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Chronic gastric ulcer healing in rats subjected to selective and non-selective cyclooxygenase-2 inhibitors

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Abstract

The influence of different nonsteroidal anti-inflammatory drugs (NSAIDs) and of a proton pump inhibitor on the healing parameters of a chronic gastric ulcer was evaluated. Wistar rats were used after the induction of a chronic acetic acid ulcer. The animals were treated orally for 8 and 15 days, twice daily, with the conventional NSAID, piroxicam (0.35 mg/kg), the non-narcotic analgesic, metamizol (33 mg/kg), the selective cyclooxygenase-2 inhibitor, celecoxib (1.8 mg/kg) and the proton pump inhibitor, omeprazole (0.35 mg/kg). Macroscopic ulcer index, myeloperoxidase activity and prostaglandin E_2 content (both biochemical parameters were evaluated in ulcerated and in intact tissue) as well as histological and immunohistochemical evaluations were carried out at 8 and 15 days. Omeprazole accelerated ulcer healing at 8 and 15 days ($P<0.05$), while celecoxib delayed healing significantly at 15 days ($P<0.01$). At 8 days, the prostaglandin E_2 content decreased with all NSAIDs at the ulcer site as well as in intact tissue. The same happened at 15 days except for celecoxib, which only diminished prostaglandins in intact mucosa. Immunohistochemistry showed differences in the location of cyclooxygenase-2 and -1. The highest cyclooxygenase-2 expression was found with piroxicam and the lowest expression was with celecoxib. *Conclusions*: Down-regulation of cyclooxygenase-2 expression as well as a possible involvement of the chemical structure of celecoxib, a 1,5-dirarylpirazole with a sulphonamide moiety, may account for the delay in ulcer healing.

Keywords: Gastric ulcer; Ulcer healing, chronic; Nonsteroidal anti-inflammatory drugs; Prostaglandin; Myeloperoxidase activity; Immunohistochemistry

1. Introduction

Chronic administration of NSAIDs is often associated with the development of adverse gastrointestinal effects, such as gastric erosions, gastric or duodenal ulceration, and severe complications, such as gastrointestinal hemorrhage or perforation (Hawkey, 1990), which often limit their widespread clinical use (Jones and Tait, 1995; Wright, 1995).

The mechanisms underlying these lesions involve ischaemic processes with subsequent oxygen free radical release (Villegas et al., 2000) and neutrophil infiltration (Beck et al., 2000). Furthermore, NSAIDs have been proven to impair the healing of previously existing gastric ulcers (Mizuno et al., 1997).

Recently, selective cyclooxygenase-2 inhibitors such as NS-398, L-745,337, celecoxib and rofecoxib have been developed in an effort to diminish gastrointestinal adverse effects, as these new drugs spare the production of prostaglandins important for the maintenance of mucosal integrity and renal blood flow (Warrer et al., 1999). However, several selective cycloxygenase-2 inhibitors have been reported to reduce significantly pain and inflammation only at doses where selectivity is lost (Wallace et al., 1998a,b). Moreover, recent studies have shown that cyclooxygenase-2 produces prostaglandins that exert anti-inflammatory actions (Gilroy et al., 1999) and that play an important role in the healing of gastric ulcers (Shigeta et al., 1998; Brzozowski et al., 2001). Therefore, these drugs may be contraindicated in circumstances of inflamed mucosa, e.g. peptic ulcer or inflammatory bowel disease (Wallace et al., 1998a,b). In this regard, some of these selective cyclooxygenase-2 inhibitors, such as celecoxib and rofecoxib, in spite of not producing toxic injuries to healthy gastrointestinal mucosa, have been proven to worsen and complicate preexisting gastric ulcers (Laudanno et al., 2001).

The aim of this study was to assess the different healing parameters of a chronic acetic acid-induced ulcer in rats

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treated with the conventional NSAID piroxicam, which inhibits predominantly cyclooxygenase-1 (Smith et al., 1994) and is rather gastrolesive, the non-narcotic analgesic metamizol, which has good gastric tolerability and has been proven to be about 10-fold more potent in vitro to inhibit the inducible rather than the constitutive isoform (Campos et al., 1999), and the selective cyclooxygenase-2 inhibitor celecoxib. Another group received the proton pump inhibitor, omeprazole.

Ulcer index, myeloperoxidase activity as index of neutrophil infiltration and prostaglandin E_2 levels were evaluated in ulcerated and in intact tissue after 8 and 15 days of treatment. Histopathological evaluation and immunohistochemical analysis of cyclooxygenase-1 and -2 were also carried out.

2. Materials and methods

2.1. Animals, drugs and doses assayed

Male and female Wistar rats supplied by Animal Services, Faculty of Medicine, University of Sevilla, Spain, and 180–250 g in body weight, were placed singly in cages with wire-net floors in a room with controlled temperature (22–24 °C) and humidity (70–75%), lighting regimen of 12L/12D) and were fed a normal laboratory diet. The rats were deprived of food for 24 h before experimentation but allowed free access to tap water throughout. They were randomly assigned to groups of 10–12 animals. Experiments followed a protocol approved by the local animal Ethics Committee and the Local Government. All experiments were in accordance with the recommendations of the European Union.

All drugs were suspended in Tween 20 (1%) and administered orally by gavage. Control groups received vehicle in a comparable volume (1 ml/100 g body weight).

The doses assayed were selected by extrapolation of the clinical human doses: metamizol (Boehringer Ingelheim) 33 mg/kg/12 h, celecoxib (Searle-Monsanto) 1.8 mg/kg/12 h, piroxicam (Cecofar) 0.35 mg/kg/12 h and omeprazole (Cepa) 0.35 mg/kg/12 h.

2.2. Ulcer induction

Gastric ulcers were induced experimentally in rats according to the method described by Okabe and Pfeiffer (1971). After anesthesia with ether, the abdomen was opened by medial incision, and 50 μl of 5% acetic acid was injected in the subserosa of the midcorpus near the antral portion of the stomach using a Hamilton syringe with a 30-gauge needle. Then the abdomen was sutured with catgut and the rats were allowed to recover and were returned to their cages with food and water ad libitum. In control animals, the abdomen was opened and closed without injection. In order to avoid postsurgical infections, the rats received penicillin i.p. 1,000,000 U/kg/12 h for the next 72 h. The treatments with metamizol, celecoxib, piroxicam and omeprazole started 24 h after surgery. Drugs were administered twice daily for 8 and 15 days.

