NK-1 antagonist reduces colonic inflammation and oxidative stress in dextran sulfate-induced colitis in rats

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NK-1 antagonist reduces colonic inflammation and oxidative stress in dextran sulfate-induced colitis in rats. Am J Physiol Gastrointest Liver Physiol 279: G1298–G1306, 2000.—Although substance P (SP) has been implicated as a mediator of neurogenic inflammation in the small intestine, little information is available regarding the role of SP in the pathogenesis of chronic ulcerative colitis. In this study, our aim was to investigate whether the intraperitoneal administration of a nonpeptide neurokinin-1 (NK-1) antagonist, CP-96345, which antagonizes the binding of SP to its NK-1 receptor, results in the attenuation of colonic inflammation induced in rats by 5% dextran sodium sulfate (DSS) in drinking water for 10 days compared with an inactive enantiomer, CP-96344. Disease activity was assessed daily for 10 days, after which colonic tissue damage was scored and myeloperoxidase activity and colon and urinary 8-isoprostanes were measured. Animals receiving DSS exhibited marked physical signs of colitis by day 5 compared with controls. Chronic administration of the NK-1 antagonist significantly reduced the disease activity index, mucosal myeloperoxidase activity, colonic tissue damage score, and mucosal and urinary levels of 8-isoprostanes compared with inactive enantiomer-injected (saline) animals receiving DSS alone. These data indicate that the administration of an NK-1 antagonist can attenuate colonic inflammation and oxidative stress and suggest a novel therapeutic approach in the treatment of chronic ulcerative colitis.

ulcerative colitis; substance P; neurokinin-1 receptors; oxidative stress; 8-isoprostane

Chronic Ulcerative Colitis (CUC) is an inflammatory bowel disease (IBD) characterized by recurrent episodes of colonic inflammation and tissue degeneration. Although its etiology remains unclear, recent evidence suggests that the overexpression of certain neuropeptides and their receptors may play a significant role in initiating and modulating the inflammation associated with CUC (37). In particular, considerable evidence supports a role for substance P (SP) (25, 33), an 11-amino acid peptide member of the tachykinin family (7). Substance P and its receptor, the neurokinin-1 (NK-1) receptor, are widely distributed throughout the gastrointestinal tract, and several lines of experimentation provide evidence that SP plays a role in regulating inflammatory and immunomodulatory responses in CUC. CUC is associated with significant increases in both colonic mucosal SP concentration (49) and NK-1 receptor expression in colonic submucosal lymph nodes and small blood vessels (24). In addition, the number of SP-immunoreactive nerve fibers in the lamina propria and the number of longitudinal muscle layers are markedly increased in colon sections obtained from patients with CUC compared with controls (17). Watanabe et al. (46) further noted that the increased SP fibers were thickened, with significantly greater linear density.

Although the aforementioned studies strongly implicate SP and its receptor in the pathophysiology of CUC and raise the possibility that NK-1 antagonists may be of therapeutic importance (23), there are only a few reports in animal models using NK-1 antagonists to elucidate mechanisms of colonic inflammation (13, 14, 26, 45). These studies used the trinitrobenzene sulfonic acid (TNBS) model to induce colitis, and they showed conflicting results. Although McCafferty et al. (26) and DiSebastiani et al. (13) demonstrated that pretreatment with an NK-1 antagonist was efficacious in reducing TNBS-induced colitis, Evangelista et al. (14) and Wallace et al. (45) failed to show a beneficial effect. Clearly, this question merits further investigation.

Because our focus was to investigate the role of SP in the pathogenesis of CUC, we chose to study the efficacy of an NK-1 antagonist in dextran sulfate sodium (DSS)-induced colitis in rats, a model not previously used to study the association of SP with CUC. Unlike the TNBS model, which induces a transmural lesion with pathological characteristics similar to Crohn’s disease, the DSS model, which induces TNBS-induced colitis, and they showed conflicting results. Although McCafferty et al. (26) and DiSebastiani et al. (13) demonstrated that pretreatment with an NK-1 antagonist was efficacious in reducing TNBS-induced colitis, Evangelista et al. (14) and Wallace et al. (45) failed to show a beneficial effect. Clearly, this question merits further investigation.

