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The effects of selective cyclooxygenase-2 inhibitors, celecoxib and rofecoxib, on experimental colitis induced by acetic acid in rats

Azza El-Medany[a], Afaf Mahgoub[a,*], Ali Mustafa[a], Maha Arafa[b], Mahmoud Morsi[c]

[a]*Department of Pharmacology, College of Medicine, King Saud University, Riyadh 11461, P.O. Box 22452, Saudi Arabia*
[b]*Pathology, College of Medicine, King Saud University, Riyadh 11461, P.O. Box 22452, Saudi Arabia*
[c]*Central Laboratories, Ministry of Health, Riyadh, Saudi Arabia*

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Abstract

Several mediators may be involved in the pathogenesis of inflammatory bowel disease, as well as in experimental colitis. The present work was conducted to investigate the effects of the two selective cyclooxygenase-2 inhibitors, celecoxib and rofecoxib, on experimentally induced colitis in rats. Rectal instillation of acetic acid was used to induce the colitis. Acetic acid treatment caused haemorrhagic diarrhoea and weight loss in rats. Celecoxib (5 mg/kg) or rofecoxib (2.5 mg/kg), when given twice daily by the oral route, reduced the degree of haemorrhagic diarrhoea and the weight loss produced. In addition, they produced a significant reduction in the degree of colonic injury, the rise in myeloperoxidase (MPO) levels, total nitric oxide synthetase (NOS) activity, platelet-activating factor (PAF), histamine levels and prostaglandin E_2 levels. In contrast, there was a significant increase in the levels of reduced glutathione (GSH). Thus, the findings of the present study provide evidence that selective cyclooxygenase-2 inhibitors may be beneficial in patients with inflammatory bowel disease.

Keywords: Celecoxib; Rofecoxib; Acetic-acid-induced colitis; Myeloperoxidase; Reduced glutathione; Total nitric oxide synthetase

1. Introduction

The cyclooxygenase pathway leads to the production of prostaglandins, which possess both pro- and antiinflammatory properties. There are two known isoforms of cyclooxygenase, namely, cyclooxygenase-1, which is a constitutive enzyme. It is present in nearly all tissues and appears to be critical in maintaining mucosal defenses and plays a key role in mediating platelet aggregation (Carty et al., 2000; Gerd and Werner, 2001). On the other hand, cyclooxygenase-2 is the inducible form of the enzyme, which is expressed in response to the stimulation of inflammatory cells, such as synoviocytes, macrophages and endothelial cells. Stimulants of this isozyme include the proinflammatory cytokines, prostaglandins and tumor necrosis factor-α (Balfour, 1994; Newberry et al., 1999).

Nonsteroidal antiinflammatory drugs (NSAIDs) inhibit the generation of prostanoids, but their effects vary in different tissues and organs. The function of the two cyclooxygenase isoforms, cyclooxygenase-1 and cyclooxygenase-2, in maintaining mucosal homeostasis and modulating inflammation in the digestive tract remains uncertain. The generation of prostanoids in the colonic mucosa is increased in both experimentally induced colitis and in inflammatory bowel disease and correlates well with disease activity (Salvatore et al., 2001; Kruidenier and Verspaget, 2002).

Indomethacin, a conventional NSAID, inhibits both cyclooxygenase isoforms, which are induced by acute and chronic enterocolitis in genetically susceptible rats (Yamada et al., 1993). This effect is attributed to the inhibition of the synthesis of prostaglandins in the gastric mucosa. These prostaglandins are known to possess cytoprotective

* Corresponding author. Tel./fax: +966 1 478 6768.
E-mail address: afaf_mahgoub@yahoo.com (A. Mahgoub).

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properties (Wallace et al., 1992). The relative contribution of cyclooxygenase-1 and cyclooxygenase-2 isoforms to the biological actions of prostaglandins in the gastrointestinal mucosa is less clear. Several investigators advocated the hypothesis that the inducible form of cyclooxygenase is the source for the proinflammatory prostaglandins released during mucosal injury. In contrast, cyclooxygenase-1 regulates gastrointestinal homeostasis through the synthesis of cytoprotective prostaglandins (Masferrer et al., 1996).

