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Effects of Oxicam Inhibitors of Cyclooxygenase on Oxidative Stress Generation in Rat Gastric Mucosa. A Comparative Study

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The aim of this study was to compare the effects of two nonsteroidal anti-inflammatory drugs (NSAID), members of the same family with a different cyclooxygenase (COX) inhibition selectivity, meloxicam, preferent COX-2 inhibitor, and piroxicam, preferent COX-1 inhibitor, on oxygen radical generation in rat gastric mucosa. Therefore, the activity of oxidative stress-related enzymes such as xanthine oxidase (XO), superoxide dismutase (SOD) and glutathione (GSH) homeostasis were studied in rats. Gastric prostaglandins (PG) were also assessed as a measure of COX-1 inhibition. Both oxicams produced a similar extent of the gastric mucosal damage and a significant decrease in PGE_2 synthesis, however only piroxicam induced an increase of both myeloperoxidase (MPO) activity and tumor necrosis factor (TNF)-α content in the gastric mucosa, indicating that neutrophil-derived free radicals were involved in gastric injury. Furthermore, both compounds reduced SOD activity and increased XO activity in gastric mucosa. Our results also revealed modifications in GSH metabolism: although glutathione peroxidase (GSH-px) activity was unaffected by meloxicam or piroxicam administration, both glutathione reductase (GSSG-rd) activity and total GSH content were significantly decreased after dosing. These results suggest that under our experimental conditions, meloxicam, preferential COX-2 inhibitor causes rates of gastric lesion in rats comparable to those seen with the traditional NSAID piroxicam, preferential COX-1 inhibitor. In addition to suppression of systemic COX activity, oxygen radicals, probably derived via the XO, and neutrophils play an important role in the production of damage induced by both oxicams. Moreover, the decrease in SOD activity and changes in glutathione homeostasis in gastric mucosa may also contribute to pathogenesis of meloxicam- or piroxicam-induced gastropathy.

Keywords: Nonsteroidal anti-inflammatory drugs (NSAID); COX-1; COX-2; Gastric damage; Oxygen radicals; Myeloperoxidase (MPO)

INTRODUCTION

A major limitation of clinical utility of nonsteroidal anti-inflammatory drugs (NSAID) is their gastrointestinal toxicity caused mainly by inhibiting synthesis of prostaglandins (PG) via cyclooxygenase (COX) enzymes. Two isoforms of COX have been recognized. COX-1 is a constitutively expressed enzyme in many tissues, including gastrointestinal tract while COX-2 is an inducible enzyme predominantly expressed at sites of inflammation.[1–3] The clinical efficacy of NSAID is primarily related to the inhibition of COX-2 activity, whereas much of the toxicity is related to COX-1 inhibition, in consequence development of NSAID that preferentially inhibit COX-2 offered the promise of relieving pain and inflammation without the adverse effects attendant to COX-1 blockade. As a result, currently available NSAID may now be characterized according to the ratio of COX-1 to COX-2 inhibition such as celecoxib, rofecoxib, nimesulide and meloxicam.[4,5] Meloxicam is a derivative of enolic acid, a member of the oxicam family, and has claimed to be a "preferential" COX-2 inhibitor, which at least in some *in vitro* and *in vivo* comparator studies may be comparable to that achieved with celecoxib.[3,6,7]

Over the last few years, a number of studies have provided evidence of an important role of reactive oxygen species (ROS) in mediating the microvascular disturbance that preceded gastric mucosal injury induced by NSAID. The available data point towards a reduction in mucosal blood flow, increased vascular

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permeability, activation of neutrophils, their enhanced adherence to the vascular endothelium and reactive radical species generation.[8,9] It has been suggested that the main chemoattractants for neutrophils are proinflammatory cytokines, such as tumor necrosis factor (TNF)-α and interleukin (IL)-1β which regulate endothelial molecules expression on endothelial cells[10,11] and promote neutrophil adherence.[12] TNF-α has recently been proposed to be involved in NSAID-induced gastrointestinal injury and its production is increased after NSAID administration.[11,13]

