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Angiogenesis, cell proliferation and apoptosis in gastric ulcer healing. Effect of a selective cox-2 inhibitor

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Abstract

To elucidate the role of cyclooxygenase-2, we compared the effects of rofecoxib, a selective cyclooxygenase-2 inhibitor, and ibuprofen, a nonselective cyclooxygenase inhibitor, on the evolution of acetic-acid-induced gastric ulcers in rats, evaluating growth factor expression, the angiogenic process, cell proliferation and cell apoptosis. Levels of basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF), angiogenesis and cell proliferation were analysed by immunohistochemical methods, and apoptosis was evaluated by an enzyme immunoassay. Both growth factors and microvessels appeared to be abundant in the granulation tissue of the ulcer bed. Rofecoxib (2.5 mg/kg/day) and ibuprofen (100 mg/kg/day) delayed ulcer healing, but only rofecoxib treatment provoked a reduction of bFGF expression and inhibition of the development of new microvessels. No changes in VEGF expression were detected. Results also showed that proliferation and apoptosis were increased in control ulcerated animals. Rofecoxib reduced significantly both processes. These findings demonstrate that a reduction of bFGF expression and an antiangiogenic action, as well as proliferation/apoptosis inhibition, are some of the mechanisms possibly implicated in the delay in ulcer healing seen after the administration of the highly selective COX-2 inhibitor rofecoxib.

Keywords: NSAID; Rofecoxib; Ibuprofen; bFGF expression; VEGF expression; Apoptosis; Proliferation

1. Introduction

The gastric mucosa is normally exposed to a wide range of aggressive insults and has developed efficient mechanisms to repair tissue injury. In the case of deeper mucosal damage, such as erosions and ulcers, both epithelial and connective tissue components, including subepithelial myofibroblasts, smooth muscle cells and vessels, are destroyed and must be regenerated (Milani and Calabro, 2001).

The reconstitution of the surface epithelium is achieved by the proliferation of undifferentiated epithelial precursors that migrate from the ulcer margin onto the granulation tissue and cover the ulcer bed. Previous studies have suggested that the balance between cell apoptosis and cell proliferation is of great importance for maintaining gastric mucosa integrity (Reed, 2000). In addition to the epithelial structures, new vessels are generated to ensure an adequate supply of oxygen and nutrients to the healing mucosa (Tarnawski et al., 2001; Guo et al., 2002).

The complex sequence of events requires a high degree of coordination and is regulated by several factors. Among them, polypeptide growth factors have received much attention in recent years because of their ability to regulate essential cell functions involved in tissue healing, including cell restoration, migration and angiogenesis (Szabo et al., 2000; Berenguer et al., 2002). Granulation tissue grows extensively under the control of basic fibroblast growth

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factor (bFGF), and vascular endothelial growth factor (VEGF) promotes angiogenesis, providing microvessels for the restoration of the microvascular network, and connective tissue, thereby contributing restoration of the mucosal architecture.

Nonsteroidal antiinflammatory drugs (NSAIDs) are a group of structurally different chemicals that have analgesic, antiinflammatory and antipyretic actions. With the recognition of two cyclooxygenase isoenzymes, i.e., constitutive cyclooxygenase-1 and the inducible form or cyclooxygenase-2, NSAIDs have been categorized into cyclooxygenase-1 or cyclooxygenase-2 inhibitors according to their ability to inhibit these isoenzymes selectively (Furst, 1999). The newly developed highly selective cyclooxygenase-2 inhibitors provide a greater reduction in clinically important gastrointestinal adverse events (Bombardier et al., 2000; Sigthorsson et al., 2000; Cannon and Breedveld, 2001; Weaver, 2001), but there are serious doubts about their safety in patients with active gastric ulcers.

It has been found that cyclooxygenase-2 inhibitors have antiproliferative and antiangiogenic activity in several types of human cancer (Masferrer et al., 2000) and also in other animal models (Majima et al., 1997). Since growth-promoting actions and angiogenesis are not only essential for tumor growth but also play a pivotal role in wound healing, selective cyclooxygenase-2 inhibitors may suppress these activities in gastric ulcer tissues and delay the healing process. Exogenous growth factors could enhance the healing of acetic-acid-induced gastric ulcers in rats and of NSAID-related ulcers in patients (Pohle et al., 1999; Konturek et al., 2001; Guo et al., 2002). However, limited information is available regarding whether NSAIDs, especially the cyclooxygenase-2 selective category, have any effect on the expression of growth factors and associated processes.

