distributed in mammalian tissues and is strongly and rapidly induced by a variety of stimuli and agents that cause oxidative stress and pathological conditions (17). HO-1 induction is important in the response of tissues to oxidative stress and inflammation (12, 45). Investigations have demonstrated (11a) that heme degradation produces a variety of biological effects. Bilirubin, an antioxidant, provides cellular protection against free radical-mediated injury (12, 36). CO regulates the generation of pro- and anti-inflammatory cytokines (27). In addition, CO leads to vasodilation and inhibition of platelet aggregation (20, 24). Unlike the action of nitric oxide (NO), which is mediated through cyclic guanosine monophosphate, the action of CO through the same nucleotide is still debatable (37). NO production in macrophages by inducible NO synthase (iNOS) has multiple tissue-damaging effects and is involved in the pathogenesis of inflammation (16). iNOS is a hemoprotein containing two heme molecules in its active site (21). The increase in HO-1 activity could reduce iNOS activity through accelerated degradation of the newly synthesized heme.

Reactive oxygen species (ROS), such as superoxide anion and hydrogen peroxide, as well as excess NO are involved in human inflammatory bowel disease (IBD) (6, 35). The IBD induced in rats by 2,4,6-trinitrobenzene-sulfonic acid (TNBS) produced acute and chronic inflammation of the colon (15, 25, 28). This was accompanied by large quantities of ROS and NO products as well as inflammatory infiltration, which pathologically resembled human IBD (15, 25, 28). To investigate whether the expression of HO-1 is an endogenous mechanism responsible for host defense against inflammatory injury in colonic tissue, we used the TNBS-induced IBD model in this study. We utilized this model to elucidate the effect of HO-1 expression and activity on the severity of colonic lesion and inflammation and to study whether HO-1 induction could protect colonic tissue from oxidative and inflammatory damage through reduction of ROS and NO products.

MATERIALS AND METHODS

Animals. The present study was approved by the University of Hong Kong Committee on the Use of Live Animals. Male Sprague-Dawley rats (180–200 g) were fed a standard diet for 1 week before experiments. Male Sprague-Dawley rats were used for these experiments because they are similar in size and body composition to human patients with inflammatory bowel disease (24, 34). Wistar rats were used as a normal control group.

Address for reprint requests and other correspondence: C. H. Cho, Dept. of Pharmacology, Faculty of Medicine, The Univ. of Hong Kong, 5 Sassoon Rd., Hong Kong (E-mail: chcho@hkusua.hku.hk).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
laboratory diet (Ralston Purina, Chicago, IL) and given tap water. The rats were kept in a room with controlled temperature (22 ± 1°C), humidity (65–70%), and a 12:12-h light-dark cycle.

Drugs and solutions. Tin mesoporphyrin IX chloride (SnMP) and ferriprotoporphyrin IX chloride (hemin) (Porphyrin Products, Logan, UT) were dissolved in 0.1 ml of 0.5 M NaOH; normal saline was added until the drug preparation equaled 0.5 ml. The solution was finally adjusted to pH 7.4. Both SnMP and hemin solutions were kept in the dark to prevent photodegradation. TNBS (Sigma, St. Louis, MO) was dissolved in 50% (vol/vol) ethanol (Merck, Darmstadt, Germany). L-Arginine (Sigma) was dissolved in normal saline.

Experimental IBD in rats. IBD was induced in rats according to the model described by Morris et al. (25). Rats were lightly anesthetized by diethyl ether (BDH, Poole, UK). Using a polyethylene catheter fitted onto a 1-ml syringe, we slowly injected 0.3 ml of 50% (vol/vol) ethanol containing 30 mg TNBS into the lumen of the colon (8 cm proximal to the anus through the rectum). Animals in the normal control group were handled similarly, but normal saline was rectally administered instead. Rats were killed at specific time points after colitis induction. The distal colon was removed, opened, and thoroughly rinsed in ice-cold normal saline. The lesion size was delineated and measured with a 1-mm square grid by an observer who was unaware of the treatment. The distal colon (length, 8 cm) was weighed, and the ratio of colon weight to body wt was obtained. This ratio was used as a parameter to assess the degree of colon edema, which reflected the severity of colonic inflammation. One piece of colonic tissue (3 × 10 mm) dissected from the most ulcerative region was excised and fixed in 10% formalin buffer for subsequent immunohistochemical studies. The rest of the colonic tissue was collected, immediately frozen in liquid nitrogen, and stored at −70°C for further biochemical investigations.

