Reduction of acute and reactivated colitis in rats by an inhibitor of neutrophil activation

JOHN L. WALLACE,1 WEBB MCKNIGHT,1 SAMUEL ASFAHA,1 AND DAVID Y. LIU2

1Intestinal Disease Research Unit, University of Calgary, Calgary, Alberta, Canada T2N 4N1; and 2Scios Inc, Sunnyvale, California 94086

Wallace, John L., Webb McKnight, Samuel Asfaha, and David Y. Liu. Reduction of acute and reactivated colitis in rats by an inhibitor of neutrophil activation. Am. J. Physiol. 274 (Gastrointest. Liver Physiol. 37): G802–G808, 1998.—Neutrophils have been implicated as major contributors to tissue injury in inflammatory bowel disease. In this study, we have assessed the effects of an inhibitor of neutrophil activation and adherence, NPC-18915 (4-[2-[(2-benzofuran-5-yl)[E]-ethenyl]benzoic acid sodium salt), in models of both acute and reactivated colitis. Acute colitis was induced by intracolonic administration of a hapten. In other rats, colitis was reactivated 6 wk after a bout of acute colitis by subcutaneous administration of the hapten. NPC-18915 given during the first 4 days after induction of acute colitis significantly reduced tissue injury and the incidence of diarrhea and adhesions. When treatment of NPC-18915 was initiated after colitis was firmly established (48 h posthapten), it did not produce a significant effect. NPC-18915 was effective at significantly reducing colonic injury and granulocyte infiltration in the reactivated colitis model, and a similar effect could be observed in rats treated with antineutrophil serum. These results demonstrate that an inhibitor of neutrophil activation is effective in both acute and reactivated colitis, although in the former case, effectiveness is only seen when the drug is given before full establishment of colitis. These results also suggest that neutrophils are a critical effector cell of hapten-induced colitis in the rat, particularly in the case of reactivated colitis.

INFLAMMATORY BOWEL DISEASE (IBD) is an umbrella term used to describe ulcerative colitis and Crohn’s disease. The etiology of these conditions is poorly understood, and consequently the therapeutic options available to patients are suboptimal. Although there are differences between the two subsets of IBD in terms of the regions of the gastrointestinal tract that are affected, a common feature is the clinical course of unpredictable relapse after periods of remission. Ulcerative colitis is characterized by inflammation of the mucosal layer of the colon, while Crohn’s disease is characterized by transmural inflammation and can occur anywhere in the gastrointestinal tract. In both conditions, neutrophils constitute one of the most prominent infiltrating cells and have been suggested to contribute significantly to the generation of tissue injury (3, 4, 9). Indeed, the anti-inflammatory effects of corticosteroids in the treatment of IBD have been ascribed, at least in part, to their ability to reduce neutrophil infiltration (2). On the other hand, 5-aminosalicylic acid, also widely used in the treatment of IBD, has been suggested to produce its beneficial effects by inhibiting the formation of and/or scavenging reactive oxygen metabolites from neutrophils (3, 8).

Given the important role of neutrophils in contributing to tissue injury in IBD, several experimental strategies have been used to reduce neutrophil infiltration into inflamed intestinal tissue. For example, immunoneutralization of the β2-integrin family (CD11/CD18) of adhesion molecules on neutrophils has been shown to reduce the severity of epithelial injury in a rabbit model of acute colitis (17). On the other hand, depletion of circulating neutrophils with an antineutrophil serum (ANS) had little effect on the severity of acute colitis induced by intracolonic acetic acid (21). Such approaches may be problematic in a clinical setting, however, since prolonged blockade of the ability of leukocytes to extravasate may render the patient susceptible to infection, and the patient may also develop antibodies directed against the injected reagent. This has led to the proposal that inhibitors of neutrophil activation might offer therapeutic advantages over the use of antibodies directed against neutrophils or their adhesion molecules. One such group of neutrophil activation inhibitors has recently been described. These compounds, referred to as “nactins” (an abbreviation of “neutrophil activation inhibitors”), are capable of suppressing CD11b/CD18-mediated neutrophil adhesion and of inducing the shedding of L-selectin, which results in a reduction in the ability of neutrophils to adhere to the vascular endothelium (4, 5). Interestingly, nactins appear to block the adhesion mediated by CD11b/CD18 without affecting the expression of this molecule on the cell surface. It has been suggested that nactins inhibit an unknown process that is permissive for CD11b/CD18-dependent adherence (5). In a previous study, we demonstrated (12) that a member of the nactin family inhibited the neutrophil adherence to mesenteric postcapillary venules induced by superfusion of these vessels with platelet-activating factor. The nactin produced this effect without altering rates of blood flow through the vessel.