In mice, cyclooxygenase-1 has been reported to play a protective role against mucosal injury of the small intestine (Cohn et al., 1997) and the colon (Tessner et al., 1998). On the other hand, cyclooxygenase-2 expression is induced during inflammation (Singer et al., 1998), and its expression is inhibited by the release of endogenous glucocorticoids (O'Bannion et al., 1991; Karmeli et al., 2000). Based on these observations, some researchers have attributed the antiinflammatory action of NSAIDs to the inhibition of cyclooxygenase-2 and their harmful effects to the inhibition of cyclooxygenase-1 (Eliakim et al., 1992; Xie et al., 1992). This association between cyclooxygenase-2 and the inflammatory events led to the development of selective cyclooxygenase-2 inhibitors, which are expected to display systemic antiinflammatory properties without the anticipated gastrointestinal toxicity. Recently, clinical trials in patients with osteoarthritis documented the safety of the selective cyclooxygenase-2 inhibitors, celecoxib and rofecoxib, on gastric mucosa as compared to the traditional NSAIDs (Goldstein et al., 2000; Martin et al., 2003).

The effect of NSAIDs in the possible modulation of colon inflammation is still controversial and remains uncertain. NSAIDs may be associated with exacerbation of ulcerative colitis and Crohn's disease (Fabia et al., 1993; Felder et al., 2000). Many patients with ulcerative colitis and Crohn's disease are advised to avoid the use of NSAIDs to prevent disease exacerbation (Bonner, 2001). However, the data in this regard have been inconsistent. There are some reports that showed no association between the use of NSAIDs and exacerbation of the underlying inflammatory bowel disease (Dominitz and Boyko, 2000; Mahadevan et al., 2001). This relative contraindication is a problem for those patients who suffer from irritable bowel disease-associated inflammatory arthropathies.

Animal models are used extensively to study the pathogenesis and pathophysiology of inflammatory bowel disease and to evaluate therapies (Morris et al., 1989; Elson et al., 1995; Wallace and Tigley, 1995). Acetic-acid-induced colitis in rats resembles human ulcerative colitis in histology, eicosanoid production and excessive oxygen-derived free radicals release by inflamed mucosa (Millar et al., 1996). Therefore, this study was undertaken to evaluate and compare the effects of two selective cyclooxygenase-2 inhibitors, namely, celecoxib and rofecoxib, on the extent and severity of colitis induced by acetic acid in rats, using macroscopic, microscopic and biochemical studies.

2. Materials and methods

2.1. Materials

Celecoxib and rofecoxib were a generous gift from Searle, USA, and Merck, USA, respectively. 5,5-Dithiobis-2-nitrobenzoic acid (DTNB), reduced glutathione (GSH), adenosine diphosphate, platelet-activating factor (PAF) and agents for myeloperoxidase (MPO) assay were purchased from Sigma, St. Louis, MO, USA. Histamine diphosphate monohydrate was purchased from BDH, U.K. L-Arginine and L-citrulline were from Amersham, Bucks, UK. All other chemicals used were of analytical grade.

2.2. Animals

Forty male Wistar albino rats (150–200 g), which were supplied from the Animal Care Centre, King Saud University, were used throughout this work. The animals were maintained in a room under standard conditions of light, feeding and temperature. The study was conducted in accordance with the standards established by the guide for the care and use of laboratory animals of the College of Medicine Research Centre at King Saud University, Riyadh. The rats were housed individually in a rack mounted with wire mesh cages to prevent coprophagia. All rats were exposed to the same environmental conditions and were maintained on a proper diet and water ad libitum. The animals were randomly divided into five groups, each consisting of eight animals: normal control group, acetic acid control group, vehicle-treated group, in which 2% gum acacia was given. The drug-treated groups received celecoxib and rofecoxib in a dose of 5 and 2.5 mg/kg, respectively. The drugs were suspended in 2% gum acacia and administered orally in a volume of 0.5 ml/100 g bodyweight. The dose was given twice daily starting 24 h after the induction of colitis and continued for five consecutive days.

2.3. Induction of experimental colitis in rats

The animals were fasted for 24 h, with access to water ad libitum before the induction of colitis. The induction of colitis was performed using a modification of the method described by Millar et al. (1996). Each rat was sedated by an intraperitoneal injection of phenobarbitone (35 mg/kg). Two milliliters of acetic acid (3% v/v in 0.9% saline) was infused for 30 s using a polyethylene tube (2 mm in diameter), which was inserted through the rectum into the colon, to a distance of 8 cm. The acetic acid was retained in the colon for 30 s, after

