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Acute effects of the cys-leukotriene-1 receptor antagonist, montelukast, on experimental colitis in rats

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Abstract

Cysteinyl leukotrienes play a part in inflammatory reactions such as inflammatory bowel diseases. The aim of the present study was to evaluate the acute effects of a cys-leukotriene-1 receptor antagonist, montelukast, on trinitrobenzene sulphonic acid (TNBS)-induced colitis in rats. Montelukast (5, 10 or 20 mg kg^{-1} day^{-1}), a 5-lipoxygenase inhibitor, zileuton (50 or 100 mg kg^{-1} day^{-1}, a positive control), or the vehicle was administered intracolonically to the rats twice daily throughout the study, starting 12 h before the induction of colitis with TNBS. The severity of colitis (macroscopic and histological assessment, as well as myeloperoxidase activity), the protein expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2, and eicosanoid production in colonic tissue incubation were assessed 24 and 72 h after colitis induction. Montelukast increased prostaglandin E_2 production at 24 h and tended to reduce the cyclooxygenase-2 protein expression at 72 h, but did not influence the severity of colitis. Zileuton failed to decrease the inflammatory reaction in spite of reduced leukotriene B_4 production at 72 h. The results suggest that drugs that block cysteinyl leukotriene receptors have limited potential to ameliorate acute TNBS-induced colitis, but that they exert some beneficial effects which make them capable of modulating the course of colitis.

Keywords: Cyclooxygenase-2; Cysteinyl leukotriene; Leukotriene B_4; Montelukast; Prostaglandin E_2; TNBS (trinitrobenzene sulphonic acid)-induced colitis; Zileuton

1. Introduction

Cysteinyl leukotrienes, leukotrienes C_4, D_4 and E_4, secreted mainly by eosinophils, mast cells, monocytes and macrophages (Jörg et al., 1982; MacGlashan et al., 1982; Damon et al., 1983; Williams et al., 1984; see Lewis and Austen, 1984), produce a variety of actions which emphasize their importance as pathogenic elements in inflammatory states, such as recruiting eosinophils and stimulating the excretion of mucus and the extravasation of plasma (Drazen et al., 1980; Hedqvist et al., 1980; Marom et al. 1982; Spada et al., 1986; see Dahlén, 1997). They also increase secretion in colonic mucosa (Jett et al., 1991) and cause smooth muscle contractions in a number of tissues, including the colon (Hedqvist et al., 1980; Goldenberg and Subers, 1982; Zijlstra et al., 1984).

Chronic inflammatory bowel diseases—Crohn's disease and ulcerative colitis—are associated with enhanced mucosal formation of cysteinyl leukotrienes (Peskar et al., 1986, 1987; Wardle et al., 1993; Casellas et al., 1994). Enhanced generation of leukotriene C_4 has been demonstrated in experimental models of colitis (Zipser et al., 1987a; Eliakim et al., 1992; Sjogren et al., 1994; Rachmilewitz et al., 1995; Yuceyar et al., 1999), where it correlates with the severity of inflammatory cell infiltration (Zipser et al., 1987a), although controversial results have also been presented (Zipser et al., 1987b; Allgayer et al., 1989; Karmeli et al., 1995; Hammerbeck and Brown, 1996).

Different leukotriene D_4 receptor antagonists have been shown to prevent early intestinal myoelectric responses and colonic transit disturbances associated with ricin or

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trinitrobenzene sulphonic acid (TNBS)-induced enteritis and colitis (Pons et al., 1992; Sjogren et al., 1994) without changes in long-term motor alterations (Morteau et al., 1993), tissue morphology or the synthesis of eicosanoids (Sjogren et al., 1994). Nishikawa et al. (1995) showed a reduction in chronic TNBS-induced colitis by a leukotriene C_4/D_4 receptor antagonist, pranlukast (ONO-1078), and in another study amelioration of ethanol-induced gastric mucosal damage by the leukotriene C_4/D_4 receptor antagonist, sodium-(βS^*,γR^*)-4-(3-(4-acetyl-3-hydroxy-2-propyl-phenoxy)-propylthio)-γ-hydroxy-β-methylbenzene butanoate (L649,923), was found (Wallace et al., 1988). There is also a case report that the selective, reversible cys-leukotriene-1 receptor antagonist, montelukast, induced a complete symptom resolution in chronic eosinophilic gastroenteritis resistant to other treatments (Neustrom, 1999).

Because of the scarcity of experimental and clinical data on the possible role of cysteinyl leukotrienes in inflammatory bowel diseases, it appeared to be of value to evaluate the effects of montelukast on the severity, markers and transmitters in colitis induced with TNBS in rats.

2. Materials and methods

2.1. Animals

Ninety-four male rats (200–260 g) of outbred HY:WIST stock (Laboratory Animal Centre, University of Helsinki, Finland) were used. They were kept under conventional conditions during the experiment. The rats were housed individually in cages in a standard laboratory at 22 ± 1 °C at a relative humidity of 40 ± 5% with a light–dark cycle of 12 h (light from 6 a.m. to 6 p.m.). The animals had free access to tap water and standard rat chow (R36; Lactamin, Stockholm, Sweden). The ethical aspects of the study protocol were approved by the Institutional Animal Care and Use Committee, University of Helsinki, Finland.

2.2. Colitis induction

Colitis was induced in halothane-anaesthetised rats using the technique described by Morris et al. (1989). Briefly, a polypropylene catheter (∅ 2.5 mm) was inserted intracolonically to a distance of 8 cm from the anus, and a 2,4,6-trinitrobenzene sulphonic acid (TNBS) (60 mg kg^{-1}, Fluka, Buchs, Switzerland) in 50% ethanol (1 ml kg^{-1}) was instilled into the colon. Healthy control rats were treated with saline.

2.3. Experimental design

The animals were randomly divided into twelve treatment groups to receive either montelukast (5, 10 or 20 mg kg^{-1} day^{-1}), or zileuton (50 or 100 mg kg^{-1} day^{-1}, a positive control), which has previously been shown to ameliorate TNBS-induced colitis (Zingarelli et al., 1993; Bertrán et al., 1996), or the vehicle. The drugs were suspended in 1% methylcellulose and administered intracolonically (1 ml kg^{-1}) directly at the site of inflammation while the rats were under halothane anaesthesia. The treatment began the night preceding the induction of colitis and was given twice a day throughout the study. The animals were weighed daily. Some of the animals were killed 24 h after colitis induction ($n = 6–10$ in each group), and the remainder were killed 72 h after induction ($n = 7–9$ in each group). The treatment groups were as follows.