2.3. Ulcer assessment

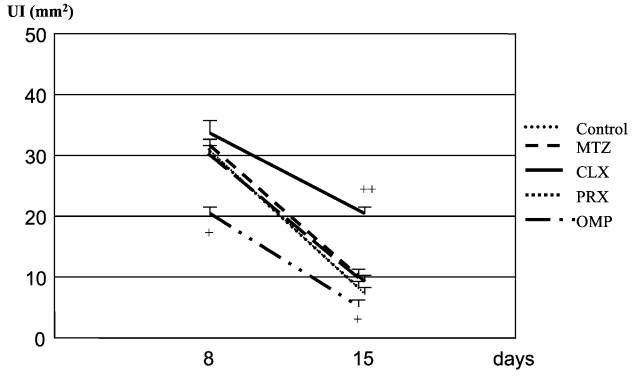
Ten animals of each group were killed at 8 and 15 days after the beginning of the treatments. The rats were killed with ether and the stomachs were rapidly dissected out, opened along the greater curvature and the mucosa was exposed for macroscopic evaluation. The size of the ulcer in square millimeters was considered the most objective measurement of macroscopic lesion. This parameter was measured with a Kodak 1D Image Analysis system.

2.4. Histopathological study

Two rats per group were killed with an overdose of ether and laparatomy was performed. The stomach was opened along the greater curvature and slightly stretched on a paraffin panel in order to prevent mucosal folding. The ulcer crater of each stomach was cut out with a scalpel and was fixed for 4 h in 4% buffered paraformaldehyde, then dehydrated gradually in ethanol and embedded in paraffin, using xylene as intermediate solvent. Serial sections (6 μm) were obtained by cutting the block in a plane perpendicular to the mucosal surface with a microtome. Coded gastric sections were stained with haematoxylin and eosin before light microscopic evaluation.

2.5. Myeloperoxidase activity

Myeloperoxidase activity was assessed as a marker of neutrophil infiltration (Grisham et al., 1990). One sample of the ulcer crater and another portion of intact gastric



+, P<0.05 and ++, P<0.01 vs ulcerated control

Fig. 1. Effect of the treatments on the ulcer index (UI), at 8 and 15 days. Drugs administered p.o. twice daily: metamizol 33 mg/kg (MTZ), celecoxib 1.8 mg/kg (CLX), piroxicam 0.35 mg/kg (PRX), omeprazole 0.35 mg/kg (OMP). Results are shown as the means±S.E.M. for 8–10 animals per group.

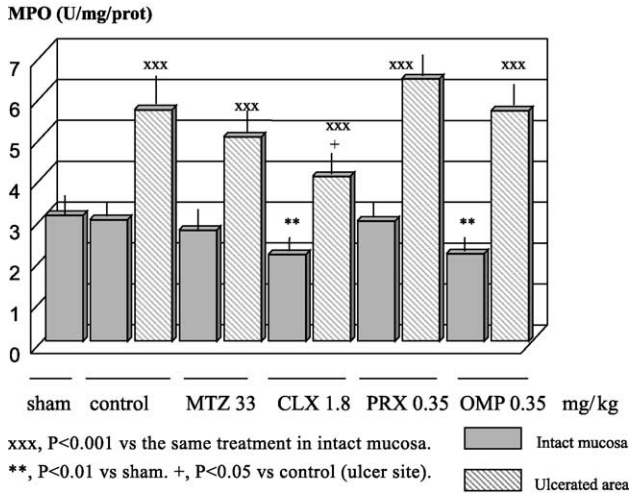


Fig. 2. Myeloperoxidase activity (MPO) as neutrophil infiltration index in ulcerated and in intact mucosa after 8 days of treatment with metamizol 33 mg/kg (MTZ), celecoxib 1.8 mg/kg (CLX), piroxicam 0.35 mg/kg (PRX) or omeprazole 0.35 mg/kg (OMP). Values are indicated as the means±S.E.M. for 8–10 rats.

mucosa were obtained from each animal. The tissue was homogenized in phosphate-buffered saline (PBS), pH=7.4 and centrifuged, and the pellet was again homogenized in PBS, pH=6.0, containing hexadecyl-trimethylammonium bromide (HETAB) and ethylendiamine tetraacetic acid. This homogenate was subjected to one cycle of freezing/thawing and a brief period of sonication. A sample of homogenate (0.5 μl) was added to a 0.5-ml reaction volume containing PBS, pH 5.4, HETAB and 3,3′,5,5′-tetramethylbenzidine. The mixture was incubated at 37 °C. The reaction was started by the addition of H_2O_2, and terminated by the sequential addition of catalase and sodium acetate, pH=3.0. The changes in absorbance at 655 nm were measured with a microplate reader (Labsystem Multiskan Ex). One unit of myeloperoxidase activity

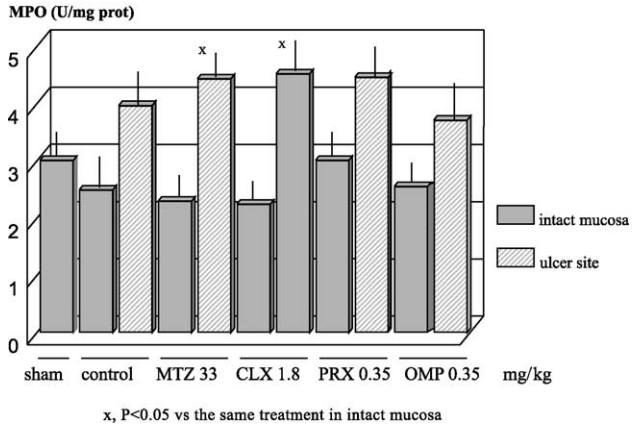


Fig. 3. Myeloperoxidase activity (MPO) at the ulcer site and in intact mucosa after 15 days of treatment with metamizol 33 mg/kg (MTZ), celecoxib 1.8 mg/kg (CLX), piroxicam 0.35 mg/kg (PRX) or omeprazole 0.35 mg/kg (OMP). Values are indicated as the means±S.E.M. for 8–10 rats.