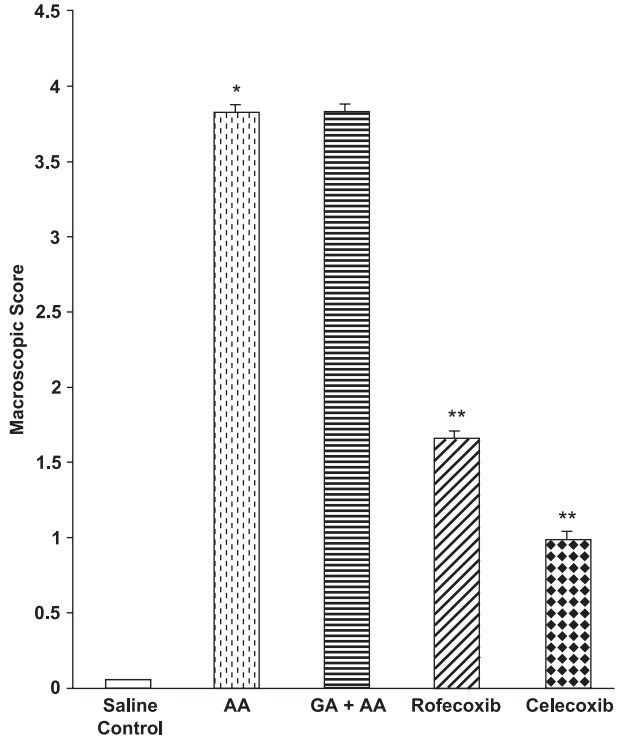

Fig. 1. Macroscopic scoring of rat rectal biopsy of normal, acetic-acid-induced colitis and after treatment with celecoxib (5 mg/kg) and rofecoxib (2.5 mg/kg) when given orally twice daily (mean±S.E.M., n=8). *P<0.05 as compared with saline control group. **P<0.05 as compared with the acetic acid group.

which the fluid was withdrawn. Following the completion of the experiments, the rats were killed using ether anaesthesia, and colonic biopsies were taken for macroscopic scoring, histopathological examination and biochemical studies.

2.4. Assessment of colitis

2.4.1. Macroscopic scoring

At postmortem laparotomy, 6 cm of colon, extending proximally for 2 cm above the anal orifice, was removed. The tissue was first split longitudinally and pinned out onto a card. An independent observer scored the macroscopic appearance of the colonic mucosa. An arbitrary scale ranging from 0–4 was used as follows:

0=no macroscopic changes
1=mucosal erythema only
2=mild mucosal oedema, slight bleeding or small erosions
3=moderate oedema, bleeding ulcers or erosions
4=severe ulceration, erosions, oedema and tissue necrosis (Millar et al., 1996).

2.4.2. Histopathological study

Full thickness biopsy specimens were fixed in 10% formol saline prior to wax embedding, sectioning and staining with haematoxylin and eosin for histological evaluation of colonic damage by light microscopy.

2.5. Biochemical study

Colonic samples were stored immediately at −20 °C until analysis. Tissue samples were homogenized in 1 ml of 10 mmol/l Tris–HCl buffer of pH 7.1, and homogenate was used for the measurement of myeloperoxidase (MPO) and total nitric oxide synthetase (NOS) activities. Also, platelet-activating factor (PAF), histamine, reduced glutathione (GSH) contents and prostaglandin E_2 levels were estimated.

2.5.1. Determination of myeloperoxidase activity

MPO activity had been used as an index of leukocyte adhesion and accumulation in several tissues, including the intestine. The principle of the method depends on the release of MPO enzyme in the homogenate of the colonic tissue used. Its level was detected using 0.3 mmol of H_2O_2 as a substrate. A unit of MPO activity is defined as that converting 1 μmol of H_2O_2 to water in 1 min at 25 °C (Bradley et al., 1982). In brief, segments of the distal colon (0.5 g) were homogenized in 10 volumes of 50 mM sodium phosphate buffer (pH 7.4) in an ice-bath using polytron homogenizer (50 mg tissue/ml). The pellet (containing 95% of the total tissue MPO activity) was resuspended in an equal volume of potassium phosphate buffer (pH 6). Another centrifugation