24 h
(1) Healthy control (vehicle i.e. 1% methylcellulose)
(2) Healthy (montelukast 10 mg kg^{-1} day^{-1})
(3) Healthy (zileuton 100 mg kg^{-1} day^{-1})
(4) Colitis control (vehicle)
(5) Colitis (montelukast 5 mg kg^{-1} day^{-1}) (low dose)
(6) Colitis (montelukast 10 mg kg^{-1} day^{-1}) (intermediate dose)
(7) Colitis (montelukast 20 mg kg^{-1} day^{-1}) (high dose)
(8) Colitis (zileuton 50 mg kg^{-1} day^{-1})
(9) Colitis (zileuton 100 mg kg^{-1} day^{-1})

72 h
(10) Colitis control (vehicle)
(11) Colitis (montelukast 10 mg kg^{-1} day^{-1})
(12) Colitis (zileuton 100 mg kg^{-1} day^{-1})

2.4. Assessment of inflammation

The animals were killed 24 or 72 h after colitis induction, by decapitation preceded by an overdose of CO_2. The colon was removed by cutting at the pubic symphysis and at the caecum, and immediately transferred into Krebs buffer (pH 7.5), which was gently bubbled with 95% O_2–5% CO_2, and the descending colon was cut 7 cm proximally. The colon was incised along its mesenteric border, gently washed, placed flat, mucosal surface upwards, on a glass dish containing the buffer, and photographed for macroscopic evaluation (colonic damage score) as previously described by Kankuri et al. (1999). The severity of inflammation was evaluated by five experienced observers unaware of the treatments, using a visual analogue scale (100 mm; minimum injury 0, maximum injury 100). The colon was then placed in a pre-weighed tube containing Krebs solution, and weighed. The colon-to-body weight ratio was calculated as an index of tissue oedema. Immediately after weighing, a 5-mm segment was cut from the macroscopically most intensively affected

region for the eicosanoid production assays, and a 2-mm segment for histological assessment was taken from the adjacent section. The remaining parts of the 7-cm segment were cut longitudinally into three strips for measurement of myeloperoxidase activity (indicator of neutrophil infiltration) (Pfeiffer and Qiu, 1995), and inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 protein expression. One unit of myeloperoxidase activity was defined as that which degraded 1 μmol of hydrogen peroxide in 1 min and was expressed as [unit g^{-1}] of tissue. The tissue samples for histology were suspended in 4% formaldehyde, embedded in paraffin, cut and stained with hematoxylin and eosin. Histological assessment by light microscopy was carried out by two observers unaware of the treatments. The degree of inflammation was graded using the criteria described by González et al. (1999), and the score represented the sum of six individual variables graded 0–3 depending upon the severity of the changes (0 = no change; 1 = mild; 2 = moderate; 3 = severe). The variables evaluated were: erosion, ulceration, necrosis, haemorrhage, oedema and inflammatory cell infiltration.

2.5. Assessment of eicosanoid production

The colon sample (whole thickness of the wall) for eicosanoid measurements (80–150 mg of healthy colon, 110–270 mg of inflamed colon) was placed in a preweighed tube containing Krebs buffer, and weighed. It was then first pre-incubated in 3 ml Krebs buffer (pre-bubbled with 95% O_2–5% CO_2) for 15 min at 37 °C to minimise the effect of sample handling, after which the buffer was discarded and the tissue was incubated in 3 ml oxygenated Krebs buffer for another 15 min at 37 °C. At the end of the incubation the media were collected, centrifuged (5 min, $2000 \times g$, at 4 °C) and stored at −80 °C until assayed by radioimmunoassay or enzyme immunoassay for the eicosanoids produced. Prostaglandin E_2 and bicyclic-prostaglandin E_2 radioimmunoassay kits were from the Institute of Isotopes (Budapest, Hungary). Bicyclic-prostaglandin E_2 (13,14-dihydro-15-keto-11β,16ε-cyclo-prostaglandin E_2) is a stable end-product of the main prostaglandin E_2 metabolite found in the circulation, 13, 14-dihydro-15-keto-prostaglandin E_2. The sensitivity of the prostaglandin E_2 radioimmunoassay is 20 pg ml^{-1} and cross-reactivity with prostaglandin E_1 100%, and negligible with other related compounds. The lower limit of detection of the bicyclic-prostaglandin E_2 assay is 10 pg ml^{-1} and cross-reactivity with other eicosanoids is negligible. The leukotriene B_4 radioimmunoassay kit was purchased from Amersham (Buckinghamshire, England). The sensitivity of this assay is 15 pg ml^{-1} and cross-reactivity with other related compounds is negligible. The thromboxane B_2 radioimmunoassay was performed as described by Saareks et al. (1999). [^{3}H]Thromboxane B_2 was obtained from NEN Life Science Products (Boston, MA, USA), unlabelled thromboxane B_2 from Cayman Chemical (Ann Arbor, MI, USA) and thromboxane B_2 antiserum from Professor C. Taube (Martin Luther University, Halle, Germany). Leukotrienes C_4, D_4 and E_4 were determined by radioimmunoassay as described by Alanko et al. (1992) and with the enzyme immunoassay kit from Amersham. The former assay was performed with [^{3}H]leukotriene C_4 (NEN Life Science Products) as the radiolabelled ligand, leukotriene E_4 (Cayman Chemical) as the non-labelled ligand, and an in-house rabbit antibody raised against the bovine serum albumin conjugate of leukotriene $C_4/D_4/E_4$. The lower limit of detection for the leukotriene $C_4/D_4/E_4$ enzyme immunoassay is 10 pg ml^{-1} and the cross-reactivity data are as follows: leukotriene C_4 100%, leukotriene D_4 100%, leukotriene E_4 70%, other eicosanoids negligible.

2.6. Western blot analysis of iNOS and cyclooxygenase-2

The colon samples (120–220 mg of healthy colon, 240–570 mg of inflamed colon) were homogenised in boiling buffer (pH 7.4) containing Tris–HCl, Na_3VO_4 and sodium dodecyl sulphate and centrifuged. Equal amounts of protein from each sample (20 μg for iNOS measurements, 30 μg (24 h samples) and 40 μg (72 h samples) for cyclooxygenase-2 measurements) were loaded onto 8% (iNOS) or 10% (cyclooxygenase-2) sodium dodecyl sulphate polyacrylamide gels and electrophoresed. The iNOS electrophoresis standard, cyclooxygenase-2 electrophoresis standard (Cayman Chemical), and prestained molecular marker proteins (Bio-Rad Laboratories, Hercules, CA, USA) were used as positive controls. The separated proteins were transferred to nitrocellulose membranes, which were blocked with powdered fat-free cow's milk (5%, in Tris-buffered saline Tween (pH 7.6)). The primary antibody used was either rabbit polyclonal anti-iNOS immunoglobulin G (Santa Cruz Biotechnology, Santa Cruz, CA, USA) or goat polyclonal anti-cyclooxygenase-2 immunoglobulin G (Santa Cruz Biotechnology), and the secondary antibody was either horseradish peroxidase-coupled anti-rabbit immunoglobulin G (Santa Cruz Biotechnology) (iNOS) or anti-goat immunoglobulin G (Zymed Laboratories, San Francisco, CA, USA) (cyclooxygenase-2). Bound antibodies were detected using an enhanced chemiluminescence reaction. The optical density of each band was quantified using specific computer programmes (GeneSnap Version 2.60.0.14 and GeneTools Version 2.10.03, Synoptics, Cambridge, UK).