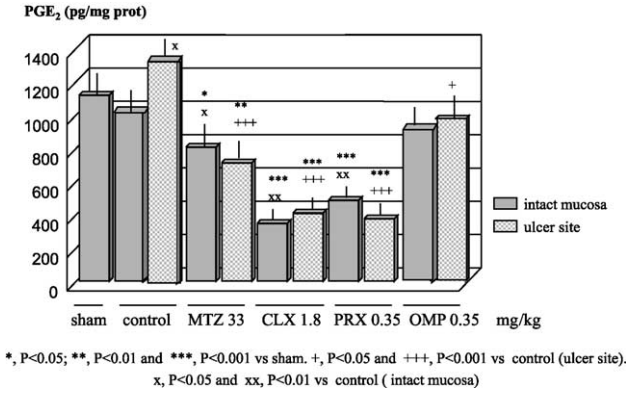


Fig. 4. Prostaglandin E_2 (PGE_2) content in intact and ulcerated mucosa after 8 days of treatment with one of the following drugs: metamizol 33 mg/kg (MTZ), celecoxib 1.8 mg/kg (CLX), piroxicam 0.35 mg/kg (PRX) or omeprazole 0.35 mg/kg (OMP). Values are indicated as the means±S.E.M. for 8–10 rats.

was defined as the amount of enzyme present that produced a change in absorbance of 1.0 U/min at 37 °C and the results were expressed as U/mg/protein. Protein content was assessed according to the method of Bradford (1976) using γ-globulin as a standard.

2.6. Mucosal prostaglandin E_2 content

Gastric mucosa was excised and rapidly rinsed with ice-cold saline. The tissue was weighed and homogenized for 15 s in 6 ml triethylamine-formic acid buffer (pH 3.24) which contained a cyclooxygenase inhibitor (lysine acetyl salicylate). The homogenate was centrifuged (3000 rpm, 10 min, 4 °C) and the supernatant was chromatographed 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 ethyl acetate, and the

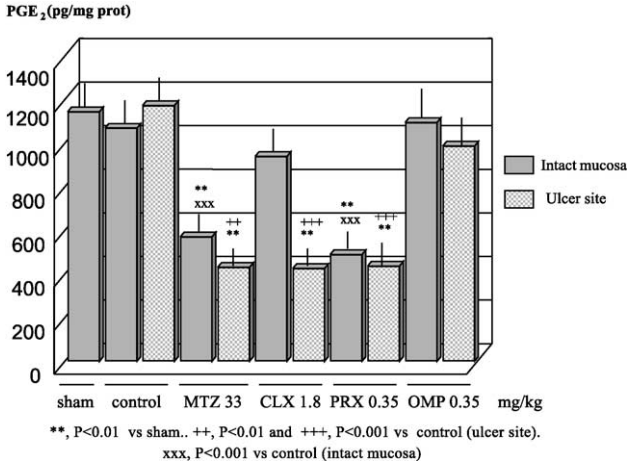


Fig. 5. Prostaglandin E_2 content in intact and ulcerated mucosa at 15 days. Drugs administered p.o. twice daily: metamizol 33 mg/kg (MTZ), celecoxib 1.8 mg/kg (CLX), piroxicam 0.35 mg/kg (PRX), omeprazole 0.35 mg/kg (OMP). Results are shown as the means±S.E.M. for 8–10 animals per group.

eluate was collected. Each fraction was evaporated with ethyl acetate, and the dry residue was redisolved in ethanol. Prostaglandin E_2 content was determined with a competitive enzyme immunoassay kit (Assay Designs) using a microplate reader (Labsystem Multiskan Ex) and the results were expressed as pg/mg protein.

2.7. Immunoreactivity of cyclooxygenase-1 and -2

Deparaffinized and rehydrated 6 μm sections were treated with blocking solution (3% bovine serum albumin) for 15 min. Then the sections were incubated separately with the primary antibodies for cyclooxygenase-1 and cyclooxygenase-2 (goat polyclonal, M-19 and M-20 of Santa Cruz Biotechnologies) at a dilution of (1:400) for 1 h. After being washed, the secondary antibody (anti-goat IgG, horseradish peroxidase conjugated) was applied at the same dilution for another hour. The slides were washed and incubated for 30 min with 3,3′ diaminobenzidine, Tris–HCl and hydrogen peroxide. The sections were examined under a light microscope (Olympus BH-2).

2.8. Statistical analysis

Values are given as arithmetic means±S.E.M. The significance of differences between means was evaluated using an analysis of variance (ANOVA) for parametric values and the Mann–Whitney *U*-test for non-parametric values.