Drug administration. Rats were administered either SnMP (20 μmol/kg) subcutaneously into posterior neck tissue 3 h before and 21 h after colitis induction or hemin (50 μmol/kg) intravenously 1 h before the TNBS enema application. L-Arginine (50 mg/kg) was administered subcutaneously once daily over 4 days before the TNBS enema. Rats were administered the TNBS enema as described above.

Determination of HO activity in colonic tissue. HO activity in colonic tissue was measured as previously described (18). Briefly, the colonic tissue was homogenized in 10 mM PBS (pH 7.2) containing 1 mM phenylmethylsulfonyl fluoride, 1.5 mM pepstatin, and 0.2 mM leupeptin. The homogenate was centrifuged at 10,000 g for 15 min at 4°C. The supernatant (100 μl) was collected for the analysis of iNOS activity and protein. The remainder was further subjected to centrifugation at 105,000 g for 1 h at 4°C. The precipitated microsome was dissolved in the above-mentioned PBS solution. The protein content was determined with a Bio-Rad assay kit, and BSA was used as the protein standard. Microsomal protein (50 μg) was used for the HO Western blot analysis and the remaining microsomal protein for HO activity analysis. HO activity was measured in a solution mixture consisting of 0.5 ml microsomal protein (6–9 mg/ml); 0.2 ml 105,000 g supernatant fraction (10 mg of protein/ml) from rat liver as the source of biliverdin reductase; an NADPH-generating system (0.8 mM β-NADPH, 0.8 mM glucose 6-phosphate, 1.5 U glucose-6-phosphate dehydrogenase, and 2 mM MgCl₂); and 20 μl of 2.5 mM heme solution. The final volume of the mixture (pH 7.4) was adjusted to 1.5 ml with 0.1 M phosphate buffer. The blank solution was similarly prepared but without the NADPH-generation system. The mixtures were prepared on ice and then aerobically incubated in the dark in a shaking incubator at 37°C for 60 min. At the end of incubation, the amount of bilirubin formed was measured spectrophotometrically at 460 to 530 nm. HO activity was expressed as nanomoles of bilirubin per 60 min per milligram of protein.

Measurement of myeloperoxidase activity in colonic tissue. Myeloperoxidase (MPO) activity was determined by a modified method as described previously by Suzuki et al. (39). In brief, colonic tissue was minced with scissors and homogenized in an ice-cold 50 mmol/l PBS (pH 6.0) solution containing 0.5% hexadecyltrimethylammonium bromide. The homogenate was freeze-thawed three times, followed by repeated sonication for 30 s each. The MPO activity assay has been described previously in detail by Guo et al. (8). The final value of MPO activity was represented as units per gram of tissue.

HO-1 immunohistochemistry in colonic tissue. The formalin-fixed colonic tissue was embedded in paraffin, and sections were de waxed. Endogenous peroxidase activity was blocked with 3% H₂O₂ in methanol for 30 min. After blocking in 0.05 mol/l Tris-buffered saline (TBS) containing 3% normal horse serum and 0.3% Triton X-100 for 30 min, sections were rinsed with TBS buffer and incubated with mouse monoclonal HO-1 antibodies (Stressgen, Victoria, BC, Canada) diluted to 1:200 overnight at 4°C. The tissue was stained for antigen-antibody complexes using a peroxidase detection system (LSAB kit, DAKO).

