Influence of Topical Rectal Application of Drugs on Dextran Sulfate-Induced Colitis in Rats

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A rat model of colitis [dextran sulfate (DSS)] was used to study the permeation of Evans blue (EB) from the lumen into the wall of proximal and distal colonic loops after exposure to the dye for 2 hr. Topical application of drugs used in human ulcerative colitis (lidocaine, mesalazine, prednisolone, or sucralfate) was given daily during induction of colitis to protect the mucosa. The mucosal changes were evaluated with special regard to peptidergic innervation [substance P (SP) and neuropeptide Y (NPY)], invasion of antigen-presenting polydendritic cells, and mucin-containing goblet cells. DSS-treatment caused a significantly increased permeation of EB. In the proximal loops a significant inhibition was obtained after treatment with lidocaine, prednisolone, or sucralfate. In the distal loops only treatment with lidocaine had a preventive effect. Immunocytochemically there was a clear hyperplasia of both mucosal SP- and NPY-immunoreactive nerve fibers in regions with crypt abnormalities. In these regions also most of the goblet cells were devoid of mucus. Like the changes in permeation, these morphological changes were most prominent in the distal loops. With induction of colitis, the mucosa and lamina propria were invaded by polydendritic cells; the visual score was markedly decreased in the proximal loops treated with lidocaine, prednisolone, or sucralfate. In the distal loops similar effects were obtained after treatment with lidocaine or prednisolone. Prevention of the influx of antigens in both loops after lidocaine treatment with reduced recruitment of polydendritic cells into the lamina propria is suggested. The nerve hyperplasia may thus be secondary to luminal challenge with antigens during induction of colitis. The discrepancy between increased permeation and absence of polydendritic cell response in the distal loops after prednisolone may reflect separate actions of steroids on the intestinal epithelium and the immune cells.

KEY WORDS: dextran sulfate; colitis; topical drugs; permeation; peptidergic nerves; polydendritic cells; goblet cells; rats.

Dextran sulfate (DSS) together with sorbitol sulfate, carboxymethyl-dextran, amylopectin sulfate, and aluminum dextran sulfate were initially investigated as potential antiulcer agents (1). Investigations in animal models demonstrated major side effects, ie, intestinal and ceccal ulcerations. DSS is poorly absorbed after oral administration, since it is a large and negatively charged molecule that cannot easily cross membranes (2). It can therefore be expected that DSS exerts its effects on the luminal surface of the colonic mucosa. One related substance, sucralfate, was devoid of side effects in animal models and has subsequently been used as an antiulcer agent. DSS has, in contrast to its presumed antiulcer effect, rather recently been used...
to induce experimental colitis in rodents (3). The induced colitis in rats primarily involves the rectum and the distal colon in analogy with ulcerative colitis in man.

An interesting histological feature of DSS-induced colitis in the rat is the appearance of subepithelial S-100-immunopositive polydendritic cells, observed within the first two days after ingestion of DSS. Polydendritic cells also appear in the colonic mucosa in patients with ulcerative colitis in the early phase of inflammation (4, 5), in contrast to the colitis seen after local exposure of the rat colonic mucosa to acetic acid (S. Björc, personal communication). Polydendritic cells are potent antigen-presenting cells. Therefore, invasion of such cells into the subepithelial layers in ulcerative colitis indicates that the epithelium has a decreased resistance to antigens, e.g., bacterial products, which may leak into the lamina propria (6). A decreased resistance of the epithelial membrane may be related to an increased intercellular permeation.

The aim of the present study was to study whether permeation was enhanced in DSS-induced colitis in rats. If so, we wanted to study the effects on permeation by topical applications of drugs used in human ulcerative colitis, i.e., lidocaine, mesalazine, prednisolone, or sucralfate. Intestinal permeation was studied by a technique utilizing Evans blue (EB) (7).

**MATERIALS AND METHODS**

**Induction of Colitis.** Male Sprague-Dawley rats (250 ± 15 g) received 5% DSS in their drinking water for seven days. They were kept at room temperature (22°C), with a controlled 12-hr light–dark cycle and with free access to a standard pellet diet and water. The animals were studied on the eighth day in acute experiments using intraluminal instillation of EB and subsequent surgical biopsies of colon (see below). The surgical procedures were performed during a period of 5–10 min of ether anesthesia. The study was approved by the Ethics Committee of the University of Göteborg.