In the present study, we have assessed the ability of a nactin, NPC-18915, to reduce the severity of colitis in an animal model. Colitis induced in rats by intracolonic administration of the hapten 2,4,6-trinitrobenzenesulfonic acid (TNBS) (13) has been widely used for assessing the effects of novel therapeutic compounds (7, 11, 16, 19). The model permits the testing of drugs in several different paradigms. In addition to testing the effects of drugs on the severity of colitis during its active stage, one can also assess the influence of a drug on reactivation of colitis once the initial injury has resolved (1). In the present study, the effects of NPC-18915 were assessed in both of these situations. For
comparison, the effects of depletion of circulating neutrophils with an ANS were also assessed.

**METHODS**

Animals. Male Wistar rats weighing 200–225 g were obtained from Charles River Breeding Farms (Montreal, QC, Canada) and were housed in plastic cages. The rats were fed standard pellet chow and water ad libitum. All experimental protocols were approved by the Animal Care Committee of the University of Calgary.

Effects of NPC-18915 on acute colitis. Colitis was induced by intracolonic instillation of the hapten TNBS (60 mg/ml) in 0.5 ml of 50% ethanol (13). The effects of NPC-18915 on severity of acute colitis were examined using two treatment paradigms. In the first protocol, NPC-18915 (0.2, 2, or 10 mg/kg) was administered intraperitoneally 1 h before and every 12 h for 4 days after induction of colitis. For the second protocol, NPC-18915 (10 mg/kg) was administered every 12 h for 4 days beginning 48 h after induction of colitis, a point at which inflammation of the colon is well established in this model (19). All rats, in a randomized order, were killed on day 10 after induction of colitis for assessment of the severity of colitis (see below). In each of these studies, each group consisted of 8–12 rats. For comparison, 5 untreated rats were killed at the same time and their colons were assessed, as described below, for damage and inflammation.

Effects of ANS in acute colitis. Groups of five rats each were given ANS intraperitoneally (2 ml of a 1:10 dilution of the serum) 24 and 2 h before induction of colitis, and then every morning for the first 4 days after induction of colitis. Controls were given the same volume of vehicle (sterile 0.9% saline) via the same route and at the same times. The rats were killed 1 or 10 days after TNBS administration for assessment of the severity of colitis, as described below. The dosing regimen for ANS was selected based on our previous findings (20), which demonstrated that ANS reduced circulating neutrophil numbers by >95%, while not significantly affecting the circulating numbers of macrophages, monocytes, lymphocytes, or eosinophils.

Effects of NPC-18915 on reactivated colitis. Colitis was induced in three groups of six rats each, as described above. Six weeks later, when the tissue injury associated with the initial colitis had healed and tissue granulocyte numbers had returned to normal levels (1), two groups of rats were given TNBS subcutaneously (10 mg/kg) every 12 h for 3 days (6 injections in total). The third group of rats received the vehicle (saline) subcutaneously in place of TNBS. Of the two groups receiving subcutaneous TNBS, one group received NPC-18915 intraperitoneally (10 mg/kg) 1 h before each of the 6 subcutaneous injections of TNBS, while the other group received the vehicle at the same times. The rats were killed 12 h after the final injection of TNBS for assessment of the severity of colitis (see below).

Effects of ANS on reactivated colitis. Colitis was induced in 15 rats by intracolonic administration of TNBS, as described above. Six weeks later, the rats were divided into three groups. In two of the groups, reactivation of colitis was induced through subcutaneous administration of TNBS, as described above. In the third group, the rats received subcutaneous saline in place of TNBS. One of the groups that received subcutaneous TNBS was given ANS (same dose as above) 24 and 2 h before the first injection of TNBS. Further injections of ANS were given 24 and 48 h after the first injection of TNBS. The other group of rats receiving subcutaneous TNBS was given the vehicle for ANS (saline) intraperitoneally at the same times. All rats were killed 12 h after the time of the final subcutaneous TNBS administration, and the severity of colitis was assessed.