In addition, NSAID affect a variety of enzyme systems resulting in increases in free-radical concentration within the cell such as lipoxygenase, glutathione peroxidase (GSH-px) and glutathione reductase (GSSG-rd), xanthine oxidase (XO) through ischemia–reperfusion, myeloperoxidase (MPO) and NADPH oxidase through white cell activation.[14–17] In a large variety of experimental models, anything that reduces the activity of GSH-px (including fasting, glutathione (GSH) deficiency or increases the rate of hydrogen peroxide (H_2O_2) production (XO activity, terbutyl hydroperoxide, etc.) increases NSAID-induced injury, whereas agents that increase availability of thiol (–SH) groups (cysteine, cysteamine, SH-generating systems), or that facilitate capture of free electrons (aminopyrine, thiourea) or increase the activity of metalloproteins that use efficient dismutation reactions in their mechanisms to detoxify ROS such as superoxide dismutase (SOD) or catalase all greatly reduces NSAID injury.[15,18,19]

The aim of this study was to compare the effects of two oxicams: meloxicam, preferential COX-2 inhibitor, and piroxicam, another oxicam family member and preferential COX-1 inhibitor,[20,21] on oxygen radical generation in rat gastric mucosa. Therefore, the activity of oxidative stress-related enzymes such as XO, SOD and GSH homeostasis were studied in rats. Gastric PG were also assessed as a measure of COX-1 inhibition.

MATERIAL AND METHODS

Animal Groups and Drug Preparation

Male and female Wistar rats supplied by Animal Services, Faculty of Medicine, University of Seville, Spain, and 180–250 g body weight; were placed singled in cages with wire-net floors in a controlled room (temperature 22–24°C, humidity 70–75%, lighting regimen of 12L/12D) and were fed a normal laboratory diet. 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 8–10 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 regarding animal experimentation (Directive of the European Counsel 86/609/EC).

Meloxicam (Boehringer Ingelheim, Barcelona, Spain) and piroxicam (Roig Farma S.A., Spain) were suspended in Tween 20 (1%) and administered p.o. in different doses to different groups of animals. Control groups received vehicle in a comparable volume (10 ml/kg body weight) also by the same route.

Gastric Mucosal Injury

The rats were fasted for 24 h and then compounds were given p.o. in equipotent doses:[22] meloxicam (7.5 or 15 mg/kg body weight) and piroxicam (10 or 20 mg/kg body weight). After drug administration the animals were killed at 9 and 6 h, respectively,[23–25] using an overdose of anesthetic. Briefly, their stomachs were removed and opened along the greater curvature. The length and width of each lesion were measured using a binocular lens and gastric damage was expressed in terms of the ulcer index (UI, score): 0—absence of lesions; 1—petechiaes; 2—from 1 to 5 pointed lesions < 3 mm; 3—more than 5 pointed lesions < 3 mm or 1 pointed lesion > 3 mm; 4—mainly lesions > 3 mm.[26] The extent of hemorrhage was also measured according to a scale (score 0–2): 0—absence, 1—slight hemorrhage, 2—severe hemorrhage. The lesions were assessed by a person unaware of the type of treatment received by the animals.

Measurement of PGE_2

Gastric mucosa was excised and rapidly rinsed with ice-cold saline. The tissue was weighed and homogenized in 6 ml TEAP buffer (pH 3.24) that contained a COX inhibitor, Inyesprin®. The homogenate was centrifuged (3000 rpm, 10 min, 4°C) and the supernatant was removed and 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 collected. Each fraction was evaporated with ethylacetate, and the dry residue redissolved in ethanol. PGE_2 was determined by a competitive PGE_2 enzyme immunoassay kit (Assay Designs, Inc.). PGE_2 levels were quantified in pg/mg protein. Comparative results were expressed as percentage of inhibition of PGE_2 synthesis respect to control groups.

Assessment of Leukocyte Involvement

Myeloperoxidase (MPO) activity was assessed as a marker of neutrophil (PMN) infiltration.[27] In all animals one sample from the body of the stomach (gastric corpus) 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% hexadecyltrimethylammonium bromide (HETAB) and 10 mM EDTA. 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 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 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 quantified in U/mg protein. Comparative data were expressed as percentage respect to control groups.