Because our focus was to investigate the role of SP in the pathogenesis of CUC, we chose to study the efficacy of an NK-1 antagonist in dextran sulfate sodium (DSS)-induced colitis in rats, a model not previously used to study the association of SP with CUC. Unlike the TNBS model, which induces a transmural lesion with pathological characteristics similar to Crohn’s disease, the DSS model produces inflammation limited to the colonic mucosa that is more closely related to CUC in humans (15). DSS-induced colitis is further characterized by a significant mucosal infiltration of neutrophils (10).

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Although the pathogenic mechanisms underlying CUC are not fully understood, the mucosal damage is thought to be mediated, in part, by neutrophil-derived oxidants (8, 48). Ample clinical and experimental data implicate oxidant-induced damage or oxidative stress in the pathogenesis of CUC (1, 22). Although the production of neutrophil-derived oxidants has been demonstrated, direct biochemical evidence of oxidative damage at the cellular level is lacking. In this study, we examined the levels of colonic and urinary 8-isoprostanate (8-IP), a specific cellular membrane lipid per-oxidation by-product that is a reliable marker of oxidative stress in vivo (20). This group of PG-like compounds, similar in structure to PGF$_{2\alpha}$, also known as 8-epi-PGF$_{2\alpha}$ or F$_2$ isoprostanes, is produced in vivo by a non-cyclooxygenase, free radical-induced lipid peroxidation of membrane-bound arachidonic acid. Although elevated levels of 8-IP were detected in tissues and urine from animals and humans with conditions associated with oxidative stress (11), the presence of these compounds has not, as yet, been explored in association with CUC.

The primary goal of this study was to explore the effects of a nonpeptide NK-1 antagonist, CP-96345, on disease activity as well as established markers of neutrophil infiltration, inflammation, and oxidative stress in the colonic mucosa of rats with DSS-induced colitis. Our results demonstrate that the administration of an NK-1 antagonist reduced colonic inflammation, oxidative stress, and tissue damage in rats with DSS-induced colitis and thus emphasize an important role for SP and its interaction with the NK-1 receptor in mediating colonic inflammation. This study further supports the possibility that NK-1 antagonists may be of therapeutic importance in CUC.

**MATERIALS AND METHODS**

Materials. All chemicals were obtained from Sigma Chemical (St. Louis, MO) unless otherwise noted. The NK-1 antagonist (2S,3S)-cis-2-(diphenylmethyl)-N-[2-(methoxyphenyl)methyl]-1-azabicyclo[2.2.2]octan-3-amine (CP-96345) and its 2R,3R-inactive enantiomer, CP-96344, which does not displace the binding of SP to its receptor, were from Pfizer Central Research (Groton, CT). The doses of CP-96345 and CP-96344 were selected on the basis of previously published dose-response studies (29, 34) showing that 2.5 mg/kg was efficacious in attenuating SP-mediated inflammation. This dose was also recommended by the manufacturer and was compatible with levels previously shown to selectively inhibit SP binding to the NK-1 receptor in vivo (42).

Animals and experimental design. Male Sprague-Dawley rats weighing between 250 and 300 g (Charles River Labs, Wilmington, MA) were housed individually in metabolic cages at a constant room temperature with 12-h light and dark cycles and fed standard rodent chow (Purina no. 5001) and water ad libitum. After a 7-day acclimation period in metabolic cages, rats were weighed and randomized into experimental groups of six. In each series of experiments, four groups of six animals each were used as follows: controls received no NK-1 antagonist, experimental controls that received 5% DSS alone, and two groups that received 5% DSS in conjunction with either the active NK-1 antagonist CP-96345 or its inactive enantiomer CP-96344. All animals were administered the NK-1 antagonist received a dose of 2.5 mg/kg intraperitoneally twice daily, beginning concurrently with DSS administration. All controls and experimental controls received equivalent volumes of vehicle (saline) injected in the same manner as the NK-1 antagonist groups for the duration of the study. DSS was administered via drinking water ad libitum. All experiments were repeated at least twice in all groups and in some instances up to four times to assess the effects of the active CP-96345 compared with its inactive enantiomer CP-96344. The specific nonpeptide NK-1 antagonist CP-96345 has nonspecific activity on calcium channels (9), whereas the inactive enantiomer has no activity on displacing SP binding from its NK-1 receptor but still has nonspecific calcium channel activity (42). Hence, any differences observed between the experimental groups receiving CP-96345 compared with CP-96344 can presumably be attributed to the direct inhibition of the binding of SP.