Assessment of severity of colitis. The colon was excised and pinned out, mucosal side up, on a wax platform. An observer unaware of the treatments the rats had received assigned a score using criteria that have been outlined in detail previously (1). Briefly, the global score consisted of a score for severity and extent of ulceration (0 to 10), summed with scores for the absence or presence of diarrhea (0 or 1; diarrhea being defined as loose or watery stool) and adhesions (0, 1, or 2), and the maximum thickness of the wall of the colon (in mm). Tissues samples were then taken from a site of macroscopically detectable inflammation (or a corresponding site in tissues with no macroscopically detectable inflammation) for measurement of myeloperoxidase (MPO) activity, as an index of tissue granulocyte numbers. The assay for MPO activity has been described in detail previously (19). Other tissue samples were taken from adjacent sites, fixed in neutral buffered Formalin, and processed by routine techniques for histological evaluation. Slides were coded to prevent observer bias. The extent of tissue injury was scored on a 0 to 3 scale using the following criteria: 0, normal appearance (intact epithelium with no apparent infiltrate of granulocytes); 1, superficial epithelial damage; 2, damage involving the muscularis mucosae but not extending through the muscularis mucosae, and infiltration of granulocytes into the mucosa; and 3, transmural ulceration and granulocyte infiltration.

Effects of NPC-18915 on leukotriene B₄ production by neutrophils. To determine if NPC-18915 inhibited the production of an inflammatory mediator by neutrophils (as an index of neutrophil activation), the following experiment was performed. A group of four rats was used for the harvesting of peritoneal neutrophils. The neutrophils were harvested 4 h after injection of type Ii oyster glycogen (Sigma Chemical, St. Louis, MO) into the peritoneum, as described previously (15). The neutrophils from each rat were divided into eight tubes, containing either vehicle, nordihydroguaiaretic acid (NDGA) at 100 µg/ml, or NPC-18915 at 0.001, 0.01, 0.1, 1.5, 10, or 100 µg/ml. NDGA was included as a positive control, as it is an inhibitor of 5-lipoxygenase, the key enzyme in leukotriene B₄ (LTB₄) synthesis. Ten minutes later, calcium ionophore (A-23187; 10 µg) was added to each tube. After gentle mixing, the tubes were placed in a shaking water bath (37°C) for 30 min. The tubes were then removed, 100 µg of NDGA was added to stop leukotriene synthesis, and after gentle mixing the tubes were centrifuged at 9,000 g (1 min). The supernatants were frozen at –20°C until LTB₄ concentrations in the supernatants were measured using a specific ELISA.

Effects of NPC-18915 on circulating leukocytes. Groups of four rats each were treated with vehicle or NPC-18915 (10 mg/kg) intraperitoneally every 12 h for 3 days (total of 6 injections). One hour after the final injection, peripheral venous blood was collected, blood smears were prepared, and the slides were stained (modified Wright stain). An observer unaware of the treatments the rats had received performed differential counts to determine the number of neutrophils, monocytes, lymphocytes, eosinophils, and basophils.

Effects of NPC-18915 on colonic blood flow. The effects of NPC-18915 on colonic blood flow were assessed using laser-Doppler flowmetry. This method has been used extensively in our laboratory (6) to determine changes in gastrointestinal blood flow after administration of a test drug. Colitis was induced by intracolonic administration of TNBS, as described above, and 72 h later the rats were anesthetized with pentobarbital sodium (60 mg/kg ip) and a laparotomy was performed. A laser-Doppler pencil probe was placed on the serosal surface of the distal colon, and blood flow was...
Table 1. Effects of NPC-18915 and ANS on colonic MPO activity in acute colitis

