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Effects of Histamine H_1 Antagonist Dithiaden on Acetic Acid-Induced Colitis in Rats

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Summary

To assess the possible involvement of mast cells and/or their mediators in inflammatory bowel diseases, the effect of the histamine H_1 antagonist Dithiaden was studied on a model of acetic acid-induced colitis in rats. Dithiaden pretreatment by intracolonic administration was found to reduce the extent of acute inflammatory colonic injury. This was manifested by a decrease in the score of gross mucosal injury, by lowered colonic wet weight and by diminished myeloperoxidase activity reflecting reduced leukocyte infiltration. Vascular permeability and gamma-glutamyl transpeptidase activity, elevated by acetic acid exposure, were decreased after Dithiaden pretreatment. The results indicate that locally administered Dithiaden may protect the colonic mucosa against an acute inflammatory attack by interfering with the action of the major mast cell mediator histamine.

Key words

Dithiaden • Acetic acid-induced colitis • Histamine • Mast cells • Rats

Introduction

Mast cell involvement has been postulated in inflammatory bowel diseases (IBD) (Hiatt and Katz 1962, Barret and Metcalfe 1984, Sommers 1996). Increased numbers of activated mast cells were found in colons of rats treated with trinitrobenzene sulfonic acid (TNB) and in the intestinal mucosa of patients with active ulcerative colitis (Chin and Barret 1990, Barret 1990). On histological examination, mast cells from active pathological regions often showed signs of degranulation (Dvorak *et al.* 1978, Balaz *et al.* 1989). Increased spontaneous mediator release as well as enhanced responsiveness of human intestinal mast cells were reported (Fox *et al.* 1990). Upon activation, mast cells generate and release various mediators, such as histamine, arachidonic acid metabolites, cytokines, chemotactic factors and neutral proteases. These mediators may be of potential relevance to the pathogenesis of intestinal inflammation and thus contribute to the features of IBD. Histamine, the main mast cell mediator known to increase vascular permeability, leukocyte infiltration and smooth muscle contraction, has been suggested to participate in intestinal inflammation. Rectal biopsies from inflamed areas in IBD spontaneously released histamine (Raithel

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et al. 1989). An increased jejunal secretion of histamine was found in Crohn's disease (Knutson *et al.* 1990). The objective of this study was to determine whether pretreatment with the histamine H_1 antagonist Dithiaden protects the colonic mucosa against an acute inflammatory attack induced by acetic acid administration in rats.

Methods

Induction of colitis

Male Wistar rats weighing 200-280 g were randomly divided into experimental groups. Each group consisted of at least seven rats. The animals were anesthetized by 50 mg/kg pentobarbital i.p. After laparotomy, a reversible ligature at the junction of the caecum and the ascending colon was made. The colon was cleansed of its luminal contents with warm saline (37 °C) and residual fluid was manually pressed out. Distal to the ligature, 2 ml of either vehicle (0.9 % saline) or Dithiaden in doses of 1, 5 or 10 mg/kg were administered. Following 30 min pretreatment, 2 ml of 4 % acetic acid was introduced into the colon and after 40 s exposure the luminal content was expelled by instillation of 10 ml of air, the ligature was then released and the abdomen was sutured. The rats were allowed to recover with food and water and the resulting injury was assessed after 48 h. Colonic vascular permeability was studied 5 min, 30 min, 60 min, and 24 h after acetic acid exposure. For gamma-glutamyl transpeptidase (GGTP) determination the intervals of 24 h, 48 h, 72 h and 7 days were used in separate experiments.

Assessment of colonic damage

The rats were weighed, inspected for the presence of diarrhea and sacrificed by an overdose of ether. The colons were excised and opened longitudinally, rinsed with cold saline and observed under a dissecting microscope. Colonic damage was scored on a 0-5 scale using the scoring system of MacPherson and Pfeiffer (1978). The scoring of colonic damage was performed by an observer unaware of the treatment. Wet weights of colons as well as their dry weights obtained after 48 h incubation at 80 °C were recorded. The colonic wet/dry weight ratio was calculated, so that an indirect index of the inflammatory reaction was obtained.

