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Ketotifen effectively prevents mucosal damage in experimental colitis

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Abstract

The effects of ketotifen, a 'mast cell stabiliser,' on two models of experimental colitis were examined. The inflammatory response elicited by either trinitrobenzene sulphonic acid or acetic acid resulted in increased colonic synthesis of platelet activating factor, prostaglandin E_2, thromboxane B_2, leukotrienes B_4 and C_4, and myeloperoxidase activity. Intragastric administration of ketotifen 100 μg/100 g twice daily significantly decreased mucosal damage when given prophylactically 48 hours before the induction of colitis and then throughout the experiment. This effect was consistent in both models and was accompanied by a significant reduction in mucosal generation of platelet activating factor, prostaglandin E_2, thromboxane B_2, and leukotrienes C_4 and B_4. Myeloperoxidase activity was reduced as well, reaching significance only in the acetic acid model. This study shows that both trinitrobenzene sulphonic acid and acetic acid colitis can be pharmacologically manipulated by ketotifen. The mechanism of action of ketotifen has not yet been determined. Ketotifen's potential in the treatment of active inflammatory bowel disease or in the prevention of exacertations, or both, remains to be elucidated.

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The inflamed mucosa in ulcerative colitis and Crohn's disease is infiltrated by equally prominent components of neutrophils, macrophages, and lymphocytes.[1-3] The presence of neutrophils along with macrophages suggests involvement of soluble chemotactic mediators causing migration of inflammatory cells into the mucosa.[4] Mast cells are involved in both the induction and amplification of the inflammatory processes in the intestine.[5] Increased numbers of activated mast cells and eosinophils are present in the active site in inflammatory bowel disease and are a component of the granuloma in Crohn's disease.[1 6-8] We and others have recently characterised mediators involved in a model of acute and chronic granulomatous colitis induced by trinitrobenzene sulphonic acid (TNB) dissolved in ethanol and administered rectally.[9 10] We have also established that ketotifen (Zaditen), a mast cell stabiliser, very effectively protects the gastric mucosa against damage induced by a variety of agents.[11]

This study aimed to assess the possible protective effect of ketotifen against the damage and inflammation in two experimental models of colitis induced by TNB and acetic acid.[12]

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Methods

All of the animal studies described here adhere to the standards established by the *Guide for the care and use of laboratory animals*.[13]

COLITIS INDUCTION TNB MODEL

Male rats (Hebrew University strain), weighing 200–250 g, and fed ad libitum were used in all the studies. Inflammation of the colon was induced under light ether anaesthesia by a single intracolonic administration of 0·25 ml of 50% ethanol containing 30 mg of TNB (Sigma, Israel) as previously described.[10] The solution was introduced via a catheter with a 0·3 mm outer diameter placed 7 cm from the anus. The rats were killed at 24 hours, 48 hours, one, two, or three weeks after the induction of colonic injury. The colon was isolated and a 10 cm segment of the distal colon proximal to the anus was resected, its lumen rinsed with ice cold saline, and weighed. A cross section was obtained for histology and the remaining mucosa was scraped, minced, and stored at 4°C. Samples of these mucosal scrapings were processed for determination of prostaglandin E_2 (PGE_2), thromboxane B2 (TxB_2), leukotriene B4 (LTB_4), leukotriene C4 (LTC_4), platelet activating factor (PAF), and myeloperoxidase (MPO) activity.

Treated rats were given ketotifen (100 μg/100 g) twice daily intragastrically 48, 24 or 12 hours before induction of damage, and, thereafter, twice daily until they were killed. In another experiment, ketotifen was given once two hours before the induction of damage and throughout the experiment until the rats were killed.

ACETIC ACID INDUCED COLITIS

Hebrew University strain male rats weighing 200–250 g were fasted for 24 hours. Under light ether anaesthesia, a midline abdominal incision was made, the colon was isolated, and the junction of caecum and ascending colon ligated. Two ml of 5% acetic acid were injected into the lumen of the colon at its proximal part through a 25 gauge needle, followed by 3 ml of air which cleared most of the acetic acid from the colon.[12] The midline incision was closed. Twenty four hours later the rats were killed and their colons removed and handled, as with the TNB model. Treated rats received ketotifen (100 μg/100 g) intragastrically, twice daily, 48 hours before the induction of damage and throughout the experiment.