After 10 days of 5% DSS and NK-1 antagonist (active or inactive) administration, animals were killed by CO$_2$ asphyxiation and their colons were immediately removed and rinsed with ice-cold phosphate-buffered saline. The excised colons were placed on ice and opened longitudinally, and mucosal samples were collected by scraping the surface with a microscope slide. Mucosal samples were aliquoted into cryovials, frozen immediately in liquid nitrogen, and stored at −70°C for further analysis of myeloperoxidase (MPO) activity and 8-IP and SP concentrations as described in MPO activity and 8-IP and SP concentrations as described in MPO activity and Mucosal and urinary 8-IP concentrations. At least two cross sections of the distal colon were also removed for histological processing and scoring as described in Induction and assessment of colitis. This study was approved by the Institutional Animal Care and Use Committee at the Boston University School of Medicine, and all procedures described were performed in accordance with recommendations outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

**Induction and assessment of colitis.** Colitis was induced by replacing normal drinking water with distilled water containing 5% DSS (wt/vol), prepared daily, mol wt 30,000–40,000; ICN Biomedicals, Costa Mesa, CA) for 10 days as indicated. Animal weights, the presence of occult (detected by Hemoccult strips; Smith Kline Diagnostics, San Jose, CA) or gross blood in the feces, and stool consistency were recorded daily for each animal by two unblinded observers. These parameters were each assigned a score based on the criteria shown in Table 1, which was used to calculate an averaged daily disease activity index (DAI) for each animal as previously described (10). The DAI has been shown to correlate well with the colon tissue damage score and with more specific measures of inflammation such as MPO activity (10). In addition, food and water consumption was recorded daily throughout the duration of each study.

<table>
<thead>
<tr>
<th>Score</th>
<th>Weight Loss</th>
<th>Stool Consistency</th>
<th>Occult/Gross Rectal Bleeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>None</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>1</td>
<td>1–5%</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>2</td>
<td>5–10%</td>
<td>Loose stools</td>
<td>Hemoccult +</td>
</tr>
<tr>
<td>3</td>
<td>10–20%</td>
<td>Diarrhea</td>
<td>Gross bleeding</td>
</tr>
<tr>
<td>4</td>
<td>&gt;20%</td>
<td>Diarrhea</td>
<td>Gross bleeding</td>
</tr>
</tbody>
</table>

The disease activity index is the combined scores of weight loss, stool consistency, and bleeding divided by 3. Adapted from Cooper et al. (10).
The extent of tissue damage was determined by assigning a numerical score to colon tissue sections obtained from each animal. After the removal of the colon, at least two cross-sections from the distal diseased area were immediately fixed in 10% neutral-buffered formalin and embedded in paraffin for histological analysis. Full-thickness sections were stained with hematoxylin and eosin and graded by a pathologist (M. O’Brien) blinded to the experimental groups according to the criteria shown in Table 2.

**MPO activity.** MPO activity has been widely accepted as an enzyme marker to quantify the degree of inflammation and estimate the accumulation of neutrophils in tissues (5). Hence, colonic mucosa was assayed in duplicate for MPO activity as described by Barone et al. (2) with the following modifications. Approximately 30–50 mg of freshly thawed mucosa was homogenized on ice with a Polytron tissue homogenizer in 4 ml of ice-cold 5 mM phosphate buffer (pH 6.0). The homogenate was centrifuged at 30,000 g for 30 min at 4°C. The resulting supernatant was discarded, and the pellet was resuspended in phosphate buffer, rehomogenized, and recentrifuged as above. This wash procedure was repeated three times to remove hemoglobin and other blood products that have been shown to markedly affect the spectrophotometric assay of MPO in leukocyte-containing tissues (47). The thrice-washed pellet was then resolubilized in 10 vol of ice-cold 0.5% hexadecyltrimethylammonium bromide in 50 mM phosphate buffer and sonified on ice at full power for 5–10 s to solubilize the enzyme. Sonification was repeated four times with 30-s cooling on ice between bursts. The sonicated extract was allowed to stand at 4°C for 20 min and then centrifuged at 12,500 g for 15 min at 4°C. MPO activity in the supernatant was then assayed by mixing 0.1 ml of the supernatant with 2.9 ml of 50 mM phosphate buffer, pH 6.0, containing 0.167 mg/ml of d-riboflavine dihydrochloride and 0.005% hydrogen peroxide. The change in absorbance at 460 nm was measured spectrophotometrically over 3 min, and one unit of MPO activity was defined as that degrading 1 µmol of hydrogen peroxide per minute.