TNF-α Levels

Gastric mucosal samples were weighed (100 mg) and homogenized, after thawing, in 0.3 ml phosphate buffer saline solution (PBS pH 7.2) at 4°C. They were centrifuged at 12,000 rpm for 10 min. Mucosal TNF-α level was assayed by a quantitative TNF-α enzyme immunoassay (ELISA) kit (Quantikine®M, R&D Systems). The TNF-α values (pg/mg protein) were expressed as percentage respect to control groups.

Xanthine Oxidase Activity (XO)

The tissue was homogenized in buffer consisting of Tris(+)-HCl, EDTA, phenylmethylsulfonyl fluoride (PMSF), dithiothreitionin and leupeptine, pH = 8.1. The homogenate was centrifuged and the supernatant was separated by Sephadex (G-25) column. Xanthine was used as substrate for XO activity studies. XO activity was assayed as uric acid production by the increase in absorbance at 294 nm in the absence of NAD^+. One unit of XO activity corresponds to the formation of 1 μM of uric acid per minute.[28] Results were expressed as percentage respect to control groups.

Superoxide Dismutase Activity (SOD)

The enzymatic activity of SOD is based on the inhibition of the reduction of cytochrome C according to the method of McCord and Fridovich.[29] Samples of gastric mucosa were homogenized in a mixture of PBS and EDTA. The homogenate was supplemented with 0.1% Triton. The assay method used cytochrome C, xanthine and sufficient milk XO to give a rate of increase in absorbance of 0.025/min at pH 7.8 and 25°C. The reaction kinetic was measured in a spectrophotometer at 550 nm at a rate of 0–80 s. Results were expressed as U/mg protein. One unit of SOD is defined as the amount of enzyme that causes 50% inhibition of cytochrome C reduction. Comparative results were expressed as percentage respect to control groups.

Glutathione Peroxidase Activity (GSH-px)

GSH-px activity was determined according to the method of Lawrence and Burk.[30] 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 EU/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 initiating 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. Absorbance at 340 nm was recorded for 5 min. The activity was the slope of the lines as nmol of NADPH oxidized per min. The blank datum (the enzyme was replaced with distilled water) was subtracted from each value. Results were expressed as percentage respect to control groups.

Glutathione Reductase Activity (GSSG-rd)

GSSG-rd reduces the oxidized glutathione (GSSG). Its activity was measured by the method of Worthington and Rosemeyer,[31] 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 percentage respect to control groups.

Total Glutathione Determination

GSH is an important constituent of intracellular protective mechanisms against a number of noxious stimuli, and it 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. Greater than 99.5% of tissue