Total RNA isolation and reverse transcriptase-PCR (RT-PCR). Total RNA was isolated from rat colonic tissue with Trizol reagent (GIBCO BRL, Gaithersburg, MD). cDNA was amplified from RNA with the use of Thermostart RT-PCR system reagent (GIBCO BRL). Primers were designed according to previously published sequences (2, 5). Sense (CAG TCG CCT CCA GAG TTC CC) and antisense primers (GTA CAA GGA GGC CAT CAC CAG C) for HO-1 (284-bp fragment), sense (ATT ACC AAG GAC CAG TCA TCT T) and antisense primers (AGG GCT TGT ACA AAC TGG GTA T) for HO-2 (251-bp fragment), sense (ATG GCT TGC CTT TGG AAG CTC) and antisense primers (TCC AGG CCA TCT TGT TGG CAA GA T) for iNOS (251-bp fragment), and sense (GTG GGG CGC CCC AGG CAC CA) and antisense primers (CTC CTT AAT GTC ACG CAT GAT TTT) for β-actin (540-bp fragment) were used. β-actin was employed as an internal control. For HO-1 and HO-2 amplification, the processes involved 30 cycles of PCR [1 min at 95°C for denaturing, 1 min at 58°C for annealing, and 2 and 7 min (final cycle) at 72°C for elongation]. For iNOS and β-actin, after denaturation at 95°C for 5 min, 30 cycles of PCR were performed with 30 s at 95°C for denaturing, 45 s at 60°C for annealing, and 1 and 7 min (final cycle) at 72°C for elongation.

Western blot analysis. Fifty micrograms of microsomal protein and the supernatants of tissue homogenate described above were respectively fractionated in a 10% or 7.5% SDS-polyacrylamide gel, then transferred to nitrocellulose membrane. After incubating in blocking buffer containing 5% skim milk dissolved in 10 mmol/l Tris-HCl, pH 7.5, 100 mmol/l NaCl, and 0.1% Tween 20, blots were probed with mouse monoclonal antibodies for HO-1 (1:400), iNOS (1:1,000), and rabbit polyclonal antibody for HO-2 (1:400) in blocking buffer for 1 h at room temperature. Antigen-antibody complexes were visualized by using horseradish peroxidase-conjugated goat anti-mouse or anti-rabbit antibodies.

Chemiluminescence assay in colonic tissue. Immediately after the colonic lesion was recorded, two full-thickness biopsy specimens (50–100 mg) were taken from the most ul-
cerative lesion and inflammatory area. The samples were initially placed in preoxygenated (95% O₂-5% CO₂ for 20 min) PBS solution with concentrations of calcium (1.13 mmol/l) and glucose (5 mmol/l) at ambient temperature and then transferred to 300 μmol/l luminol immediately before assessment of the chemiluminescence response. The chemiluminescence was measured in a liquid scintillation counter (2000 CA, Packard) with an analyzer operated in “the out-of-coincidence” mode for 2 min. The final result of each colonic determination was the value after subtraction of background counts. The value was expressed as the number of photons per minute per milligram of wet tissue (34).

Determination of iNOS activity in colonic tissue. iNOS activity in colonic tissue was measured as previously described (3) by the conversion of [³H]arginine to citrulline. To determine iNOS activity, EGTA (91 mmol/l) was used to inhibit the activity of calcium-dependent (constitutive) NOS. The final result was expressed as picomoles of [³H]citrulline per minute per milligram of protein.

Statistical analysis. Results were expressed as means ± SE. Differences between two groups were examined using one-way ANOVA followed by unpaired Student’s t-test unless specified. The correlation between the two parameters was tested using the Pearson correlation test. P < 0.05 was considered statistically significant.

RESULTS

Effects of HO activity on colonic lesion and MPO activity in TNBS-induced IBD in rats. Intracolonic administration of TNBS-ethanol resulted in noticeable hyperemia and exfoliation of epithelial lining at 2 and 6 h, and maximal mucosal lesion and inflammation in the distal colon (e.g., extensive ulceration, bowel wall thickening, and hyperemia) were grossly and also histologically evident at 24 and 48 h. The weight of the distal colon was also markedly increased because of tissue edema.

Figure 1 shows that the TNBS enema markedly increased HO activity in the colonic tissue at 6 and 24 h, with maximal activity level reached at 72 h. Administration of 20 μmol SnMP 3 h before and 21 h after TNBS induction remarkably inhibited TNBS-induced HO activity. The colonic lesion area in animals receiving SnMP was significantly potentiated (Fig. 2). MPO activity was dramatically increased at 6, 24, and 72 h after TNBS administration. SnMP administration enhanced MPO activity stimulated by the TNBS enema. However, SnMP alone did not change MPO activity compared with the normal control group (Fig. 3).