**Mucosal and urinary 8-IP concentrations.** Mucosal and urinary concentrations of 8-IP were used as indexes or markers of lipid peroxidation and oxidative stress (20). Total 8-IP concentrations were determined in colonic mucosa and urine with a commercially available enzyme immunoassay (EIA) kit (catalog no. 516351; Cayman Chemical, Ann Arbor, MI) essentially according to the manufacturer’s specifications with the following modifications. Approximately 30–50 mg of freshly thawed mucosa were added to 10 vol of chloroform-methanol (2:1, vol/vol) containing 0.005% butylated hydroxytoluene and placed in a Teflon-capped glass tube and sealed under argon. The sample was vortexed vigorously for 1 min and then placed on a lab rocker at 30 rpm overnight at room temperature. The extract was then filtered through glass wool that was rinsed twice with chloroform-methanol (2:1, vol/vol) and pooled with the original extract. The entire extract, containing both free and esterified 8-IP, was evaporated to dryness under a stream of nitrogen and resuspended in 1.0 ml of HPLC-grade methanol by vortexing. The sample was then saponified by the addition of 1.0 ml of 15% potassium hydroxide and incubated at 40°C for 60 min. After the adjustment of the pH to 2.0 with HCl, the sample was purified on a preconditioned reverse-phase C18 column (Extract-Clean RC Octadecylsilyl C18 column; Alltech Associates, Deerfield, IL) according to the manufacturer’s specifications using a 12-sample Visiprep manifold vacuum chamber (Supelco, Bellefonte, PA). The 8-IP was eluted off the column with HPLC-grade ethyl acetate containing 1% HPLC-grade methanol and collected into glass tubes. Each sample was passed over the C18 column twice to ensure adequate recovery with minimal interfering contamination. Pooled eluates were evaporated to dryness under a stream of nitrogen, reconstituted in EIA buffer, and assayed according to the manufacturer’s recommendations.

Urinary 8-IP concentrations were determined after an overnight collection in metabolic cages. Animals were fasted, and fine screens were inserted into the cages during the collection period to eliminate contamination. One milliliter of urine, acidified to pH 3.0 with HCl, was centrifuged at 400 g for 10 min. The supernatant was purified on a reverse-phase C18 column preconditioned with methanol and acidified ultrapure water (pH 3.0) as above with the following exceptions. After elution of the sample with 10 ml of ethyl acetate-hexane (1:1, vol/vol), 2.5 g of sodium sulfate was added to the eluate. The supernatant was decanted and placed onto a silica cartridge (Alltech Associates) preconditioned with methanol and ethyl acetate. The sample was then eluted with 5 ml of ethyl acetate-methanol (1:1 vol/vol), evaporated to dryness under a stream of nitrogen, reconstituted in 1.0 ml of EIA buffer, and assayed according to the manufacturer’s specifications. Urinary 8-IP levels were normalized to urine creatinine concentrations, which were measured using a Sigma creatinine kit (no. 555A; Sigma Diagnostics, St. Louis, MO).

**Mucosal SP concentrations.** Mucosa was obtained and stored as described in Animals and experimental design. SP was extracted from mucosal samples as described by Nilsson et al. (32). SP concentrations were determined in colonic mucosa with a commercially available EIA kit (catalog no. 583751, Cayman Chemical) essentially according to the manufacturer’s specifications.

**Statistics.** Group differences were determined by ANOVA followed by a post hoc Tukey’s multiple mean separation test. All data are expressed as means ± SE.