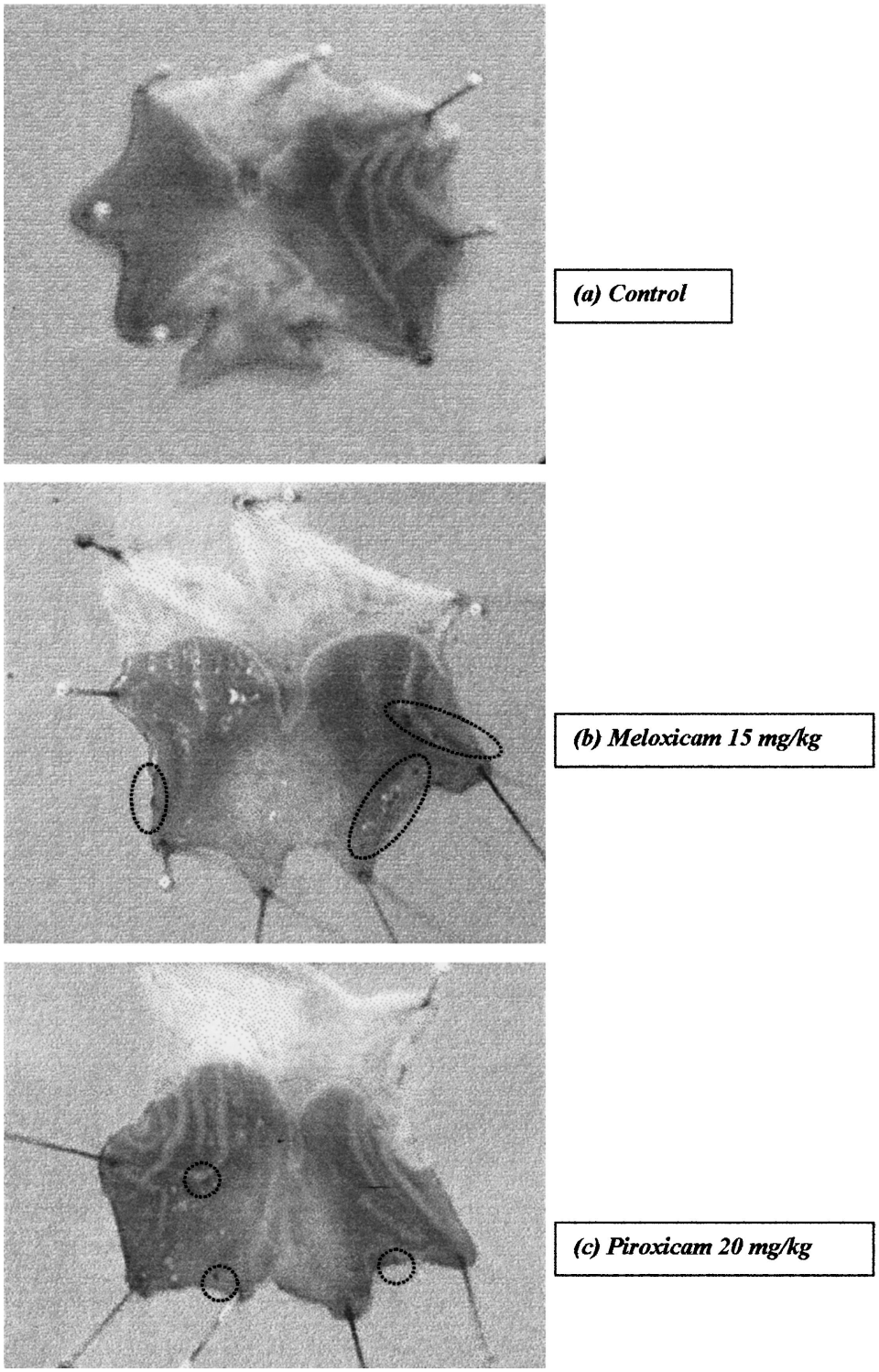

FIGURE 1 Aspect of macroscopic damage in gastric mucosa of rats: (a) control, (b) meloxicam 15 mg/kg, and (c) piroxicam 20 mg/kg.

"total glutathione" is in form of GSH.[32] The tissue was homogenized in trichloroacetic acid (TCA), the homogenate was centrifuged and the supernatant solutions are stored at 4°C until assayed. GSH is oxidized by 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) to give GSSG witch stoichiometric formation of reduced DTNB. GSSG is reduced to GSH by the action of the highly specific glutathione reductase and NADPH. The rate of reduced DTNB formation is followed at 412 nm and is proportional to the sum, of GSH and GSSG present. Results were expressed as percentage respect to control groups.