Colonic myeloperoxidase activity

Myeloperoxidase (MPO) activity was determined in colonic mucosal scrapings 48 h after induction of colitis by the modified method of Bradley *et al.* (1982). MPO activity was assayed spectrophotometrically by determining the decomposition of hydrogen peroxide using o-dianisidine as the hydrogen donor. Tissue samples of approximately 50 mg were weighed and homogenized three times for 30 s at 4 °C in 1 ml of ice-cold 0.5 % hexadecyltrimethylammonium bromide in a 50 mmol/l phosphate buffer (pH 6). The homogenate was subjected to three freeze/thaw cycles and centrifuged for 15 min at 40 000 x *g*. MPO activity was determined spectrophotometrically by the addition of 0.1 ml of the supernatant to 2.9 ml of the 50 mmol/l phosphate buffer containing 0.167 mg/ml o-dianisidine dihydrochloride and 0.0005 % w/v hydrogen peroxide. The change in absorbance at 460 nm over a 5 min period was measured at 25 °C. The data are expressed as the mean absorbance ± S.E.M. at 5 min/g wet weight (Noronha-Blob *et al.* 1993).

Colonic vascular permeability

The vascular permeability of the colon was studied by a modified method of Erickson *et al.* (1992). The rats were anesthetized, a femoral vein catheter was inserted, 1 % Evans blue in 0.9 % saline was injected in a volume of 0.2 ml/100 g b.w. and 30 min later the rats were sacrificed by decapitation. The colons were removed, opened longitudinally and rinsed in saline. Approximately 200 mg segments were taken, weighed and put into formamide in a shaking water bath at 37 °C for 48 h. The amount of Evans blue extracted into the formamide from the tissue sample was then measured spectrophotometrically at 610 nm using Hewlett Packard Vectra 286/12 model spectrophotometer. The amount of extravasated dye was calculated from the standard curve. Tissue Evans blue concentrations were expressed as μg/g wet weight.

Colonic gamma/glutamyl transpeptidase activity

In separate experiments, gamma-glutamyl transpeptidase (GGTP) activity was assessed after 24 h, 48 h, 72 h, and 7 days according to Orlowski and Meister (1970). Briefly, the assay was performed under linear reaction conditions using gamma-glutamyl-p-nitroanilide as donor substrate and methionine as acceptor. Homogenates (1:9 wt/vol) of the colonic mucosa were prepared in 2 ml of homogenizing media consisting of (in mM): NaH_2PO_4 2.6, Na_2HPO_4 50, EDTA 15, NaCl 68, pH 8.1. Samples were homogenized for 3x20 s at 0 °C by means of an Ultra Turax homogenizer (Janke and Kunkel, Germany). Aliquots (500 μl) were incubated at 37 °C for 60 min in 200 μl

buffer substrate solution of 8.7 mM of gamma-glutamyl-p-nitroanilide and 44 mM methionine in isopropyl-alcohol (65 %). The reaction was terminated with 2.3 ml of cold methanol. The tubes were cooled in ice-cold water, the samples filtrated and read at 405 nm in a spectrophotometer (Hewlett Packard Vectra 286/12) against a blank. Reaction mixtures in the absence of either the substrate or the acceptor were used as reference samples.

Drugs

The following drugs were used: Dithiaden® 4-[3-(dimethylamino) propylidene]-4, 9-dihydrothieno [2, 3-b]benzo[e]thiepin (Léčiva, Praha), Evans Blue, reagents in MPO and GGTP assay from Sigma (St. Louis, MO, USA), other chemicals were of analytical grade.

Statistical analysis

The results are expressed as means ± S.E.M. Student's t-test was used for statistical analysis, $P<0.05$ was considered significant. Non-parametric data were analyzed by the Mann-Whitney U test.

