Disruption of intestinal barrier function associated with experimental colitis: possible role of mast cells

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Disruption of intestinal barrier function associated with experimental colitis: possible role of mast cells. Am. J. Physiol. 274 (Gastrointest. Liver Physiol. 37): G203–G209, 1998.—The objective was to characterize changes in barrier and transport function in an experimental model of colitis, and to determine whether mast cells contribute to these changes. Colitis was induced in rats with intracolonic 2,4,6-trinitrobenzenesulfonic acid (TNBS, 30 mg) in 50% ethanol. Controls received 0.9% saline or the ethanol vehicle alone. In vivo loop perfusion was used to assess colonic water flux (in \( \mu l \cdot cm^{-1} \cdot h^{-1} \)) and lumen-to-blood \(^{51}\)Cr-labeled EDTA clearance (% administered dose) after TNBS. Myeloperoxidase (MPO) was used as an index of granulocyte influx. TNBS or its vehicle caused a marked decrease in water absorption and an increase in permeability at 4 h after administration compared with saline. Neither dexamethasone (anti-inflammatory control) nor doxantrazole (mast cell stabilizer) was able to attenuate these early changes likely caused by the vehicle. In contrast, at later times, TNBS (but not its vehicle) also increased \(^{51}\)Cr-EDTA permeability and decreased water absorption; both effects were significantly attenuated by dexamethasone or doxantrazole. These drugs also significantly reduced TNBS-induced MPO accumulation and release of rat mast cell protease II. We conclude that experimental colitis is associated with severe defects in intestinal transport and barrier functions and that mast cells may contribute to the pathogenesis of these changes.

intestinal transport; permeability; inflammation; doxantrazole; dexamethasone

ALTERATIONS IN INTESTINAL barrier function and water absorption have been described in a large number of investigations of inflammatory bowel diseases (IBD) spanning back more than 25 years (9, 15). It is presently controversial whether an increase in intestinal permeability precedes the development of the inflammatory process or is the consequence of it. Some investigators have argued that an abnormally high intestinal permeability may play a crucial role in the development of IBD, by enhancing both uptake of luminal antigens and granulocyte migration (16). These authors and others have likewise proposed that increased intestinal permeability is a primary etiologic factor in IBD, since first degree relatives of IBD patients, without evidence of disease, also showed abnormal intestinal permeability (16, 23). However, other studies have disputed these findings (1, 19, 33, 36). Nevertheless, whether changes in barrier and transport function that occur in IBD are primary or secondary phenomena, they certainly have the capacity to contribute to symptomatic disease perpetuation. Thus alterations in active electrolyte transport by the diseased epithelium would result in altered water flux, and could thereby contribute to the secretory diarrhea that is a frequent symptom of IBD. Furthermore, diminished barrier function of the diseased epithelium would allow the enhanced passage of luminal antigens, bacterial products, and microorganisms into the mucosa, thereby enhancing the inflammatory process.

Despite the substantial relevance of altered transport and barrier function to the generation of disease in IBD patients, relatively little is understood regarding the mechanisms of these changes. Over the past several years, it has become increasingly appreciated that various cellular components of the immune system and their products (including inflammatory mediators and cytokines) are likely to be key regulators of intestinal epithelial function (2, 8, 27, 28). Likewise, mast cells have long been suspected to play a key role in a variety of chronic inflammatory processes, including the inflammation seen in IBD (3, 4). Mast cell numbers have been reported by a number of studies to be increased in tissues from IBD patients (10, 24). These mast cells also appear to be activated. They release greater quantities of mediators both spontaneously and in response to epithelial cell antigens than do mast cells obtained from normal tissues or uninfamed tissues from IBD subjects (11, 12). Several studies have also clearly shown that mast cell activation alters intestinal epithelial functions including ion transport, mucus secretion, and permeability (2, 7, 30–32).