**Surgical Procedures.** The distal ileum was ligated at the junction with the cecum. The proximal cecum was punctured with a needle connected to a 50-ml syringe filled with phosphate-buffered saline (PBS) at room temperature. Gentle rinsing of the colon was performed to evacuate the fecal contents. A proximal loop (length 4 cm), including the ascending and transverse colon, and a distal loop (length 5 cm), including the descending colon and rectum to a level 4 cm proximal to the anal orifice, were tied off with silk ligatures. A solution of EB (3%) in PBS (total volume 1 ml) was injected into each loop through a fine-needle syringe (0.4 mm outer diameter). The abdomen was then closed with sutures and the colonic loops were exposed to EB for 120 min. The animals were killed by cardiac puncture under ether anesthesia. The colon was rapidly dissected out, and the amount of EB permeated into the gut wall was assayed.

**Assay of EB Permeation.** The colonic loops were opened and rinsed for 1 min × 3 in 6 mM acetylsteine in PBS. After the last rinse, there was only minimal coloring of the solution. The midportions of each loop (length 15–25 mm) were then removed, dried on filter paper to remove excess fluid, weighed, and incubated with 4 ml of formamide (NHO₂) at 50°C for 24 hr in a shaking waterbath. Colorimetric measurements were performed in a spectrophotometer at the peak absorption of 612 nm. Three measurements were performed on each sample, and the mean value was used for calculations, based on external standards in formamide. All analyses were performed in a blinded protocol.

**Immunohistochemistry.** Specimens (N = 3) from both loops in all animals were taken and immediately immersed in cold 4% paraformaldehyde in PBS at pH 7.4. After 4 hr of fixation, the specimens were rinsed several times in PBS and in PBS with 10% sucrose, added for cryoprotection. The specimens were frozen and cryostat-sectioned at 10 µm. The sections were thawed onto gelatin-coated glass slides and incubated for immunofluorescence using the indirect method of Coons using fluorescein isothiocyanate (FITC)-labeled secondary antibodies (8). Peptidic nerves were demonstrated using well-characterized polyclonal antisera against neuropeptide Y (NPY, 1:200 from Dr. Elvar Theodorssör, Linköping) and substance P (SP, 1:200, RPN 1572 Amersham, UK). A polyclonal antiserum directed against the Schwann cell protein (S-100, 1:1000, from Dr. Kenneth Haglund, Göteborg) was also used to demonstrate nerves, perineuronal Schwann cell sheaths, and polydendritic cells (see reference 4). As a control of specificity of the immunoreaction, sections were regularly incubated omitting the primary antisera, ie, only incubated with secondary antisera. In such sections no specific FITC fluorescence was seen over nerves. Occasional cells in lymphoid follicles were labeled with FITC, probably corresponding to Fc-binding receptors, but these cells did not obscure the evaluation of the true presence of polydendritic cells. All sections (coded) contained longitudinal profiles of the villi. The sections were evaluated independently by two investigators. The visual impression of fluorescence intensity and density of nerves in certain locations (mucosa, submucosa, myenteric plexus, and circular muscle layer) and the relative abundance of polydendritic cells in the mucosa were estimated. Semi-quantitative scores for whole individual sections, regarding fluorescence intensity and density of immunoreactive nerves, were often misleading, since DSS treatment induced nerve hyperplasia in limited areas of crypt abnormalities. Between these regions the innervation pattern was normal.

**Other Histological Procedures.** Specimens were prepared from controls and DSS-treated animals with and without lidocaine treatment. After the perfusion with EB and subsequent washing with acetylsteine, small biopsies of each colonic loop were frozen in liquid nitrogen. Cryostat sections, 5 µm, were cut and mounted in DPX after drying. The sections were then viewed in an epifluorescence microscope (Nikon Microphot-FX), using a filter combination optimal for the red EB fluorescence (100-W halogen illuminator, 580-nm dichroic mirror, 510- to 560-nm excitation filter and 590-nm barrier filter) to analyze the permeation into control and experimental tissues.

Sections were also prepared from each colonic loop after