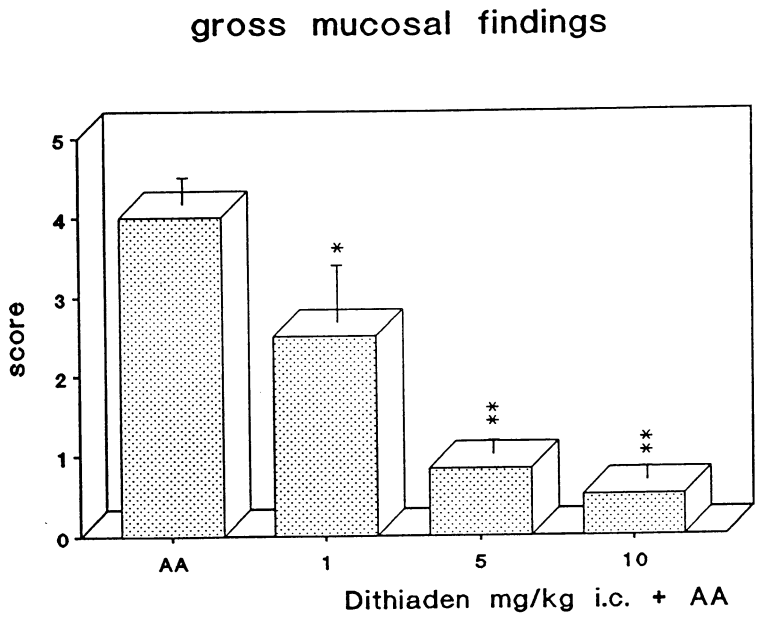

Fig. 1. *Effect of Dithiaden on the score of gross mucosal injury 48 h after induction of colitis by intracolonic administration of 4 % acetic acid (AA). Dithiaden (in doses of 1, 5 or 10 mg/kg) was injected into the ligated colon after laparotomy 30 min before AA. Colonic damage was scored using the criteria of MacPherson and Pfeiffer (1978). Values are means ± S.E.M., * $P<0.05$, ** $P<0.01$ versus acetic acid (vehicle-treated) group.*

Table 1. Effect of Dithiaden on colonic wet weight and wet/dry weight ratio in acetic acid-induced colitis in rats

Treatment	dose mg/kg	wet weight mg/cm	wet/dry weight ratio
sham-operated		65.8±5.7	4.21±0.17
acetic acid + vehicle		97.8±4.0**	4.89±0.07**
acetic acid + Dithiaden	1	95.7±5.3*	4.65±0.10*
acetic acid + Dithiaden	5	91.4±6.7*	4.44±0.21
acetic acid + Dithiaden	10	84.5±6.8	4.40±0.09++

* $p<0.05$, ** $p<0.01$ *versus control (sham-operated) rats,* ++$p<0.01$ *vehicle versus Dithiaden-treated rats*