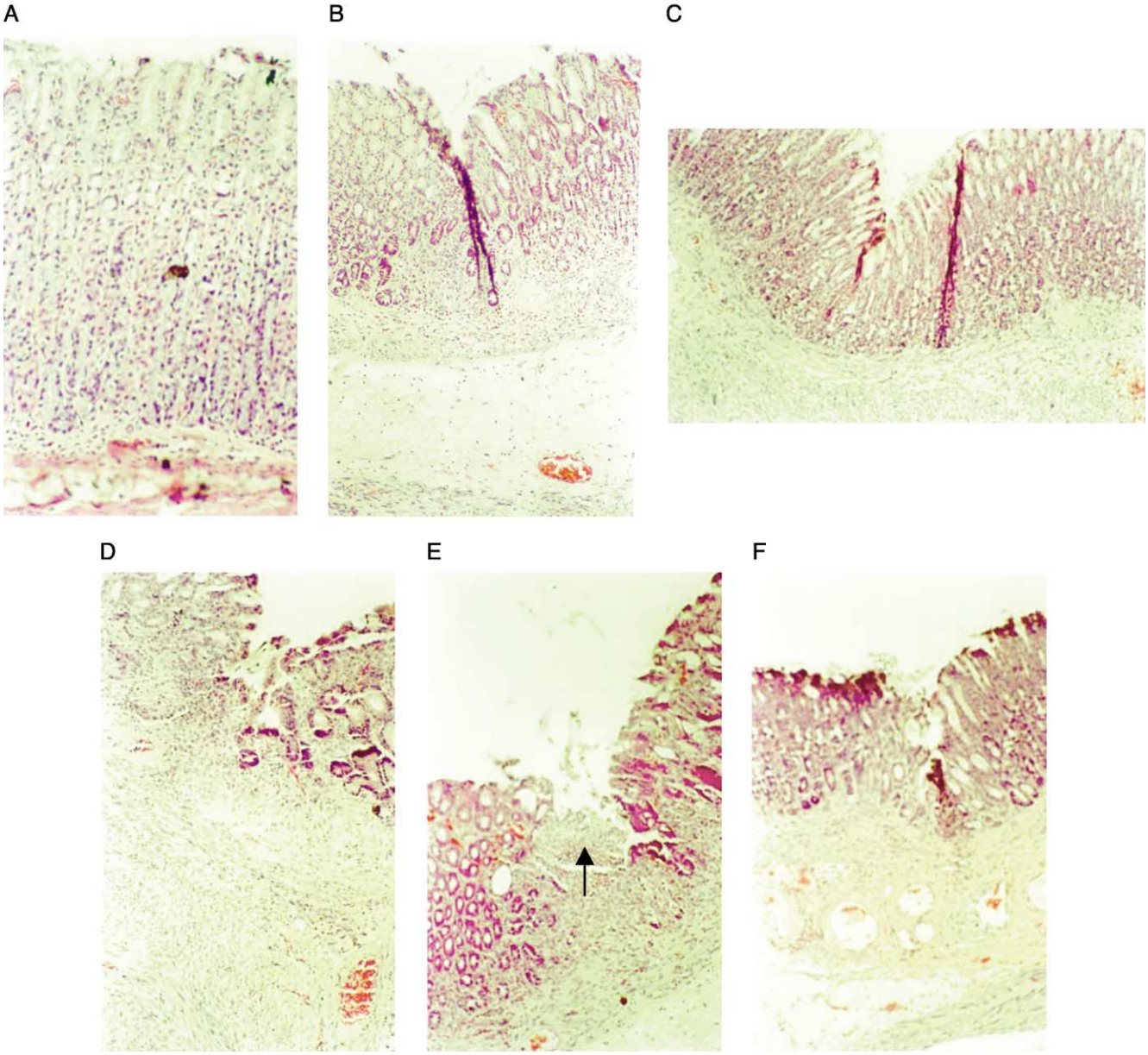


Fig. 6. Histological analysis of the 5% acetic acid-induced ulcer after 8 days of treatment. (A) Sham; (B) control; (C) metamizol; (D) celecoxib; (E) piroxicam; (F) omeprazole. Piroxicam showed a clearly detectable neutrophil infiltration at the ulcer site (arrows). Haematoxylin and eosin (10×).

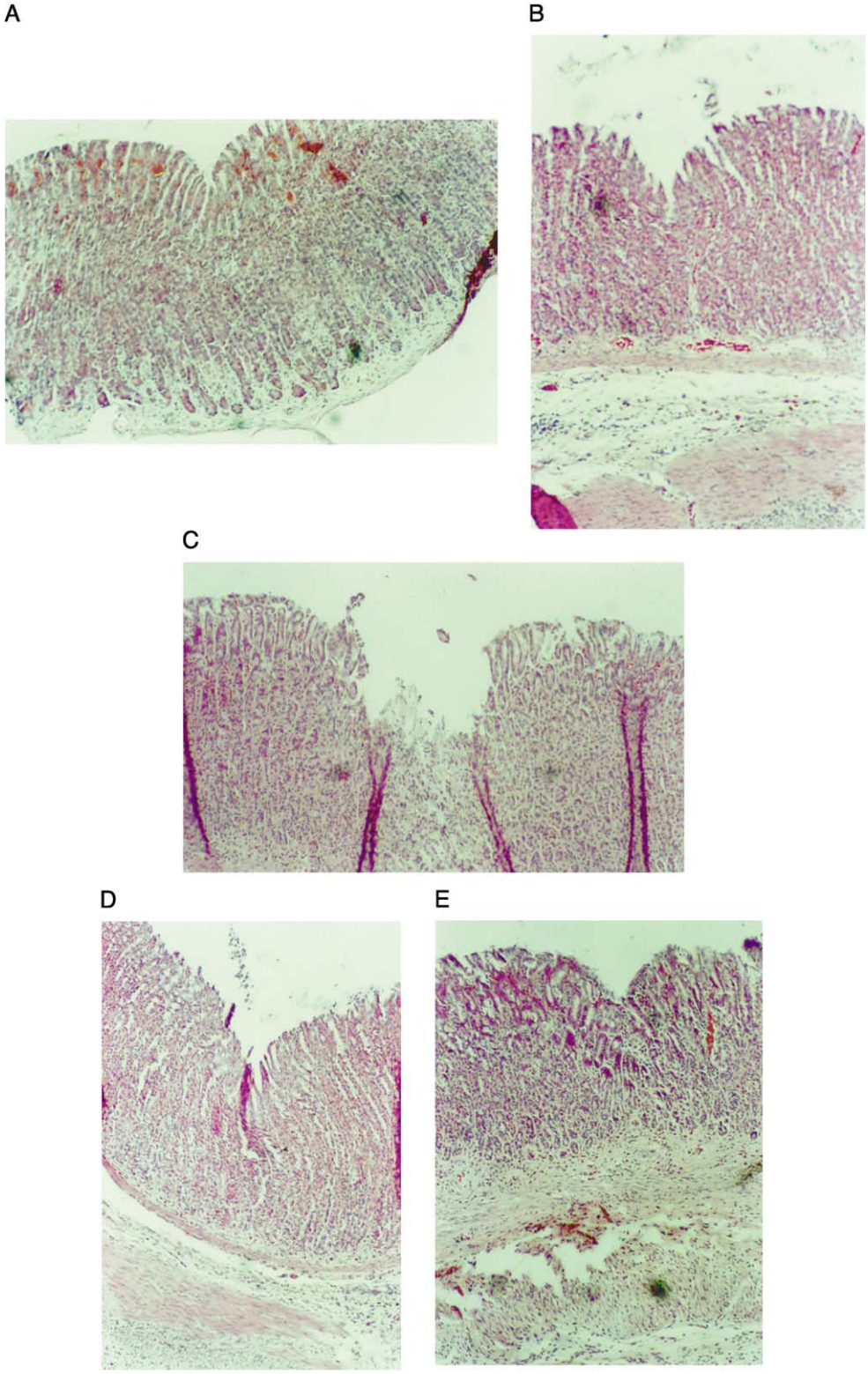


Fig. 7. Histological analysis of the ulcer crater after 15 days of treatment. (A) Control; (B) metamizol; (C) celecoxib; (D) piroxicam; (E) omeprazole. Haematoxylin and eosin (10×).