2.7. Drugs

Montelukast was kindly donated by Merck (Rahway, NJ, USA). Abbott Laboratories (Chicago, Il, USA) generously supplied us with zileuton.

Table 1
Body weight change, colon wet weight and histological score for the colon 24 and 72 h after colitis induction

	n	Body weight change (%)	Colon wet weight (g kg^{-1})[a]	*n*	Histological score (0–18)[b]
24 h					
Healthy control	8	−1 ± 1	4.0 ± 0.2	4	0.0 ± 0.0
Colitis control	10	−7 ± 1	9.6 ± 0.6	4	11.8 ± 1.3
Montelukast 5 mg kg^{-1}	7	−9 ± 1	9.3 ± 0.7	4	11.3 ± 1.0
Montelukast 10 mg kg^{-1}	6	−8 ± 1	8.8 ± 0.6	4	12.3 ± 1.8
Montelukast 20 mg kg^{-1}	6	−8 ± 1	8.5 ± 0.5	4	11.0 ± 0.7
Zileuton 100 mg kg^{-1}	10	−8 ± 1	9.5 ± 0.4	4	12.0 ± 0.7
72 h					
Colitis control	7	−14 ± 1	9.9 ± 0.3	4	13.0 ± 1.0
Montelukast 10 mg kg^{-1}	9	−13 ± 1	9.1 ± 0.4	4	12.8 ± 0.6
Zileuton 100 mg kg^{-1}	9	−15 ± 1	10.1 ± 0.3	4	11.0 ± 2.4

Data are means ± S.E.M.
[a]Weight of 7-cm segment of colon related to body weight.
[b]Histological score: minimum injury, 0; maximal injury, 18. Colitis control values are significantly different from the healthy control values ($P < 0.05$).

2.8. Statistical analysis

Data are expressed as means ± S.E.M. Statistical analyses were carried out using the Mann–Whitney *U*-test.

3. Results

3.1. Severity of inflammation

Body weight was reduced equally in all the colitis groups during the 24- and 72-h periods after colitis induction (Table 1). The TNBS treatment induced severe macroscopic and microscopic inflammation in the colon, as assessed from the colonic damage score (Fig. 1) and the histological score (Table 1). Histological assessment showed necrosis, haemorrhage, oedema and inflammatory cell infiltration, but also erosion and ulceration in the inflamed colon. None of the treatments reduced the score of the macroscopic or histological damage. Colonic inflammation was also associated with oedema, as measured by increased colon wet weight (Table 1), and neutrophil accumulation, as assessed from increased myeloperoxidase activity (Fig. 2). The two treatments (montelukast and zileuton) affected oedema formation similarly as compared with the colitis control group. Montelukast (or zileuton) did not influence the macroscopic severity of inflammation at any of the three doses. However, at 72 h, montelukast reduced the mean value for myeloperoxidase activity by 50% in comparison with the colitis control ($P = 0.27$).

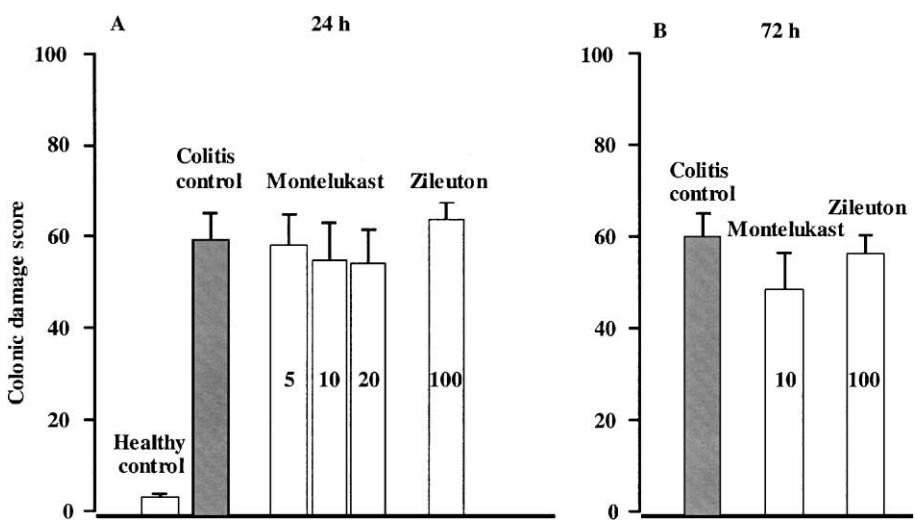

Fig. 1. Colonic damage score (A) 24 and (B) 72 h after colitis induction. Minimum injury, 0; maximum injury, 100. Data are means ± S.E.M. ($n = 6$–10 in each group). Values in the bars indicate the drug doses (mg kg^{-1}).

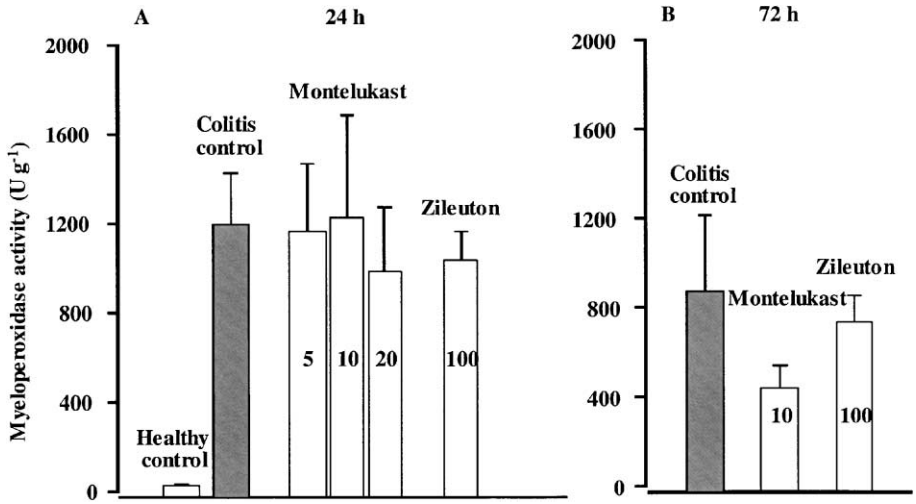

Fig. 2. Myeloperoxidase activity in the colon (A) 24 and (B) 72 h after colitis induction. Data are means ± S.E.M. ($n = 6–10$ in each group). Values in the bars indicate the drug doses (mg kg^{-1}).

