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Effects of food intake and oxidative stress on intestinal lesions caused by meloxicam and piroxicam in rats

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Abstract

Large intestinal ulcers, bleeding and perforation are occasionally due to non-steroidal anti-inflammatory drugs (NSAID). In addition to suppression of prostaglandins synthesis, a number of factors have been implicated, including enterohepatic recirculation, food intake and vascular injury with oxygen free-radical generation. The present study aimed to determine the effect of food intake and the role of oxidative stress in the pathogenesis of intestinal injury induced by oral administration of meloxicam (preferential cyclooxygenase-2 inhibitor) vs. piroxicam (preferential cyclooxygenase-1 inhibitor). Therefore, the activity of oxidative stress-related enzymes such as myeloperoxidase, xanthine oxidase and superoxide dismutase, as well as levels of lipid peroxides and glutathione homeostasis were studied in an experimental model using re-fed rats. The animals treated with piroxicam (10–20 mg/kg) had a dose-dependent increase in the severity of intestinal lesions, but only the highest dose of meloxicam (15 mg/kg) caused macroscopic damage. The severity of piroxicam and meloxicam-induced damage was correlated with a significant increase of xantine oxidase activity and a decrease of superoxide dismutase activity and glutathione levels ($P < 0.05$ and $P < 0.001$ vs. control). In contrast, there was no significant neutrophil infiltration of the intestine after dosing. Our results support the hypothesis that oxygen free radicals, probably derived via the action of xantine oxidase, the decrease in superoxide dismutase activity, and depletion of mucosal glutathione contribute to the pathogenesis of meloxicam and piroxicam-induced intestinal ulceration in re-fed rats.

Keywords: Non-steroidal anti-inflammatory drug (NSAID); Meloxicam; Piroxicam; Gastric mucosal injury; Oxygen radical

1. Introduction

The gastrointestinal tract is the most common location for side effects of non-steroidal anti-inflammatory drugs (NSAID). Large intestinal ulcers, bleeding and perforation are occasionally observed, in addition to chronic blood and protein loss. In addition to structural lesions, NSAID can cause a clinical entity of diffuse intestinal inflammation and increased intestinal mucosal permeability (Bjarnason et al., 1993).

Although it is generally accepted that the pathogenesis of NSAID enteropathy is complex, there is consensus that there is both an important initiating "topical" aspect of the damage and a systemic one (Somasundaram et al., 1995; Wallace, 1997), mainly due to suppression of prostaglandin synthesis (Reuter et al., 1997). Other factors, such as enteric bacteria and feeding conditions, have also been implicated (Satoh et al., 1981; Weissenborn et al., 1985). In this context, it has been reported that feeding animals after NSAID dose clearly enhances the small intestine macroscopic damage (Satoh et al., 1981).

Biliary excretion and enterohepatic circulation of NSAID (Reuter et al., 1997) can also be factors involved in intestinal lesions. This is partly because of the longer exposure of gastrointestinal tract to the drug, partly because of the reflux of the bile together with NSAID, which can further damage the gastric mucosa by breaking the gastric mucosal barrier (Brune et al., 1986; Rainsford, 1986).

On the other hand, it has been proposed that neutrophil- and oxygen radical-dependent microvascular injuries may be important prime events that lead to intestinal injury induced by NSAID (Wallace, 1994; Scarpignato, 1995).

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Nygard et al. (1994, 1995) also observed neutrophil margination in the small bowel in response to acute NSAID administration. In a large variety of experimental models, NSAID-induced gastrointestinal injury is greatly reduced by several factors: agents that facilitate capture of free radicals, or increase thiol availability, enhancement of antioxidant defences such as administration of catalase or superoxide dismutase, and finally, by the inhibition of xanthine oxidase by treatment with allopurinol (Buzas et al., 1991; Vaananen et al., 1991; Wallace, 1994). These findings indicate a potential contribution of free radicals to NSAID-induced gastrointestinal effects. Nevertheless, the effects on the activity of certain related enzymes have not been extensively investigated.