3. Results

Eight days after acetic acid ulcer induction the size of the ulcer crater in the control group was 30.32±12.41 mm^2. Oral administration of metamizol 33 mg/kg, celecoxib 1.8 mg/kg or piroxicam 0.35 mg/kg scarcely modified this ulcer index. However, omeprazole 0.35 mg/kg diminished the ulcerated area significantly ($P<0.05$). After 15 days, the healing of the lesion had progressed significantly (9.37± 2.20 mm^2; $P<0.001$ vs. 8 days control). Again, omeprazole showed the most favorable behaviour, resulting in an ulcer area of 4.75±0.98 mm^2; $P<0.05$ vs. 15 days control), whereas celecoxib delayed ulcer healing very markedly ($P<0.001$). Again, neither metamizol nor piroxicam affected the healing process (Fig. 1).

Myeloperoxidase activity as an index of neutrophil infiltration was assessed in intact tissue samples as well as in specimens obtained from the ulcer crater. No significant changes were observed vs. sham and control rats in the intact tissue with any of the treatments at 8 days or at 15 days. On the contrary, a strong increase in enzymatic activity was observed at the ulcer site after 8 days with all treatments ($P<0.001$ vs. intact tissue) (Fig. 2). At 15 days, myeloperoxidase activity remained higher in the ulcerated area than in intact mucosa with all treatments, but only metamizol and celecoxib produced significantly different effects in ulcerated and in non-ulcerated tissue (Fig. 3).

Prostaglandin E_2 content at 8 days increased significantly in the ulcerated area of the control group (1244.21±118.0 pg/mg protein) when compared with intact tissue of the same group (887.77±97.33 pg/mg protein; $P<0.05$). However, prostaglandin values decreased with all treatments in the intact as well as in the ulcerated mucosa. In both intact and ulcerated mucosa, the smallest decreases occurred with

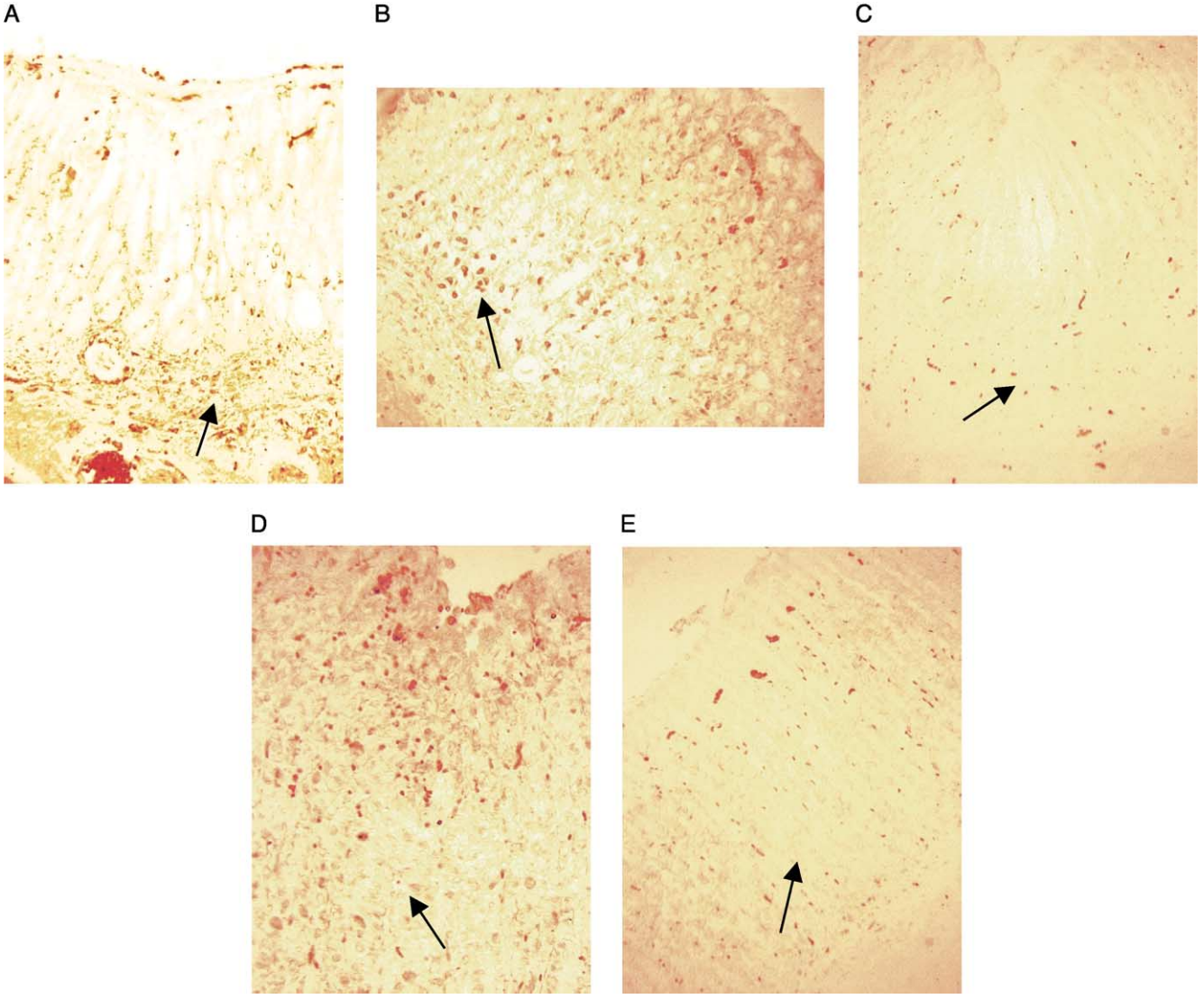


Fig. 8. Cycloxygenase-1 expression adjacent to the ulcer after 8 days. (A) Control; (B) metamizol; (C) celecoxib; (D) piroxicam; (E) omeprazole. Cyclooxygenase-1 was detected by immunohistochemistry mainly in mucous neck cells and in the base of the gastric glands (arrows), (20×).

metamizol ($P<0.05$ and $P<0.01$, respectively, vs. sham) and omeprazole (no statistical significance vs. sham), while the lowest values for mucosal prostaglandin content were obtained with celecoxib and piroxicam ($P<0.001$ vs. sham with both drugs) (Fig. 4). At 15 days, no differences in prostaglandin content between sham, intact mucosa and ulcer crater of the control and of the omeprazole group were observed. However, metamizol, celecoxib and piroxicam decreased the prostanoid levels in the ulcer crater to the same extent ($P<0.01$ vs. sham). In intact mucosa, celecoxib only slightly diminished the prostaglandin E_2 content (no significant difference vs. sham), whereas metamizol and piroxicam remained at low levels ($P<0.01$ vs. sham) (Fig. 5).