The aim of the present study was to evaluate the effect of the cyclooxygenase-2 selective inhibitor, rofecoxib, in comparison with a classic nonselective cyclooxygenase inhibitor, ibuprofen, on ulcer healing after the induction of chronic gastric ulcers in terms of the expression of the growth factors bFGF and VEGF during angiogenesis, cell apoptosis and cell proliferation in a rat model.

2. Material and methods

2.1. Reagents and drugs

All chemicals and reagents were purchased from Sigma (St. Louis, MO) unless otherwise specified. The doses administered in all treatments were 100 mg/kg body weight of ibuprofen and 2.5 mg/kg body weight of rofecoxib (Vioxx®, Merck Sharp and Dohme). The selection of the single doses for both products was based on our own experience (Jiménez et al., 2002; Martín et al., 2003; Jiménez et al., 2004) and also on data from other groups (Pajdo et al., 2001; Kato et al., 2002; Melarange et al., 1994). The doses used were in line with the clinical used in humans.

2.2. Animals and induction of gastric chronic ulcer

The study was approved by the Ethics Committee for Experimental Research of the Faculty of Pharmacy of the University of Seville, and all experiments were in accordance with the recommendations of the European Union. Male Wistar rats (150–180 g) were supplied by the Animal Service (Faculty of Pharmacy, Seville, Spain). The rats were fed on standard laboratory chow and tap water ad libitum. The animals were deprived of food for 18–20 h before experiments but had free access to water. They were randomly assigned to groups.

Gastric ulcers were induced by luminal application of acetic acid, as described previously by Motilva et al. (1996), with slight modifications. A medial laparotomy was carried out on diethyl ether-anaesthetized rats, the stomach was exposed, and at the junction of the corpus and antrum, 50 μl of 20% acetic acid was injected using a microsyringe into the subserosal layer of the anterior wall of the stomach. In sham-operated animals, the abdomen was opened and closed without injection. This procedure produced histologically characteristic ulcers, 2–3 days after injection, with a benign spontaneous evolution.

2.3. Assessment of ulcer size

There were four treatment groups with eight rats in each group: sham group without ulcer; the other groups with ulcer; vehicle (distilled water) treatment as control; and ibuprofen and rofecoxib. Rats were given 1 ml/kg orally of either the vehicle or NSAID, using an orogastric tube. They were given the treatment once daily for 7 and 14 days starting 1 day after administration of acetic acid. Animals were killed, and the stomach was cut along its greater curvature and rinsed thoroughly with normal saline to remove the attached debris. For the histology study, a longitudinal section of stomach along the greater curvature, which included the ulcer base and both sides of the ulcer margin, was taken and fixed in 4% formalin for 24 h at 4 °C and embedded in paraffin. Morphological examination was performed with haematoxylin and eosin staining for histological changes during gastric ulcer healing. To calculate the ulcer size (mm^2), a video camera coupled to an automatic image analysis system (Motic Images 2000, 1.1, System Microscopes) was used.

2.4. Immunohistochemistry with bFGF and VEGF

Formalin-fixed and paraffin-embedded tissue sections (5 μm) were incubated in an oven at 55 °C for hour and then deparaffinized in two changes of xylene and hydrated.

Staining for bFGF was carried out by immunohistochemistry according to the peroxidase–antiperoxidase (PAP) method. Prior to PAP treatment, sections were dipped in a solution of 3% hydrogen peroxide for 20 min at room temperature to inhibit endogenous peroxidase activity. After being washed in several changes of phosphate-buffered saline (PBS), the sections were treated for 20 min with a protein blocking agent, 3% bovine serum albumin in PBS, at room temperature to block nonspecific protein binding sites. Then the sections were incubated at 4 °C overnight with the polyclonal rabbit anti-bFGF antibody (Oncogene™ Research Products) at dilution of 1:20 in PBS. After being rinsed in several changes of PBS, the sections were transferred for 30 min to antirabbit immunoglobulin G (IgG) antibody (Calbiochem®) diluted 1:100 in PBS at room temperature.