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Treatment Days</th>
<th>MPO Activity (U/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>Days 0–4</td>
<td>65.2 ± 9.6</td>
</tr>
<tr>
<td>NPC-18915 (0.2 mg/kg)</td>
<td>Days 0–4</td>
<td>69.8 ± 6.0</td>
</tr>
<tr>
<td>NPC-18915 (2 mg/kg)</td>
<td>Days 0–4</td>
<td>54.2 ± 12.1</td>
</tr>
<tr>
<td>NPC-18915 (10 mg/kg)</td>
<td>Days 0–4</td>
<td>64.7 ± 13.7</td>
</tr>
<tr>
<td>Vehicle</td>
<td>Days 2–6</td>
<td>68.8 ± 7.6</td>
</tr>
<tr>
<td>NPC-18915 (10 mg/kg)</td>
<td>Days 2–6</td>
<td>45.8 ± 11.4</td>
</tr>
<tr>
<td>Vehicle</td>
<td>Days 0–4</td>
<td>72.3 ± 10.0</td>
</tr>
<tr>
<td>ANS</td>
<td>Days 0–4</td>
<td>69.3 ± 12.6</td>
</tr>
</tbody>
</table>

Values are means ± SE; each group consisted of 5–12 rats. All rats were killed on day 10 after induction of colitis. In rats in which colitis was not induced (untreated), the mean colonic myeloperoxidase (MPO) activity was 2.2 ± 0.5 U/mg. ANS, antineutrophil serum.

Recorded for 15 min. An intraperitoneal injection of either NPC-18915 (10 mg/kg) or vehicle (n = 4/group) was then performed, and blood flow was measured for a further 90 min. Changes in blood flow after this injection were expressed as a percentage of the basal flow in each rat.

Statistical analysis. All data are expressed as means ± SE. Groups of data were compared using a one-way ANOVA followed by a Student-Newman-Keuls test (damage scores). The incidence of diarrhea and adhesions was compared across groups using Fisher’s exact test. With all analyses, an associated P value of <0.05 was considered significant.

Materials. TNBS was obtained from Fluka Chemica (Buchs, Switzerland). ANS was purchased from Caymen Chemical (Ann Arbor, MI). NDGA was obtained from VWR Scientific (Edmonton, AB, Canada).

RESULTS

Effects of NPC-18915 in acute colitis. As described previously (13, 19), intracolonic administration of TNBS resulted in widespread hemorrhagic damage to the distal colon. In addition to ulceration, massive infiltration of granulocytes, extending through the mucosa, submucosa, and muscularis propria, was evident. In rats killed 10 days after TNBS administration, the colonic damage score was 15.67 ± 0.42. Diarrhea was observed in all 12 rats, as were adhesions between the colon and other organs. The thickness of the wall of the colon was greatly enlarged (4.25 ± 0.53 mm) over that observed in normal rats (1.2 ± 0.2 mm). Consistent with the histological evidence of granulocyte infiltration, MPO activity of the colon was profoundly increased above that of normal rats (~30-fold) (Table 1).

Treatment with NPC-18915 beginning just before TNBS administration and continuing for 4 days resulted in a significant reduction in the severity of colitis. As shown in Fig. 1, the doses of 2 and 10 mg/kg produced statistically significant reductions in the colonic damage score, while the lower dose (0.2 mg/kg) was ineffective. The incidence of diarrhea was significantly reduced with the 10 mg/kg dose of NPC-18915 (4 of 8 rats; P < 0.05), while the thickness of the wall of the colon was significantly reduced by both the 2 and 10 mg/kg doses of NPC-18915 (1.76 ± 0.23 and 2.25 ± 0.21 mm, respectively; P < 0.05). Despite reducing the severity of colitis, NPC-18915 did not significantly affect tissue MPO activity at any of the doses tested (Table 1).

When treatment with NPC-18915 (10 mg/kg) was initiated 2 days after induction of colitis and continued for 4 days, it did not significantly affect the severity of the colitis (Fig. 2). None of the components of the global colonic damage score (e.g., incidence of diarrhea or adhesions, thickness of the wall of the colon) were affected by NPC-18915 treatment. Colonic MPO activity was also unaffected by treatment with NPC-18915 over days 2–6 after induction of colitis (Table 1).

Effects of ANS on acute colitis. In rats killed 1 day after induction of colitis, treatment with ANS produced a profound reduction in colonic granulocyte content. The mean MPO activity in rats treated with saline in place of ANS was 65.8 ± 10.0 U/mg. Treatment with

Fig. 1. Effects of intraperitoneal administration of NPC-18915 on severity of acute colitis. NPC-18915 was administered at 1 of 3 doses 1 h before induction of colitis and every 12 h thereafter for 4 days. Severity of colonic damage was scored on the 10th day after induction of colitis. Each point represents colonic damage score for a single rat, while horizontal dashed lines represent the mean score for each group. ★ P < 0.001 compared with vehicle-treated group.