Histological examination of tissues revealed a lesion penetrating the full thickness of the gastric wall (mucosa to serosa) 3 days after subserosal acetic acid injection (pic-

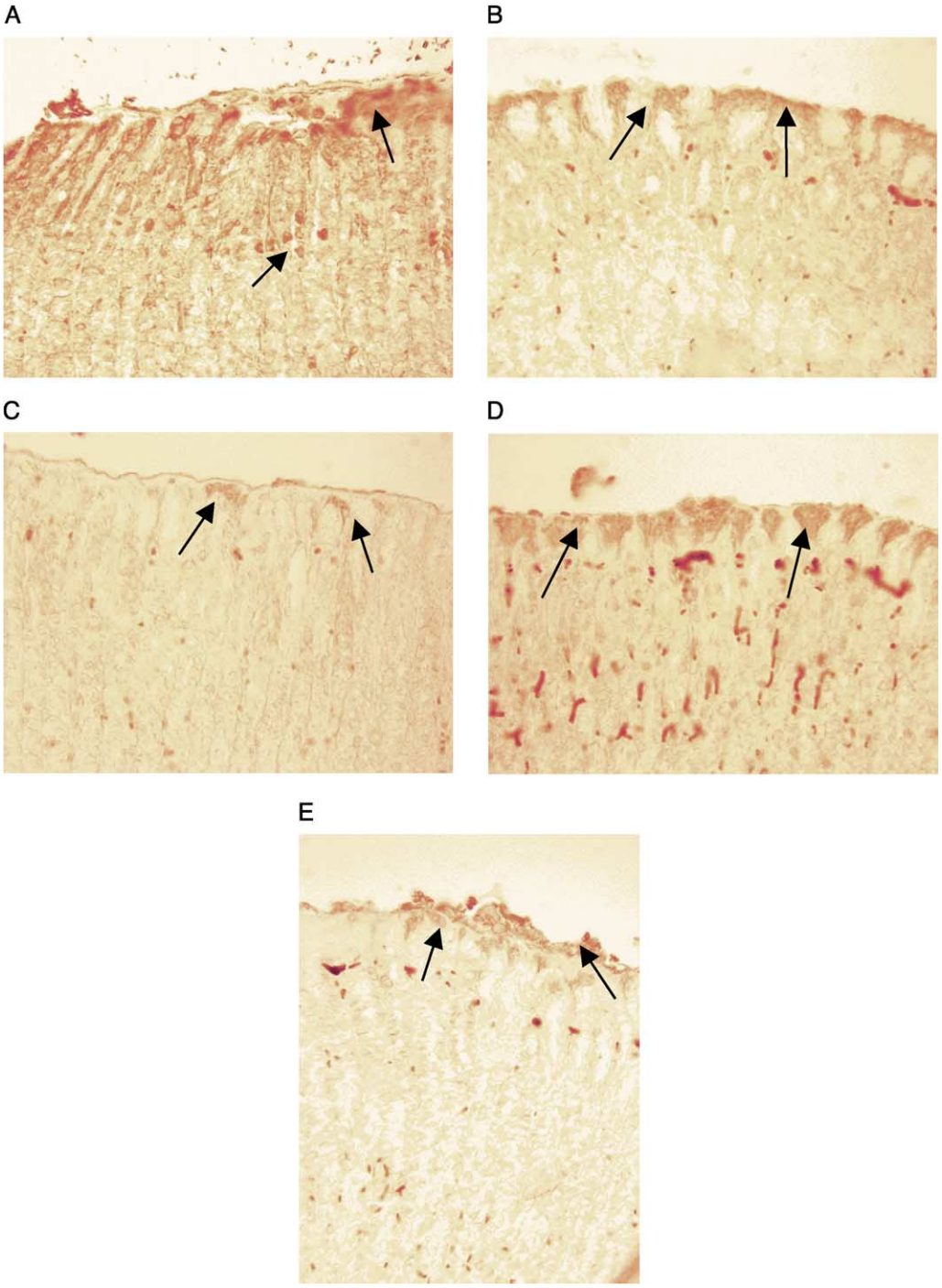


Fig. 9. Cyclooxygenase-2 expression detected by immunohistochemistry adjacent to the ulcer at 8 days. (A) Control; (B) metamizol; (C) celecoxib; (D) piroxicam; (E) omeprazole. Cyclooxygenase-2 expression was found in mucous surface cells and mucous cells of the foveoles (arrows), (20×).