It is generally accepted that there are two isoforms of cyclooxygenase enzyme: one, constitutive or cyclooxygenase-1, which produces prostaglandins for physiological reactions including the maintenance of mucosal integrity, gastric microcirculation, secretory activity and motor functions, and the other, inducible or cyclooxygenase-2, which is triggered by various cytokines, growth factors and endotoxins. While prostaglandins produced by cyclooxygenase-1 appear to contribute to the physiological control of mucosal integrity, cyclooxygenase-2 products have been implicated in inflammatory reactions (Smith and DeWitt, 1995; Vane and Botting, 1996).

In recent years, various approaches to the development of new gastrointestinal safe NSAID have emerged. Intensive efforts are now being made to develop selective inhibitors of cyclooxygenase-2, assuming that these agents will inhibit this isoform when it is induced at sites of inflammation, but will not inhibit prostaglandin synthesis in other tissues such as the stomach, were cyclooxygenase-1 is constitutively expressed (Vane and Botting, 1998).

Several cyclooxygenase-2 inhibitors have been developed such as celecoxib, rofecoxib and meloxicam (Hawkey, 1999). The latter is a new NSAID belonging to the enolic acid group of the oxicam family (Albengres et al., 1993), and has been estimated to have a 3- to 77-fold greater affinity for cyclooxygenase-2 than for cyclooxygenase-1 (Patrignani et al., 1997; Cryer and Dubois, 1998). This limited selectivity has led to meloxicam's description as a preferential cyclooxygenase-2 inhibitor. Some studies in the rat have shown that meloxicam shows particularly good gastric tolerance (Engelhardt et al., 1995), although a previous study by Villegas et al. (2000) demonstrated that it can induce gastric lesions associated with an increase of oxidative metabolism. In addition, short term studies assessing intestinal permeability, which appear to give predictive information on the longer term small intestinal tolerability, show that meloxicam increases intestinal permeability (Bjarnason, 1999).

Piroxicam, another oxicam family member and preferential cyclooxygenase-1 inhibitor (Smith et al., 1994; Cryer and Dubois, 1998), is a widely used NSAID with anti-inflammatory, analgesic, and antipyretic activities, but previous studies in rats have shown the formation of severe gastric erosions on intragastric administration of this drug as acute treatment (Ávila et al., 1996).

The aim of the present study was to determine the effect of food intake and the role of oxidative stress in the pathogenesis of intestinal injury induced by oral administration of meloxicam (preferential cyclooxygenase-2 inhibitor) vs. piroxicam (preferential cyclooxygenase-1 inhibitor). Therefore, the activity of oxidative stress-related enzymes such as myeloperoxidase, xanthine oxidase and superoxide dismutase, as well as levels of lipid peroxides and glutathione homeostasis were studied in re-fed rats.

2. Material and methods

2.1. Animals groups and drug preparation

Male and female Wistar rats supplied by Animal Services (Faculty of Medicine, University of Seville, Spain), 8–10 each group and 180–250 g body weight, were placed singly in cages with wire-net floors in a room with controlled temperature (22–24°C) and humidity (70–75%) with a lighting regimen of 12 h/12 h 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. Experiments followed a protocol approved by the local animal Ethics Committee and the Local Goverment. All experiments were in accordance with the recommendations of the European Union regarding animal experimentation (Directive of the European Counsel 86/609/EC).

Meloxicam (Boehringer Ingelheim, Barcelona, Spain) and piroxicam (Roig Farma, Spain) were suspended in Tween-20 (1%) and administered p.o. in different doses to different groups of animals. Results were compared with those from two control groups: fasted rats and re-fed rats. These groups received vehicle in a comparable volume (10 ml/kg body weight) also by the same route.

2.2. Induction of intestinal damage

The rats were fasted for 24 h and then compounds were given p.o. in equipotent doses (Barner, 1996): meloxicam (7.5 or 15 mg/kg body weight) and piroxicam (10 or 20 mg/kg body weight). After drug administration, the animals were re-fed for 24 h until killing with an ether overdose (Satoh et al., 1981). The small intestine was spread out on filter paper, opened by a longitudinal incision along the antimesenteric side, and the contents were removed. The length and width of each lesion were measured using a binocular lens and the sum of the products (areas sum total) was expressed in terms of the ulcer index (UI, mm^2). The extent of haemorrhage was also measured according to a scale (score 0–2): 0—absence, 1—slight haemorrhage, 2—severe haemorrhage. The lesions were

assessed by a person unaware of the type of treatment received by the animals.