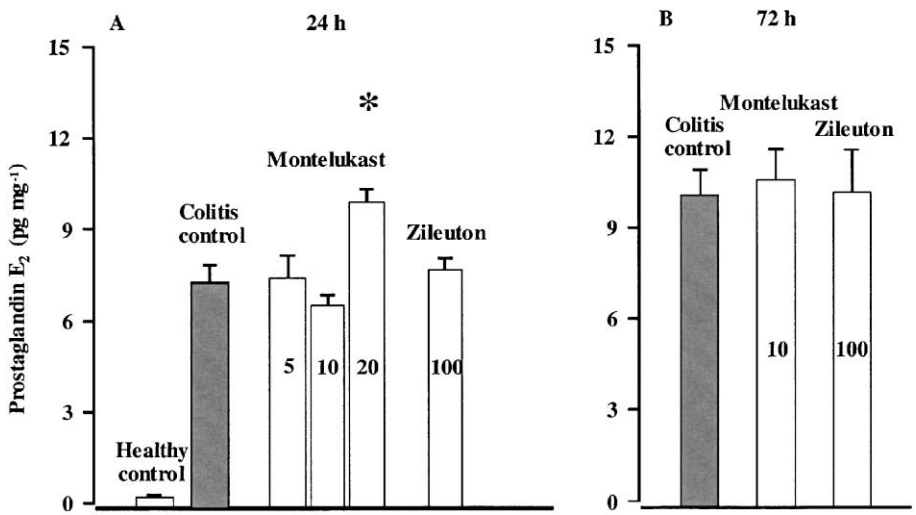

Fig. 3. Prostaglandin E_2 production by colonic tissue in vitro (A) 24 and (B) 72 h after colitis induction. Data are means ± S.E.M. ($n = 6–10$ in each group). Values in the bars indicate the drug doses (mg kg^{-1}). $^{*}P < 0.05$ vs. colitis control.

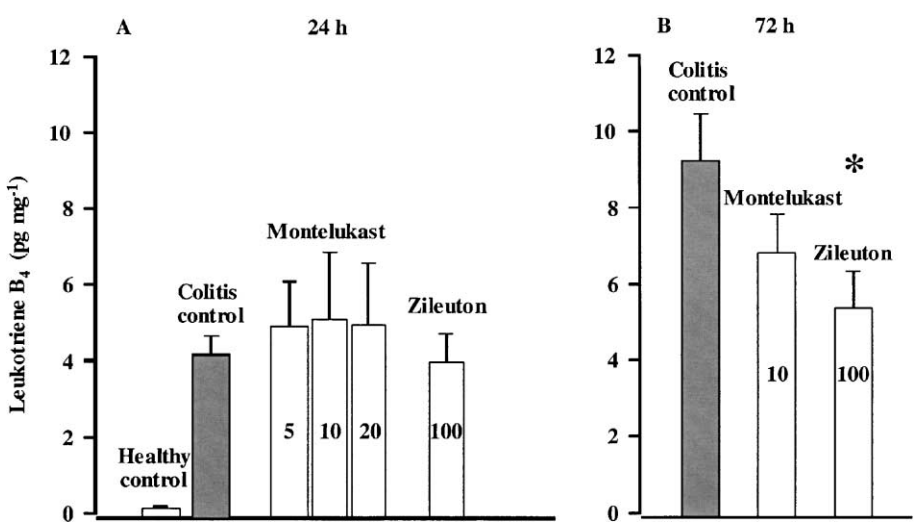

Fig. 4. Leukotriene B_4 production by colonic tissue in vitro (A) 24 and (B) 72 h after colitis induction. Data are means ± S.E.M. ($n = 6–10$ in each group). Values in the bars indicate the drug doses (mg kg^{-1}). $^{*}P < 0.05$ vs. colitis control.

Table 2
Bicyclic-prostaglandin E_2 and thromboxane B_2 production in the colon in vitro 24 and 72 h after colitis induction

	n	Bicyclic-prostaglandin E_2 production	Thromboxane B_2 production
24 h		*(pg mg^{-1})*	*(pg mg^{-1})*
Healthy control	7	7.9 ± 1.1	4 ± 0
Colitis control	10	4.4 ± 0.3	36 ± 2
Montelukast 5 mg kg^{-1}	7	5.9 ± 0.7	44 ± 6
Montelukast 10 mg kg^{-1}	6	4.4 ± 0.8	39 ± 9
Montelukast 20 mg kg^{-1}	6	5.1 ± 0.4	37 ± 2
Zileuton 100 mg kg^{-1}	10	4.6 ± 0.5	43 ± 3
72 h		*(%)*[a]	*(pg mg^{-1})*
Colitis control	7	100 ± 9	39 ± 5
Montelukast 10 mg kg^{-1}	9	93 ± 10	33 ± 2
Zileuton 100 mg kg^{-1}	9	114 ± 9	43 ± 4

Data are means ± S.E.M.
[a] Percentage of the colitis control average. Colitis control values are significantly different from the healthy control values ($P < 0.01$).

The lower dose of zileuton did not reduce the inflammatory reaction (or eicosanoid production) though it reduced myeloperoxidase activity compared with the colitis control (722 ± 144 vs. 1197 ± 229 U g^{-1}, $P = 0.07$). The colon samples from the healthy groups that received either montelukast or zileuton did not differ from the healthy control group as to any of the parameters measured (data not shown).

3.2. Eicosanoid production

The in vitro colonic production of prostaglandin E_2 (Fig. 3), leukotriene B_4 (Fig. 4) and thromboxane B_2 (Table 2) increased after TNBS application both at 24 h and at 72 h, whereas the production of bicyclic-prostaglandin E_2, which is a stable end-product of the main circulating metabolite of prostaglandin E_2, was higher in the healthy control group than in the colitis control group (Table 2). When the prostaglandin E_2 and bicyclic-prostaglandin E_2 values were summarized, the inflammed colon samples produced over 40% more prostaglandin E_2 than did the healthy samples (11.7 ± 0.7 vs. 8.1 ± 1.1 pg mg^{-1}, $P < 0.05$).