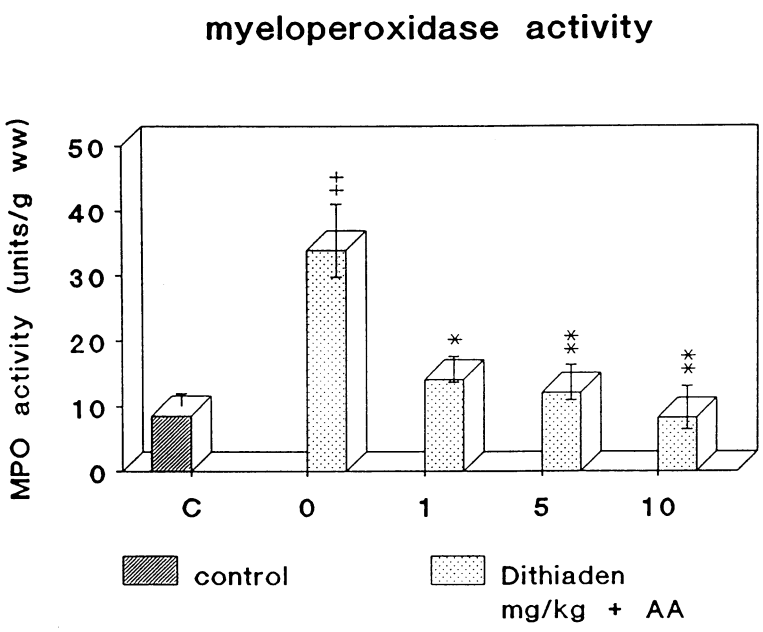

Fig. 2. *Effect of Dithiaden on myeloperoxidase (MPO) activity measured in colonic mucosal scrapings 48 h after induction of colitis by intracolonic administration of 4 % acetic acid (AA). For further details of Dithiaden pretreatment see Fig. 1. Values are means ± S.E.M., expressed as units MPO/g wet weight, ++ P<0.01 versus control (sham-operated) rats, * P<0.05, ** P<0.01 vehicle versus Dithiaden-treated rats.*

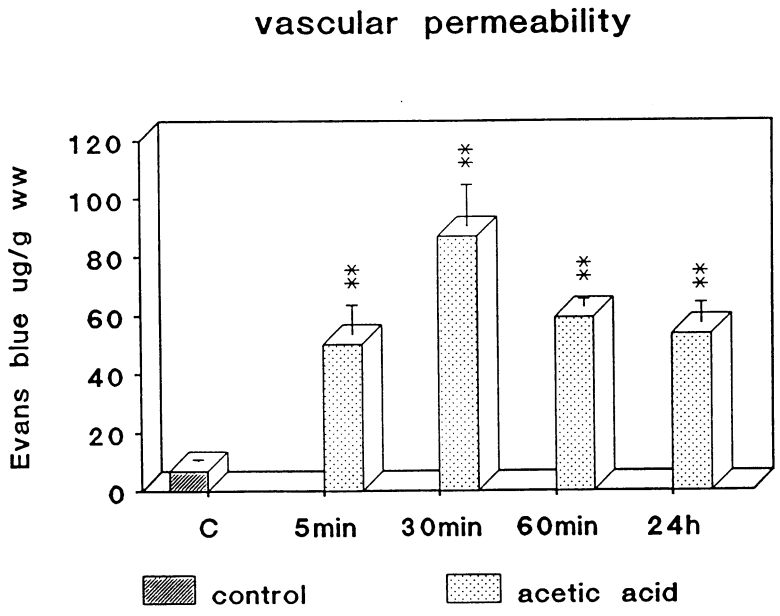

Fig. 3. *Vascular permeability measured by the extravasation of i.v. injected Evans blue in the rat colon during the development of colitis induced by the administration of 4 % acetic acid in anesthetized rats. Vascular permeability was measured 5 min, 30 min, 60 min, and 24 h after the induction of colitis. Values are means ± S.E.M., expressed as μg Evans blue/g wet weight, **P<0.01 versus control (sham-operated) rats.*

Results

Intracolonic administration of 4 % acetic acid induced an acute inflammatory reaction in control (vehicle-treated) rats. Diffuse hyperemia and bleeding with erosions and ulcerations were present in the colon 48 h after acetic acid administration. Dithiaden pretreatment significantly reduced the gross mucosal injury in a dose-dependent manner (Fig. 1). The mean score of 4.0 in vehicle-treated rats decreased to 2.5 after 1 mg/kg Dithiaden, to 0.83 after 5 mg/kg and to 0.5 after 10 mg/kg Dithiaden. The incidence of diarrhea was also diminished after Dithiaden pretreatment.