The immunostaining of VEGF was carried out using the streptavidin–biotin–peroxidase method. A pressure-cooking antigen retrieval system in which the slides were boiled in 10 mM Tris (pH 10.0) at maximum pressure for 1 min and cooled at room temperature for 20 min was used. The endogenous peroxidase activity was inhibited, and then the sections were incubated in normal horse serum (Vectastain® Kit, Vector Laborartories, Burlingame, CA) for 20 min to reduce nonspecific staining and successively incubated with monoclonal mouse anti-VEGF antibody (Oncogene™ Research Products) at dilution of 1:20 overnight at 4 °C. Subsequently, slides were treated with antimouse IgG antibody (Vectastain® Kit, Vector Laborartories, Burlingame, CA) for 30 min and incubated with the streptavidin–peroxidase complex (Vectastain® Kit, Vector Laborartories, Burlingame, CA) for 30 min.

Binding was demonstrated with 3,3′ -diaminobenzidine (DAB), and the sections were counterstained with haematoxylin to enhance colour contrast. Negative control sections were treated in the same way, but the primary antibody was omitted.

Results were classified as negative, few, intermediate, and high number of cells was stained and converted using a semiquantitative scale into 0–3 arbitrary units. Two investigators examined the slides independently. In case of disagreement, a third investigator also examined it.

2.5. Determination of angiogenesis in granulation tissue

The number of microvessels in the ulcer as an indicator of angiogenesis was assessed by means of an immunohistochemical method for von Willebrand factor (Weidner et al., 1991). The sections were digested with 0.1% trypsin (Difco Laboratories, Detroit, MI) in distilled water for 20 min at 37 °C. After inhibition of endogenous peroxidase activity and nonspecific protein binding sites, the sections were incubated with the primary antibody, polyclonal rabbit antihuman Factor VIII-related antigen (DAKO, USA), for 1 h at room temperature, and the streptavidin–biotin–peroxidase method was used.

Individual microvessels were counted. Any positive cells or cells clusters that were clearly separate from adjacent microvessels and other connective tissue elements were considered as single, countable microvessels. Occasional immunopositive leukocytes were excluded on morphological grounds. The vascular areas immediately adjacent to the normal tissue of the stomach were not considered in the vessel counts. However, these microvessels did serve as internal quality controls for Factor VIII-relates antigen immunostaining. In all of the samples, the number of microvessels counted in five microscopic fields in the ulcer base at ×200 magnification was averaged.

2.6. Immunohistochemistry with proliferating cell nuclear antigen (PCNA) and apoptosis study

Mucosal cell proliferation was measured by immunostaining with PCNA. Tissue was stained with an anti-PCNA antiserum, which recognizes a nuclear antigen expressed in all stages of the cell cycle. The basic methodology used was the same as that for VEGF. The buffer used in the pressure-cooking antigen retrieval system was citrate buffer (pH 6.0). Monoclonal mouse anti-PCNA (DAKO) at dilution of 1:1000 in PBS and antimouse IgG antibody (Vectastain® Kit, Vector Laborartories, Burlingame, CA) were used as primary and secondary antibodies.

For each ulcer, the PCNA-positive cells in the ulcer margin and in the granulation tissue were counted, as defined by Yamada et al. (1992). The PCNA labelling index was determined by calculating the percentage or ratio of the number of positive cells to the total number of cells. A cell was deemed to be positive only if it showed positive nuclear staining. Three observers made the evaluations independently.

Apoptosis was measured by means of the cell death detection enzyme-linked immunosorbent assay^{plus} kit (Roche Molecular Biochemicals). The assay is based on a quantitative sandwich enzyme immunoassay using mouse monoclonal antibodies directed against DNA and histones, respectively. This allows the specific determination of mono- and oligonucleosomes in the cytoplasmic fraction of cell lysates. The values are expressed as mU/g tissue (mU=absorbance [10^{-3}]).

2.7. Statistical analysis

The results are expressed as means±S.E.M. Statistical comparisons were evaluated by one-way analysis of variance (ANOVA test) followed by the Fisher test for parametric data. Statistical analysis was performed using Graph-Pad Prism 2.01. Values of probability $p<0.05$ were considered significant.