HO-1 immunohistochemistry in TNBS-induced colitis. HO-1 immunoreactivity was absent in normal colon tissue (Fig. 4A). HO-1 immunoreactivity was detected in the vascular endothelial, epithelial, and smooth muscle cells of colonic tissues 2 h after application of the TNBS-ethanol enema (Fig. 4, B and C). This was accompanied by some positive-stained infiltrated inflammatory cells. The HO-1 staining was evident and predominantly localized in the submucosa underneath the mucosal lesion area in the tissue 24 h after colitis induction (Fig. 4D). The cells contributing to an increase in HO-1 expression were infiltrated neutrophils, macrophages, and other inflammatory cells (Fig. 4D, inset).

Expression profiles of HO mRNA and proteins in TNBS-induced colitis. Total RNA and protein were isolated at various times after colitis induction. As shown in Fig. 5A, RT-PCR yielded a 284-bp product for HO-1 and a 242-bp product for HO-2 when HO-1- and HO-2-specific primers, respectively, were used. The level of HO-1 mRNA was low in normal colonic tissue. After TNBS administration, HO-1 mRNA transcripts rapidly increased starting at 6 h and peaked at 3 days with an ~25-fold increase over the normal level (Fig. 5A). On the other hand, there was no significant change for HO-2 mRNA expression during the inflammation period (Fig. 5B). The pattern of HO-1 protein accumulation showed a trend similar to that of mRNA expression. HO-1 protein level was elevated starting at 6 h and reached its peak at day 3 (Fig. 6A). Again, the protein level of HO-2 was not significantly changed during the inflammation period (Fig. 6B). However, it was expressed more during this period of time compared with normal tissue.

Elevation of luminol-amplified chemiluminescence by SnMP treatment in TNBS-induced colitis. Luminol reacts with oxidants such as ROS, to form 3-aminophthalate. Electrons in the luminol are raised to higher energy levels during the reaction with oxidants. On reverting to the basal state, the energy is released in the form of photons (chemiluminescence), which are detected by the photomultiplier tubes of a scintillation counter.
counter (6). The changes in chemiluminescence can reflect ROS production in tissues. Luminol-amplified chemiluminescence in colonic tissue was markedly increased at 6 h and even more at 24 h after the TNBS enema. Pretreatment with SnMP produced significantly more luminol-amplified chemiluminescence in rats with TNBS-induced colitis and did not increase chemiluminescence in normal rats (Fig. 7).

Inhibition of iNOS activity and expression with HO-1 induction. The TNBS enema led to a significant increase in iNOS activity in colonic tissue at 6, 24, and 72 h. SnMP administration further elevated iNOS activity compared with the respective TNBS control groups. Pretreatment with SnMP alone did not cause the alteration of iNOS activity in normal rats (Fig. 8). RT-PCR showed that iNOS mRNA expression was increased by SnMP administration compared with the TNBS control at 24 h (Fig. 9A, lanes 3 and 4 vs. lanes 1 and 2). The protein level of iNOS in Western blots coincided with its mRNA expression at 24 h (Fig. 9B, lanes 3 and 4 vs. lanes 1 and 2). To confirm further the effect of HO activity on iNOS, the expression of iNOS mRNA and protein was examined in the TNBS-treated colonic tissue pretreated with hemin, a stimulator for HO. Administration of hemin increased the HO activity by about twofold (from 5.34 to 10.43 nmol bilirubin·h\(^{-1}\)·mg protein\(^{-1}\)) 24 h after TNBS administration and reduced iNOS mRNA expression and protein production in colonic tissue (Fig. 9, A and B, lanes 5 and 6 vs. lanes 1 and 2).

Aggravated colonic damage by L-arginine pretreatment in TNBS-induced colitis. There was a strong correlation between iNOS activity and colonic lesion area (Pearson correlation coefficient = 0.816, \(P < 0.01\); data not shown). Supplemented with the substrate of iNOS for the production of NO, L-arginine further exacerbated the severity of the mucosal lesion induced by the TNBS enema and also the injury action of SnMP at 24 and 72 h after TNBS administration (Fig. 10).