In the vehicle-treated rats, the mean value of the colonic wet/dry weight ratio increased significantly from 4.2 to 4.9 48 h after acetic acid exposure. In the rats pretreated with 1, 5 and 10 mg/kg Dithiaden, the respective mean values of 4.65, 4.44, and 4.40 were

obtained. Similarly, there was a significant increase of colonic wet weight after acetic acid administration which was reduced by Dithiaden pretreatment (Table 1).

Colonic MPO activity was used to quantify neutrophil infiltration. The results showed a pronounced increase of MPO activity 48 h after the induction of colitis which was diminished by Dithiaden pretreatment (Fig. 2).

Vascular integrity of the colon was assessed by i.v. Evans blue administration. An increase of colonic vascular permeability was observed 5 min, 30 min, 60 min, and 24 h after acetic acid administration. Maximal extravasation was reached 30 min after acetic acid exposure (Fig. 3). Dithiaden was found to attenuate this enhanced permeability in a dose-dependent manner (Fig. 4). The protective effect of Dithiaden was also detected at the 24 h interval (data not shown). Dithiaden (10 mg/kg) reduced the Evans blue values from 53.4 μg/g in untreated rats to 23.6 μg/g in treated rats.

The activity of GGTP increased gradually during the inflammatory reaction. Elevated levels were reached within 48 h after acetic acid administration and remained significantly higher for further 7 days (Fig. 5). Dithiaden in doses of 5 and 10 mg/kg prevented the maximal increase in GGTP measured 72 h after acetic acid exposure (Fig. 6).

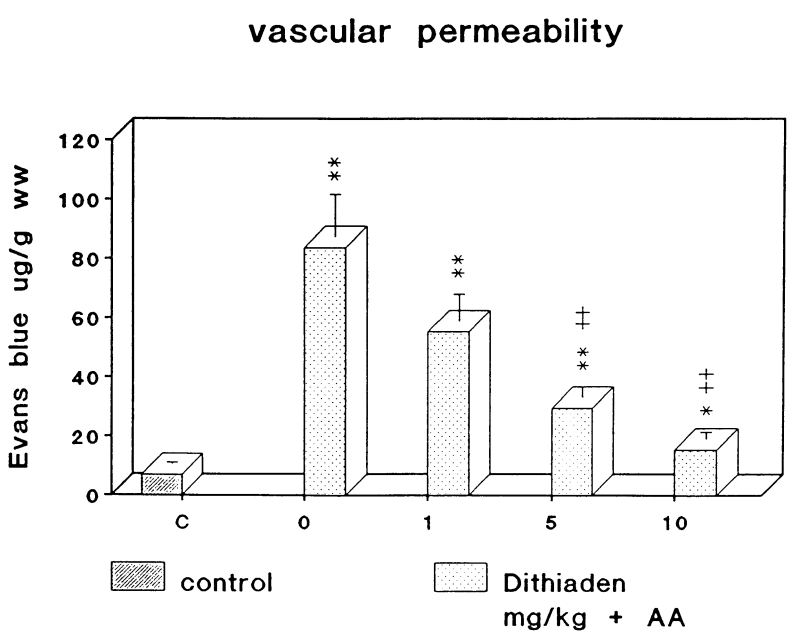

Fig. 4. *Effect of Dithiaden on vascular permeability measured in the rat colon 30 min after induction of colitis by intracolonic administration of 4 % acetic acid (AA). For further details of Dithiaden pretreatment see previous figures. Values are means ± S.E.M., expressed as μg Evans blue/g wet weight, * P<0.05, ** P<0.01 versus control (sham-operated) rats, ++ P<0.01 vehicle versus Dithiaden-treated rats.*