3. Results

3.1. Effect of selective cyclooxygenase-2 inhibitor, rofecoxib, in gastric ulcer healing

Histological analysis showed characteristic features of healing after ulceration. As was observed in the tissue of the control group, at 7 days after ulceration, the acetic acid ulcer consisted of epithelial cells and granulation tissue at the ulcer base, which is a connective tissue component consisting of fibroblasts, macrophages and proliferating endothelial cells forming microvessels. These cells adjacent to the ulcer crater constitute a region with maximal repair activity. At 14 days, some epithelial cells in the ulcer margin had proliferated and migrated over and into the ulcer crater, which was strongly infiltrated by inflammatory cells, fibroblasts and endothelial cells (Fig. 1). Because ulcer healing was maximal at day 14, this time point was selected for further study.

NSAIDs treatment delayed ulcer healing at day 14 compared to that in the control group, indicating a slower ulcer healing process at this time (Fig. 2A).

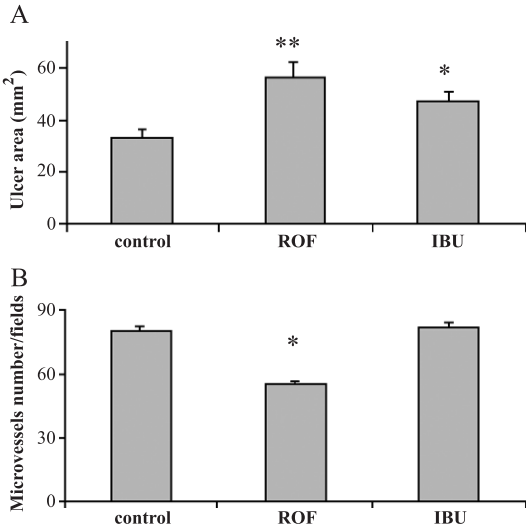

Fig. 2. Effects of rofecoxib (ROF) and ibuprofen (IBU) on ulcer area (A) and angiogenesis (B) in chronic gastric ulcers on day 14 after ulcer induction. The ulcers in the ROF and IBU treatment groups were significantly larger than those in the control group. Microvessels were identified by immunohistochemistry according to the streptavidin–biotin–peroxidase method. The number of microvessels counted in five microscopic fields in the ulcer base at ×200 magnification was averaged. Data are expressed as means±S.E.M. for eight animals. *p<0.05 and **p<0.01 when compared with the corresponding control group.

3.2. Effect of selective cyclooxygenase-2 inhibitor, rofecoxib, on bFGF and VEGF expression in chronic gastric ulcers

bFGF and VEGF support ulcer healing. To determine how a selective cyclooxygenase-2 inhibitor affected ulcer healing, we measured these growth factors in the acetic-acid-induced ulcers of rats treated with rofecoxib in comparison with ibuprofen. In chronic gastric ulcers, immunoreactivity for bFGF was detected in the ulcer margin and in granulation tissue in the ulcer bed and was markedly increased in the regenerating tissues around the acetic-acid-induced ulcer. At higher magnification, the bFGF immunoreactivity was found in most of the typical fibroblasts, granulocytes and smooth muscle cells in the ulcer crater, in granulation tissue and in the stromal cells in the lamina propia on the ulcer margin (Fig. 3). In the NSAID-treated cases, less immunoreactive bFGF was detected than in the control group. However, only in the group treated with rofecoxib was this significant (p<0.05) (Fig. 4).

VEGF immunoreactivity was augmented in granulation tissue from control rats and was detected in granulocytes, fibroblasts and smooth muscle (Fig. 3). Fig. 4 shows levels of VEGF that immunoreactivity were not significantly affected by either NSAID.

3.3. Effect of selective cyclooxygenase-2 inhibitor, rofecoxib, on angiogenesis in chronic gastric ulcers

In asmuch as angiogenesis plays a pivotal role in wound healing, we examined the number of microvessels in chronic ulcers with or without NSAIDs treatments. The growth of new blood vessels was denser in granulation tissue and in