**RESULTS**

Disease activity was reduced by CP-96345. Oral administration of 5% DSS to rats over a 10-day period induced a predictable course of colitis that has been

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**Table 2. Scoring criteria of full-thickness distal colon sections**

<table>
<thead>
<tr>
<th>Mucosal epithelium</th>
<th>Neutrophil infiltrate</th>
<th>Lymphoid infiltrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crypts</td>
<td></td>
<td>Crypt Mitotic Activity</td>
</tr>
<tr>
<td>Lower third (0); mild mid third (1); Moderate mid third (2); upper third (3)</td>
<td>Crypt epithelium—neutrophil infiltrate</td>
<td>Mucus depletion</td>
</tr>
<tr>
<td>Stroma</td>
<td>Plasmacytoid infiltrate</td>
<td>Neutrophil infiltrate</td>
</tr>
<tr>
<td>Vascularity</td>
<td>Ulceration</td>
<td>Ulceration</td>
</tr>
<tr>
<td>Fibrin deposition</td>
<td>None (0); mucosal (1); submucosal (2); transmural (3)</td>
<td>None (0); mild surface (1); moderate (2); extensive—full thickness (3)</td>
</tr>
<tr>
<td>Submucosal neutrophils</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Submucosal edema</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Scoring scale: 0, none; 1, mild; 2, moderate; 3, severe; maximum score = 36.
well documented in the literature (see Ref. 15). Initially, the animals receiving either DSS alone or DSS plus the inactive enantiomer CP-96344 developed loose stools that were eventually accompanied by the presence of occult blood after 3 or 4 days. By 5 or 6 days, gross rectal bleeding ensued that was associated with a 15% reduction in food intake from baseline and a failure to gain weight at a rate similar to controls. By 8 or 9 days, gross rectal bleeding was detected in all animals receiving 5% DSS or 5% DSS plus the inactive enantiomer. In these two groups, food consumption was further reduced to 24% of baseline and weight loss averaged 4% from baseline. There were no significant differences in water consumption among all the groups over the 10 days.

The calculation of a disease activity index (DAI) based on data collected by two unblinded observers indicated that those animals receiving either 5% DSS or 5% DSS plus the inactive enantiomer developed significant physical signs associated with DSS-induced colitis compared with either animals receiving 5% DSS plus CP-96345 or controls (Fig. 1). In contrast, the animals receiving 5% DSS plus CP-96345 had a significantly attenuated response on days 8, 9, and 10 compared with animals receiving 5% DSS alone or 5% DSS plus CP-96344. In separate experiments, the administration of either CP-96345 (n = 6 animals) or CP-96344 (n = 6 animals) alone over a 10-day period showed no changes in the DAI (data not shown). These two groups were not included in subsequent experiments because they behaved like controls.

**Colonic infiltration of neutrophils was reduced by CP-96345.** One of the hallmarks of DSS-induced colitis is the marked infiltration of neutrophils into the mucosa (15), whose content can be readily estimated by measuring tissue MPO activity (5). In parallel with the increased number of neutrophils infiltrating the colonic mucosa and lamina propria, the oral administration of 5% DSS alone or DSS plus the inactive enantiomer over a 10-day period produced a significant elevation in mucosal MPO activity compared with control untreated animals (Fig. 2). Although the administration of 5% DSS plus the active antagonist CP-96345 resulted in a 64% reduction (P < 0.05) in MPO activity compared with 5% DSS alone, MPO levels were significantly higher than those in controls (Fig. 2). MPO activity was also significantly elevated in the animals receiving 5% DSS plus CP-96344 compared with either the 5% DSS plus CP-96345 or control groups.

**Tissue damage was attenuated by CP-96345.** Neutrophils and other inflammatory cells have been shown to initiate mucosal damage by releasing a host of reactive oxygen metabolites and oxygen free radicals (48). As indicated by the significantly elevated MPO activities, the inflammatory response induced by 5% DSS was characterized by a diffuse neutrophil infiltration into the colonic mucosa. The photomicrographs shown in Fig. 3 represent full-thickness rat colon sections. Figure 3A shows representative colonic mucosa from a control rat. The crypts show a normal architecture and goblet cell component. There are no inflammatory changes present. Colonic mucosa from rats receiving CP-96344 and CP-96345 alone without 5% DSS were similar (not shown). Figure 3B represents colonic mucosa from a rat administered 5% DSS for 10 days. Mucosal height was significantly reduced compared with that in controls. Full-thickness mucosal ulceration was evident. The granulation replacing the mucosa was heavily infiltrated primarily with neutrophils as well as other inflammatory cells, which were also numerous within the submucosa. The neutrophil infiltration undoubtedly accounted for the increased MPO activity observed in this group. Figure 3C shows colonic mucosa from a rat consuming 5% DSS and the active NK-1 receptor antagonist CP-96345 for 10 days. The crypts show a nearly normal architecture and
goblet cell component compared with rats consuming 5% DSS. Although some neutrophils are dispersed within the mucosa, accompanied by a minor reduction in mucosal height, no significant inflammatory changes are evident in the majority of the sections scored. Figure 3D shows representative colonic mucosa from a rat consuming 5% DSS and the inactive enantiomer for 10 days. Mucosal height is significantly reduced compared with controls as is full-thickness mucosal ulceration. The necrotic mucosa was heavily infiltrated with neutrophils, which again accounts for the elevated MPO activity in this group.