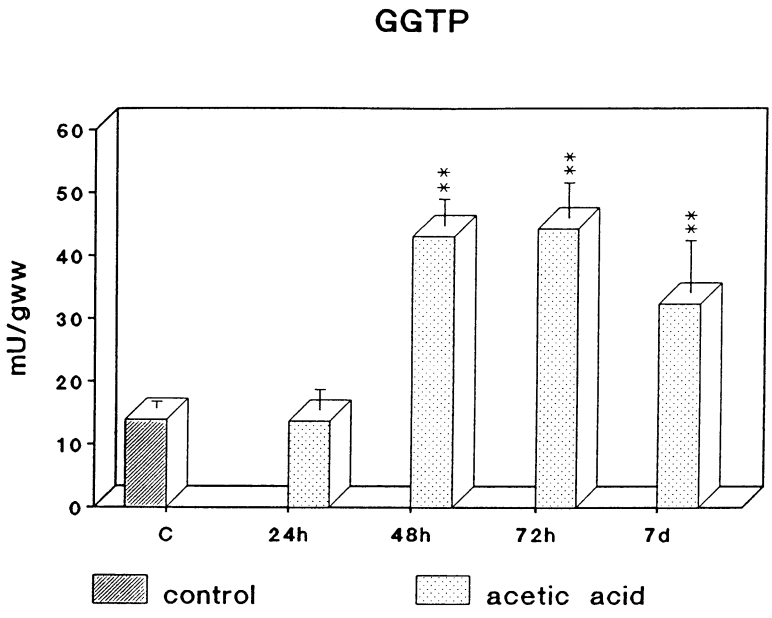

Fig. 5. *Gamma-glutamyl transpeptidase (GGTP) activity in the rat colonic mucosa during the development of acetic acid-induced inflammation. GGTP was measured 24 h, 48 h, 72 h, and 7 days after intracolonic administration of 4 % acetic acid to anesthetized rats. Values are means ± S.E.M., expressed in nmol/min/g wet weight, ** P<0.01 versus control (sham-operated) rats.*

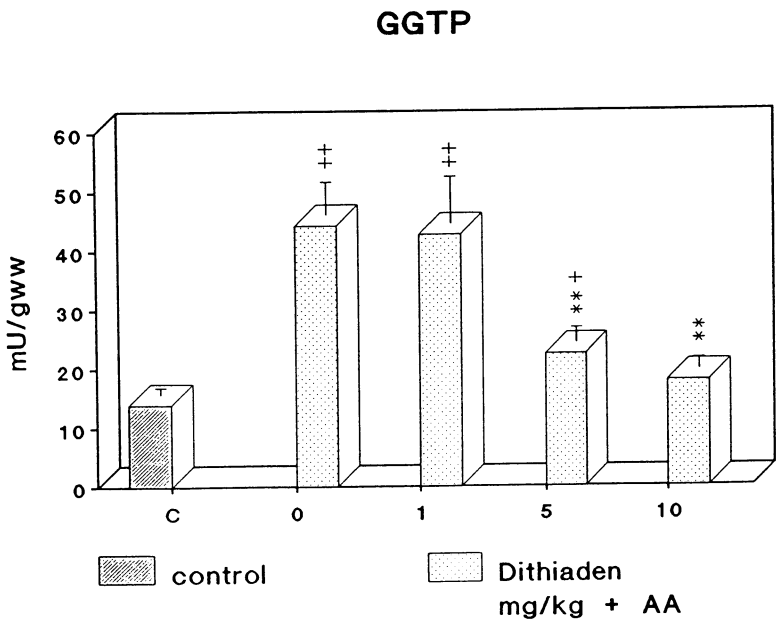

Fig. 6. *Effect of Dithiaden on the activity of gamma-glutamyl transpeptidase (GGTP) in the colonic mucosa of rats with colitis induced by 4 % acetic acid (AA). Dithiaden was administered in doses of 1, 5 and 10 mg/kg into the colon 30 min before acetic acid and GGTP activity was determined 72 h afterwards. Values are means ± S.E.M., expressed in nmol/min/g wet weight, ++ P<0.01 versus control (sham-operated rats), ** P<0.01 vehicle versus Dithiaden-treated rats.*