2.3. Assessment of leukocyte involvement

Myeloperoxidase activity was assessed as a marker of neutrophil infiltration (Grisham et al., 1990). In all animals, one sample from the small intestine was obtained. Samples were excised from each animal and rapidly rinsed with ice-cold saline, blotted dry, and frozen at −70°C. The tissue was thawed, weighed and homogenized in 10 volumes 50 mM phosphate-buffered saline (PBS), pH = 7.4. The homogenate was centrifuged at 20,000 × *g*, 20 min, 4°C. The pellet was again homogenized in 10 volumes 50 mM PBS, pH = 6.0, containing 0.5% hexadecyl-trimethylammonium bromide (HETAB) and 10 mM EDTA. This homogenate was subjected to one cycle of freezing/thawing and brief sonication. A sample of homogenate (0.5 μl) was added to a 0.5 ml reaction volume containing 80 mM PBS, pH 5.4, 0.5% HETAB and 1.6 mM 3,3′,5,5′-tetramethylbenzidine (TMB). The mixture was incubated at 37°C for 5 min and the reaction was started by the addition of 0.3 mM H_2O_2.

Each tube containing the complete reaction mixture was incubated for exactly 3 min at 37°C. The reaction was terminated by the sequential addition of catalase (20 μg/ml) and 2 ml 0.2 M sodium acetate, pH = 3.0. The changes in absorbance at 655 nm were measured with a spectrophotometer. One unit of MPO activity was defined as the amount of enzyme present that produced a change in absorbance of 1.0 U/min at 37°C in the final reaction volume containing the acetate. Results were expressed as U/mg protein.

2.4. Xanthine oxidase activity

Xantine oxidase is the enzyme responsible for the conversion of xanthine and hipoxanthine to uric acid. The enzyme occurs in two forms: the NAD^+-dependent dehydrogenase, which reduces NAD^+ to NADH, and NAD^+-independent oxidase, which reduces molecular oxygen to superoxide. The tissue was homogenized in buffer consisting of Tris–HCl, EDTA, phenylmethylsulphonyl fluoride, dithiothreitionin and leupeptine, pH = 8.1. The homogenate was centrifuged and the supernatant was separated on a Sephadex (G-25) column. Xanthine was used as substrate for xantine oxidase activity studies. Xantine oxidase activity was assayed as uric acid production, based on the increase in absorbance at 294 nm in the absence of NAD^+. One unit of xantine oxidase activity corresponds to the formation of 1 μM of uric acid per minute (Devenyi et al., 1987). Results were expressed as U/mg protein/min.

2.5. Superoxide dismutase activity

Measurement of the enzymatic activity of superoxide dismutase is based on the inhibition of the reduction of cytochrome C according to the method of McCord and Fridovich (1969). Samples of small intestine were homogenized in a mixture of PBS and EDTA. The homogenate was supplemented with 0.1% Triton. The assay method used ferricytochrome *c*, xanthine, as source of O_2^-, and sufficient milk xantine oxidase to give a rate of increase in absorbance of 0.025/min at pH 7.8 and 25°C. Reaction kinetics were measured in a spectrophotometer at 550 nm during 60 s at intervals of 10 s. Results were expressed as U/mg protein. One unit of superoxide dismutase is defined as the amount of enzyme that causes 50% inhibition of cytocrome *c* reduction.

2.6. Glutathione-peroxidase activity (GSH-peroxidase)

GSH-peroxidase activity in the mucosa of the small intestine of rats after administration of meloxicam was determined according to the method of Lawrence and Burk (1976). The reaction mixture consisted of 50 mM potassium phosphate buffer (pH 7.0), 1 mM EDTA, 1 mM NaN_3, 0.2 mM reduced nicotinamide adenine dinucleotide phosphate (NADPH), 1 E.U./ml oxidized glutathione (GSSG)-reductase, 1 mM GSH, and 0.25 mM H_2O_2. Samples were added to 0.8 ml of the above mixture and incubated for 5 min at 25°C before initiation of the reaction with the addition of peroxide solution. A sample of supernatant fluid with 10% homogenate solution and 1.15% KCl was prepared by centrifugation at 4000 × *g* for 10 min at 4°C. The absorbance at 340 nm was recorded for 5 min. The activity was the slope of the lines as micromoles of NADPH oxidized per minutes. The blank value (the enzyme was replaced with distilled water) was subtracted from each value. Results were expressed as nmol/mg protein/min.