Studies of barrier and transport function in human IBD patients may be complicated by the difficulty of studying a disease with frequent relapses and remissions, as well as in a heterogeneous patient population that may be taking medications that have the potential to alter epithelial function. For these reasons, in this study we have used a rat model of chronic colitis (26) to assess the involvement of mast cells in the disturbance of intestinal water transport and barrier function occurring in IBD. This colitis model displays histopathological features that are similar to those observed in human Crohn’s disease, including changes in epithelial barrier function (39). The use of such a model, where the initiation of inflammation can be controlled, allows the investigation of factors that contribute to changes in epithelial function at various stages of disease. We have also employed the mast cell-stabilizing drug doxantrazole, which is a known inhibitor of rat mucosal mast cell activation (29). Our goal, therefore, was to characterize changes in epithelial barrier and transport function that accompany intestinal inflammation and to determine the role of mast cells in any such changes.
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MATERIALS AND METHODS

Animals. All studies were performed with male Sprague-Dawley rats (Charles River) weighing ~230–280 g. The animals were housed in the University of California, San Diego (UCSD), School of Medicine Animal Care Facility in rack-mounted cages. They received standard pelleted chow and water ad libitum. All studies received the approval of the UCSD Committee on Investigations Involving Animal Subjects.

Induction of colitis. Colitis was induced according to the method of Morris et al. (26) by the intrarectal administration of 0.5 ml of a solution of 2,4,6-trinitrobenzenesulfonic acid (TNBS, 30 mg) in 50% ethanol. Control rats received either 0.5 ml of the 50% ethanol vehicle or 0.5 ml of 0.9% saline. All solutions were delivered via a soft catheter introduced 8 cm beyond the anus. TNBS-, ethanol-, and saline-treated rats were further divided into various groups according to pretreatment with either doxantrazole (5 mg ip, given 2 h prior to intracolonic treatments), dexamethasone (1 mg ip, given 24 and 2 h prior to intracolonic treatments), or a single intraperitoneal injection of 0.5 ml 0.9% saline 2 h prior to induction of colitis. Each group comprised 4–6 rats. Animals were assessed for intestinal barrier and transport function at 4 and 12 h and 2, 4, and 7 days after the induction of colitis or the intracolonic administration of control solutions, as described below.

Epithelial permeability and water flux measurements. Rats were anesthetized initially with an intraperitoneal injection of pentobarbitone sodium (50 mg/kg) and placed on a heating pad. This was thermostatically controlled via a rectal thermometer to maintain the animal’s temperature at 37°C throughout the experiment. A tracheostomy was then performed, and the jugular vein was cannulated to maintain anesthesia and hydration. The abdominal cavity was opened, and the bowel was exteriorized. Beginning 8 cm proximal to the anus, a 5-cm loop of colon (to include the site of TNBS-induced colitis) was cannulated with polyethylene tubing. The tubing was inserted into small incisions, facing the mesentery, at the proximal and distal ends of the segment. Care was taken to maintain the vascular supply to the cannulated segment. The cannulated segment was rinsed and, with the remainder of the bowel, was replaced in the abdominal cavity, and the incision was covered with a moist swab. The technical aspects of this perfusion system have been described by Meddings and Westergaard (25).

A prewarmed, isosmotic solution [140 mM NaCl, 10 mM N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES), pH 7.5] was then perfused through the loop for 10 min at a rate of 3 ml/min and discarded. Following this, the test solution (85 mM NaCl, 10 mM HEPES, 23 mg/l phenol red, 5 mM KCl, 5 mM CaCl2, 20 mM mannitol, 10 µCi/ml 51Cr-labeled EDTA; pH 7.5) was perfused through the segment at a rate of 0.5 ml/min and in a recirculating fashion, by means of a peristaltic pump. The total perfusion time with the test solution was 360 min. Samples (0.5 ml) of the test perfusate were taken before and at the end of the perfusion period for analysis.

To assess epithelial permeability, lumen-to-blood clearance of 51Cr-EDTA was measured. This was accomplished by sampling blood from the femoral vein before the start of the perfusion period and at hourly intervals thereafter. After 3 h, urine was also collected with a syringe, the bladder was washed twice with 0.5 ml of saline, and these washings were pooled with collected urine. Radioactivity was measured directly in urine samples and in serum prepared from sampled blood, by means of a gamma counter. 51Cr-EDTA recoveries were expressed as a percentage of the administered dose. Net water fluxes were calculated by comparing the initial and final volumes of the recirculating perfusate, as well as the respective concentrations of phenol red in these samples, and expressed as microliters per centimeter per hour.