Discussion

The results demonstrated the protective effect of Dithiaden in a model of acute colitis in rats. The blockade of histamine H_1 receptors prevented the development of early inflammatory reaction, suggesting the involvement of mast cells and their major mediator histamine in this process. Our findings are in agreement with the observation of Binder and Huidberg (1967), Raithel *et al.* (1989), Fox *et al.* (1990) and Knutson *et al.* (1990), who reported an increased histamine content and/or release in the intestinal samples from patients with active ulcerative colitis and/or Crohn's disease. Inflamed tissue in Crohn's disease was found to contain lower amounts of diamine oxidase activity, a main catabolic enzyme for histamine, thus suggesting an accumulation of released histamine at the sites of inflammation (Schmidt *et al.* 1990). Mast cell involvement was also observed in gastritis with or without *Helicobacter pylori* infection. A significantly greater density of mast cells was reported in the mucosa of patients with gastritis than in the gastric mucosa of non-infected subjects. After eliminating the infection, the mast cell density decreased. Mast cell degranulation was demonstrated by electron microscopy in *Helicobacter pylori* infected mucosa (Nakajima *et al.* 1997). The administration of a mast cell stabilizer ketotifen in two models of colitis induced by TNB or acetic acid in rats proved to be effective in preventing mucosal damage and an inflammatory reaction. The protection provided by ketotifen was accompanied by a significant decrease in the release of some mediators from mast cells, i.e. LTB_4, PAF, PGE_2, and TXB_2 (Eliakim *et al.* 1992, 1995).

However, inflammation could still be induced by TNB in mast cell-deficient W/W^V mice, suggesting that mast cells were not essential for the expression of the inflammatory response (Chin and Barret 1994). Nevertheless, the colons of W/W^V mice still contained measurable amounts of histamine at approximately 10-20 % of the levels in littermate controls (Gelbmann *et al.* 1995). Thus, other sources of histamine besides mast cells might also be available. The possibility cannot be ruled out that in the mast cell-deficient mice other cell types would take over the role normally played by mast cells (Minocha *et al.* 1995).

The fact that many of the mediators produced by mast cells are not specific for these cells, but can be synthesized by different cells, may account for the problems arising in studying the role of mast cells and their mediators in inflammatory bowel disease (Gelbmann and Barret 1995). In rodents, histamine is stored within enterochromaffin-like cells restricted mainly to the stomach, while in other gastrointestinal regions there is a correlation between mast cell number and tissue histamine content (Saavedra-Delgado *et al.* 1984).

The effects of Dithiaden observed in our experiments indicate that this agent might be effective in the treatment of ulcerative colitis by blocking the action of histamine, as reflected by the reduction of increased vascular permeability and edema formation, by attenuation of increased MPO activity, thus suppressing leukocyte infiltration, as well as by other indices of inflammation. Measurement of GGTP activity in inflammation proved to be a valuable biochemical marker for the assessment of anti-inflammatory activity

of drugs *in vivo* (Singh *et al.* 1986). GGTP plays an important role in the turnover of glutathione and in protein biosynthesis. Since both catabolic and anabolic steps are activated in inflammation, GGTP activity is altered during the development of the inflammatory process. The increase in GGTP activity observed in our experiments on the colitis model corresponds to the findings on other inflammatory models (such as carrageenin edema, cotton pellet granuloma, adjuvant arthritis), in which protective effects similar to that of Dithiaden were found using different anti-inflammatory drugs, e.g. indomethacin or prednisolone.

The mechanism of Dithiaden anti-inflammatory action is not yet fully elucidated. The possibility of H_1 receptor-independent effects of Dithiaden, e.g. anticholinergic, local anesthetic effect, or inhibition of platelet aggregation should also be taken into account. Since other drugs effective in IBD, e.g. sulfasalazine, 5-aminosalicylic acid and steroids, were also shown to affect mast cells (Goldsmith *et al.* 1990, Fox *et al.* 1991), the blockade of mast cell mediator(s) release and/or action seems to be a promising approach to the treatment of colitis.

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