Fig. 2. Effects of intraperitoneal administration of NPC-18915 at a dose of 10 mg/kg on severity of acute colitis in the rat. The drug was given either over the first 4 days after induction of colitis or during the period between days 2 and 6 after induction of colitis. All rats were killed on day 10 after induction of colitis for assessment of the severity of damage. Each point represents colonic damage score for a single rat, while horizontal dashed lines represent the mean score for each group. ★★ P < 0.001 compared with vehicle-treated group.
ANS reduced MPO activity to 4.2 ± 0.8 U/mg (P < 0.001). This depletion of neutrophils did not significantly affect the severity of colitis in rats killed 1 day after its induction, as damage scores in the ANS-treated rats (10.7 ± 0.9) did not differ from those in the control group (11.5 ± 0.5).

Continued daily treatment with ANS over a period of 4 days after induction of colitis did not significantly affect the severity of colitis relative to controls when assessed on day 10 after TNBS administration. The mean colonic damage score (15.8 ± 0.4) was not significantly different from that observed in rats receiving vehicle (15.5 ± 0.5). MPO activity in the ANS-treated rats did not differ significantly from vehicle-treated rats (Table 1). The rats treated over a 4-day period with ANS were lethargic and cachexic (1 of the 5 rats died before completion of the experiment).

Effects of NPC-18915 on reactivated colitis. Reactivation of colitis involves systemic administration of the hapten (TNBS) to rats in which colitis was induced 6 wk earlier. At this time point, the colitis had resolved to such a point that the colonic damage score averaged 2.7 ± 0.6 and MPO activity averaged 5.9 ± 1.1. In rats receiving subcutaneous TNBS, a significant (P < 0.001) increase in colonic damage score was observed (Fig. 3), accompanied by a significant (P < 0.05) increase in colonic MPO activity (Fig. 4). Treatment with NPC-18915 (10 mg/kg) during the reactivation procedure resulted in a significant reduction of both the colonic damage score (macroscopic and histological) and colonic MPO activity. In fact, NPC-18915 reduced the colonic damage score and MPO activity to levels not significantly different from those in rats that did not receive the reactivating doses of TNBS (Figs. 3 and 4).

Effects of ANS on reactivated colitis. Administration of ANS during the reactivation procedure significantly reduced the colonic damage score (macroscopic and histological) and colonic MPO activity relative to the TNBS-treated control group (Fig. 4).

Effects of NPC-18915 on neutrophil LTB4 synthesis. As shown in Table 2, NPC-18915 virtually abolished calcium ionophore-induced LTB4 synthesis by peritoneal neutrophils at concentrations of 10 and 100 µg/ml. At lower concentrations, it had no effect. NDGA (100 µg/ml) also abolished LTB4 synthesis.

Effects of NPC-18915 on circulating leukocyte numbers. Injection of NPC-18915 every 12 h over a 3-day period did not significantly affect the total numbers of circulating leukocytes or the relative proportions of neutrophils, eosinophils, lymphocytes, basophils, or monocytes. For example, in vehicle-treated rats, neutrophils accounted for 17 ± 3% of the circulating leukocytes, which was not significantly different from the 13 ± 4% observed in rats treated with NPC-18915.

Effects of NPC-18915 on colonic blood flow. Injection of vehicle or NPC-18915 (10 mg/kg) into the peritoneum had no significant effect on colonic blood flow. In each case, the injection of either NPC-18915 or vehicle caused a small (~10%) reduction of blood flow that recovered to basal levels within a few minutes. Mean blood flow 30, 60, and 90 min after administration of NPC-18915 was 91 ± 5%, 90 ± 4%, and 88 ± 6% of basal levels, which was not significantly different from the rates of blood flow at the corresponding times in the vehicle-treated group (94 ± 4%, 90 ± 5%, and 90 ± 3% of basal levels, respectively).