At 24 h the high dose of montelukast increased prostaglandin E_2 production by almost 40% ($P < 0.05$), while zileuton had no effect (Fig. 3A). At the same time, the colonic tissue samples of the rats treated with the low dose of montelukast showed a 30% increase in bicyclic-prostaglandin E_2 production ($P = 0.051$), whereas treatment with zileuton yielded no increase (Table 2). At 24 h, there were no differences between treatment groups for leukotriene B_4 production (Fig. 4A). However, zileuton decreased it by 40% at 72 h ($P < 0.05$) (Fig. 4B). Montelukast did not modify leukotriene B_4 production. No differences in thromboxane B_2 production were found between the treatment groups and the colitis control (Table 2). Surprisingly, no leukotriene C_4, D_4 or E_4 in vitro production by the colonic tissue was detected.

3.3. Colonic iNOS and cyclooxygenase-2 protein contents

iNOS (Fig. 5) and cyclooxygenase-2 (Fig. 6) protein expression increased in the colon after colitis induction, but there were no statistically significant differences between the treatment groups and the colitis control group. High-dose montelukast reduced iNOS expression by 50% in comparison with zileuton treatment at 24 h ($P = 0.065$).

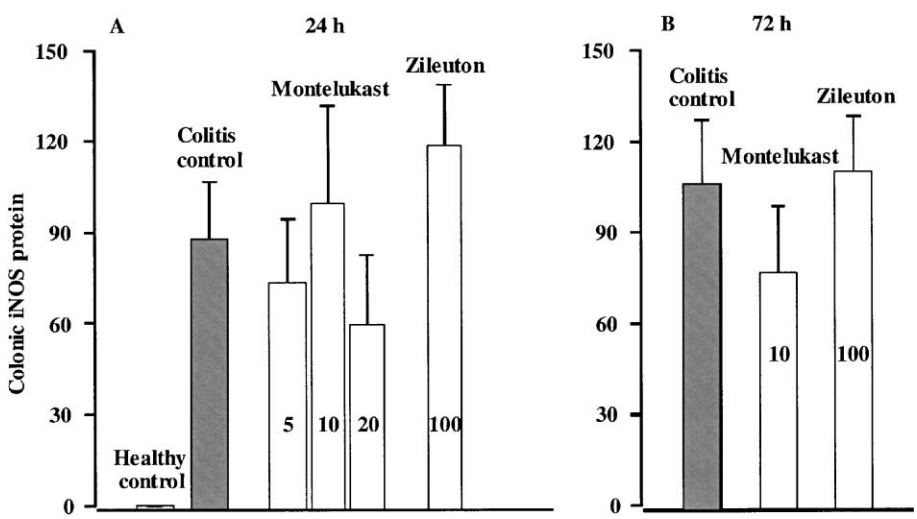

Fig. 5. iNOS protein expression of colon tissue (A) 24 and (B) 72 h after colitis induction. iNOS protein was assessed by Western blotting, and the results were quantified using a computer. Data are means ± S.E.M. ($n = 6$–10 in each group). Values in the bars indicate the drug doses (mg kg^{-1}).

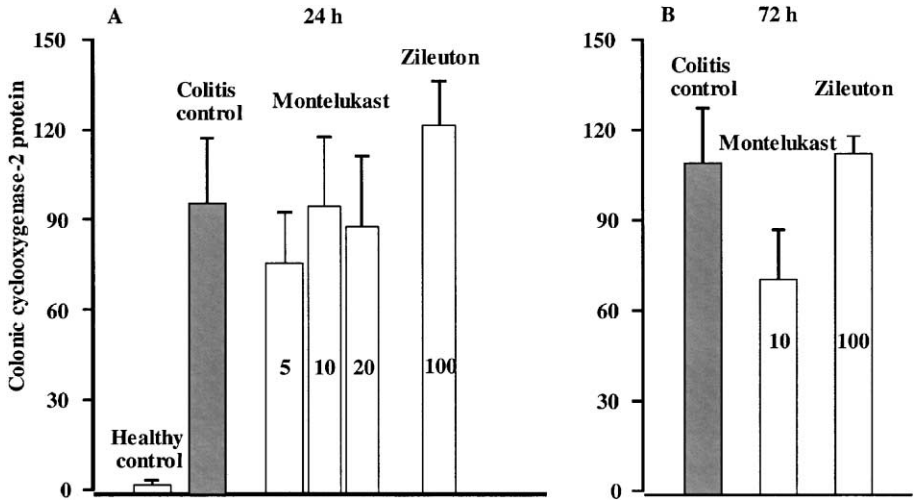

Fig. 6. Cyclooxygenase-2 protein expression of colon tissue (A) 24 and (B) 72 h after colitis induction. Cyclooxygenase-2 protein was assessed by Western blotting, and the results were quantified using a computer. Data are means ± S.E.M. ($n = 6–10$ in each group). Values in the bars indicate the drug doses (mg kg^{-1}).

An almost 40% decrease in cyclooxygenase-2 expression at 72 h ($P = 0.07$ vs. zileuton; $P = 0.15$ vs. colitis control) was also seen with montelukast.

4. Discussion

In the present study, we investigated the effects of the local intracolonic administration of the cys-leukotriene-1 receptor antagonist, montelukast, in acute TNBS-induced colitis in rats. On the basis of reports in the literature, the 5-lipoxygenase inhibitor, zileuton, was used as a positive control. We found that the montelukast treatment did not ameliorate the severe inflammatory reaction, although it increased prostaglandin E_2 production, which has beneficial effects in colitis models, and slightly decreased cyclooxygenase-2 protein expression, the enzyme induced in inflammation.

Cysteinyl leukotrienes are secreted by different inflammatory cells (Goetzl and Payan, 1984; see Lewis and Austen, 1984). In addition, endothelial cells, platelets and smooth muscle cells generate cysteinyl leukotrienes from leukotriene A_4 provided by other cells (Feinmark and Cannon 1986, 1987; Maclouf and Murphy, 1988; see Müller-Peddinghaus, 1999). These cells are important contributors to the pathogenesis of TNBS-induced colitis (Morris et al., 1989; Vilaseca et al., 1990), as well as of human inflammatory bowel diseases (see Stenson, 1995).

On incubation of colonic tissue for 15 min, no cysteinyl leukotriene production was detected. However, enhanced cysteinyl leukotriene production in this model has previously been shown by others (Eliakim et al., 1992; Yuceyar et al., 1999). It is possible that cysteinyl leukotrienes were rapidly degraded during the incubation.