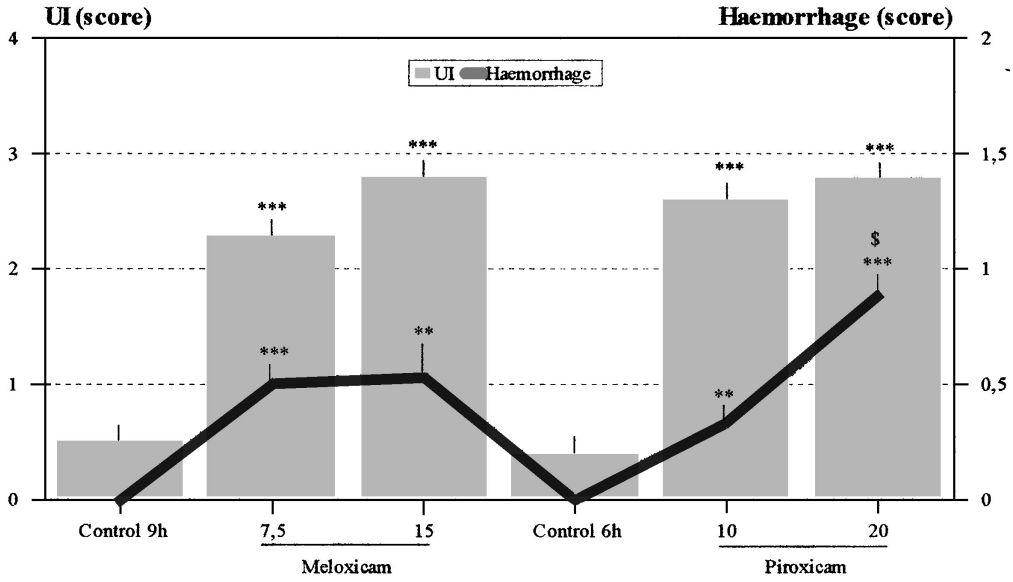

FIGURE 2 Gastric ulcer index (UI, score) and hemorrhage (score) in rats treated with meloxicam (7.5–15 mg/kg) and piroxicam (10–20 mg/kg). The data are shown as mean ± SEM (**$p < 0.01$ and ***$p < 0.001$ vs. control; $$p < 0.05$ vs. piroxicam 10 mg/kg).

Statistical Analysis

Values are given as arithmetic means ± SEM. The significance of differences between means was evaluated by the Mann–Whitney *U*-test.

RESULTS

Oral administration of both drugs induced gastric hemorrhage erosions. The lesions were linear or dotted in shape and were often located on the upper part of the mucosal folds. The animals treated with meloxicam had a dose-dependent increase in the severity of the gastric lesions (Fig. 1b), reaching an ulcer index (UI, score) of 2.8 ± 0.2 at the dose of 15 mg/kg (Fig. 2). Piroxicam had a similar damaging effect on gastric mucosa as compared to meloxicam (Fig. 1c) and at the dose of 20 mg/kg, reached a value of the area of gastric damage, about 2.8 ± 0.1 (Fig. 2). Figure 3 shows the percentage difference between gastric PGE_2 levels in controls and treated rats. Under our experimental conditions there was a significant reduction in PGE_2 concentration in treated groups compared to control animals (up to 77.7% and 85.0% after meloxicam and piroxicam

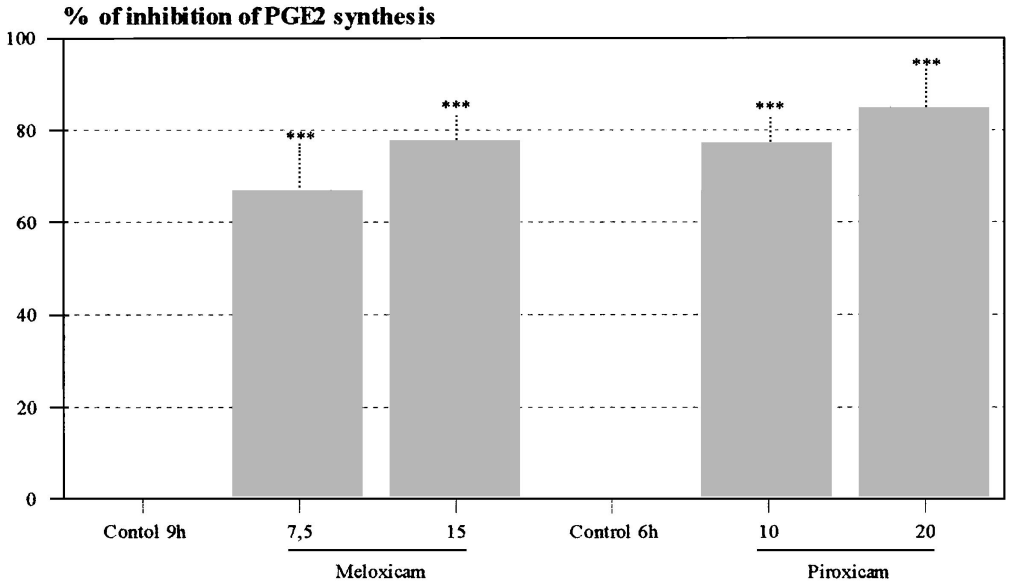

FIGURE 3 Changes in gastric PGE_2 levels after meloxicam (7.5–15 mg/kg) and piroxicam (10–20 mg/kg) treatments. Results shown are expressed as inhibition percentages of the control values (***$p < 0.001$ vs. control).