Myeloperoxidase assay. Intestinal myeloperoxidase (MPO) activity was measured as an index of granulocyte infiltration. At the end of the perfusion experiments, full-thickness tissue samples (~30–100 mg) from macroscopically inflamed areas or from corresponding locations in control rats were weighed and then placed in a solution of hexadecyltrimethylammonium bromide (0.5%, wt/vol; 1 ml/50 mg tissue). The tissue samples were homogenized (11,000 rpm, 30 s) in this solution using a Tissue Tearor (Biospec Products, Bartleville, OK), and the resulting homogenate was subjected to three cycles of freezing and thawing. The samples were centrifuged (14,000 rpm in a microcentrifuge, 20 min) to remove insoluble material, and the supernatants were removed to clean tubes and stored at ~20°C until assayed. MPO was assayed according to Bradley et al. (6) with hydrogen peroxide as substrate. One unit of MPO was defined as that which converts 1 µmol of hydrogen peroxide to water in 1 min at 22°C.

Rat mast cell protease II assay. Serum levels of rat mast cell protease II (RMCPII) were measured as a specific marker of ongoing degranulation of mucosal mast cells. Four days after the induction of colitis, blood samples were obtained from rats by cardiac puncture at the end of the perfusion experiments described above. Serum from these samples was stored at ~20°C until assay, then assayed for RMCPII using a commercially available enzyme-linked immunosorbent assay (More- dund Institute, Edinburgh, UK) according to the methods of Huntsley et al. (17).

Statistical analysis. Data are presented as means ± SD for a given number of animals. Statistically significant differences between values were assessed by means of Student’s t-test, for either paired or unpaired samples, or by analysis of variance (ANOVA), as appropriate. Values were considered to be significantly different if P < 0.05.

Materials. TNBS (ICN Pharmaceuticals, Cleveland, OH), doxantrazole (3-[5-tetrazolyl]-thioxanthone 10,10-dioxide; Aldrich Chemical, Milwaukee, WI), and 51Cr-EDTA (specific activity 1.38 Ci/mol; New England Nuclear, Boston, MA) were purchased from the sources indicated. Reagents utilized in the MPO assay were obtained from Sigma Chemical (St. Louis, MO). All other chemicals were of analytical grade and were obtained from Fisher Scientific.

RESULTS

Effect of colitis on net water absorption. At early time points, administration of TNBS or its vehicle (50% ethanol) resulted in marked alterations in net water transport compared with animals receiving intracolonic saline. Four hours after the administration of TNBS, net water absorption decreased from a control value of 154 ± 32 to 0.82 ± 0.12 µl·cm⁻¹·h⁻¹ in the colitis group (Fig. 1A). These early changes were most likely induced by the ethanol vehicle, since similar decreases in water absorption were seen in the rats receiving 50% ethanol without TNBS (data not shown). In contrast, by 12 h after the administration of the ethanol vehicle, values for water absorption did not differ significantly from those seen in saline-treated controls (not shown). However, net water absorption remained markedly depressed in TNBS-treated animals at 12 h. Water absorption in the colitis group
progressively increased thereafter, but even at 7 days after the induction of colitis, it remained significantly different from control values (Fig. 1B).

Neither dexamethasone nor doxantrazole was able to significantly attenuate the decrease in water absorption induced by TNBS at 4 h (Fig. 1A). As noted above, these early changes were most likely attributable to the ethanol vehicle. Thus the inability of either the anti-inflammatory control, dexamethasone, or the mast cell stabilizer, doxantrazole, to reverse these changes is not very surprising. However, in contrast to their lack of effect on early transport changes, both dexamethasone and doxantrazole significantly reduced the later effects of TNBS on water transport (Fig. 1B). Both drugs accelerated the recovery of water absorption and to a similar extent, such that by 1 wk after the induction of colitis, values for water absorption in rats treated with dexamethasone or doxantrazole did not differ significantly from those in control animals in which colitis was not induced. These values also differed significantly from water absorption values in colitic rats in the absence of dexamethasone or doxantrazole pretreatment.