DETERMINATION OF MUCOSAL DAMAGE

Mucosal damage was quantitated by the scoring system of Wallace *et al.*[14] In this system: 0=no damage; 1=hyperaemia, no ulcers; 2=linear ulceration without hyperaemia or bowel wall thickening; 3=linear ulcer with inflammation at one site; 4=two or more sites of ulceration and inflammation; 5=two or more sites of major ulceration and inflammation, or one major site of damage extending more than 1 cm along the length of the colon; 6–10=when area of ulceration and inflammation extend more than 2 cm along the length of the colon, the score is increased by one mark for each additional cm of involvement. Mucosal damage was also measured macroscopically and expressed in mm^2/rat. All scoring and measurements of damage were performed by two observers using a stereomicroscope. For the purpose of scoring, inflammation was defined as hyperaemia and thickening of the bowel wall.

MORPHOLOGICAL STUDIES

Sections of colon were obtained from the same areas of the large intestine during autopsy. They were fixed in phosphate buffered formaldehyde, embedded in paraffin, and routine 5 μm sections were prepared. Tissues were routinely stained with haematoxylin and eosin and were evaluated by light microscopy.

DETERMINATION OF MPO ACTIVITY

Two hundred mg of mucosal scrapings were homogenised three times for 30 seconds at 4°C with a polytron (Kinematica GmbH, Kriens-Luzern, Switzerland) in 1·0 ml of ice cold 0·5% hexadecyltrimethylammonium bromide in 50 mM phosphate buffer, pH 6·0. The polytron probe was rinsed twice with 1·0 ml of the buffer and the washings were added to the homogenate. The homogenate was then sonicated for 10 seconds, freeze thawed three times, and centrifuged for 15 minutes at 40 000 g. An aliquot of the supernatant was taken for determination of the enzyme activity, according to Bradley *et al.*[15]

DETERMINATION OF CYCLO-OXYGENASE AND LIPO-OXYGENASE PRODUCTS

One hundred and fifty mg of mucosa were placed in preweighed tubes containing 1 ml of phosphate buffer (50 mM, pH 7·4). The mucosa was minced with scissors and centrifuged in an Eppendorf centrifuge for 10 seconds. The pellet was resuspended in 1 ml of the above buffer, incubated for one minute in a vortex mixer, indomethacin was added, and the tubes centrifuged for 60 seconds. The supernatants were kept at −20°C until radioimmunoassays were performed. The capability of the mucosa to generate PGE_2, TxB_2, LTC_4, and LTB_4 was expressed as ng/g wet tissue weight.

DETERMINATION OF PGE_2

PGE_2 was determined by a modification of the radioimmunoassay previously reported.[16] Briefly, 0·1 ml of the specific monoclonal anti-PGE_2 was added to either 0·1 ml of standards or to the tested solutions, followed by the addition of 0·1 ml of $^3HPGE_2$ (4500–5500 cpm). After overnight incubation at 4°C, the antibody bound to $^3HPGE_2$ was separated from the free PGE_2 by adding 0·2 ml of the mixture of 1% activated charcoal (Fisher Scientific Co, Fair Lawn, NJ) and 0·1% Dextran T-70 (Pharmacia, Uppsala, Sweden) in PBS to each sample. After vortexing, the mixture was centrifuged at 3000 rpm for 10 minutes in a refrigerated centrifuge (4°C). The supernatant was transferred into scintillation vials to which 7 ml of scintillation fluid were added and counted for one minute each in a beta scintillator counter. PGE_2 (Sigma, Petach Tikva, Israel) was prepared as a stock solution (1 mg/ml absolute ethanol) and kept at −20°C. A working dilution (10 ng/ml) was freshly prepared in PBS for standards ranging from 0·15–10 ng/ml. Specific monoclonal anti-PGE_2 was purchased from Interpharm (Rehovot, Israel). Scintillation fluid was prepared by mixing 1:2 (vol/vol) lumax (Lumac, Landgraff, Netherlands) and toluene (Sigma). TxB_2 values were determined via a commercial radioimmunoassay kit (RIA) (Amersham, England).