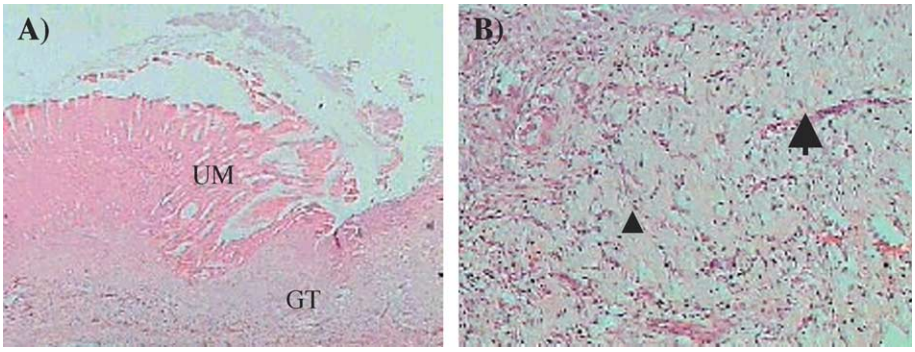

Fig. 1. Haematoxylin and eosin staining of acetic-acid-induced gastric ulcers on day 14 after ulcer induction. Ulcer margin (UM) and granulation tissue (GT) at the ulcer base are shown (A). Granulation tissue is composed of macrophages, fibroblasts (▲) and proliferating endothelial cells forming microvessels (▲) (B).

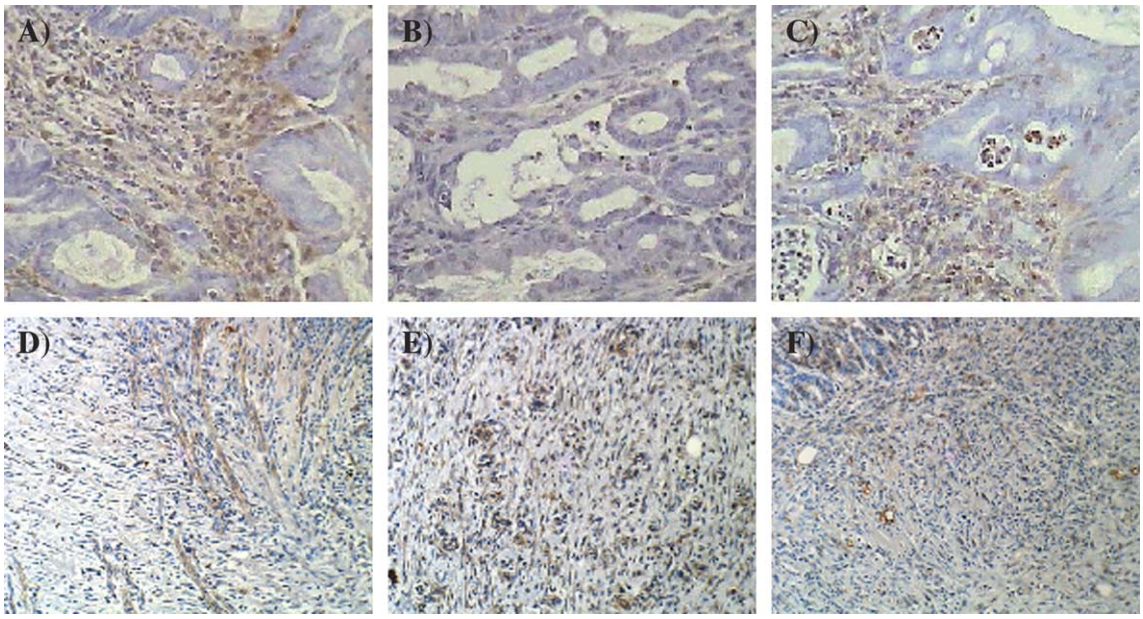

Fig. 3. Immunohistochemical staining of basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) in chronic gastric ulcers of rats. bFGF expression in the control group (A), rofecoxib group (B) and ibuprofen group (C). VEGF expression in the control group (D), rofecoxib group (E) and ibuprofen group (F). bFGF and VEGF immunostaining was carried out according to the peroxidase–antiperoxidase and streptavidin–biotin–peroxidase methods, respectively. Original magnification ×200.

control group rats. The number of microvessels in rats treated with either NSAID was lower than that of the control group, although this difference was significant only with rofecoxib treatment ($p<0.05$), indicating that the selective cyclooxygenase-2 inhibitor inhibited angiogenesis during ulcer healing (Fig. 2B).