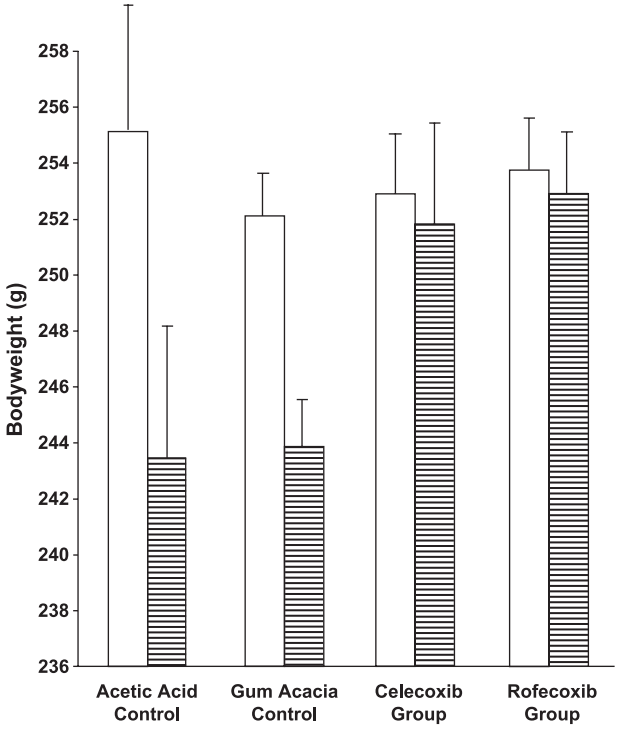

Fig. 2. The effect of acetic-acid-induced colitis on the body weight of rats before and after treatment with celecoxib (5 mg/kg) and rofecoxib (2.5 mg/kg) when given orally twice daily for 5 days. (Before treatment=open bar; after treatment=lined bar).

step for a period of 20 min at 16,000×*g* was done. The resultant supernatant was used for MPO assay using tetramethylbenzidine. The activity of MPO was measured at 655 nm using a Jenway 6505 UV-Vis spectrophotometer.

2.5.2. Determination of reduced glutathione

Reduced glutathione was determined using the method previously described by Owens and Belcher (1965), which is based on the reaction of 5,5-dithiobis-(2-nitrobenzoic acid) with the GSH present. The absorbance was measured at 412 nm in an Schimadzu double-beam spectrophotometer. The amount of glutathione present in the sample was calculated using a standard solution of GSH containing 1 mg of GSH/1 ml 3% metaphosphoric acid. The increase in the extinction at 412 nm is proportional to the amount of GSH present.

2.5.3. Determination of platelet-activating factor

The assay of PAF activity in the different samples was measured in an aggregometer by the aggregation of aspirin-treated washed rabbit platelets in the presence of adenosine diphosphate scavengers after preparing the platelets by differential centrifugation. PAF activity is presented as pg/10 mg tissue (Wallace et al., 1989).

2.5.4. Determination of total nitric oxide synthetase enzyme activity

The NOS enzyme activity was determined by conversion of L-[^{3}H] arginine to L-[^{3}H] citrulline. It was performed by a modification of the method described by McNaughton et al. (1998). Frozen colon specimens were homogenized in an ice-cold HEPES buffer (100 mg/ml). The homogenate was centrifuged at 11,300×*g* for 10 min, and supernatants were