MEASUREMENT OF LTB_4

LTB_4 immunoreactivity was determined by a RIA kit (Amersham, TRK 940). The assay combines the use of a high specific activity leukotriene B_4 tracer, an antiserum specific for LTB_4 (cross reactivity 100%) and a leukotriene standard (range 1·6 to 200 pg/tube). The specific binding of tracer is 42·5%, non-specific binding 2·4%. Fifty per cent B/Bo displacement is obtained with 15 μg/tube and 90% B/Bo displacement with 2·2 pg/tube of LTB_4.

MEASUREMENT OF CYSTEINYL LEUKOTRIENES

Monoclonal rat antileukotrienes (C_4, D_4, and E_4) were used for the quantification of cysteinyl containing leukotrienes by RIA. The monoclonal rat antileukotriene reacts specifically with LTC_4, LTD_4, and LTE_4 using dextran coated charcoal RIA. It shows the following cross reactivity in competitive RIA: per cent cross reactivity at 50% displacement for LTA_4 <2, LTB_4 <1, LTC_4= 100, LTD_4, and LTE_4 >80. The buffer used in RIA is phosphate buffer, 10 mM, pH 7·4 containing 130 mM NaCl, 0·1% N_3Na, and 3% normal horse serum. The working dilution for the monoclonal antileukotriene was 1:1000. Standard stock solutions of leukotriene (10 μg/ml) were prepared in absolute ethanol. Working standard was diluted in phosphate buffer to a concentration of 10 ng/ml and further doubling dilutions were prepared (range from 10 ng/ml to 0·078 ng/ml). Radiolabelled leukotriene, a fresh solution of 50 000–10 000 dpm/ml of tritiated leukotriene, was prepared in phosphate buffer. Dextran coated charcoal was prepared as follows: 1% (w/v) Norit A activated charcoal and 0·1% (w/v) dextran T-70 in buffer without horse serum. The scintillant used was toluene:lumax (2:1).

RIA was performed as follows: One hundred μl of either standard or sample buffer were

pipetted into assay tubes and 100 μl of antibody was added to all tubes except for total and blank tubes. The tubes were incubated at 4°C for 30 minutes. One hundred μl tritiated leukotriene were added to all tubes and incubated at 4°C overnight. Two hundred μl cold buffer were added, followed by 200 μl dextran coated charcoal solution, excluding the total tube to which 200 μl buffer was added. The tubes were vortexed, incubated at 4°C for 10 minutes, and centrifuged in a refrigerated Beckman centrifuge at 3000 rpm for 10 minutes. The supernatant was transferred to scintillation vials to which 7 ml scintillation fluid was added and was counted in a Kontron liquid scintillation spectrometer.

PAF DETERMINATION

Extraction of PAF from colonic mucosa

To extract PAF from the mucosa, 0·5 ml of ethanol 80% was added to preweighed gastric mucosa (20–30 mg) 24 hours before performance of the aggregation assay.

Platelet preparation

Fifty ml of rabbit venous blood were collected into Falcon tubes, mixed with 1 ml 0·2 M ethylenediaminetetraacetic acid and centrifuged for 20 minutes at 1600 rpm. Plasma was transferred to another tube and centrifuged for 15 minutes at 3000 rpm. The platelet pellet was reconstituted with a washing buffer (pH 6·5) containing 2·6 mM KCl, 1 mM $MgCl_2$, 137 mM NaCl, 0·2 mM ethyleneglycol-bis(beta-aminoethylether)-N,N′-tetra-acetic acid, 5·5 mM glucose, and 0·25% gelatine and centrifuged for 15 minutes at 3000 rpm. The platelet pellet was resuspended in the above buffer and treated with 0·1 mM aspirin for 15 minutes at room temperature, followed by another centrifugation for 15 minutes at 3000 rpm. Platelets were resuspended in washing buffer at appropriate concentrations for the measurement of platelet aggregation.