3.4. Effect of selective cyclooxygenase-2 inhibitor, rofecoxib, on PCNA-positive cells staining and apoptosis in chronic gastric ulcers

To investigate the effect of rofecoxib on cell proliferation in chronic gastric ulcers, cell proliferation was quantified by immunostaining. In the sham group, the immunoreaction for PCNA was observed as a dark accumulation of DAB reaction products in the nuclei of the middle and lower parts of the crypts in the normal gastric mucosa. In the control ulcerated group, PCNA-positive nuclei were distributed in epithelial cells in the ulcer margin and in fibroblasts in granulation tissue (Fig. 5). Fig. 6A shows that the PCNA labelling index was significantly lower in rofecoxib-treated animals ($p<0.05$) than in control animals. Ibuprofen did not decrease cell proliferation significantly compared to control.

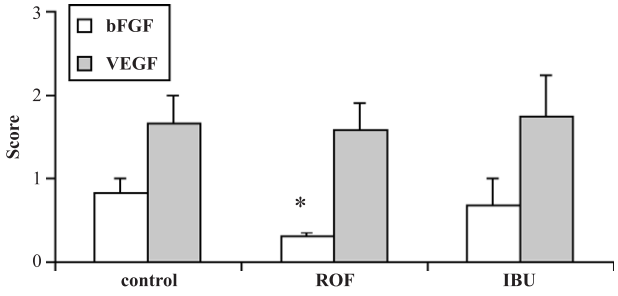

Fig. 4. Effect of rofecoxib (ROF) and ibuprofen (IBU) on basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) expression in chronic gastric ulcer after 14 days. bFGF and VEGF immunostaining was carried out according to the peroxidase–antiperoxidase (PAP) and streptavidin–biotin–peroxidase methods, respectively. Immunostaining was converted by a semiquantitative scale into 0–3 arbitrary units. Data are expressed as the means±S.E.M. for eight animals. *$p<0.05$ when compared with the corresponding control group.

Because gastric mucosal inflammation contributes to cell turnover, we investigated whether apoptosis was altered by chronic gastric ulcers and how these apoptotic effects were affected by rofecoxib in comparison with ibuprofen. The decreased proliferation observed in the rofecoxib-treated group was also correlated with a decrease in apoptosis after ulcer induction ($p<0.05$; Fig. 6B).

To determine the healing capacity of the stomach, the ratio of proliferation to apoptosis was calculated. This ratio was a significantly lower with rofecoxib and ibuprofen treatments ($p<0.001$ and $p<0.05$, respectively). Moreover, the proliferation:apoptosis ratio was statistically significant for the selective cyclooxygenase-2 inhibitor versus ibuprofen ($p<0.05$; Fig. 6C).

4. Discussion

In the last years, it has been proven that NSAIDs that are selective inhibitors of cyclooxygenase-2 have a reduced capacity to cause severe ulceration compared with conventional NSAIDs. However, under certain conditions, such as preexisting lesions including experimental chronic gastric ulcers, cyclooxygenase-2 inhibitors have shown a similar or

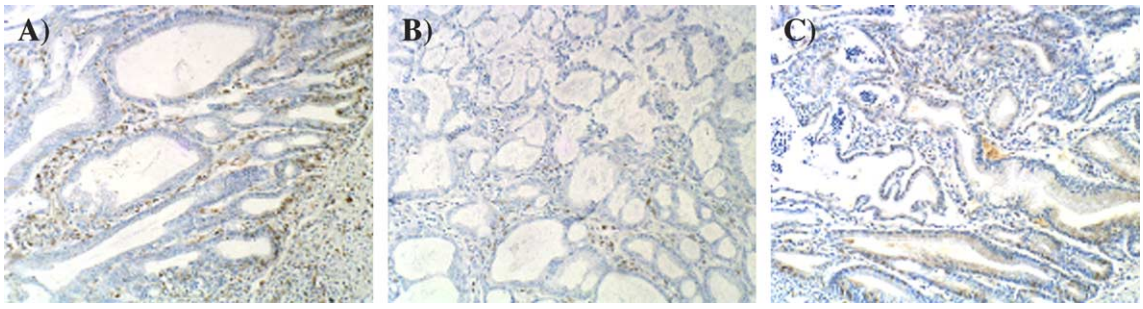

Fig. 5. Immunohistochemical staining of proliferating cell nuclear antigen (PCNA) in the ulcer margin of animals treated with vehicle (A), rofecoxib (B) and ibuprofen (C).