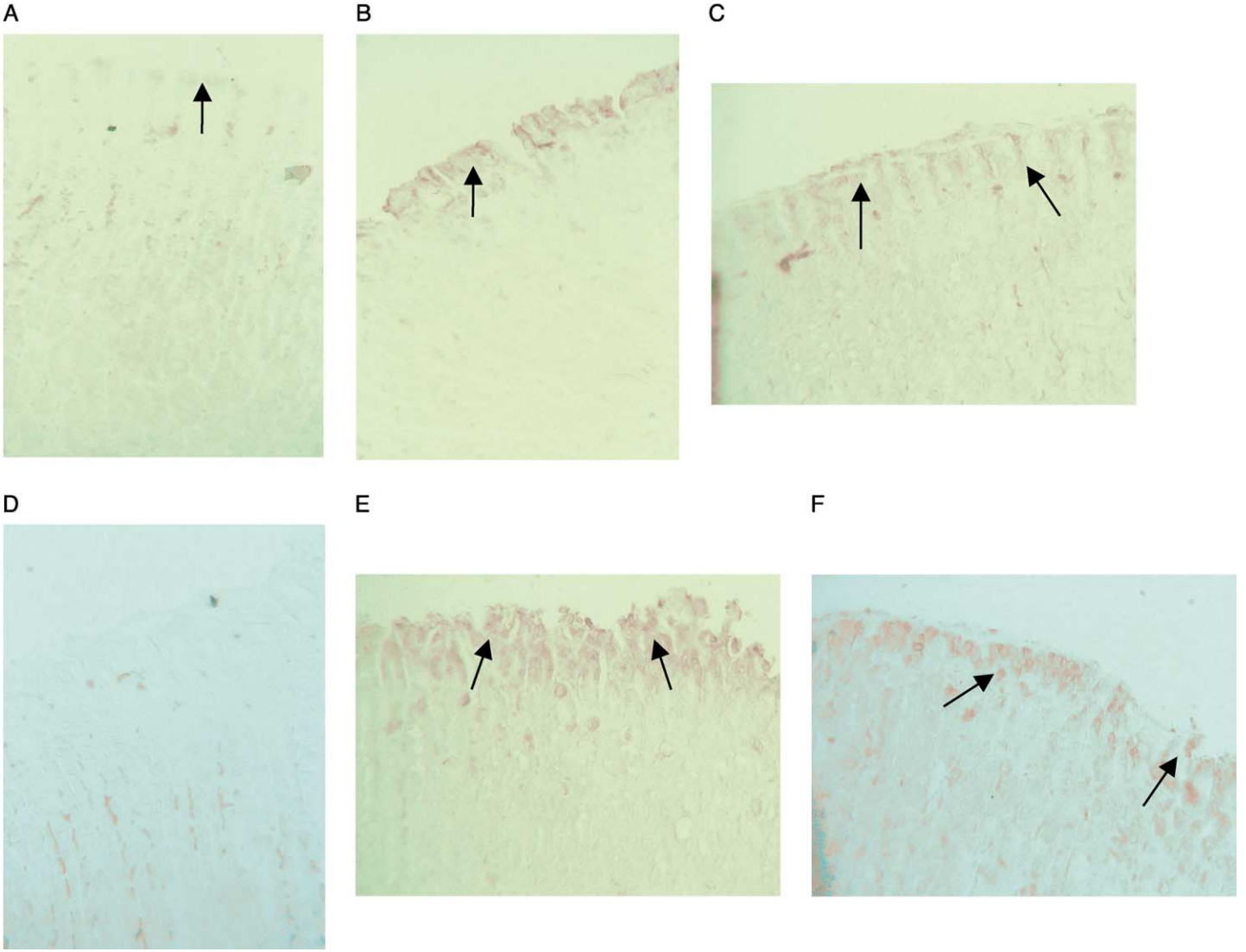


Fig. 10. Immnunohistochemistry for cyclooxygenase-2 expression at 15 days, (arrows), (20×). (A) Sham; (B) control; (C) metamizol; (D) celecoxib; (E) piroxicam; (F) omeprazole.

tures not shown). Acute inflammation with a fluid exudate rich in neutrophils was observed. Necrotic tissue was also present at this stage, whereas the gastric mucosa around the ulcer crater remained unchanged. After 8 days, the serosa was thickened externally and exhibited fibrous adhesions to the underlying liver. The ulcer crater was in an advanced healing stage. Fibrous granulation and scar tissue were observed in the ulcer base, and the regenerating epithelium contained glands that were cystic and hyperproliferative. The muscularis mucosa around the ulcer margin was replaced by granulation tissue. The thickness of the granulation tissue was greatest in animals receiving piroxicam, whereas omeprazole-treated rats showed the thinnest granulation tissue. The ulcerated control group and the one treated with omeprazole had a high density of microvessels in the ulcer base. On the contrary, microvessels were absent in the ulcerated tissue of rats that had received metamizol, celecoxib or piroxicam. Piroxicam was the only treatment which was followed by neutrophil infiltration clearly visible under light microscopy in the ulcer crater (Fig. 6).

After 15 days, the ulcer scar had undergone very extensive healing and restoring although regeneration of the mucosa on the ulcer base was apparently prevented by celecoxib (Fig. 7).

Immunohistochemical studies revealed that cyclooxygenase-1 and -2 proteins are located in different sites (Figs. 8 and 9). The constitutive isoform was expressed mainly in mucous neck cells and in the base of the gastric glands, whereas cyclooxygenase-2 was detected in mucous surface cells and mucous cells lining the foveoles adjacent to the ulcer crater. Cyclooxygenase-1 expression reflected no important differences among individual groups. On the contrary, cyclooxygenase-2 was scarcely found in the mucosa of the sham group, but was clearly present in the ulcerated control after 8 days. At this period, the strongest expression of this isoenzyme was observed in the animals treated with piroxicam, while the slightest expression corresponded to celecoxib. Metamizol-treated rats showed an intermediate level of expression, similar to that of the ulcerated control and to that following omeprazole.

At 15 days, an appreciable amount of cyclooxygenase-2 expression was still visualized in the tissue adjacent to the ulcer crater of the control group as well as in animals subjected to drug therapies (Fig. 10). Once again, celecoxib-treated rats exhibited the lowest cyclooxygenase-2 expression, while control and the other treatments showed similar expression levels. Constitutive isoform expression remained unaffected in all groups (pictures not shown).

4. Discussion

The mechanisms suggested to be responsible for the delay in ulcer healing caused by NSAIDs are: (1) inhibition of prostaglandin synthesis, which is crucial for gastrointestinal defense (Ligumsky et al., 1983; Wang et al., 1989); (2) inhibition of epithelial cell proliferation in the ulcer margin, which is critical for the re-epithelization of the ulcer crater (Levi et al., 1990); (3) inhibition of angiogenesis, which is essential for oxygen and nutrient supply to the ulcer bed (Hirose et al., 1991); and (4) inhibition of proliferation and function of myofibroblasts, which are important for the remodelling and contraction of the granulation tissue in the ulcer bed (Ogihara and Okabe, 1993).

In order to study these aspects related to ulcer healing in rats subjected to a concurrent NSAID treatment, we selected the chronic ulcer model proposed by Okabe and Pfeiffer (1971), which uses a relatively low acetic acid concentration in comparison to those used by other authors who inject the ulcerogenic agent at a much higher concentration: 20% (Fujita et al., 1998), or even 100% (Lesch et al., 1998). However, the model used by us made possible the production of well-characterized ulcers with a benign spontaneous ulcer healing (Navarro et al., 1990). In control animals killed after 1 and 3 days, we confirmed the formation of a well-defined ulcer. The original lesion undergoes a healing process of approximately 3 weeks (Navarro et al., 1990) and is found macroscopically healed in 50% of the animals at the end of this period. In spite of the macroscopic improvement, the lesions are persistent microscopically, which confirms the chronicity of these ulcers.