Aggregation assay

PAF activity was measured by platelet aggregation using a Chrono-log Corporation aggregometer. Platelets were stirred in 400 μl buffer containing 2·6 mM KCl, 1 mM $MgCl_2$, 137 mM NaCl, 12 mM $NaHCO_3$, 1·5 mM $CaCl_2$, 5·5 mM glucose, 0·25% gelatine, 1 mM creatine phosphate, and 10 U/ml creatine phosphokinase (pH 7·4). Samples of 10 μl were added and aggregation recorded. WEB 2086 BS inhibited dose dependently (0·17–17 μM) the PAF induced platelet aggregation (r=0·891; y=140–8·8x).

STATISTICAL ANALYSIS

Data are expressed as the mean (SEM). Statistical analysis for significant differences was performed according to the Student's *t* test for unpaired data and the non-parametric Mann-Whitney U test.

MATERIALS

Acetic acid (Frutarom, Israel); aspirin (Aspegic, Lab Egic, Amilly, France); creatine phosphate, creatine phosphokinase, fatty acid free bovine serum albumin LTC_4, indomethacin, trinitrobenzene sulfonic acid (all from Sigma Laboratories, Israel); PAF (C18) (Bachem, Switzerland); ketotifen (Wander Ltd, Berne, Switzerland); LTB_4 RIA; ^{3}H-LTC_4 (Amersham, England); LTC_4 antibody (Biomakor, Israel); TxB_2 RIA (Amersham, England).

Results

TNB/ETHANOL INDUCED COLITIS

Mucosal damage

Intracolonic administration of TNB/ethanol resulted in extensive haemorrhagic and ulcerative damage to the proximal colon, as reported previously.[9,10] By 24 hours, the damage was localised to the distal colon, lesion score being 9·0 (0·6) and the lesion area 604 (61) mm² (Table

TABLE I *Effect of ketotifen on trinitrobenzene sulphonic acid (TNB) induced colitis in rats killed 24 and 48 hours and 3 weeks after induction of injury (Values, mean (SEM) (range))*

		24 h		48 h		3 wk	
	Control	TNB/ethanol	TNB/ethanol +ketotifen	TNB/ethanol	TNB/ethanol +ketotifen	TNB/ethanol	TNB/ethanol +ketotifen
No	9	6	9	15	7	5–11	12
Lesion score	0	9·0 (0·6)* (7–10)	4·8 (1·0)** (1–10)	7·6 (0·6)* (0–10)	3·0 (0·9)** (0–7)	7·4 (0·7)* (3–10)	0·8 (0·1)** (0–2)
Lesion area (mm²)	0	604 (61)* (400–825)	314 (61)** (25–600)	386 (64)* (0–900)	85 (40)** (0–324)	624 (98)* (230–1392)	152 (34)** (0–400)
Weight (g/10 cm)	0·7 (0·02) (0·6–0·8)	1·4 (0·07)* (1·1–1·6)	1·3 (0·07) (1·1–1·8)	1·5 (0·1) (0·7–2·4)	1·2 (0·1) (0·8–1·6)	1·73 (0·18)* (0·9–2·8)	1·7 (0·1) (0·9–2·5)
MPO (U/g)	0·6 (0·06) (0·2–0·9)	4·1 (0·65)* (1·6–6·4)	4·7 (0·1) (1·2–9·3)	6·4 (1·0)* (1·0–16·5)	5·2 (1·3) (0·9–9·7)	3·0 (0·7)* (1·3–5·2)	1·6 (0·4) (0·1–4·3)
LTB_4 (ng/g)	3·2 (0·4) (1·6–5·1)	4·7 (0·4)* (3·1–5·8)	6·2 (1·01) (2·2–12·0)	14·4 (1·4)* (5·1–27·1)	12·8 (2·0) (3·0–27·1)	12·4 (2·0)* (3·8–21·4)	5·2 (0·5)** (0·9–7·6)
LTC_4 (ng/g)	2·4 (0·2) (1·1–4·5)	8·6 (2·4)* (2·6–20·0)	4·2 (1·0) (1·3–12·1)	11·6 (2·3)* (2·3–36·0)	8·3 (1·6) (1·5–16·3)	16·0 (2·5)* (7·4–40·3)	3·1 (0·5)** (0·4–6·5)
PAF (pg/10 mg)†	17·3 (1·45) (9·8–58·3)	21·0 (4·5) (5·5–40·0)	11·3 (1·5)** (5·6–17·0)	27·2 (6·2)* (9·6–105·0)	10·7 (1·5)** (6·4–15·8)	20·8 (3·4) (3·5–41·5)	21·4 (0·8) (17·8–41·5)
PGE_2 (ng/g)	29·9 (1·1) (23·0–36·0)	18·6 (3·3)* (8·5–32·0)	23·5 (1·4) (16·4–31·0)	63·3 (9·5)* (17·0–157·0)	78·9 (10·0) (47·6–125·0)	35·0 (2·7) (28·2–62·0)	32·6 (1·5) (22·0–41·0)
TxB (μg/g)	0·44 (0·02) (0·2–0·5)	0·28 (0·02)* (0·2–0·4)	0·35 (0·03) (0·2–0·5)	1·1 (0·2)* (0·7–2·3)	1·2 (0·1) (0·8–1·5)	0·8 (0·1)* (0·6–1·3)	0·4 (0·016)** (0·3–0·5)