In the present study, montelukast increased colonic prostaglandin E_2 production in the early phase of inflammation. Previous studies have also shown that different leukotriene C_4/D_4 receptor antagonists increase prostanoid synthesis in gastric damage (Wallace et al., 1988) and in experimental colitis (Sjogren et al., 1994; Nishikawa et al., 1995). Exogenous prostaglandins have been shown to ameliorate intestinal damage in experimental models of colitis (Wallace et al., 1985; Allgayer et al., 1989; Fedorak et al., 1990). Thus, montelukast has the potential to attenuate inflammatory reactions in the colon.

The role of cyclooxygenase-2—the enzyme induced at sites of inflammation (see Whittle, 2000)—in colitis is not clear. Although its messenger RNA and protein expression increases in the TNBS model (Reuter et al., 1996), the selective inhibition of cyclooxygenase-2 activity has been reported both to have no effect (Lesch et al., 1999) and to exacerbate the inflammatory reaction in the colon (Reuter et al., 1996). Preferential cyclooxygenase-2 inhibitors have been shown to abolish experimental colitis in other models (Karmeli et al., 2000; Kankuri et al., 2001b). In the present study, montelukast had no statistically significant effect on cyclooxygenase-2 protein expression. However, cyclooxygenase-2 expression in the montelukast group 72 h after the colitis induction was clearly lower than that in the colitis control group. This is supported by a recent finding by Öhd et al. (2000), who showed increased cyclooxygenase-2 expression as well as prostaglandin E_2 production by leukotriene D_4 in human and rat intestinal epithelial cell lines. Our results do not indicate any direct effect of montelukast on cyclooxygenase-2 induction, but they do imply that montelukast reduces cyclooxygenase-2 expression as a reaction secondary to the amelioration of inflammation.

Increased prostaglandin E_2 production in the montelukast group early in the inflammatory phase is not inconsistent with the decreased cyclooxygenase-2 protein

expression in the same group at a later phase, when the different timepoints and the different aspects they evaluate are taken into account: the tissue sample for prostaglandin E_2 measurements was taken from the most intensively inflamed region whereas an entire colon length sample was taken for the cyclooxygenase-2 expression measurement. This means that the former is a measure of a very local intense inflammatory reaction whereas the latter measures the extension of the inflammatory lesions longitudinally in the colon.

Although the role of iNOS—another enzyme induced in inflammatory reactions—remains to be discovered (see Kubes, 2000), high levels of nitric oxide, produced by iNOS, are considered to cause intestinal injury (see Perner and Rask-Madsen, 1999). This is consistent with the recent study, where a selective iNOS inhibitor reduced damage in TNBS-induced colitis in rats, whereas a non-selective inhibitor was ineffective (Kankuri et al., 2001a). In the present study, iNOS was induced after TNBS treatment, but the different treatments had no effect on its expression in the colon.

Although zileuton has not proved effective in the treatment of ulcerative colitis in clinical trials (Laursen et al., 1994; Hawkey et al., 1997), it has been shown to reduce the severity of experimental colitis in rats in the same colitis model and with same doses as we used (Zingarelli et al., 1993; Bertrán et al., 1996). In contrast, in the present study, zileuton failed to reduce the macroscopic inflammatory reaction in TNBS-induced colitis in spite of the fact that it reduced leukotriene B_4 production at 72 h. The lack of a decrease in leukotriene B_4 production by zileuton at 24 h in the present study is supported by previous results, where intracolonically administered zileuton or another 5-lipoxygenase inhibitor, 4-bromo-2,7-dimethoxy-3*H*-phenothiazin-3-one (L651,392), did not antagonize increased leukotriene B_4 production until three days after TNBS application (Wallace et al., 1989; Bertrán et al., 1996), and orally administered zileuton only slightly decreased leukotriene B_4 production 2 days after TNBS treatment (Zingarelli et al., 1993). Leukotriene B_4 is a potent chemotactic agent for neutrophils (see Ford-Hutchinson, 1990). Thus, the observation that zileuton modified leukotriene B_4 production without having any effect on myeloperoxidase activity at 72 h, is worth noting. This is consistent with results of the previous study, where an inhibitor of leukotriene synthesis reduced leukotriene B_4 production in the colon 96 h after TNBS application, but had no effect on myeloperoxidase activity at that time (Wallace et al., 1989). It has been suggested that leukotriene B_4 is not an important chemotactic factor for neutrophils in TNBS model during the acute phase (Wallace and Keenan, 1990). In the present study, zileuton did not modify the colonic damage score or the histological picture at 24 or at 72 h. However, when administered intracolonically, it has been shown to accelerate the healing of TNBS-induced colitis four weeks after colitis induction (Bertrán et al., 1996), which implies that leukotriene B_4 might be more important during a chronic than an acute phase of a TNBS model.

In conclusion, we suggest that drugs that block cysteinyl leukotriene receptors have limited potential to ameliorate acute colitis in this severe TNBS model. However, they are able to modify the inflammatory reaction, possibly via increased production of prostaglandins, which can exert cytoprotective effects in the gastrointestinal tract.

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References

Alanko, J., Riutta, A., Mucha, I., Kerttula, T., Kaukinen, S., Vapaatalo, H., Metsä-Ketelä, T., Seppälä, E., 1992. Adrenaline stimulates thromboxane and inhibits leukotriene synthesis in man. Eicosanoids 5, 169–175.

Allgayer, H., Deschryver, K., Stenson, W.F., 1989. Treatment with 16,16′-dimethyl prostaglandin E_2 before and after induction of colitis with trinitrobenzenesulfonic acid in rats decreases inflammation. Gastroenterology 96, 1290–1300.

Bertrán, X., Mañé, J., Fernández-Bañares, F., Castellá, E., Bartolí, R., Ojanguren, I., Esteve, M., Gassull, M.A., 1996. Intracolonic administration of zileuton, a selective 5-lipoxygenase inhibitor, accelerates healing in a rat model of chronic colitis. Gut 38, 899–904.

Casellas, F., Guarner, F., Antolín, M., Rodríguez, R., Salas, A., Malagelada, J.-R., 1994. Abnormal leukotriene C_4 release by unaffected jejunal mucosa in patients with inactive Crohn's disease. Gut 35, 517–522.

Dahlén, S.-E., 1997. Leukotriene receptors and their antagonists. In: Holgate, S., Dahlén, S.-E. (Eds.), SRS-A to Leukotrienes. The Dawning of a New Treatment. Blackwell Science, London, pp. 155–166.

Damon, M., Chavis, C., Godard, P., Michel, F.B., Crastes de Paulet, A., 1983. Purification and mass spectrometry identification of leukotriene D_4 synthesized by human alveolar macrophages. Biochem. Biophys. Res. Commun. 111, 518–524.