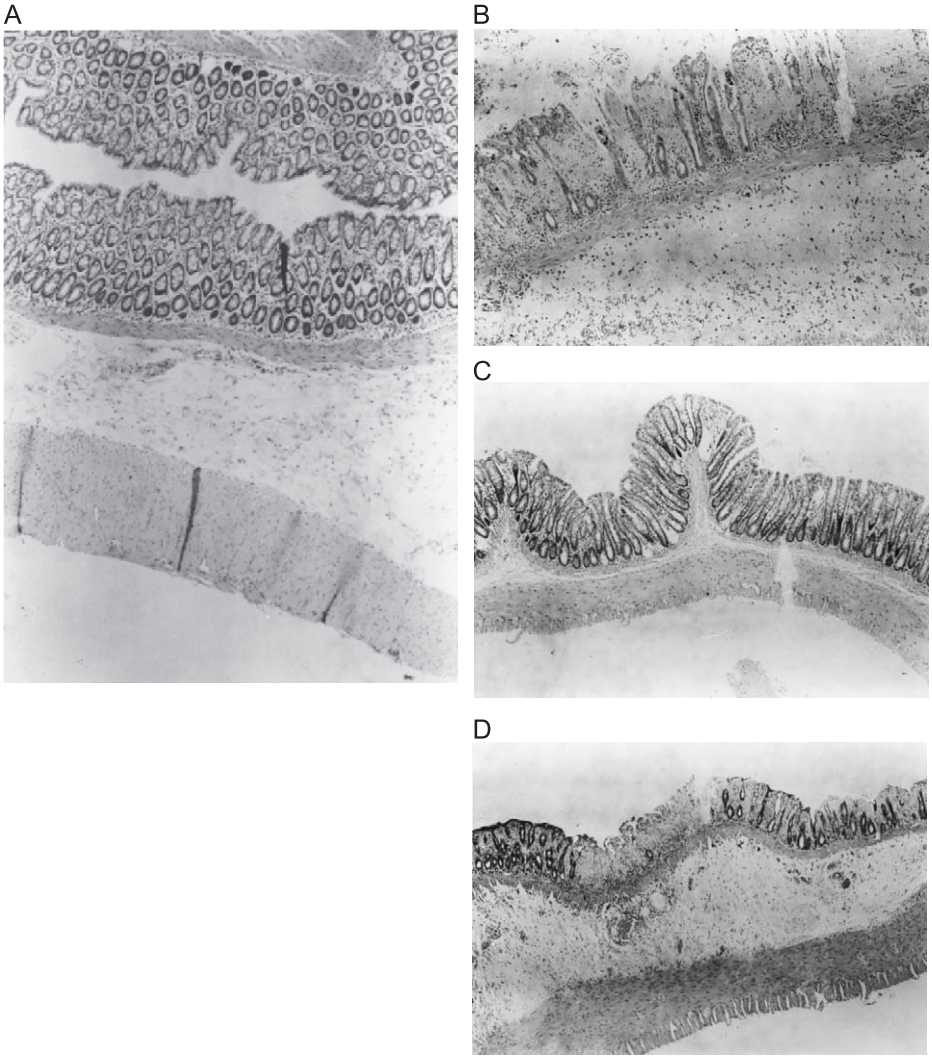

Fig. 3. (A) Photomicrograph of the haematoxylin and eosin stained section of rat colon (Normal control group); ×100. (B) Photomicrograph of haematoxylin and eosin stained section of the rat colon. It shows the acetic-acid-induced areas of massive necrotic destruction of epithelium, submucosal oedema, areas of haemorrhages and inflammatory cellular infiltration; ×100. (C) Photomicrograph of the haematoxylin and eosin stained section of the rat colon, which had been treated with celecoxib (5 mg/kg) given orally twice daily. The mucosa appears intact; ×100. (D) Photomicrograph of haematoxylin and eosin stained section of the rat colon, which was treated with rofecoxib (2.5 mg/kg) given orally twice daily. It shows areas of mild cell infiltration and congestion. The epithelial surface appears intact; ×100.

collected. Aliquots (50 μl) of the supernatant were mixed with a calcium-dependent assay mixture, for total NOS activity in a final volume of 200 μl, and controls were prepared with distilled water instead of homogenate supernatants. The incubation was performed at 37 °C for 10 min. The final mixture was incubated for 30 min at 4 °C after the addition of 0.5 ml distilled water. The tubes containing the final mixture were centrifuged at 1500 rpm, and 0.7 ml aliquots of supernatant were counted in a Beckman L-S-7500 scintillation counter. The activity was calculated as picomoles of citrulline produced per minute per milligram of protein.

2.5.5. Fluorometric assay of colonic histamine content

Colonic tissue (0.5 g) was homogenized with 0.4 N perchloric acid using the dismembranator. An aliquot of the supernatant fluid (4 ml) was transferred to a 25 ml glass-stoppered tube containing 0.5 ml of 5 N NaOH, 1.5 g of solid NaCl and 10 ml *n*-butanol. The tubes were shaken to extract histamine into the butanol layer. After centrifugation, the aqueous phase was removed by aspiration. The histamine formed was measured with an Aminco-Bowman spectrophotofluorometer, with an excitation wavelength of 360 nm and fluorescence wavelength of 450 nm (Lorenz et al., 1971).

2.5.6. Measurement of prostaglandin E_2 production

A piece of colonic mucosa was excised and rapidly rinsed with ice-cold saline. The tissue was weighed and homogenized in triethylammonium phosphate (TEAP) buffer (pH 3.3), which contained a cyclooxygenase inhibitor, Inyesprin®. The homogenate was centrifuged at 3000 rpm for 10 min at 4 °C, and the supernatant was removed. The supernatant was passed through a reverse-phase octadecylsilica C18 Sep Pak cartridge, which was washed with 10 ml distilled water, 10 ml 15% ethanol, 10 ml hexane and 10 ml ethylacetate, and the eluate was collected. Each fraction was evaporated with ethylacetate, and the dry residue was redissolved in ethanol. Prostaglandin E_2 was measured with a competitive enzyme immunoassay kit. Results are expressed as pg/mg tissue (Martin et al., 2003).

Table 2
Effects of celecoxib and rofecoxib on nitric oxide synthetase (NOS) activity in the colonic tissue of rats

Treatment	*n*	Dose (mg/kg, p.o.)	Nitric oxide synthetase activity (nmol/g tissue)
Saline control	8	–	1.08±0.11
3% Acetic acid	8	–	4.35±0.50[a]
2% Gum acacia	8	–	4.15±0.40
Celecoxib	8	5	2.05±0.10[b]
Rofecoxib	8	2.5	3.00±0.18[b]

Results are expressed as mean±S.E.M. of eight observations.
The vehicle or drugs were administered orally twice daily for 5 days, 24 h after the induction of colitis; *n*=number of animals used.
[a] $P<0.05$ as compared to the saline control group.
[b] $P<0.05$ as compared to the acetic acid group.