TABLE I Effects of meloxicam (7.5–15 mg/kg) and piroxicam (10–20 mg/kg) p.o. after 9 and 6 h of treatment, respectively, on myeloperoxidase (MPO), xanthine oxidase (XO), Superoxide dismutase (SOD) activities, and tumor necrosis factor (TNF)-α, expressed as percentage of enzymatic activities or TNF content in gastric mucosa of rat. Comparative data are expressed as percentage respect to control groups, and are shown as means ± SEM

Doses (mg/kg)	MPO (%)	TNF-α (%)	XO (%)	SOD (%)
Control 9 h	99.9 ± 10.1	99.9 ± 7.7	99.9 ± 14.8	99.9 ± 4.2
Meloxicam 7.5	96.3 ± 21.6	92.3 ± 25.7	107.4 ± 15.1	72.2 ± 2.4***
Meloxicam 15	118.4 ± 23.7	99.9 ± 12.4	222.0 ± 52.9*$^{+}$	76.9 ± 4.4***
Control 6 h	99.9 ± 11.3	99.9 ± 7.7	99.9 ± 5.7	99.9 ± 16.1
Piroxicam 10	127.5 ± 13.1*	106.4 ± 10.6	110.6 ± 39.1	42.3 ± 5.1**$^{+++}$
Piroxicam 20	136.1 ± 11.8*	139.9 ± 18.5*	277.4 ± 29.7**$^{\$\$\$}$	30.2 ± 2.5***$^{\#\#\#}$

*$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ vs. respective control; $^{+}p < 0.05$ and $^{+++}p < 0.001$ vs. meloxicam 7.5 mg/kg; $^{\#\#\#}p < 0.001$ vs. meloxicam 15 mg/kg and $^{\$}p < 0.05$ and $^{\$\$\$}p < 0.001$ vs. piroxicam 10 mg/kg.

administration, respectively ($p < 0.001$). Nevertheless, no significant differences between the tested compounds were found.

Table I shows the difference between XO oxidase activity percentage in controls and treated rats. Oral administration of the highest doses of both oxicams resulted in a significant increase in XO activity in relationship to rats treated only with the vehicle reaching a percentage of 222.0% ($p < 0.05$) with meloxicam and a percentage of 277.4% ($p < 0.01$) in the case of piroxicam. There were, however, no significant differences in this parameter between meloxicam- and piroxicam-treated groups. Our results show that MPO activity was significantly increased from control in piroxicam-treated group ($p < 0.05$). These results indicate that there was a substantial neutrophil migration into the gastric mucosa. However, neutrophil infiltration, i.e. MPO activity, did not change significantly in rats treated with meloxicam when compared with controls (Table I).

Table I also presents the comparative data on the production of gastric mucosal TNF-α during the oxicams-induced gastric injury. Piroxicam-induced damage was accompanied by a 140% ($p < 0.05$) increase in TNF-α expression over that of the controls whereas no significant changes vs. control occurred in the animals treated with meloxicam.

TABLE II Effects on total GSH (nmol/mg protein), expressed as percentage respect to control groups, and activities percentage of GSH-px (nmol/mg protein/min) and GSSG-rd (nmol/mg protein/min) in gastric mucosa of rat induced by piroxicam (10–20 mg/kg) and meloxicam (7.5–15 mg/kg) after 6 and 9 h of treatment, respectively. Comparative data are expressed as percentage respect to control groups, and are shown as means ± SEM

Doses (mg/kg)	GSHt (%)	GSSG-rd (%)	GSH-px (%)
Control 9 h	99.9 ± 11.7	99.9 ± 12.7	99.9 ± 12.7
Meloxicam 7.5	51.5 ± 5.2***	68.4 ± 5.8*	103.2 ± 9.5
Meloxicam 15	33.0 ± 5.0***$^{++}$	71.6 ± 7.3*	107.3 ± 10.3
Control 6 h	99.9 ± 12.8	99.9 ± 13.0	99.9 ± 15.5
Piroxicam 10	79.4 ± 8.1	73.3 ± 5.1*	95.8 ± 6.5
Piroxicam 20	71.9 ± 3.7*	64.9 ± 6.4*	88.1 ± 4.8

*$p < 0.05$ and ***$p < 0.001$ vs. control; $^{++}p < 0.01$ vs. meloxicam 7.5 mg/kg.