*Significantly different from control rats $p<0·05$; **significantly different from TNB treated rats only at the respective time interval $p<0·05$ (Mann-Whitney U test); †for control n=17; ‡for control n=16.
MPO=myeloperoxidase activity; LT=leukotriene; PAF=platelet activating factor; PGE_2=prostaglandin E_2; TxB_2=thromboxane B_2.

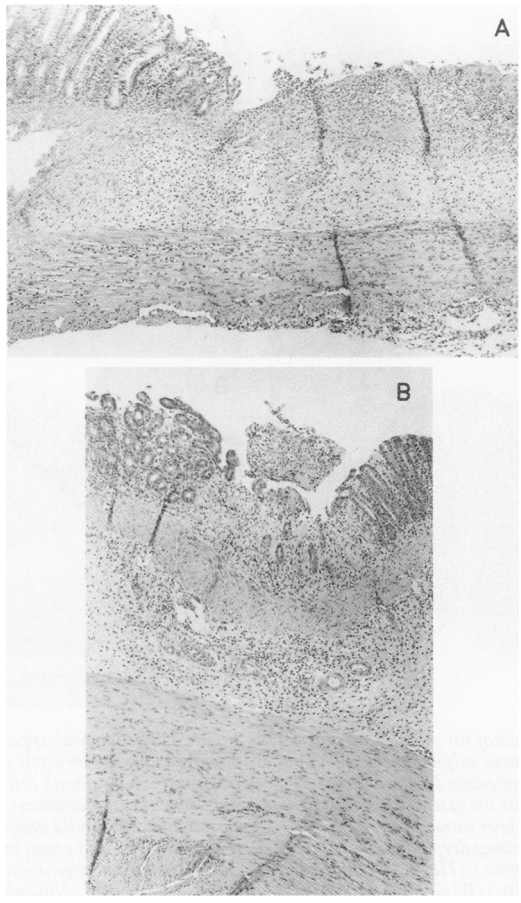

Figure 1: Histological appearance of rat colon 48 hours after damage induction by trinitrobenzene sulphonic acid (TNB) ethanol. (A) Sections from TNB treated large intestine showing a severe wide ulceration accompanying an extensive inflammatory cell infiltrate. Note that the inflammatory cells invade the muscularis propria (right lower corner). (Haematoxylin and eosin, original magnification ×10.) (B) Sections from ketotifen protected large intestine showing a small ulcer accompanied by an acute inflammatory cell infiltrate confined to the mucosa and submucosa. The muscularis propria is spared. (Haematoxylin and eosin, original magnification ×10.)