2.7. Glutathione reductase activity (GSSG-reductase)

GSSG-reductase reduces the oxidized glutathione (GSSG). Its activity was measured by the method of Worthington and Rosemeyer (1974), following the decrease in absorbance at 340 nm induced by oxidized glutathione in the presence of NADPH in PBS buffer, pH 7.8. Results were expressed as nmol/mg protein/min.

2.8. Total glutathione determination

GSH is an important constituent of intracellular protective mechanisms against a number of noxious stimuli, and is known to be a major low molecular weight scavenger of free radicals in cytoplasm. GSH and GSSG occur in tissues, GSH is by far the predominant form. More than 99.5% of tissue "total glutathione" is in form of GSH (Anderson, 1985).

The tissue was homogenized in trichloroacetic acid, the homogenate was centrifuged and the supernatant solutions were stored at 4°C until assayed. GSH is oxidized by 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) to give GSSG

with stoichiometric formation of TNB. GSSG is reduced to GSH by the action of the highly specific glutathione reductase and NADPH. The rate of TNB formation is followed at 412 nm and is proportional to the sum of GSH and GSSG present. Results were expressed as nmol/mg protein.

2.9. Statistical analysis

The values are given as arithmetic means ± S.E.M. The significance of differences between means was evaluated with the non-parametric Mann–Whitney *U*-test.

3. Results

Oral administration of meloxicam or piroxicam induced multiple intestinal lesions. Ulcers occurred mainly on the mesenteric side of the middle and distal intestine and appeared as small round ulcers or as longitudinal ulcers. No lesions were observed in control animals (Fig. 1a).

The animals treated with piroxicam (10 or 20 mg/kg) had a dose-dependent increase in the severity of intestinal lesions (Fig. 1b) reaching an ulcer index (UI) of 3.02 ± 1.21 ($P < 0.01$) and 19.54 ± 3.57 mm^2 ($P < 0.001$), respec-

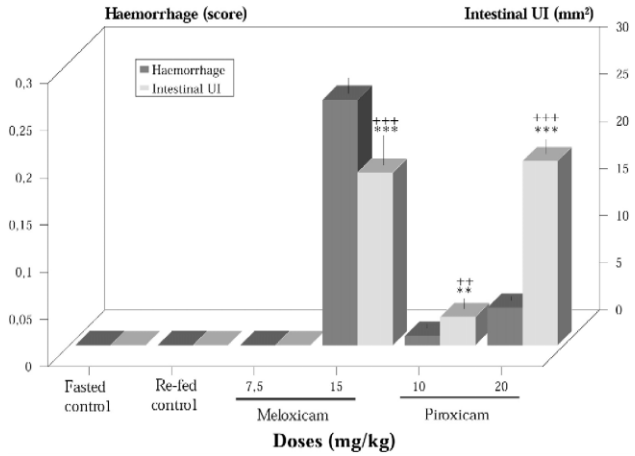

Fig. 2. Haemorrhage (score) and intestinal ulcer index (UI, mm^2) in re-fed rats treated with meloxicam (7.5–15 mg/kg) and piroxicam (10–20 mg/kg). The data are shown as means ± S.E.M. (+ + $P < 0.01$ and + + + $P < 0.001$ vs. fasted control; * * $P < 0.01$ and * * * $P < 0.001$ vs. re-fed control).