Drazen, J.M., Austen, K.F., Lewis, R.A., Clark, D.A., Goto, G., Marfat, A., Corey, E.J., 1980. Comparative airway and vascular activities of leukotrienes C-1 and D in vivo and in vitro. Proc. Natl. Acad. Sci. U.S.A. 77, 4354–4358.

Eliakim, R., Karmeli, F., Okon, E., Rachmilewitz, D., 1992. Ketotifen effectively prevents mucosal damage in experimental colitis. Gut 33, 1498–1503.

Fedorak, R.N., Empey, L.R., MacArthur, C., Jewell, L.D., 1990. Misoprostol provides a colonic mucosal protective effect during acetic acid-induced colitis in rats. Gastroenterology 98, 615–625.

Feinmark, S.J., Cannon, P.J., 1986. Endothelial cell leukotriene C_4 synthesis results from intercellular transfer of leukotriene A_4 synthesized by polymorphonuclear leukocytes. J. Biol. Chem. 261, 16466–16472.

Feinmark, S.J., Cannon, P.J., 1987. Vascular smooth muscle cell leukotriene C_4 synthesis: requirement for transcellular leukotriene A_4 metabolism. Biochim. Biophys. Acta 922, 125–135.

Ford-Hutchinson, A.W., 1990. Leukotriene B_4 in inflammation. Crit. Rev. Immunol. 10, 1–12.

Goetzl, E.J., Payan, D.G., 1984. Inhibition by somatostatin of the release of mediators from human basophils and rat leukemic basophils. J. Immunol. 133, 3255–3259.

Goldenberg, M.M., Subers, E.M., 1982. The reactivity of rat isolated gastrointestinal tissues to leukotrienes. Eur. J. Pharmacol. 78, 463–466.

González, R., Rodríguez, S., Romay, C., Ancheta, O., González, A., Armesto, J., Remirez, D., Merino, N., 1999. Anti-inflammatory activity of phycocyanin extract in acetic acid-induced colitis in rats. Pharmacol. Res. 39, 55–59.

Hammerbeck, D.M., Brown, D.R., 1996. Presence of immunocytes and sulfidopeptide leukotrienes in the inflamed guinea pig distal colon. Inflammation 20, 413–425.

Hawkey, C.J., Dube, L.M., Rountree, L.V., Linnen, P.J., Lancaster, J.F., 1997. A trial of zileuton versus mesalazine or placebo in the maintenance of remission of ulcerative colitis. Gastroenterology 112, 718–724.

Hedqvist, P., Dahlén, S.-E., Gustafsson, L., Hammarström, S., Samuelsson, B., 1980. Biological profile of leukotrienes C_4 and D_4. Acta Physiol. Scand. 110, 331–333.

Jett, M.-F., Marshall, P., Fondacaro, J.D., Smith, P.L., 1991. Action of peptidoleukotrienes on ion transport in rabbit distal colon in vitro. J. Pharmacol. Exp. Ther. 257, 698–705.

Jörg, A., Henderson, W.R., Murphy, R.C., Klebanoff, S.J., 1982. Leukotriene generation by eosinophils. J. Exp. Med. 155, 390–402.

Kankuri, E., Asmawi, M.Z., Korpela, R., Vapaatalo, H., Moilanen, E., 1999. Induction of iNOS in a rat model of acute colitis. Inflammation 23, 141–152.

Kankuri, E., Vaali, K., Knowles, R.G., Lähde, M., Korpela, R., Vapaatalo, H., Moilanen, E., 2001a. Suppression of acute experimental colitis by a highly selective inducible nitric-oxide synthase inhibitor, N-[3-aminomethyl/benzyl] acetamidine. J. Pharmacol. Exp. Ther. 298, 1128–1132.

Kankuri, E., Vaali, K., Korpela, R., Paakkari, I., Vapaatalo, H., Moilanen, E., 2001b. Effects of a COX-2 preferential agent nimesalide on TNBS-induced acute inflammation in the gut. Inflammation (in press).

Karmeli, F., Eliakim, R., Okon, E., Samuni, A., Rachmilewitz, D., 1995. A stable nitroxide radical effectively decreases mucosal damage in experimental colitis. Gut 37, 386–393.

Karmeli, F., Cohen, P., Rachmilewitz, D., 2000. Cyclo-oxygenase-2 inhibitors ameliorate the severity of experimental colitis in rats. Eur. J. Gastroenterol. Hepatol. 12, 223–231.

Kubes, P., 2000. Inducible nitric oxide synthase: a little bit of good in all of us. Gut 47, 6–9.

Laursen, L.S., Lauritsen, K., Bukhave, K., Rask-Madsen, J., Jacobsen, O., Næsdal, J., Goebell, H., Peskar, B., Rubin, P.D., Swanson, L.J., Kesterson, J.W., Kashavarzian, A., Kozarek, R., Hanauer, S., Cort, D., Stenson, W.F., 1994. Selective 5-lipoxygenase inhibition by zileuton in the treatment of relapsing ulcerative colitis: a randomized double-blind placebo-controlled multicentre trial. Eur. J. Gastroenterol. Hepatol. 6, 209–215.

Lesch, C.A., Kraus, E.R., Sanchez, B., Gilbertsen, R., Guglietta, A., 1999. Lack of beneficial effect of COX-2 inhibitors in an experimental model of colitis. Methods Find. Exp. Clin. Pharmacol. 21, 99–104.

Lewis, R.A., Austen, K.F., 1984. The biologically active leukotrienes. Biosynthesis, metabolism, receptors, functions, and pharmacology. J. Clin. Invest. 73, 889–897.

MacGlashan, D.W., Schleimer, R.P., Peters, S.P., Schulman, E.S., Adams III, G.K., Newball, H.H., Lichtenstein, L.M., 1982. Generation of leukotrienes by purified human lung mast cells. J. Clin. Invest. 70, 747–751.

Maclouf, J.A., Murphy, R.C., 1988. Transcellular metabolism of neutrophil-derived leukotriene A_4 by human platelets. A potential cellular source of leukotriene C_4. J. Biol. Chem. 263, 174–181.

Marom, Z., Shelhamer, J.H., Bach, M.K., Morton, D.R., Kaliner, M., 1982. Slow-reacting substances, leukotrienes C_4 and D_4, increase the release of mucus from human airways in vitro. Am. Rev. Respir. Dis. 126, 449–451.

Morris, G.P., Beck, P.L., Herridge, M.S., Depew, W.T., Szewczuk, M.R., Wallace, J.L., 1989. Hapten-induced model of chronic inflammation and ulceration in the rat colon. Gastroenterology 96, 795–803.