I). The lesion area increased to 878 (206) mm² a week after induction of injury, and fell to 624 (98) mm² after three weeks, the score being 7·4 (0·7) (Table I). MPO activity paralleled the acute inflammation, reaching a peak of 6·4 (1·0) U/g 48 hours after induction of injury (Table I). Mucosal damage was accompanied by diarrhoea in all the untreated rats.

Effects of ketotifen on mucosal damage

Ketotifen had no effect on the severity of mucosal damage when given two hours beforehand and continued after the induction of colonic inflammation by TNB – (lesion scores 8·9 (0·4) (n=9) and 9·0 (0·6) (n=6) and lesion areas 969 (97) mm² and 604 (61) mm², respectively in ketotifen treated and control rats sacrificed 24 hours after the induction of damage. In rats sacrificed 48 hours after the induction of injury, no protection by ketotifen was observed as well. In another experiment rats were given ketotifen (100 μg/100 g twice daily) 24 and 12 hours beforehand and continued after the induction of injury, and were sacrificed three days later. No significant effect was seen on the severity of mucosal damage: lesion scores 7·0 (1·6) and 7·3 (1·4) (n=5), respectively, in the 24 and 12 hour ketotifen groups *versus* 7·0 (0·6) (n=10) in the TNB group only. In contrast, ketotifen (100 μg/100 g twice daily) given intragastrically 48 hours beforehand and continued after the induction of injury, significantly decreased mucosal damage at all time intervals examined (Table I).

The protective effect of ketotifen was not accompanied by a significant reduction in MPO activity, although there was a trend towards lower MPO values after three weeks (Table I). Mucosal LTB_4, LTC_4, and TxB_2 generation were significantly reduced in ketotifen treated rats three weeks after damage induction (Table I), whereas mucosal PGE_2 generation was significantly lower only at two weeks: 28·4 (4·2) (n=6) compared with 52·6 (6·6) in TNB treated rats ($p<0·05$). Twenty four and 48 hours after damage induction, mucosal PAF values were significantly lower in ketotifen treated rats (Table I). Three weeks after the induction of damage by TNB all rats had diarrhoea (defined as watery stool), compared with only 10% of rats in the group treated with ketotifen. The protective effect was not accompanied by a reduction in the wet weight of the involved segment.

TABLE II *Effect of ketotifen on acetic acid induced colitis in rats killed 24 hours after induction of colitis (values mean (SEM) (range))*

	Treatment		
	None	*Acetic acid*	*Acetic acid +ketotifen*
No	9	9	8
Weight (g/10 cm)	0·7 (0·02) (0·6–0·8)	1·0 (0·1)* (0·7–1·5)	0·9 (0·04) (0·6–1·0)
Lesion score	– –	5·8 (0·6)* (3·0–9·0)	1·8 (0·4)** (0–4)
Lesion area (mm²)	– –	213 (46)* (11·0–508·0)	26·0 (9·5)** (0–82·0)
MPO (U/g)	0·6 (0·1) (0·2–0·9)	3·7 (0·7)* (1·0–7·7)	1·9 (0·3)** (1·1–2·2)
PGE_2 (ng/g)	29·9 (1·1) (23·0–36·0)	46·2 (4·1)* (28·0–70·0)	32·2 (1·4)** (26·0–40·0)
LTB_4 (ng/g)	3·2 (0·4) (1·6–5·1)	5·0 (1·1) (0·8–11·6)	5·7 (0·4) (4·4–7·8)
LTC_4 (ng/g)†	2·4 (0·2) (1·1–4·5)	3·6 (0·4)* (2·0–5·2)	1·9 (0·3)** (1·2–2·3)
TxB_2 (μg/g)	0·4 (0·02) (0·2–0·5)	0·5 (0·02) (0·4–0·6)	0·5 (0·02) (0·4–0·6)

*Significantly different from control rats, $p<0·05$.
**Significantly different from rats treated with acetic acid only, $p<0·05$ (non-parametric Mann-Whitney U test).
†For LTC_4 controls n=17.