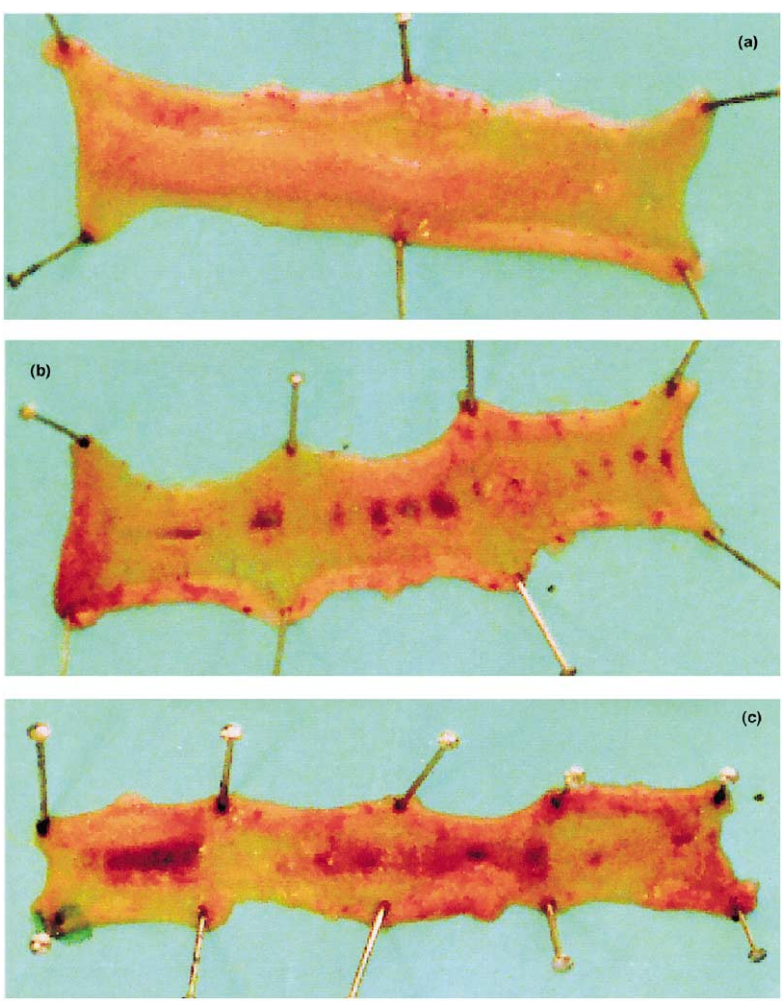

Fig. 1. Aspect of macroscopic damage in intestinal mucosa of re-fed rats: (a) re-fed control, (b) piroxicam 10 mg/kg, and (c) meloxicam 15 mg/kg.

Table 1
Activity of myeloperoxidase, superoxide dismutase, and xanthine oxidase after oral administration of meloxicam (7.5–15 mg/kg) or of piroxicam (10–20 mg/kg) in intestinal mucosa of re-fed rats. The data are shown as means ± S.E.M.

Doses (mg/kg)	Myeloperoxidase (U/mg protein)	Superoxide dismutase (U/mg protein/min)	Xantine oxidase · 10^{-4} (U/mg protein/min)
Fasted control	0.48 ± 0.03	4.70 ± 0.57	1.35 ± 0.23
Refed control	0.45 ± 0.06	5.54 ± 0.40	1.25 ± 0.14
Meloxicam 7.5	0.40 ± 0.10	4.28 ± 0.68[a]	1.11 ± 0.11
Meloxicam 15	0.39 ± 0.06	3.29 ± 0.27[a,b]	1.63 ± 0.09[a]
Piroxicam 10	0.35 ± 0.04	4.73 ± 0.33	1.43 ± 0.29
Piroxicam 20	0.43 ± 0.09	4.22 ± 0.48[a]	1.64 ± 0.11[a]

[a] Indicates a significant change as compared to re-fed control.
[b] Indicates a significant change as compared to fasted control.

tively, as can be seen in Fig. 2. Only the highest dose of meloxicam (15 mg/kg) caused macroscopic damage (Fig. 1c). The lesions were similar in location, aspect and severity to those induced by piroxicam (20 mg/kg). Neither meloxicam nor piroxicam produced a significant increase in the haemorrhagic index (Fig. 2).