**DISCUSSION**

Neutrophils have been proposed to be major effector cells of tissue injury in both human IBD (3, 9) and animal models of colitis (17, 21). The tissue injury produced by these cells has been attributed to their ability to liberate a variety of reactive oxygen metabolites (17) and to the disruption of epithelial barrier function when these cells migrate into the lumen (14). Consistent with the latter hypothesis, prevention of neutrophil extravasation, through administration of an immunoneutralizing antibody directed against the β2-integrin family, was shown to reduce the permeability changes normally associated with colitis in a rabbit model (21). The results of the present study demonstrate that NPC-18915, a compound with inhibitory
effects on neutrophil activation and adherence (4, 5), was able to significantly reduce the severity of acute colitis and also markedly reduce the severity of colonic injury and granulocyte infiltration in a model in which the inflammatory reaction was reactivated. The beneficial effects of NPC-18915 in acute colitis were only observed when the compound was administered at the onset of the inflammatory reaction; delaying the start of therapy for 2 days, a point at which granulocyte infiltration is near maximal (13, 19), resulted in a loss of activity.

The reactivation model of colitis offers some clear advantages over the original TNBS model in terms of the testing of potential therapeutic agents. The colitis initially induced by TNBS involves the intracolonic administration of this hapten in a vehicle of ethanol. This solution is highly cytotoxic; its contact with the colonic epithelium leads to extensive mucosal injury that is largely independent of any immune activation. As a result, pharmacological or immunologic modulation of the tissue injury is difficult to discern. On the other hand, the systemic administration of TNBS to rats 6 wk after the initial bout of colitis results in a recurrence of inflammation and ulceration at the site of the initial injury (1). This inflammatory reaction can be inhibited by administration of corticosteroids, 5-aminosalicylic acid, or cyclosporin A (to varying degrees) (1). Although the mechanisms underlying reactivation of colitis by systemic TNBS administration are not yet clear, the results of the present study suggest that the neutrophil is a primary effector cell in this model. Administration of NPC-18915 reduced the severity of injury in the reactivation model significantly; indeed, the colonic damage scores were not significantly different from those in rats that did not receive the reactivating doses of TNBS. Moreover, NPC-18915 inhibited the granulocyte recruitment into the colon after systemic TNBS administration. A causal link between the reduction of granulocyte infiltration and the reduction of tissue injury is supported by the observation that treatment with ANS, which prevented granulocyte infiltration, also prevented the reactivation of colitis (i.e., tissue injury) after systemic TNBS administration.

NPC-18915 failed to significantly affect the severity of colonic damage when treatment was initiated after inflammation and injury were already established. This was mostly likely attributable to two factors. First, as tissue injury was already very severe when treatment was started, a beneficial effect of the test drug would only be expected if it accelerated the healing process. Second, if a primary mechanism by which NPC-18915 reduced the severity of colitis was through suppression of granulocyte recruitment, one would not expect to see beneficial effects when the drug is given at a time when granulocyte infiltration has already occurred. This latter explanation raises another issue. When tissue MPO activity was examined in rats treated with NPC-18915 vs. those treated with vehicle, no significant differences were observed. This was the case even when NPC-18915 significantly reduced the severity of colitis. At first examination, this could be taken as evidence that NPC-18915 did not influence granulocyte recruitment. However, it must be considered that tissue samples for MPO measurement were taken from regions of macroscopically visible injury. Thus it is possible that granulocyte recruitment into the distal colon as a whole could be reduced without the levels at a site of injury being affected. Moreover, the tissue samples for MPO measurements

Table 2. Effects of NPC-18915 on LTB₄ synthesis

<table>
<thead>
<tr>
<th>Treatment</th>
<th>LTB₄ Synthesis, pg/ml</th>
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<tbody>
<tr>
<td>Vehicle</td>
<td>274 ± 70</td>
</tr>
<tr>
<td>NPC-18915 (0.001 µg/ml)</td>
<td>318 ± 41</td>
</tr>
<tr>
<td>NPC-18915 (0.01 µg/ml)</td>
<td>198 ± 7</td>
</tr>
<tr>
<td>NPC-18915 (0.1 µg/ml)</td>
<td>300 ± 32</td>
</tr>
<tr>
<td>NPC-18915 (1 µg/ml)</td>
<td>249 ± 53</td>
</tr>
<tr>
<td>NPC-18915 (10 µg/ml)</td>
<td>9 ± 4*</td>
</tr>
<tr>
<td>NPC-18915 (100 µg/ml)</td>
<td>2 ± 1*</td>
</tr>
<tr>
<td>NDGA (100 µg/ml)</td>
<td>2 ± 1*</td>
</tr>
</tbody>
</table>