Histology

Sections from the large intestine of rats sacrificed 48 hours after the induction of injury (Fig 1A) showed severe widespread ulcerations with extensive inflammatory cell exudate consisting mainly of polymorphonuclear leukocytes. The inflammatory exudate infiltrated all layers of the intestine, including the muscularis propria. Sections from the large intestine of rats treated with ketotifen showed much smaller ulcerations (Fig 1B) with a milder inflammatory response and infiltration into the lamina propria. The TNB treated rats which were followed for 21 days and killed afterwards, showed extensive and widespread ulcerations of the mucosa, as well as extensive infiltration of the inflammatory cells with production of granulation tissue (Fig 2A). On the other hand, ketotifen protected rats that were killed after 21 days showed almost normal mucosa (Fig 2B).

ACETIC ACID INDUCED COLITIS

Mucosal damage

As in the TNB model, and as previously reported,[12] acetic acid induced extensive colitis. Twenty four hours after administration the

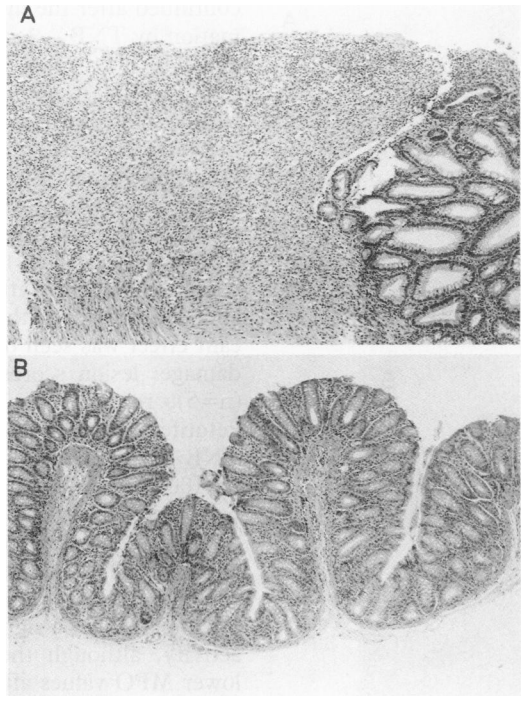

Figure 2: Histological appearance of rat colon 21 days after damage induction by trinitrobenzene sulphonic acid (TNB) ethanol. (A) Section from large intestine 21 days after induction of colitis by TNB. Note the extensive and deep ulceration of the mucosa (right upper corner) with the widespread inflammation penetrating deeply into the muscularis propria (left lower corner). (Haematoxylin and eosin, original magnification ×10.) (B) Section from large intestine of a ketotifen protected rat 21 days after induction of colitis by TNB. The mucosa looks normal without ulcerations with only a very mild inflammatory response confined to the mucosa. (Haematoxylin and eosin, original magnification ×10.)

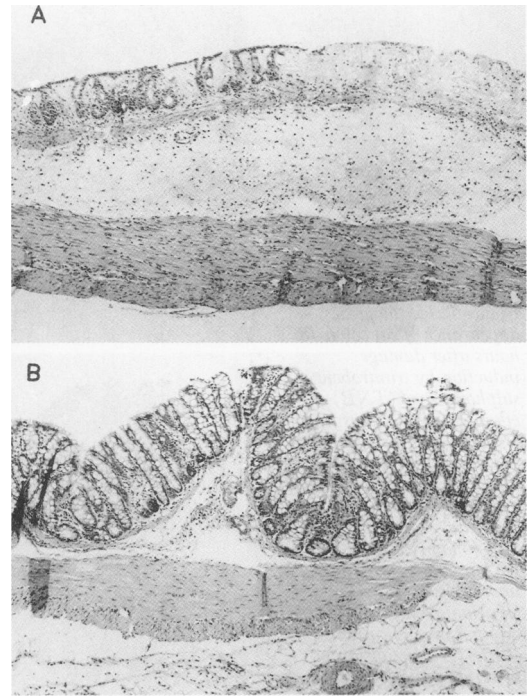

Figure 3: Histological appearance of rat colon 24 hours after damage induction by acetic acid. (A) Sections of large intestine from a rat with colitis induced by acetic acid. Note small superficial ulceration (right upper corner) with extensive edema and only a mild acute inflammatory response. (Haematoxylin and eosin, original magnification ×10.) (B) Section from large intestine of ketotifen protected acetic acid induced colitis. Note almost normal mucosa with only very mild inflammation. (Haematoxylin and eosin, original magnification ×10.)

lesion score reached 5·8 (0·6) and the lesion area was 213 (46) mm^2. MPO activity increased from 0·6 (0·1) U/g in normal controls to 3·7 (0·7) U/g in the acetic acid treated rats. All treated rats experienced diarrhoea.