Table 1 compares myeloperoxidase activity as an index of neutrophil infiltration in control mucosal samples with the activity after p.o. administration of meloxicam or piroxicam. Our data show that food ingestion did not induce modifications in myeloperoxidase activity as an index of neutrophil infiltration. Similarly, there was no significant enzymatic activity of the intestine after dosing. These results indicate that there was no substantial neutrophil influx into the mucosa in response to drug-induced injury. On the other hand, oral administration of the highest doses of both compounds resulted in a significant increase in xantine oxidase activity from a basal concentration of 1.25 ± 0.14 to 1.63 ± 0.09 and 1.64 ± 0.11 U/mg protein/min × 10^{-4} ($P < 0.05$), respectively (Table 1). The activity of superoxide dismutase in the intestinal mucosa of re-fed rats treated with the vehicle was 5.54 ± 0.40 U/mg protein/min. Our data showed that superoxide dismutase activity decreased significantly following oral treatment with meloxicam 7.5 and 15 mg/kg ($P < 0.05$ and $P < 0.001$, respectively), as well as with piroxicam 20 mg/kg ($P < 0.05$) (Table 1).

Fig. 3 shows the changes in GSH metabolism. Concentrations of reduced GSH in the intestinal mucosa of re-fed control rats were 8.29 ± 0.67 nmol/mg protein. After administration of meloxicam (15 mg/kg) or piroxicam (10 or 20 mg/kg), the level of GSH was significantly ($P < 0.01$ and $P < 0.001$) and dose dependently decreased to 5.74 ± 0.35 nmol/mg protein, 5.70 ± 0.59 and 5.00 ± 0.24 nmol/mg protein, respectively (Fig. 4). In addition, under our experimental conditions, treatment with either drug, meloxicam at the dose of 15 mg/kg and piroxicam at 10 or 20 mg/kg, lowered the activity of GSSG-reductase significantly ($P < 0.05$, $P < 0.01$), compared with that in control rats. In contrast, no changes in the activity of GSH-peroxidase were observed (Fig. 3).

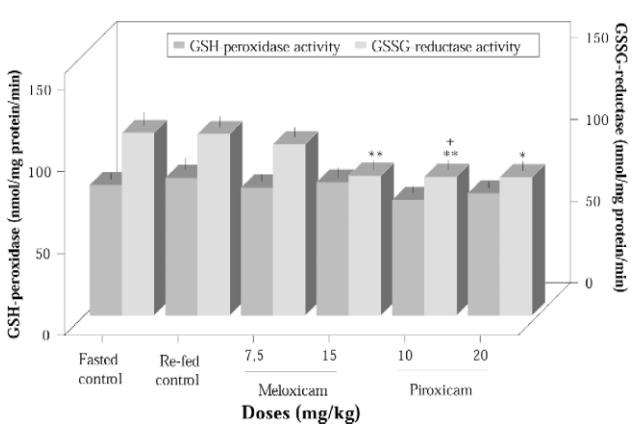

Fig. 3. Glutation peroxidase (GSH-peroxidase) and glutation reductase (GSSG-reductase) activities (nmol/mg protein/min) induced in intestinal mucosa of re-fed rats by meloxicam (7.5–15 mg/kg) and piroxicam (10–20 mg/kg) treatment. The data are shown as means ± S.E.M. (+ $P <$ 0.05 vs. fasted control; * $P < 0.05$ and * * $P < 0.01$ vs. re-fed control).

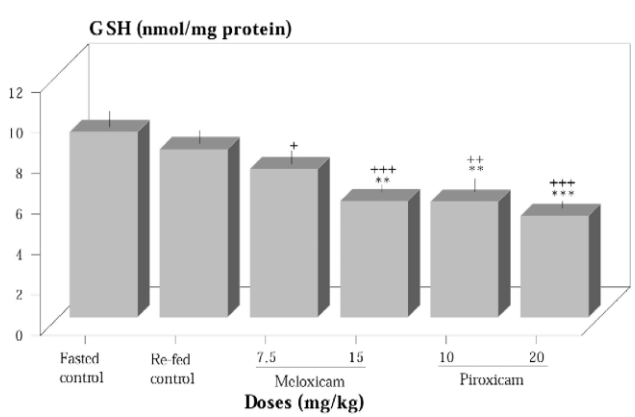

Fig. 4. Total GSH (nmol/mg protein) induced in intestinal mucosa of re-fed rats by meloxicam (7.5–15 mg/kg) and piroxicam (10–20 mg/kg) treatment. The data are shown as means ± S.E.M. (+ $P < 0.05$, + + $P <$ 0.01 and + + + $P < 0.001$ vs. fasted control; * * $P < 0.01$ and * * * $P <$ 0.001 vs. re-fed control).