2.6. Statistical analysis

Data are expressed as mean±standard error of the mean (S.E.M.) for eight rats per experimental group. Statistical group analysis was performed with SPSS 10.0 statistical software. One-way analysis of variance (ANOVA) was used to compare the mean values of quantitative variables among the groups. Duncan's multiple range tests was used to identify the significance of pair-wise comparisons of mean values, among the groups.

3. Results

3.1. Histological results

3.1.1. Macroscopic

Twenty-four hours after the administration of 3% acetic acid into the colon, macroscopic inspection showed

Table 1
Effects of celecoxib and rofecoxib on myeloperoxidase (MPO) levels in the colonic tissue of rats

Treatment	*n*	Dose (mg/kg, p.o.)	Myeloperoxidase levels (units/mg tissue)
Saline control	8	–	0.58±0.02
3% Acetic acid	8	–	1.05±0.05[a]
2% Gum acacia	8	–	1.03±0.04
Celecoxib	8	5	0.64±0.02[b]
Rofecoxib	8	2.5	0.75±0.02[b]

Results are expressed as mean±S.E.M. of eight observations.
The vehicle or drugs were administered orally twice daily for 5 days, 24 h after the induction of colitis; *n*=number of animals used.
[a] $P<0.05$ as compared to the saline control group.
[b] $P<0.05$ as compared to the acetic acid group.

Table 3
Effects of celecoxib and rofecoxib on reduced glutathione (GSH) content in the colonic tissue of rats

Treatment	*n*	Dose (mg/kg, p.o.)	Reduced glutathione content (nmol/g tissue)
Saline control	8	–	1121.17±7.15
3% Acetic acid	8	–	847.67±11.33[a]
2% Gum acacia	8	–	845.83±8.89
Celecoxib	8	5	1118.83±3.53[b]
Rofecoxib	8	2.5	1008.33±4.89[b]

Results are expressed as mean±S.E.M. of eight observations.
The vehicle or drugs were administered orally twice daily for 5 days, 24 h after the induction of colitis; *n*=number of animals used.
[a] $P<0.05$ as compared to the saline control group.
[b] $P<0.05$ as compared to the acetic acid group.

Table 4
Effects of celecoxib and rofecoxib on platelet activating factor (PAF) content in the colonic tissue of rats

Treatment	n	Dose (mg/kg, p.o.)	Platelet activating factor (pg/10 mg tissue)
Saline control	8	–	12.5±0.5
3% Acetic acid	8	–	25.63±1.37[a]
2% Gum acacia	8	–	25.27±1.39
Celecoxib	8	5	14.07±0.70[b]
Rofecoxib	8	2.5	17.43±0.62[b]

Results are expressed as mean±S.E.M. of eight observations.
The vehicle or drugs were administered orally twice daily for 5 days, 24 h after the induction of colitis; *n*=number of animals used.
[a] $P<0.05$ as compared to the saline control group.
[b] $P<0.05$ as compared to the acetic acid group.

Table 6
Effects of celecoxib and rofecoxib on prostaglandin E_2 levels in the colonic tissue of rats

Treatment	n	Dose (mg/kg, p.o.)	Prostaglandin E_2 (pg/mg tissue)
Saline control	8	–	441.76±15.21
3% Acetic acid	8	–	882.17±17.98[a]
2% Gum acacia	8	–	876.89±16.69
Celecoxib	8	5	443.96±13.74[b]
Rofecoxib	8	2.5	445.24±19.08[b]

Results are expressed as mean±S.E.M. of eight observations.
The vehicle or drugs were administered orally twice daily for 5 days, 24 h after the induction of colitis; *n*=number of animals used.
[a] $P<0.001$ as compared to the saline control group.
[b] $P<0.001$ as compared to the acetic acid group.

evidence of damage, namely, the presence of mucosal congestion, extensive disruption and linear and deep haemorrhagic ulceration (Fig. 1). In this experimental group, there was evidence of haemorrhagic diarrhoea and weight loss (Fig. 2).

Treatment of rats with either rofecoxib or celecoxib significantly attenuated the extent and severity of the colonic injury and reduced the score of the macroscopic damage (Fig. 1). In addition, the drugs attenuated the degree of haemorrhagic diarrhoea and weight loss that was observed in acetic acid-treated rats (Fig. 2).