Effects of ketotifen on mucosal damage

Pretreatment for 48 hours with ketotifen reduced the lesion area by about 85% and the lesion score by 68%. The protection provided by ketotifen was accompanied by significant decreases in diarrhoea, in mucosal MPO activity , and in PGE_2 and LTC_4 generation. Mucosal TxB_2 and LTB_4 values were similar in acetic acid treated rats regardless of pretreatment with ketotifen (Table II).

Histology

Sections of the large intestine from the rats with acetic acid induced colitis showed superficial, small mucosal ulcerations with extensive oedema accompanied only by a mild inflammatory cell infiltration (Fig 3A). Ketotifen treated rats showed almost normal mucosa without ulceration and a very mild inflammatory response (Fig 3B).

Discussion

Ketotifen is an oral tricyclic benzochlohepta-thiophene agent used in allergic conditions such as asthma and atopic dermatitis. Its principal pharmacological effects are to block the release of chemical mediators from mast cells and other inflammatory cells and to block their effects on target organs.[17-20]

Mast cell involvement has been postulated in inflammatory bowel disease.[1 5-8 21 22] Increased numbers of activated mast cells are present in the sites of active ulcerative colitis and are part of the granuloma in Crohn's disease.[1 5-8 21 22] Increased histamine concentrations have been found in the inflamed colonic mucosa of TNB treated rats.[22] However, in W/Wv mast cell deficient mice inflammation can still be induced by TNB,[23] thus suggesting that mast cells are not mandatory for the full expression of the inflammatory response. Ketotifen's impressive protection in the two models tested here can be attributed not only to its effects on mast cell mediators but also to its known effects on neutrophils and eosinophils.[1-3 24] The fact that ketotifen was effective only when given at least 48 hours before damage induction suggests that part of its effects might be due to inhibition of mediators release and, thus, of further recruitment of inflammatory cells to the site of damage. We have shown that ketotifen protects rat gastric mucosa against ethanol induced damage.[11]

The protective effect of ketotifen was accompanied by reduction of mucosal PGE_2 and LTC_4 values in the acetic acid model and reduction of PAF, PGE_2, LTC_4, LTB_4 and $Tx\ B_2$ values in the TNB model. PAF and LTB_4 values were found

to be increased in colonic mucosa of patients with active ulcerative colitis.[25 26] Inhibition of PAF and LTB_4 synthesis in the TNB model of colitis accelerated healing.[10 14 27 28] The same was shown in the acetic acid model.[29]

Colonic PGE_2 concentrations increased in both models of colitis, reaching peak levels two days after damage induction in the TNB model. Mucosal protection was accompanied by significant reduction in mucosal PGE_2 values in the acetic acid model. Allgayer *et al* have shown that treatment with 16,16'dimethyl PGE_2 decreases inflammation in the TNB model of colitis,[30] raising the question of whether the decreased PGE_2 values observed here after ketotifen treatment were merely secondary to the reduction in inflammation.

In summary, the present study shows that both acetic acid and TNB colitis can be pharmacologically manipulated by ketotifen, and that the microscopical and macroscopical inflammatory response, MPO activity and mediator release responded to prophylactic ketotifen administration. Neither models mimic the human diseases of ulcerative colitis and Crohn's disease, but despite this drawback, they are useful for testing the potential effect of experimental drugs for the treatment of non-specific inflammatory bowel disease. The results reported herein suggest exploring the potential usage of ketotifen in the prevention and treatment of inflammatory bowel disease.

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