4. Discussion

The present data show that under our experimental conditions meloxicam, a preferential cyclooxygenase-2 inhibitor, caused levels of intestinal toxicity comparable to those seen with traditional NSAID in an experimental model using re-fed rats. The macroscopic damage observed was similar for the highest dose of both drugs.

The cyclooxygenase-2 inhibitors constitute a new NSAID generation which, besides its clinical effectiveness in certain inflammatory processes such as rheumatoid arthritis or osteoarthritis, shows a lower incidence of gastrointestinal toxicity such as adverse digestive reactions (Davies and Wallace, 1997). Nevertheless, some experimental results indicate that although these drugs produce less gastrointestinal damage than do the classic NSAID (Simon et al., 1998), they may not be as safe on several experimental models or on previously lesioned mucosa (Wallace, 1999). Worsening of the damage when selective cyclooxygenase-2 inhibitors are administered, and a delay in the healing of experimentally induced ulcers in rodents have been observed (Mizuno et al., 1997), as well as exacerbation of inflammation-associated colonic injury in rats through inhibition of cyclooxygenase-2 (Reuter et al., 1996).

Several factors are likely to be involved in the intestinal lesions induced by NSAID. It has been emphasized that kinetic factors are important in the intestinal toxicity of these compounds; enterohepatic recirculation of NSAID promotes lesion formation in the small intestine of experimental animals (Brune et al., 1986; Rainsford, 1986; Reuter et al., 1997), and luminal bile acids may also contribute. The mean plasma half-life ($t_{1/2}$) of meloxicam is about 20 h, while the piroxicam $t_{1/2}$ is about 50 h, and both drugs undergo gastrointestinal recirculation (Brune et al., 1987; Türck et al., 1997).

Several studies, including ours, support the view that food ingestion may play an important role in the pathogenesis of experimental enteropathy induced by NSAIDs. Satoh et al. (1981) and Weissenborn et al. (1985) observed that indomethacin administered p.o. to rats caused no intestinal lesions when the animals were starved before and after treatment; it produced moderate lesions when the animals were fed continuously and maximal lesions when the animals were fed in the postdrug period after starvation in the predrug period. In our study, as in the above-cited papers, lesions were detected predominantly in the small intestine of re-fed rats. All these findings suggest that the ulcerogenic action of NSAIDs may be modified by physiologic alterations related to feeding conditions.

Regarding oxidative stress, the results of the present study are consistent with the hypothesis that free radicals play an important role in the production of mucosal damage. Quantitatively, the main free radical in tissues is superoxide that can be produced by both activated neutrophils via myeloperoxidase and by endothelial cells through xantine oxidase. This latter enzyme is found in tissues but not in neutrophils, and is known to play a crucial role in ischaemia-reperfusion injury (Van der Vliet and Bast, 1991).

Gastrointestinal mucosa is particularly rich in xantine oxidase. During ischaemia, ATP is degraded to hypoxanthine, and xanthine dehydrogenase is converted to xantine oxidase. On reperfusion, xantine oxidase catalyzes the conversion of hypoxanthine to uric acid with the release of superoxide radicals (Van der Vliet and Bast, 1991). The severity of the intestinal damage induced by meloxicam and piroxicam was related to a significant increase of xantine oxidase activity. The increase of xantine oxidase on administration of both drugs indicated that the free radicals derived via action of xantine oxidase were in part responsible for the oxidative tissue damage of intestinal mucosa, suggesting that xantine oxidase may be involved in gastric oxyradical formation in situations other than ischaemia-reperfusion. These findings are consistent with those from other studies which show that xantine oxidase activity is also increased after administration of certain NSAID such as indomethacin (Tanaka and Yuda, 1996).