3.1.2. Microscopic results

The histopathological features included transmural necrosis, oedema and diffuse inflammatory cell infiltration in the mucosa, desquamated areas and loss of the epithelium. An infiltrate consisting of polymorphonuclear leukocytes, lymphocytes and eosinophils was observed as compared to normal control (Fig. 3A and B). Celecoxib attenuated the extent and severity of the histological signs of cell damage. Additionally, we did not see inflammatory cells in the lamina propria. In some areas, the epithelium was still intact (Fig. 3C and D).

Table 5
Effects of celecoxib and rofecoxib on histamine concentrations in the colonic tissue of rats

Treatment	n	Dose (mg/kg, p.o.)	Histamine concentration (μg/g tissue)
Saline control	8	–	17.67±0.67
3% Acetic acid	8	–	26.83±0.79[a]
2% Gum acacia	8	–	26.83±1.17
Celecoxib	8	5	18.17±0.60[b]
Rofecoxib	8	2.5	20.33±0.99[b]

Results are expressed as mean±S.E.M. of eight observations.
The vehicle or drugs were administered orally twice daily for 5 days, 24 h after the induction of colitis; *n*=number of animals used.
[a] $P<0.05$ as compared to the saline control group.
[b] $P<0.05$ as compared to the acetic acid group.

3.2. Biochemical results

The levels of MPO activity in the tissues showed a statistically significant difference among the five tested groups ($P<0.001$). By performing pair-wise comparisons among these five groups, we can infer that the mean value of acetic acid control group was significantly increased ($P<0.05$) as compared to that of the normal control group. On the other hand, after treatment with celecoxib or rofecoxib, a significant reduction in MPO activity was observed, as compared with the acetic acid control group ($P<0.01$; Table 1).

The level of NOS activity was significantly elevated in the colonic tissue 24 h following acetic acid instillation ($P<0.05$) as compared to that of the normal control group. After treatment with celecoxib or rofecoxib, NOS activity was substantially decreased ($P<0.05$; Table 2).

The tissue levels of GSH were significantly decreased after the induction of colitis as compared to that of the normal control. After treatment with celecoxib or rofecoxib, there was a significant increase in the GSH concentration (Table 3).

Both PAF and histamine contents were increased significantly in the colonic tissue of the acetic acid group as compared to that of the normal control animals ($P<0.01$). Treatment with rofecoxib or celecoxib, however, produced a statistically significant reduction in the levels of both histamine and PAF, as shown in Tables 4 and 5, respectively.

The levels of prostaglandin E_2 were significantly elevated in the colon, 24 h after acetic acid instillation ($P<0.001$). However, the levels of this prostanoid were significantly decreased ($P<0.001$) in rats treated with either celecoxib or rofecoxib, as compared to that of acetic acid group ($P<0.001$; Table 6).

4. Discussion

The present study showed that treatment with the cyclooxygenase-2 inhibitors rofecoxib and celecoxib reduced the inflammation and the damage to the colon,

induced by acetic acid, as verified by our macroscopic, histological and biochemical findings.

The results of the present study clearly demonstrated that celecoxib and rofecoxib exert a significant attenuation of the extent and severity of the histological signs of cell damage. These results are in agreement with Martin et al. (2003), who showed that rofecoxib decreased the mucosal damage induced by TNBS in rats. Furthermore, Cuzzocrea et al. (2001) provided evidence for the potential protective effect of celecoxib in reducing the severity of colonic injury induced by dinitrobenzene sulfonic acid (DNBS). Additionally, Karmeli et al. (2000) also reported that nimesulide, a nonsteroidal antiinflammatory, ameliorates the extent of tissue damage in acetic acid and iodoacetamide-treated rats.

There is good evidence that an enhanced formation of reactive oxygen species contributes to the pathophysiology of inflammatory bowel disease (Guo et al., 1999; Kruidenier and Verspaget, 2002). Quantitatively, the principal free radical in tissues is superoxide anion (O_2^-), which is converted to H_2O_2 by superoxide dismutase. Superoxide anion (O_2^-) can be produced by activated neutrophils through NADPH oxidase, which reduces molecular oxygen to the O_2^- radical through the enzyme myeloperoxidase. In our present study, the increase in myeloperoxidase activity in the colon was reduced by celecoxib or rofecoxib. This finding is in agreement with the results previously reported by other investigators (Cuzzocrea et al., 2001; Kankuri et al., 2001), which showed that cyclooxygenase-2 inhibitors, such as celecoxib or nimesulide, markedly reduce cellular infiltration and inflammation of the colon.