Effect of colitis on $^{51}$Cr-EDTA permeability. The induction of colitis was associated with a significant increase in intestinal permeability as assessed during a 3-h perfusion experiment. Initial studies examined permeability in rats starting at 4 h after the administration of TNBS. In such animals, the blood concentration of luminally administered $^{51}$Cr-EDTA was markedly and significantly increased compared with saline-treated control animals without colonic injury (Fig. 2). Likewise, urine recovery and luminal disappearance rate of $^{51}$Cr-EDTA were also significantly enhanced in colitic vs. control rats (not shown). As observed for the changes in water absorption seen at this early time point, changes in permeability were likely attributable to the TNBS vehicle, since changes of similar magnitude were seen when rats were treated intracolonically with 50% ethanol alone (not shown). Likewise, the early effects of TNBS on $^{51}$Cr-EDTA permeability could not be attenuated by either dexamethasone or doxantrazole (Fig. 2).

In contrast to the early effects of TNBS on lumen-to-blood clearance of $^{51}$Cr-EDTA, which could be ascribed to effects of the vehicle, permeability changes occurring at later time points after TNBS administration were not seen if rats were treated with 50% ethanol alone (not shown). Furthermore, while the enhanced permeability associated with colitis slowly began to resolve over the 7 days after the administration of TNBS, it...
still remained significantly elevated (Fig. 3). However, if rats were pretreated with either dexamethasone or doxantrazole, the permeability defect ascribable to TNBS was less severe to begin with and resolved more rapidly than the changes seen in rats with no pretreatment (Fig. 3). Both dexamethasone and doxantrazole appeared to resolve the changes in permeability to a similar degree. Furthermore, at 1 wk after the induction of colitis, rats receiving either doxantrazole or dexamethasone, followed by the induction of colitis with TNBS, displayed permeability values that did not differ significantly from those in healthy control animals.

Colonic MPO activity. MPO was measured in colonic tissue samples as a measure of granulocyte infiltration. TNBS, but not ethanol or saline, induced an increase in MPO in tissues that became significant at 8 h after the induction of colitis. There was a modest, but not statistically significant, increase in MPO levels at 4 h. Histological inspection of the tissues revealed that the source of the MPO in inflamed tissue was likely to be predominantly neutrophils. Representative data for MPO levels obtained at 4 days after the induction of colitis (or the administration of control solutions) are shown in Fig. 4. As expected, tissues treated with TNBS showed significantly higher MPO levels than those treated with saline or the ethanol vehicle alone. Moreover, both dexamethasone and doxantrazole were able to suppress completely the increase in MPO induced by TNBS. In data not shown, dexamethasone and doxantrazole failed to alter the modest increase in MPO induced by TNBS at the 4 h time point and also did not appear to alter neutrophil influx at this time. However, as early as 8 h after the induction of colitis, dexamethasone and doxantrazole significantly reduced the increases in MPO associated with the induction of colitis by TNBS. Control experiments revealed that none of the compounds used in the experiments interfered with the MPO assay.

Mucosal mast cell activation. Serum RMCPII levels were measured 4 days after the induction of colitis as a measure of ongoing mucosal mast cell degranulation. In control rats, the mean basal level of RMCPII was ~270 ng/ml, and this was not significantly altered by either dexamethasone or doxantrazole (Fig. 5). Induction of colitis with TNBS led to an approximately threefold increase in serum RMCPII levels at 4 days after induction of colitis with TNBS.
(P < 0.01, Fig. 5). This rise in RMCPII was completely abolished by pretreatment of the animals, prior to the induction of colitis, with either dexamethasone or doxantrazole (Fig. 5). These data are consistent with prior reports showing that doxantrazole inhibits mucosal mast cell activation, as assessed histologically (37), and that dexamethasone leads to a depletion of the mucosal mast cell population (36).

**DISCUSSION**

Our studies, presented here, have shown that the induction of inflammation in a widely used animal model of colitis is associated with marked defects in epithelial barrier and transport function. The early phase of these changes was produced by the ethanol vehicle, which is used in this colitis model precisely because it is a “barrier breaker” (26). However, the effects of ethanol on both barrier function and water absorption were transient and were only observed at the 4 h time point. Thereafter, decrements in both epithelial functions were only seen in animals that received both ethanol and TNBS, and not in animals receiving the ethanol vehicle or in saline-treated controls. They were, therefore, specific for the inflammatory condition.