On the other hand, intragastric administration of either meloxicam or piroxicam significantly decreased the activity of the antioxidant enzyme SOD in gastric mucosa ($p < 0.001$; $p < 0.01$) (Table I). Furthermore, the reduction of SOD activity after piroxicam treatment was more accentuated and significantly different to that of meloxicam group ($p < 0.001$) (Table I).

Table II shows the changes in GSH metabolism. Six hours after administration of 20 mg/kg of piroxicam, the level of GSH was significantly ($p < 0.05$) decreased, reaching a percentage of 71.9% of the control value. After 9 h of treatment with meloxicam; the decrease of the endogenous thiol levels was dose-dependently reaching a reduction of a 51.5–33.0% ($p < 0.001$). In addition, pretreatment with both drugs significantly lowered the activity of GSSG-rd ($p < 0.05$) whereas no changes in the activity of GSH-px were observed after dosing.

DISCUSSION

This study shows that, at equipotent dosage, meloxicam caused levels of gastric damage comparable to those seen with traditional NSAID. In recent years, the role of PG synthesis inhibition in the pathogenesis of NSAID-gastropathy has been reconsidered, highlighting the immunomodulatory and proinflammatory consequences of prostanoids suppression. PG play a key role in modulating gastric defense and also are important for epithelial restitution and repair of mucosal injury. The awareness that mucosal damage is due to the non-discriminatory effect of NSAID on COX isoenzymes has lead to the development of more selective, COX-2 inhibitors. Nevertheless, there are a number of uncertainties remaining about gastroduodenal toxicity associated with COX-2 inhibitors. These include: ulcer healing[33] and ulceration[34] induces gastric mucosal COX-2 and retards healing in animal models,[35] likewise exacerbation of inflammation-associated colonic injury in rats through inhibition of COX-2.[36] Previous reports have confirmed that

meloxicam at low doses showed good gastric tolerance and did not cause significant suppression of gastric PGE_2 levels.[37,38] However, under our experimental conditions meloxicam as well as piroxicam inhibited PGE_2 biosynthesis, and these results were in concordance with those obtained by Tegeder *et al*.[39]

In human studies, the efficacy of both drugs was equivalent in osteoarthritis patients but meloxicam exhibited gastrointestinal adverse events in 10% of them.[40,41] In animal models, there was ample evidence to support the claim that selective inhibitors of COX-2 produced less gastrointestinal damage than standard NSAID when administered acutely to healthy animals.[42] In contrast, when administered for altered gastrointestinal mucosa, they aggravated and complicated gastric ulcers as well as necrosis in the small intestine,[36,42] consequently restricting their clinical use. Moreover, recent findings suggest that the situation is more complex than initially anticipated. Thus, constitutive expression of COX-2 was found in many tissues and there is now considerable evidence that this isoform performs important physiological functions too.[43–48] Therefore, suppression of COX-2 with selective and preferential inhibitors should not be expected to be without some adverse consequences. In recent years, many studies have suggested that COX-2 can contribute to gastric mucosal defense[49,50] and also appears to play an important role in promoting the healing of ulcers in the stomach,[35,51,52] at least in some circumstances. So, Gretzer *et al*.[49] reported that selective COX-2 inhibitors interfered with adaptive response of the gastric mucosa to a topical irritant. On the other hand, a role of COX-2-derived prostaglandins in gastric ulcer healing is supported by studies in experimental models,[33,35,53] and it is possible that inhibition of both COX-1 and COX-2 in the gastric mucosa contributes to the generation of erosions and ulcers. The study performed by Wallace *et al*.[50] shows that selective inhibition of either COX-1 or COX-2 does not elicit gastric damage in the rat. But because all conventional NSAID inhibit both isoforms when administered at doses effective in reducing inflammation and pain, inhibition of both COX-1 and COX-2 is required for NSAID-induced damage to develop.[3,54]