even greater capacity to aggravate ulcer healing (Ma et al., 2002). Moreover, evidence that the cyclooxygenase-2 enzyme plays several physiological roles in addition to mediating pain and inflammation is increasing (Wallace and Muscara, 2001). Cyclooxygenase-2-derived prostaglandins promote epithelial cell proliferation and granulation tissue formation by improving blood flow and stimulating the expression of growth factors (Brozozowski et al., 2001; Dannhardt and Kiefer, 2001; Ma et al., 2002). Numerous authors have demonstrated an up-regulation of cyclooxygenase-2 expression and activity in the ulcer base not only during the acute phase of inflammation but also in the ulcer healing stage and especially in areas of intense tissue repair (Schmassmann et al., 1998; To et al., 2001; Berenguer et al., 2002). The present study corroborates previous data showing the deleterious effect of NSAIDs, classic and highly specific COX-2 inhibitors, on the healing of preexisting ulcers without there being significant differences between them.

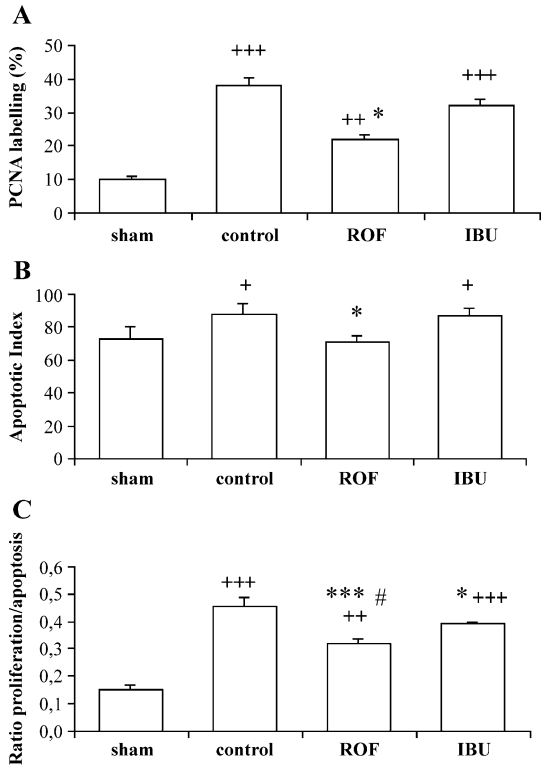

Fig. 6. Effects of rofecoxib (ROF) and ibuprofen (IBU) on PCNA-positive cell staining (A), apoptosis (B) and ratio of proliferation/apoptosis (C) in chronic gastric ulcers on day 14 after ulcer induction. PCNA-positive cells were identified by immunohistochemistry according to the streptavidin–biotin–peroxidase method. Immunoreactions for PCNA were observed as a dark accumulation of DAB reaction products in the nuclei of cells in the ulcer margin and in granulation tissue. The assay of apoptosis is based on a quantitative sandwich enzyme immunoassay using mouse monoclonal antibodies directed against DNA and histones, respectively. Data are expressed as the means±S.E.M. for eight animals.+p<0.05, ++p<0.01 and +++p<0.001 when compared sham group; *p<0.05 and ***p<0.001 when compared with control group and #p<0.05 when compared with ibuprofen group.

bFGF belongs to a large family of at least 18 members that are able to control several distinct activities via different signal transduction pathways, depending on the specific cellular localization (Milani and Calabro, 2001). This growth factor has mitogenic activity on fibroblasts, which are known to produce extracellular matrix components, such as collagen, and an increase in its level is considered to compensate for the tissue defect due to ulceration, hence, leading to ulcer healing (Yabu et al., 1993). Experimental studies have demonstrated that stimulation of angiogenesis in granulation tissue by bFGF accelerates the healing of experimental gastric ulcers in rats (Ernst et al., 2001; Tarnawski et al., 2001). VEGF is another growth factor that has also been implicated in mucosal protection. It has a variety of effects on the vascular endothelium, including the ability to promote endothelial cell viability, mitogenesis, chemotaxis and vascular permeability (Ferrara, 2000; Tarnawski et al., 2001). Szabo et al. (2000) showed that VEGF expression is increased during ulcer healing, suggesting that VEGF might also play an important role in mucosal repair.