Neutrophils appear to be the main effector cells in NSAID-induced small intestinal damage, and the microbial invasion of the mucosa may provide neutrophil chemoattractants. These agents provoke a burst of reactive radical species generation (Bjarnason et al., 1993). These leukocytes contain a NADPH oxidase, which reduces molecular oxygen to the superoxide anion radical, and also secrete the enzyme, myeloperoxidase, which catalyzes the formation of a potent cytotoxic oxidant such as hypochlorous acid (HOCl) from H_2O_2 and chloride ions and *N*-chloramines. Nevertheless, the level of myeloperoxidase activity was not significantly increased by either drug, indicating that neutrophil-derived free radicals were possibly not involved in intestinal injury. However, recent studies in experimental animals using a pulsed Doppler flowmetry applied to the mesenteric circulation have shown that certain NSAID such as indomethacin (Battarbee et al., 1996) promote leukocyte adherence and emigration in postcapillary venules. In a previous study by our experimental group another NSAID, dexketoprofen (Alarcón de la Lastra et al., 2000), increased myeloperoxidase activity as the index of neutrophil infiltration in intestinal mucosa, indicating that there was a substantial neutrophil influx into the mucosa in response to dexketoprofen-induced injury. In addition, previous experimental studies (García-Vicuña et al., 1997) showed that piroxicam and meloxicam were able to interfere with events of neutrophil function, such as their degranulation and cytokine-mediated activation changes in adhesion molecules. It is possible that both drugs could have a direct effect on the accumulation of neutrophils in inflammed tissue.

In order to protect tissues against the deleterious effects of oxygen radicals, all cells possess numerous antioxidant enzymes and free radical scavengers. Primary defences

include the enzymes, superoxide dismutase, catalase, and GSH-peroxidase. Superoxide dismutase is an intracellular metalloenzyme that catalyzes the dismutation of the superoxide radical with subsequent oxidative damage. In addition to removing a highly reactive species that might directly contribute to tissue necrosis, the dismutation of superoxide anion by superoxide dismutase may be protective by extending the half-life of the endothelium-derived relaxing factor, nitric oxide (Gryglewski et al., 1986). A role for superoxide anion in the present model of intestinal injury is supported by the observation that superoxide dismutase activity was significantly reduced by both treatments.

GSH is an important constituent of intracellular protective mechanisms against a number of noxious stimuli, including oxidative stress (Pihan et al., 1987). Additionally, GSH scavenges O_2^- and protects protein thiol groups from oxidation. The GSH redox cycle catalyzed by the endogenous antioxidative enzyme, GSH-peroxidase, reduces H_2O_2, thus breaking the chain reaction leading from O_2^- to the highly reactive •OH. At the same time, the antioxidant activity of GSH-peroxidase is coupled with the oxidation of GSH to GSSG, which can subsequently be reduced by GSSG-reductase with NADPH as the reducing agent (Halliwell and Gutteridge, 1989). Previous reports described the effect of some NSAID on glutathione levels in various organs of the rat (Micheli et al., 1992a,b). Our results revealed modifications in GSH metabolism: although glutation peroxidase activity was unaffected by meloxicam or piroxicam administration, GSSG-reductase activity and total GSH content were significantly decreased, probably due to GSH consumption during oxidative stress.

In conclusion, the results of the present study are consistent with the hypothesis that oxygen radicals, probably derived via the xantine oxidase pathway, play an important role in the production of damage induced by meloxicam, a preferential cyclooxygenase-2 inhibitor, as well as by piroxicam, a preferential cyclooxygenase-1 inhibitor. Furthermore, the decrease in superoxide dismutase activity and changes in glutathione homeostasis in intestinal mucosa may also contribute to the pathogenesis of meloxicam- or piroxicam-induced enteropathy.

It can also be concluded, therefore, that, under our experimental conditions, meloxicam causes rates of intestinal lesioning in rats comparable to those seen with traditional NSAID. Therefore, we propose that further studies be performed to demonstrate clearly the advantages of preferential or selective cyclooxygenase-2 inhibitors vs. classic NSAID.

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