Moreover, there is strong evidence that COX-1 contributes to inflammation and pain,[55] so selective inhibitors of COX-2 will not necessarily produce the same degree of efficacy that is seen with mixed inhibitors of COX-1 and COX-2.[50]

There is good evidence for a role of ROS in experimental NSAID gastropathy.[13,18] Quantitatively, the principal free radical in tissues is superoxide anion (O_2^-) which is converted to the secondary oxidant H_2O_2 by SOD. O_2^- can be produced by both endothelial cells through XO and activated neutrophils through NADPH oxidase, which reduces molecular oxygen to the O_2^- radical, and through the enzyme MPO. This enzyme catalyzes the formation of such potent cytotoxic oxidant as hypochlorous acid (HOCl) from H_2O_2 and chloride ions and *N*-chloramines. In addition, neutrophils can also release proteases, lactoferrin and lipid mediators that can contribute to gastric injury.[14] TNF-α is a cytokine with marked chemotactic function and may be a key signal for NSAID-induced neutrophil adherence within the gastrointestinal microcirculation.[13,56] The level of MPO activity was significantly increased solely by piroxicam administration, indicating that neutrophil-derived free radicals were involved in gastric injury. These data were also associated with a significant increase of TNF-α levels. Our results are in line with previous reports, indicating that up-regulation of TNF-α gastrointestinal production was correlated with the development of indomethacin-induced intestinal injury.[57] Our data were also in accordance with another observation in which the cytokine was similarly enhanced after administration of the chiral NSAID flurbiprofen to rats.[58]

XO is found in tissues but not in neutrophils and is known to play a crucial role in ischemia–reperfusion injury. Gastrointestinal mucosa is particularly rich in XO, during ischemia, ATP is degraded to hypoxanthine and xanthine dehydrogenase is converted to XO. In the reperfusion state, XO catalyzes the reaction of hypoxanthine or xanthine and molecular oxygen to superoxide radicals O_2^-. These radicals rapidly react with the free radical nitric oxide and peroxynitrite anion and other reactive species.[59] The increase in XO suggests that ROS derived from the XO pathway are involved in the lesions. These findings are consistent with other studies, which show that XO activity is also increased after administration of certain NSAID, such as indomethacin[19] or ketoprofen.[60]

SOD is an intracellular metalloenzyme which owes its antioxidant properties to its elevated capacity of scavenging O_2^- radicals. The decrease in SOD activity may enhance lipid peroxidation (LP) as well as aggravate the injury to the gastric mucosa.[61] In the present study, SOD activity was significantly decreased by both meloxicam and piroxicam. This finding would explain in part a role for O_2^- in oxicams-induced gastric injury. This result is also in line with previous studies that reported a significant reduction of SOD activity after indomethacin,[18,34] diclofenac[17] or ibuprofen administration.[16]

GSH is an important component of detoxication systems and it is present in high concentrations in the stomach. Therefore, its depletion in gastric mucosa, after an ischemia/reperfusion process or ethanol administration, results in lipid peroxidation

erosions and ulceration.[23,62] The GSH redox cycle catalyzed by an endogenous antioxidative enzyme GSH-px, reduces H_2O_2 thus breaking the chain reaction leading from O_2^- to the highly reactive OH. On the other hand, the antioxidant activity of GSH-px is coupled with the oxidation of GSH to GSSG, which can subsequently be reduced by GSSG-rd with NADPH as the reducing agent.[63] Changes in GSH metabolism have been reported after administration of indomethacin[18,19,64] or diclofenac.[17] Our results revealed modifications in GSH metabolism: although GSH-px activity was unaffected by meloxicam or piroxicam administration, GSSG-rd activity and total GSH content was significantly decreased, probably due to its consumption during oxidative stress.

In conclusion under our experimental conditions, meloxicam, preferential COX-2 inhibitor causes rates of gastric lesion in rats comparable to those seen with the traditional NSAID piroxicam, preferential COX-1 inhibitor. In addition to suppression of systemic COX activity, oxygen radicals probably derived via the XO and neutrophils play an important role in the production of damage induced by both oxicams. Furthermore, the decrease in SOD activity and changes in GSH homeostasis in gastric mucosa may also contribute to pathogenesis of meloxicam- or piroxicam-induced damage.

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