Nitric oxide (NO), a reactive free radical gas, is generated enzymatically in a variety of cells from the L-arginine pathway by three isoforms of NO synthetase (Yue et al., 2001). In the gastrointestinal tract, NO can be either protective or damaging to tissues, depending on what type of NOS is involved in the pathological condition. In experimental colitis, NO derived from iNOS, together with other free radicals, contribute significantly to the inflammatory response in the colon. The mechanism for this inflammatory response is likely explained by the interaction of NO with superoxide to produce peroxynitrite, which is a strong oxidizing agent that initiates lipid peroxidation. High concentrations of NO are known to exhibit toxic effects through the formation of nitroso derivatives (Rachmilewitz et al., 1995a). The reduction in NOS activity, which was observed in the present study, following treatment with either rofecoxib or celecoxib, corroborates the previously reported findings of Miller et al. (1993), Rachmilewitz et al. (1995b) and Cho (2001), who showed a reduction in NOS activity following treatment with nonselective cyclooxygenase inhibitors. This reduction, which we had observed, may be attributed mainly to the antiinflammatory effect and to, some extent, to the antioxidant activity of these two cyclooxygenase-2 inhibitors.

The chondroitin sulfate-E-containing mast cells, which synthesize platelet activating factor (PAF), are present in human colon and gastric mucosa. The release of PAF is increased in active ulcerative colitis (Eliakim et al., 1988). It had been proposed by Rosam et al. (1986) that PAF acts as an endogenous mediator of gastric ulceration. It induces extensive mucosal haemorrhages and erosions. In the present study, a significant elevation of mucosal PAF levels was observed in the acetic-acid-treated rats. Similar increases in PAF had been reported using the same acetic acid model (Mahgoub, 2003; Mahgoub et al., 2003) or other models (Barret, 1990; Eliakim et al., 1992; Fabia et al., 1993). This elevation was significantly reduced after pretreatment of rats with either celecoxib orrofecoxib. It had been reported that 5-aminosalicylic acid, a known antiinflammatory drug, inhibited the synthesis of PAF (Eliakim et al., 1988). This effect was explained by the inhibition of the synthesis of lipooxygenase products (Billah et al., 1985). Similarly, the reduction in colonic PAF levels by celecoxib or rofecoxib could be explained by the same mechanism as was suggested for 5-aminosalicylic acid by Billah et al. (1985).

Reactive oxygen species contribute to tissue injury in inflammatory bowel disease. The tripeptide glutathione is the most important intracellular antioxidant. GSH deficiency signifies an excessive production of reactive oxygen species (Sido et al., 1998). Millar et al. (1996) used the acetic acid model to test the antioxidant potential of 5-aminosalicylic acid. Other investigators (Grisham et al., 1991; Barret, 1990) had shown a reduction in GSH levels following the use of TNBS as a model of experimental colitis. They suggested that the levels of GSH were reduced in tissues when the antioxidant was neutralized by the liberated oxygen-derived free radicals. Treatment of rats in the present study with celecoxib or rofecoxib increased the GSH levels in colonic mucosa. Therefore, it may be suggested that the elevated levels of GSH, which we had observed, may be explained to the radical scavenging capacity of rofecoxib and celecoxib.

The colitis induced by acetic acid in the present study was accompanied by a significant increase in colonic histamine levels. Indeed, it had been shown that TNBS-induced colitis in rats resulted in a significant increase in colonic histamine, which was accompanied by an increase in ulcer index score and myeloperoxidase enzyme activity (Barret, 1990). The decrease in the histamine level in the colon observed after celecoxib and rofecoxib treatments is in agreement with the work of Karmeli et al. (1995), who reported a decline in histamine levels following the treatment of rats with ketotifen.

Endogenous prostaglandin E_2 is produced by mononuclear cells in the lamina propria and is dependent on cyclooxygenase-2 expression. It modulates the intestinal immune response, including the differentiation of T cells and the production and release of proinflammatory cytokines (Newberry et al., 1999). During the course of inflammatory bowel disease and experimental colitis, some prostanoids are released and subsequently modulate the

course of the disease (Carty et al., 2000). Our results showed an elevation of prostaglandin E_2 in the acetic acid group. The administration of the cyclooxygenase-2 inhibitors, rofecoxib and celecoxib, produced a significant reduction in tissue prostaglandin E_2 production. Our present data are in accord with the previous studies of Guo et al. (2001) and Kankuri et al. (2001), who showed a reduction in prostaglandin E_2 production following the administration of nimesulide. Therefore, pretreatment with cyclooxygenase-2 inhibitors may attenuate the inflammation in experimental colitis by reducing the levels of prostaglandin E_2.

In conclusion, our data suggest that cyclooxygenase-2 selective inhibitors may have a therapeutic role in ulcerative colitis.

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