Results are means ± SE release of leukotriene B₄ (LTB₄) by peritoneal neutrophils from the rat (n = 4). Neutrophils were incubated in the presence of 1 of the test drugs or vehicle for 10 min, stimulated through addition of 10 µg/ml calcium ionophore (A-23187), and incubated for a further 30 min. NDGA, nordihydroguaiaretic acid. * P < 0.001 compared with vehicle-treated group.
were taken on day 10 after induction of colitis, whereas the treatment with NPC-18915 that reduced colonic injury was only carried out to day 4. Therefore it is possible that any inhibitory effect on NPC-18915 on granulocyte recruitment was no longer evident. In support of this latter possibility is the observation that treatment with ANS, which produced a profound reduction in tissue MPO activity during the period of treatment, failed to reduce tissue MPO activity when measured at day 10 (6 days after the final dose). In contrast to the results in the acute colitis model, the results from the studies performed in the reactivation model of colitis suggest that NPC-18915 does suppress granulocyte recruitment into the distal colon. Thus it is possible that NPC-18915 is more effective at inhibiting granulocyte recruitment initiated through immune events than that triggered by the widespread mucosal injury induced by intracolonic administration of TNBS and ethanol.

The failure of ANS to significantly affect the severity of colonic damage in the acute colitis model is consistent with the reports of Higa et al. (10) and Yamada et al. (21) using the acetic acid colitis model in rats and mice. The failure of the profound reduction of circulating neutrophil numbers to influence the severity of colitis may be attributable to the fact that the rats were moribund when this treatment was given in close temporal association with intracolonic administration of TNBS and ethanol. In contrast, administration of the same dose of ANS in the reactivation model of colitis did not appear to alter the general well-being of the animals. In the former case, it is likely that the massive disruption of colonic epithelial integrity renders the animal susceptible to bacterial translocation. In combination with a profound reduction in circulating neutrophil numbers, the rats would undoubtedly be at increased risk of endotoxemia, which would account for their observed moribund state.

NPC-18915 belongs to a family of compounds collectively referred to as nactins. Nactins have previously been shown to increase the shedding of L-selectin from neutrophils, an event associated with inhibition of neutrophil adherence. In a previous study (12), we demonstrated that another nactin (NPC-17923) was capable of inhibiting neutrophil adherence to the mesenteric vascular endothelium induced by platelet-activating factor. In both the present and the previous study (12), nactins were found not to significantly affect blood flow. We also demonstrated in the present study that NPC-18915 did not produce its beneficial effects through depletion of circulating leukocytes; however, this drug did significantly affect LTB₄ synthesis by neutrophils. Although it is not possible to say with certainty how NPC-18915 reduced the severity of colitis, it seems most likely that inhibitory effects on neutrophil extravasation, perhaps as a consequence of increased shedding of L-selectin, accounted for the observed reduction in granulocyte infiltration and tissue injury. It is also possible that inhibition of LTB₄ synthesis by NPC-18915 contributed to its beneficial effects, at least in the acute colitis model. Previously, it was found (18, 19) that inhibitors of leukotriene synthesis accelerate healing in acute colitis. However, the same inhibitors failed to significantly affect the severity of colitis in the reactivation model (1).

In summary, an inhibitor of neutrophil activation and adherence was capable of significantly inhibiting reactivation of experimental colitis and of dose dependently reducing the severity of acute colitis. The former observation, together with an observed beneficial effect of depletion of circulating neutrophils, suggests that the neutrophil is a key effector cell of tissue injury in the reactivation model. These results support the hypothesis that the neutrophil may represent a rational target for novel therapies aimed at reducing the severity or preventing relapses of IBD.

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Address for reprint requests: J. L. Wallace, Dept. of Pharmacology and Therapeutics, Univ. of Calgary, 3330 Hospital Dr. NW, Calgary, Alberta, Canada T2N 4N1.

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