Tissue damage scores of the photomicrographs graded according to the criteria in Table 2 are shown in Fig. 4. Consistent with their marked histopathology and dense neutrophil infiltrate, animals consuming 5% DSS had significantly elevated damage scores compared with controls. Although the administration of CP-96345 with 5% DSS reduced the score of the tissue damage by nearly 50% \( (P < 0.05) \) compared with animals consuming 5% DSS alone, the scores in this group were significantly higher than controls (Fig. 4). Tissue damage scores were also significantly elevated in the animals receiving 5% DSS plus the inactive enantiomer compared with either the 5% DSS plus CP-96345 or control groups.

Oxidative stress was reduced by CP-96345. As mentioned above, non-cyclooxygenase-derived tissue and urinary 8-IPs have been shown to be reliable markers of free radical-induced lipid peroxidation and oxidative stress in vivo (20). Consistent with the marked mucosal infiltration of neutrophils and other oxidant-producing inflammatory cells, animals consuming 5% DSS had significantly elevated mucosal 8-IP levels compared with controls (Fig. 5, top). The administration of CP-96345 with 5% DSS reduced the mucosal levels of 8-IP by >85% \( (P < 0.05) \) compared with animals consuming 5% DSS alone. Animals receiving 5% DSS plus the inactive enantiomer also had significantly elevated mucosal levels of 8-IP compared with either the 5% DSS plus CP-96345 or control groups (Fig. 5, top).

Once cleaved from phospholipids, tissue-derived 8-IPs are thought to be cleared by the kidney and excreted in the urine (20). Hence, measuring urinary 8-IP levels may represent a noninvasive whole body assessment of lipid peroxidation in diseases such as ulcerative colitis in which oxidative stress is thought to contribute significantly to the pathology (20). Consistent with the marked elevation of mucosal 8-IP, animals consuming 5% DSS had a threefold increase (\( P <

![Figure 3](image-url)

**Fig. 3.** Representative photomicrographs of full-thickness rat colon sections fixed in 10% neutral-buffered formalin, paraffin embedded, and stained with hematoxylin and eosin from control (A), 5% DSS alone (B), 5% DSS plus the active antagonist CP-96345 (C), and 5% DSS plus the inactive enantiomer CP-96344 (D) rats. Magnification: \( \times 150 \).
0.05) in urinary 8-IP levels compared with controls (Fig. 5, bottom). The administration of CP-96345 with 5% DSS reduced urinary 8-IP excretion by 65% (P < 0.05) compared with animals consuming 5% DSS alone. Animals receiving 5% DSS plus the inactive enantiomer also had significantly elevated urinary levels of 8-IP compared with either the 5% DSS plus CP-96345 or control groups (Fig. 5, bottom).

Mucosal SP concentrations were elevated by DSS. In control rats (n = 6), the mucosal level of SP was 0.048 ± 0.01 pmol/µg protein. Treatment with 5% DSS (n = 6) resulted in a significant elevation (P < 0.05) in this level to 0.084 ± 0.01 pmol/µg protein.

Markers of oxidative stress correlate with colon inflammation and disease activity. Pearson correlations derived by pooling data from all the experiments described here as well as additional data on MPO and disease activity from preliminary studies show that colon inflammation, as assessed by MPO activity, correlates significantly with both the tissue damage score (r = 0.75; P = 0.000001) and DAI (r = 0.80; P = 0.000001) as well as with tissue 8-IP levels (r = 0.70; P = 0.000001). The tissue damage score was also significantly related to tissue (r = 0.69; P = 0.00001) and urinary (r = 0.37; P = 0.013) 8-IP as well as the DAI (r = 0.76; P = 0.000001). Tissue 8-IP was significantly related to both urinary 8-IP (r = 0.41; P = 0.008) and the DAI (r = 0.79; P = 0.000001), and urinary 8-IP was also significantly related to the DAI (r = 0.42; P = 0.0012).