Morteau, O., More, J., Pons, L., Bueno, L., 1993. Platelet-activating factor and interleukin 1 are involved in colonic dysmotility in experimental colitis in rats. Gastroenterology 104, 47–56.

Müller-Peddinghaus, R., 1999. Pharmacological aspects of 5-lipoxygenase inhibition. Adv. Exp. Med. Biol. 447, 117–121.

Neustrom, M.R., 1999. Treatment of eosinophilic gastroenteritis with montelukast. J. Allergy Clin. Immunol. 104, 506.

Nishikawa, M., Hikasa, Y., Hori, K., Tanida, N., Shimoyama, T., 1995. Effect of leukotriene C_4D_4 antagonist on colonic damage induced by intracolonic administration of trinitrobenzene sulfonic acid in rats. J. Gastroenterol. 30, 34–40.

Öhd, J.F., Wikström, K., Sjölander, A., 2000. Leukotrienes induce cell-survival signaling in intestinal epithelial cells. Gastroenterology 119, 1007–1018.

Perner, A., Rask-Madsen, J., 1999. Review article: the potential role of nitric oxide in chronic inflammatory bowel disorders. Aliment. Pharmacol. Ther. 13, 135–144.

Peskar, B.M., Dreyling, K.W., Peskar, B.A., May, B., Goebell, H., 1986. Enhanced formation of sulfidopeptide-leukotrienes in ulcerative colitis and Crohn's disease: inhibition by sulfasalazine and 5-aminosalicylic acid. Agents Actions 18, 381–383.

Peskar, B.M., Dreyling, K.W., May, B., Schaarschmidt, K., Goebell, H., 1987. Possible mode of action of 5-aminosalicylic acid. Dig. Dis. Sci. 32, 51S–56S.

Pfeiffer, C.J., Qiu, B.S., 1995. Effects of chronic nitric oxide synthase inhibition on TNB-induced colitis in rats. J. Pharm. Pharmacol. 47, 827–832.

Pons, L., Droy-Lefaix, M.-T., Bueno, L., 1992. Leukotriene D_4 participates in colonic transit disturbances induced by intracolonic administration of trinitrobenzene sulfonic acid in rats. Gastroenterology 102, 149–156.

Rachmilewitz, D., Karmeli, F., Okon, E., 1995. Sulfhydryl blocker-induced rat colonic inflammation is ameliorated by inhibition of nitric oxide synthase. Gastroenterology 109, 98–106.

Reuter, B.K., Asfaha, S., Buret, A., Sharkey, K.A., Wallace, J.L., 1996. Exacerbation of inflammation-associated colonic injury in rat through inhibition of cyclooxygenase-2. J. Clin. Invest. 98, 2076–2085.

Saareks, V., Mucha, I., Sievi, E., Riutta, A., 1999. Nicotinic acid and pyridoxine modulate arachidonic acid metabolism in vitro and ex vivo in man. Pharmacol. Toxicol. 84, 274–280.

Sjogren, R.W., Colleton, C., Shea-Donohue, T., 1994. Intestinal myoelectric response in two different models of acute enteric inflammation. Am. J. Physiol. 267, G329–G337.

Spada, C.S., Woodward, D.F., Hawley, S.B., Nieves, A.L., 1986. Leukotrienes cause eosinophil emigration into conjunctival tissue. Prostaglandins 31, 795–809.

Stenson, W.F., 1995. The tissue reaction in inflammatory bowel disease. In: Kirsner, J.B., Shorter, R.G. (Eds.), Inflammatory Bowel Disease. Williams and Wilkins, Baltimore, MD, USA, pp. 177–189.

Vilaseca, J., Salas, A., Guarner, F., Rodriguez, R., Malagelada, J.-R., 1990. Participation of thromboxane and other eicosanoid synthesis in the course of experimental inflammatory colitis. Gastroenterology 98, 269–277.

Wallace, J.L., Keenan, C.M., 1990. An orally active inhibitor of leukotriene synthesis accelerates healing in a rat model of colitis. Am. J. Physiol. 258, G527–G534.

Wallace, J.L., Whittle, B.J.R., Boughton-Smith, N.K., 1985. Prostaglandin protection of rat colonic mucosa from damage induced by ethanol. Dig. Dis. Sci. 30, 866–876.

Wallace, J.L., Beck, P.L., Morris, G.P., 1988. Is there a role for leukotrienes as mediators of ethanol-induced gastric mucosal damage? Am. J. Physiol. 254, G117–G123.

Wallace, J.L., MacNaughton, W.K., Morris, G.P., Beck, P.L., 1989. Inhibition of leukotriene synthesis markedly accelerates healing in a rat model of inflammatory bowel disease. Gastroenterology 96, 29–36.

Wardle, T.D., Hall, L., Turnberg, L.A., 1993. Inter-relationships between inflammatory mediators released from colonic mucosa in ulcerative colitis and their effects on colonic secretion. Gut 34, 503.

Whittle, B.J.R., 1978. COX-1 and COX-2 products in the gut: therapeutic impact of COX-2 inhibitors. Gut 47, 320–325.

Williams, J.D., Czop, J.K., Austen, K.F., 1984. Release of leukotrienes by human monocytes on stimulation of their phagocytic receptor for particulate activators. J. Immunol. 132, 3034–3040.

Yuceyar, H., Ozutemiz, O., Huseyinov, A., Saruc, M., Alkanat, M., Bor, S., Coker, I., Batur, Y., 1999. Is administration of n−3 fatty acids by mucosal enema protective against trinitrobenzene-induced colitis in rats? Prostaglandins Leukotrienes Essent. Fatty Acids 61, 339–345.

Zijlstra, F.J., Vincent, J.E., Bonta, I.L., 1984. The effects of leukotrienes, thromboxane A_2 and phospholipase A_2 on human, porcine and guinea pig lung parenchyma. Agents Actions 14, 88–92.

Zingarelli, B., Squadrito, F., Graziani, P., Camerini, R., Caputi, A.P., 1993. Effects of zileuton, a new 5-lipoxygenase inhibitor, in experimentally induced colitis in rats. Agents Actions 39, 150–156.

Zipser, R.D., Nast, C.C., Lee, M., Kao, H.W., Duke, R., 1987a. In vivo production of leukotriene B_4 and leukotriene C_4 in rabbit colitis. Relationship to inflammation. Gastroenterology 92, 33–39.

Zipser, R.D., Patterson, J.B., LeDuc, L.E., 1987b. Chemotactic peptide stimulation of leukotrienes from healthy and inflamed rabbit colons. J. Pharmacol. Exp. Ther. 241, 218–222.