These data suggested that both peptides are essential for tissue healing, stimulating the development of new vessels to supply oxygen and nutrients to the healing mucosa and also providing the matrix substrate for the proliferation and differentiation of epithelial structures. The results obtained in our present study confirm this inasmuch as levels of bFGF and VEGF were high in the granulation tissue of control ulcerated rats, contributing in this way to normal recuperation. Ibuprofen did not disturb growth factor production or

microvessel development. In contrast, rofecoxib treatment significantly decreased the number of new vessels and bFGF-protein expression. Other studies have also demonstrated that products of cyclooxygenase-2 activity play a pivotal role in promoting ulcer healing and angiogenesis via bFGF (Suzuki et al., 1998; Guo et al., 2002).

Apoptosis, also known as programmed cell death, is a form of genetically regulated cell death under tight control. The important function of apoptosis, besides the regulation of physiological cell turnover, is the removal of damaged cells. This form of active cell death is found in all tissues including the gastrointestinal epithelium (Wong et al., 1999) and can be induced by a wide variety of physiological and pathological stimuli. Under normal physiological conditions, the balance between gastric epithelial cell proliferation and death is of great importance in maintaining gastric mucosal integrity. Disturbance of this balance could result in either cell loss, leading to mucosal damage and ulcer formation, or cell accumulation, leading to cancer development (Moss, 1998; Khoda et al., 1999). Therefore, assessment of these processes provides an intimate insight into the mucosal repair process including ulcer healing.

Previous results showed that following exposure to water immersion stress or in the ethanol-induced ulcer model, the apoptotic rate in the gastric epithelium is increased (Piotrowski et al., 1997; Konturek et al., 2001; Liu et al., 2001). In experimental studies or human assays of *Helicobacter pylori* infection, apoptosis and the proliferation of epithelial cells are also increased (Zhu et al., 1998; Leung et al., 2000; Kim et al., 2001). It would be reasonable to speculate that these increases in proliferation/apoptosis are the result of a direct stimulatory effect of the injurious agents or perhaps as a compensatory mechanism that favours cell turnover (Kim et al., 2001). Apoptosis and cell proliferation in chronic ulcers induced by acetic acid have been studied less. Our results showed that both indexes were augmented in the control ulcerated groups. These results are consistent with the abovementioned studies, and in our opinion, apoptosis together with proliferation is crucial in the complex reparative machinery that an active organism develops to counteract damage to restore the homeostatic state as quickly as possible.

Emerging epidemiological, clinical and animal data have shown that NSAIDs have an antineoplasic effect in colon or gastric cancer (Church et al., 2003). The mechanisms responsible for these remarkable antineoplasic effects are not completely understood, but several lines of evidence indicate that NSAIDs induce inhibition of cell turnover, such as induction of apoptosis and inhibition of cell proliferation, and that cyclooxygenase enzymes, specifically cyclooxygenase-2, might be an important molecular target in the treatment of both early and late stages of some cancers (Anderson et al., 2003; Gasparini et al., 2003; Huls et al., 2003). However, independent of the cancer pathological process and analysis of the responses associated with NSAID administration in models of gastric damage, the results may be controversial. In experimental studies, some authors have shown that NSAID administration drives gastric epithelial cells to apoptosis (Fiorucci et al., 2001). However, other authors did not observe any important changes in apoptotic and proliferative activities after NSAID treatment for 10 days (Sant et al., 1995; Kim et al., 2001). NSAIDs may reverse the increased apoptosis and proliferation of epithelial cells during *H. pylori* infection (Zhu et al., 1998; Kim et al., 2001). We demonstrated that the apoptosis and proliferation observed in control animals were inhibited by rofecoxib. Ibuprofen did not significantly modify any index analysed. Moreover, the ratio of proliferation to apoptosis diminished with both NSAIDs although the effect of rofecoxib was significantly different from that of ibuprofen. The delay in healing combined with the decrease in the proliferation/apoptosis ratio observed after rofecoxib treatment could suggest the importance of cyclooxygenase-2 products in the regulation of cell turnover and in gastric mucosa function.

Together, these findings show that the selective cyclooxygenase-2 inhibitor, rofecoxib, delays the healing of acetic acid ulcer by exerting an antiangiogenic action possibly by bFGF inhibition and by inhibition of cell proliferation and apoptosis, indicating that cyclooxygenase-2 and its products may be actively implicated in the control of reparative mechanisms.

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