DISCUSSION

In these studies, we used the specific NK-1 receptor antagonist CP-96345 and its inactive enantiomer, CP-96344, to test whether the chronic administration of an NK-1 antagonist would attenuate an inflammatory response in the colon. The studies reported here represent new findings in a model not previously used to study the association of SP with CUC. The rat model of DSS-induced colitis was used because it not only is a well-characterized model with a predictable disease progression but also has numerous clinical, biochemical, and histological features that closely recapitulate CUC in humans (10, 15). The major finding of this study is that a specific nonpeptide NK-1 receptor antagonist, CP-96345, can attenuate the progression of DSS-induced colitis when administered prophylactically and continued over the course of a 10-day study. Even before any biochemical or histological data were obtained, it could be observed that the animals receiving the active NK-1 antagonist had significantly reduced “clinical signs” of colitis as determined by the disease activity index (DAI) (Fig. 1). The significant correlation between the DAI and other more quantitative markers of disease activity such as tissue MPO activity (r = 0.80; P = 0.000001) and tissue damage score (r = 0.76; P = 0.000001) further underscores this observation. In addition, these studies demonstrated that tissue and urinary levels of 8-IP, a unique marker of oxidative stress not previously associated with colitis, are reduced by administration of an NK-1 antagonist.

The increased mucosal MPO activity in the animals receiving either 5% DSS or 5% DSS plus the inactive enantiomer indicates that the substantial neutrophil infiltration that occurs in response to DSS (Figs. 2 and 3, B and D, respectively) is significantly reduced by the administration of the active NK-1 antagonist (Figs. 2 and 3C). There is ample experimental and clinical evidence to suggest that the inflamed colon undergoes substantial oxidative stress by neutrophil-derived oxidants, which represent a significant mechanism for tissue damage during chronic intestinal inflammation (8, 48). These highly reactive oxidants can directly damage or alter the function of essential cellular components such as lipids, DNA (48), and key protein thiols responsible for maintaining cellular redox state in colonic epithelial cells (28). The redox-sensitive transcriptional factor nuclear factor-κB (NF-κB) is upregulated in IBD (38), as are other redox-sensitive genes (8), which undoubtedly facilitates the production of proinflammatory cytokines and chemokines such as interleukin-8 (IL-8) (41) that are associated with colonic inflammation (18). Additional reports also
showed that the specific NK-1 antagonist CP-96345 similarly reduces the acute inflammatory response in rat ileal loops exposed to Clostridium difficile toxin A (34) and that reactive oxygen metabolites released primarily from neutrophils invading the inflamed ileum contribute to the pathophysiology (35), again suggesting the involvement of an NK-1 receptor-dependent mechanism. The significant correlation between tissue MPO and 8-IP levels (r = 0.70; P = 0.000001) suggests that neutrophils invading the inflamed tissue are associated with increased oxidative stress. Furthermore, the reduction in both colon mucosal (Fig. 5, top) and urinary (Fig. 5, bottom) 8-IP levels in rats receiving the active NK-1 antagonist provides additional evidence that oxidative stress has been reduced by an NK-1 receptor-dependent pathway.

Neutrophil-derived MPO may further contribute to tissue damage, in and of itself, by virtue of its ability to oxidize, chlorinate, and nitrosylate proteins in the presence of neutrophil-derived oxidants and nitric oxide (16). The production of reactive oxygen metabolites and MPO by the excess infiltration of neutrophils, in concert with a reduction in colonic antioxidants and antioxidant defenses in the inflamed colon (6, 22), underscores the potential for the extensive neutrophil-mediated tissue damage shown in Fig. 3, B and D. Because there is enhanced binding of neutrophils to intestinal microvascular endothelium in patients with IBD (4) and SP has been shown to increase the expression of both vascular cell adhesion molecule-1 (VCAM-1) (36) and intracellular adhesion molecule-1 (ICAM-1) (30) in endothelial cells, it appears that the significant reduction in neutrophil-induced tissue damage in the animals receiving the active NK-1 antagonist (Fig. 2) was the direct result of the inhibition of neutrophil extravasation by a NK-1 receptor-dependent mechanism. Interestingly, another DSS-induced colitis study in which the extravasation of neutrophils into the colon was blocked with a specific ICAM-1 antisense oligonucleotide showed similar reductions in tissue damage (3), further demonstrating the destructive nature of neutrophils.