DISCUSSION

Recent studies (14, 27, 29, 42, 45) with HO-1-knockout mice have shown that induction of HO-1 helps to ameliorate tissue injury or inflammation changes in a variety of experimental models. However, the exact role of HO-1 in IBD is unclear. In this study, HO-1 was induced in colonic tissue damaged by the TNBS-ethanol enema. Prior administration with the HO activity inhibitor SnMP potentiated the colonic damage. The protective effect of HO could result from radical scavenging and inhibition of iNOS expression.

TNBS significantly increased HO activity in the colonic tissue. Administration of SnMP 3 h before the TNBS enema resulted in an apparent decrease of HO activity (Fig. 1). The changes in HO activity were shown to be associated with the severity of mucosal macroscopic lesion (Fig. 2). MPO activity was used as an index of quantitative inflammation and neutrophil infiltration in tissues (30). SnMP administration was able to aggravate neutrophil infiltration and inflammation (Fig. 3). These results suggest that generation of endogenous HO-1 may possibly protect colonic tissue against inflammation. RT-PCR showed a trace of HO-1...
mRNA in normal colon, which was below the detection limit of Western immunoblotting. The TNBS-ethanol enema increased HO-1 mRNA transcription and protein level starting at 6 h and peaked at 72 h (Figs. 5A and 6A). On the other hand, the expression of HO-2 mRNA and protein was slightly elevated in TNBS-induced IBD; however, there was no significant change at different time points (Figs. 5B and 6B). These results indicate that the altered activity and probable protective effect of HO are mainly due to HO-1 induction in IBD. HO-1 is a stress-response protein and can be induced by a variety of oxidant stimuli that facili-

Fig. 4. HO-1 immunohistochemistry in rat colonic tissue. A: normal colon. B: mucosa and submucosa of colon 2 h after a TNBS-ethanol enema; inset: positive-stained HO-1 endothelial cells shown by the arrow. C: muscle and serosa layers of colon 2 h after a TNBS-ethanol enema; inset: positive-stained HO-1 smooth muscle cells shown by the arrow. D: mucosa and submucosa of colon 24 h after a TNBS-ethanol enema; inset: positive-stained HO-1 polymorphonuclear and monomorphonuclear cells shown by the arrow. Magnification, ×200.

Fig. 5. RT-PCR analysis of HO-1 (A) and HO-2 mRNA (B) from colonic tissues at different times after TNBS administration. N, normal; d, day.
tate the generation of ROS. TNBS can be rapidly metabolized to generate ROS such as superoxide anion in rat colonocytes (7). The increase in HO-1 expression could be a protective response against the ROS-generated injury induced by TNBS. HO-1 immunolocation was observed in the vascular endothelium and smooth muscle layers of the colon as early as 2 h (Fig. 4, B and C). Hayashi et al. (11) have reported that HO-1 induction in endothelial cells can suppress oxidant-induced microvascular leukocyte adhesion through the action of bilirubin. The present study demonstrated that changes in HO activity caused by SnMP affected MPO activity, which is an index of neutrophil infiltration. As the colonic damage advanced, colitis was characterized by increasing inflammatory infiltrates in the colonic lamina propria. HO-1-immunoreactive cells were found to be in both polymorphonuclear and monomorphonuclear cells (Fig. 4D). ROS in the insulted mucosa cause an adverse reaction with tissue macromolecules, e.g., DNA, protein, and lipid, resulting in pathophysiological changes. ROS production within the colonic interstitium therefore represents an important pathway by which TNBS mediates the pathogenesis of IBD. The increase in oxidant-induced HO activity can increase cellular capacity to catalyze free heme, which generates into bilirubin and its precursor biliverdin to scavenge ROS (36). HO inhibition of SnMP treatment enhanced chemiluminescence response in TNBS-induced colonic tissue injury, suggesting that HO-1 induction is a physiological defense against oxidant damage in colonic tissue.

Experimental data, including our previous study (8), have demonstrated that TNBS-induced colitis is associated with increased expression and activity of iNOS in infiltrated inflammatory cells, especially neutrophils and macrophages. High NO concentrations from activated iNOS are toxic and can damage tissues, because NO reacts with superoxide anion to form peroxynitrite in the course of inflammation (3, 8, 31). Peroxynitrite, a highly cytotoxic oxidant, promotes oxidative and nitrosative injury of lipids, proteins, DNA, and other cellular biomolecules, leading to changes of cellular structure and function. Thus excess NO may be important in the pathogenesis of IBD. Indeed, aminoguanidine (an iNOS inhibitor) treatment ameliorated colonic damage (23); however, HO-1 expression was not significantly reduced (unpublished observations). These findings suggest that unlike a high NO level in tissues (5), a subthreshold NO concentration could not modulate HO-1 expression.