The mechanism whereby TNBS induces colitis in this model is still a matter of some debate. Although the model has been presumed to involve an immune, hapten-mediated component, the fact that chronic inflammation can be induced with a single administration of the hapten argues that the colitis may actually result from erosive mechanisms. In any event, our current findings and those of other investigators (39) demonstrate the utility of this model to study the initiation of changes in barrier function that may contribute to the pathogenesis of symptoms in IBD. An increase in epithelial permeability would be expected to allow for the perpetuation of intestinal inflammation, by allowing the increased passage of microbial products and antigenic proteins into the inflamed mucosa. Likewise, we observed that water absorption in the inflamed mucosa was markedly diminished. This effect would be expected to contribute to the diarrhea that occurs not only in this animal model but also in human IBD. Water fluxes in the gastrointestinal tract are passive and driven by a balance between active secretion and absorption of ionic species and nutrients. Furthermore, the extent of water transport will also depend on the overall permeability of the paracellular pathway. In the complex setting of the intact animal, it is not possible to assess the precise contributions of increased solute secretion, decreased absorption, and increased paracellular permeability to the marked decrease in water absorption that we have seen in these studies. Nevertheless, the changes that we have described here clearly can account for the diarrheal symptoms that are prominent in this colitis model, as well as in human IBD.

The results presented in this study are consistent with the hypothesis that mast cells and their mediators contribute to the pathogenesis of intestinal inflammation, as well as to the epithelial dysfunction that occurs in the setting of such inflammation. The findings are in keeping with previous reports of mast cells in human IBD, where most investigators have found evidence for mast cell involvement as indicated by an increase in mast cell numbers, or evidence of cell activation, or both (10–12, 24). Doxantrazole was used in these experiments because it is a known inhibitor of mediator release from both connective tissue and mucosal-type mast cells (29). This is an important consideration, since many mast cell-stabilizing drugs are without significant activity on mast cells of the mucosal phenotype, as found in the mucosa of the gastrointestinal tract (5). Moreover, doxantrazole has been employed by other investigators, at doses equivalent to those studied here, to implicate mucosal mast cell activation in changes in mucosal blood flow and neutrophil migration associated with an ischemia-reperfusion model of intestinal injury (18). Measurements of RMCPII in the current study specifically indicated that doxantrazole stabilized mast cells in this model.

It is unclear as yet whether the ability of doxantrazole to attenuate permeability and transport defects associated with colitis reflects a primary role for mast cell products in producing these changes. Certainly a number of mast cell mediators (including histamine, cytokines, and platelet activating factor) whose release would be blocked by doxantrazole can increase mucosal permeability directly (13, 14, 20, 22, 40). Alternatively, mast cell products could increase permeability indirectly in this model, perhaps by stimulating the influx of inflammatory cells such as neutrophils. These cells, in turn, could release cytotoxic and other products that would alter epithelial properties (21, 28). This interpretation is consistent with our finding that doxantrazole not only attenuated changes in epithelial barrier and transport functions in this study but also significantly decreased neutrophil infiltration into the mucosa, as assessed by measurements of MPO. In either event, activation of mast cells in response to the initiation of the inflammatory response would be central to the development of later epithelial changes. In fact, direct antigenic challenge of intestinal mucosal mast cells in sensitized rats also led to an increase in paracellular permeability (38).

Dexamethasone was used in these experiments as a positive anti-inflammatory control. Steroids in general are known to exert a large number of anti-inflammatory effects on a variety of cell types. Of note, they significantly suppress cytokine synthesis, as well as altering the release of both preformed and newly generated mediators from many cells (34). However, it is likely that at least a part of the efficacy of dexamethasone in reducing epithelial dysfunction could be ascribed to an effect on mast cells in this model, since dexamethasone has been shown to selectively deplete the mucosal mast cell population under conditions very similar to those used here (35). This interpretation is supported by our finding that TNBS-induced colitis was not associated with an increase in serum RMCPII in animals that had been pretreated with dexamethasone. Indeed, the RMCPII data in both doxantrazole- and dexamethasone-
treated animals are suggestive of (but do not prove) the possibility that these drugs inhibit at least one component of the initiation of the inflammatory response rather than accelerating repair. However, distinguishing between these alternatives will require further study.

We report here that the development of colitis in a rat model is associated with significant changes in epithelial transport and barrier function. Furthermore, the data provide evidence that alterations in epithelial function may be produced, either directly or indirectly, by products released from activated mast cells. Our study may shed light on the development of diarrheal illness in patients with IBD or other forms of intestinal inflammation. Likewise, they raise the possibility for new routes to intervene therapeutically against this disabling and ubiquitous symptom.

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