Omeprazole accelerated ulcer healing as soon as after 8 days of treatment and this was also evident after 15 days. This drug facilitates ulcer healing by inhibiting acid secretion and augmenting luminal pH (Dent and Chir, 1998), thereby decreasing acid and pepsin damage to the ulcerated mucosa as well as reducing basic fibroblast growth factor degradation (Shigeta et al., 1998).

Omeprazole-treated rats exhibited a flat ulcer margin and this may have been due to the protection of the newly formed epithelial cells in the ulcer margin and granulation tissue (including microvessels in the ulcer bed) from direct gastric acid-pepsin damage (Shigeta et al., 1998). Whether omeprazole-induced hypergastrinemia also has an effect on ulcer healing cannot be excluded (Schmassmann, 1998). These results are consistent with results of studies indicating that proton pump inhibitors promote ulcer re-epitheliation despite further intake of NSAIDs (Folkman et al., 1991). Moreover, omeprazole-treated rats showed a thinner granulation tissue together with a higher density of microvessels in the ulcer base when compared with NSAID-treated animals. This observation is consistent with the hypothesis that stimulation of angiogenesis in the ulcer base may be one of the main mechanisms of the acceleration of ulcer healing (Schmassmann et al., 1996), as increased angiogenesis has the potential to deliver the correct supply of oxygen and nutrients to the granulation tissue, allowing accelerated re-epithelization and restoration of glandular structures (Walan et al., 1989).

With NSAIDs treatments, thick granulation tissue was found after 8 days and was still evident after 15 days. This tissue contains many inflammatory cells and macrophages

and is relatively poor in microvessels. This finding may be explained by the inhibition of both angiogenesis and the development and function of myofibroblasts, which impedes the ability of the granulation tissues to contract (Schmassmann, 1998).

In the group of animals treated with celecoxib, the delay in lesion healing was not detected macroscopically after 8 days, but was very significant at 15 days, which is consistent with the results obtained by Lesch et al. (1998), Schmassmann (1998) and Schmassmann et al. (1996, 1998) using other cyclooxygenase-2 selective inhibitors: NS-398 and L-745,337, respectively.

The delay in ulcer healing observed during NSAID treatment may be explained by the fall of gastric prostaglandin E_2 levels in the ulcerated area, whereas in control animals the concentration of these prostanoids increases in the ulcer crater and remains at sham levels in intact mucosa (Shigeta et al., 1998; Lesch et al., 1998). Shigeta et al. (1998) found a decrease in prostaglandin E_2 in ulcerated tissue only when low doses of the selective inhibitor NS-398 were administered. However, a higher dose of the same substance inhibited prostaglandin synthesis in both ulcerated and intact tissue. These results may reflect a dose-dependent loss of selectivity for cyclooxygenase-2 inhibition. Furthermore, Wallace et al. (1998b) confirmed that a single dose of L-745,337 did not inhibit gastric prostaglandins in rats, while repeated administration for 1 week provoked a significant inhibitory effect on cyclooxygenase-1. The dose of celecoxib used in this chronic ulcer model, 1.8 mg/kg/12 h, corresponds with a daily human dose of 200 mg. It is possible that, at this dose, and administered repeatedly, celecoxib is not acting as a selective inhibitor in rats.

After 15 days, when the difference in ulcer healing of the celecoxib-treated group compared to that in the control was maximum, the decrease in prostaglandin E_2 content was only evident in the lesioned area, while the levels in the intact mucosa were much higher and similar to those in the control group.

On the other hand, Brzozowski et al. (2001) have studied the effects of a selective cyclooxygenase-1 inhibitor, reveratrol, a classic NSAID, indomethacin, and two cyclooxygenase-2 selective NSAIDs, NS-398 and rofecoxib, in a model of 100% acetic acid-induced ulcer. They observed a delay in ulcer healing and a decrease in prostaglandin content at the ulcer site in all cases. These results suggest that both isoforms are responsible for the production of prostanoids important for ulcer healing.

Inhibition of cyclooxygenase-2 activity during healing processes is detrimental, as it has been proven that this isoenzyme also exerts anti-inflammatory effects (Gilroy et al., 1999) and both isoforms are necessary for the amelioration of the lesion (Mizuno et al., 1997; Wallace et al., 1998a).

Furthermore, Lesch et al. (1998) have proposed that the chemical structure with a sulphonamide moiety may account for the healing delay, although the possible mechanism for this impairment is not known. The authors base this hypothesis on the results of their experiment as well as on data from Mizuno et al. (1997): in both cases, a significant delay in acetic acid ulcer healing was observed on the assay of the selective cyclooxygenase-2 inhibitor, NS-398, which possesses the sulphonamide group. On the contrary, another selective NSAID, PD 138387, of different chemical structure, failed to affect the restoring process. Moreover, Schmassmann et al. (1996, 1998) found a longer healing period when using the selective inhibitor, L-745,337, which belongs to the same sulphonamide group.

Our results from immunohistochemistry after 8 and 15 days of ulcer induction revealed that cyclooxygenase-1 expression does not show important changes among the different treatments, as it would be expected for a constitutive enzyme.

However, the cyclo-oxygenase-2 showed different expression levels among the different groups. This isoform is strongly induced in the gastrointestinal tract under inflammatory conditions. In our experiment, the strongest cyclooxygenase-2 expression in the ulcer margins was obtained in piroxicam-treated animals, whereas the celecoxib group exhibited the faintest expression, much less than in the ulcerated control. This suggests that down-regulation of cyclooxygenase-2 expression might also affect the healing rate.

Regarding the neutrophil infiltration levels, our biochemical and histological results showed that piroxicam is the drug which mostly increased the inflammatory infiltrate after 1 week. According to Fujita et al. (1998), the delay in ulcer healing may be due to a decrease in antichemotactic activity, characteristic of normal gastric mucosa and also due to an increase in the chemotactic activity in the ulcerated tissue, leading to persistent neutrophil infiltration. Moreover, it has been proposed that the infiltration of inflammatory cells such as neutrophils and mononuclear cells plays a key role in the recurrence of gastric ulcers (Arakawa et al., 1998).

Concluding, the present data show that the selective cyclooxygenase-2 inhibitor celecoxib delays chronic ulcer healing, whereas metamizol and the preferential cyclooxygenase-1 inhibitor, piroxicam, do not affect the evolution of the 5% acetic acid-induced ulcer. On the contrary, the proton pump inhibitor, omeprazole, accelerates the healing of the lesion.

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