The exact mechanism whereby upregulation of HO-1 attenuates iNOS expression requires exploration. Because iNOS contains heme, a substrate for HO, in its interactions.
active sites, it is postulated that catabolism of cellular heme by HO would limit iNOS synthesis (1, 4). It has been shown that inducible macrophage NOS is a cytochrome P-450-type hemoprotein and contains a protoheme IX in its active site (44). HO degrades protoheme IX by cleaving its α-methene bridge into CO, iron, and biliverdin IXα (40). In addition, both HO and NOS systems require NADPH as an electron donor, thus competition to consume NADPH by increasing HO-1 molecules might impair NO synthesis in the NOS system. In the present study, SnMP administration enhanced mRNA and protein expression of iNOS in TNBS-induced colitis. To validate the possibility that alteration of HO activity affects iNOS mRNA expression, we examined the effect of an HO inducer on iNOS mRNA transcription. The administration of HO activity inducers such as hemin lowered iNOS mRNA and protein expression in the TNBS-inflamed colon (Fig. 9). HO-1 inducers such as hemin and SNP have been reported (1, 4) to abolish iNOS mRNA and protein expression both in vitro and vivo, and the action could be indirect through the substances released from heme metabolism. Iron released from the process of heme degradation would attenuate the NO level by down-regulating mRNA nuclear transcription and protein of iNOS initiated by inflammatory factors (13, 43). The improper overexpression of HO-1 would, however, reverse the HO-1-related protective effect, because large amounts of iron released from heme are harmful and are recognized to increase cellular susceptibility to oxidative stress (38). It is crucial to determine the concentration of HO-1 inducer needed to promote protective HO-1 expression. Therefore, appropriate HO-1 induction should be important in providing protection in tissues. This phenomenon requires further investigation.

CO released in the process of heme catabolism was also shown to inactivate existing iNOS by interacting with its heme iron moiety (21). iNOS expression is primarily from activated macrophages that increase their infiltration from 6 h after injury and reach the maximum at 72 h (32). The significant positive correlation between iNOS activity and colonic lesion area indicated that increased iNOS activity and expression contributed to colonic lesion formation induced by TNBS in rats. Furthermore, exogenous administration of L-arginine followed by SnMP pretreatment aggravated the colonic damage by the TNBS enema (Fig. 10). Thus iNOS downregulation by HO-1 induction may ameliorate the colonic lesion. It is likely that cells may possess an intrinsic system to attenuate NO-mediated cytotoxicity, and HO-1 may be a potential candidate. Excessive NO production originating from intrinsic
endothelial cells and monocytes can induce HO-1 gene expression. Both transcriptional and posttranscriptional mechanisms have been implicated in this action (5, 46). To lessen the extent of NO-induced injury, cells can rapidly recruit HO-1 and defend against NO-mediated oxidative injury as a negative feedback regulation mechanism. Our results indicate that the negative modulation of iNOS possibly occurs at transcription and enzyme activity levels.

Experimental evidence has demonstrated that both biliverdin and bilirubin possess antimcomplement effects and inhibit the cytotoxic activity of human T lymphocytes (10, 26). CO generated from HO-1 expression inhibits the release of proinflammatory cytokines, such as tumor necrosis factor-α and interleukin-1β, and increases the release of anti-inflammatory cytokines, such as cyclooxygenase, could also be modulated by HO activity. Thus modulation of HO-1 activity may represent a therapeutic strategy to ameliorate damages in IBD. However, it would be prudent to evaluate the beneficial effect of HO-1.

In conclusion, our data demonstrate that HO-1 expression is a self-defense mechanism against experimental colitis induced by TNBS. The increase of heme degradation by HO-1 induction could scavenge ROS products and decrease NO production by inhibiting iNOS expression and activity.

REFERENCES