Unlike other less-specific markers of lipid peroxidation in vivo such as malondialdehyde or 4-hydroxynonenal, whose pathobiological effects are unclear (11), the production of 8-IP may have potential pathophysiological implications in mediating oxidative stress. 8-IP is a potent vasoconstrictor (39) and may exacerbate oxidative stress in the inflamed colon by inducing mucosal ischemia. More recently, 8-IP has been shown to enhance neutrophil adhesion to endothelial cells (50), further facilitating the extravasation of neutrophils. Although the production of colonic 8-IP does not appear to be through a direct NK-1-dependent mechanism, its effects on neutrophil adhesion to the endothelium may augment the actions of SP during colonic inflammation. Hence, not only is 8-IP a by-product of oxidative stress, but it appears that this bioactive lipid itself may have potential pathophysiological relevance in IBD.

Because cell surface NK-1 receptors are G protein coupled and increase intracellular levels of both inositol trisphosphate and diacylglycerol after the binding of SP (19), there are a host of diverse intracellular signal transduction pathways that may be impinged by the antagonism of the NK-1 receptors. SP, like oxidative stress, also induces the activation of NF-κB and plays a role in regulating the synthesis and activity of various proinflammatory mediators, cell surface receptors, transcription factors, and adhesion molecules that are implicated with the pathogenesis of ulcerative colitis. SP-induced NF-κB activation also has been associated with an increased mRNA expression and secretion of IL-8 (21). The stimulatory effect of SP was specific, because a selective NK-1 receptor antagonist completely prevented NF-κB activation (21). In addition, the activation of NF-κB in patients with active IBD (38) suggests that a NK-1 receptor-dependent pathway may be involved in the regulation of NF-κB activity in chronic colonic inflammation.

Recent studies have shown that both acute and chronic inflammation in the colon are associated with a significant increase in mucosal SP concentrations (44, 49). In the present study, we also demonstrated that rats receiving 5% DSS had significantly elevated levels of colon mucosal SP concentrations compared with controls. Interestingly, the overexpression of SP in the colon may potentiate the inflammatory response by several independent mechanisms. Recent studies by Sterner-Kock et al. (43) showed that SP stimulates the production of reactive oxygen species and nitric oxide in human neutrophils, thereby exacerbating the tissue-damaging potential of invading neutrophils. In addition, SP has been shown to directly stimulate and perpetuate the synthesis and secretion of IL-8 from neutrophils, further facilitating neutrophil recruitment into inflamed tissues (40). Treatment of animals with an NK-1 antagonist has also been shown to down-regulate the tumor necrosis factor-α response as well, further implicating SP in contributing to the induction of other proinflammatory cytokines also associated with the pathogenesis of colonic inflammation (12).

The overexpression of SP not only facilitates the recruitment, extravasation, and transendothelial migration of neutrophils by the upregulation of endothelial adhesion molecules (30, 36) but also increases both plasma leakage in postcapillary and collecting venules (27) and paracellular permeability (31), suggesting a role for SP in the maintenance of endothelial gap junction integrity. These studies emphasize the importance of SP in modulating microenvironmental vascular changes in inflamed colon including endothelial cell permeability and activation and may again explain, in part, the significant reduction in neutrophils and other inflammatory cells in the presence of the active NK-1 antagonist (Fig. 3C).

There is abundant evidence to suggest that SP is a mediator of neurogenic inflammation in the inflamed colon (17, 24, 44, 46, 49). Although our studies demonstrate that an NK-1 receptor antagonist was efficacious in attenuating DSS-induced colitis and suggest that an NK-1 receptor-dependent pathway may participate at key points in the inflammatory response, the deline-
tion of the intracellular pathways responsible for the actions of NK-1 antagonists in preventing the inflammatory response requires further investigation. Hence, it appears that the antagonism of SP activity may play a significant role in reducing colonic inflammation and oxidative stress and, more importantly, raises the possibility that NK-1 antagonists may be of therapeutic importance in chronic intestinal inflammation.

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REFERENCES


