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Role of L-Arginine in Ibuprofen-induced Oxidative Stress and Neutrophil Infiltration in Gastric Mucosa

M.D. JIMÉNEZ[a], M.J. MARTÍN[a,*], C. ALARCÓN DE LA LASTRA[a], L. BRUSEGHINI[b], A. ESTERAS[b], J.M. HERRERÍAS[c] and V. MOTILVA[a]

[a]*Department of Pharmacology, Faculty of Pharmacy, University of Seville, 41012 Seville, Spain;* [b]*Zambon Group, Barcelona, Spain;* [c]*Department of Gastroenterology, Virgen Macarena Hospital, University of Seville, Seville, Spain*

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It has been proposed that neutrophil and oxygen dependent microvascular injuries may be important prime events in gastrointestinal (GI) toxicity of non-steroidal antiinflammatory drugs (NSAIDs). L-arginine (L-ARG) is an essential amino acid which participates in many important biochemical reactions associated to the normal physiology of the organism. In these experimentations, we studied the role of L-ARG, aminoacid precursor of NO synthesis, on ibuprofen (IB) induced gastric lesions, and also on the inflammatory and oxidative mechanisms related to mucosal damage.

Oral administration of IB ($100\,mg\,kg^{-1}$), produced severe damage on gastric mucosa, which was more important after 6 h test-period, and was accompanied by a significant increment in myeloperoxidase (MPO) activity, as index of neutrophil activation, as well as lipid peroxidation (LP) levels and xanthine oxidase (XO) activity. However, no changes were observed in total mucosal glutathione (tGSH), nor glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) activity. Simultaneous treatment with equimolar doses of L-ARG (oral and i.p.), considerably reduced the number and intensity of lesions, and at the same time (6 h) the maximum protection was also observed. In addition, L-ARG inhibited the IB-induced LP and XO enhancement, but did not produce changes in leukocyte infiltration, tGSH, GSH-Px and SOD activity. These findings suggest that (1) L-ARG protective effect on gastric mucosa against IB-induced mucosal lesions could be explained by a local effect and also might be due to the systemic action of the aminoacid; (2) the active oxygen species, derived both from XO and activated neutrophils, could play a role in the pathogenesis of gastric injury induced by IB, (3) L-ARG exhibit a protective effect against IB-induced mucosal damage, probably through the inhibition of oxidative stress derived via xanthine-XO, but it does not block the oxygen free radical production through polymorphe nuclear leukocytes.

Keywords: L-arginine (L-ARG); Nonsteroidal anti-inflammatory drugs (NSAID); Ibuprofen (IB); Gastric damage; Neutrophils; Oxidative stress

INTRODUCTION

Nonsteroidal antiinflammatory drugs (NSAID) although used frequently for the treatment of arthritis and musculoskeletal disorders, may produce deleterious effects related to the gastrointestinal (GI) tract, including dyspeptic symptoms, erosions, ulcers, and serious GI complications (i.e. bleeding, perforation, and gastric outlet obstruction). Conservative calculations estimate that approximately 20–50% of NSAIDs users develop digestive complications at some time, and 1–2% of those on continuous treatment, are annually hospitalized.[1,2]

In addition to deficiency of prostaglandins (PG) due to inhibition of cyclooxygenase (COX), it has been proposed that neutrophil and oxygen radical-dependent microvascular injuries may be important prime events that lead to mucosal damage induced by NSAID.[3,4] Development of damage is based on the demonstrable ability of these agents to cause leukocyte adherence to endothelial cells, blocking capillaries causing local decrease in mucosal blood flow, and harming the endothelial integrity through the release of elastases, leukotrienes, and active oxidants. Lipid peroxidation (LP) mediated by oxygen radicals plays also

*Corresponding author. E-mail: calero@fafar.us.es

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an important role in the pathogenesis of the gastric damage after NSAID administration.[5–7] In this aspect, it has been demonstrated that several drugs, such as metamizole or acetaminophen, without pathogenic effect on gastric mucosa, do not induced oxidative stress,[8] furthermore, the protective effect of melatonin on indomethacin-induced gastric damage has been related to its antioxidant properties.[9]

There is plenty of evidence that nitric oxide (NO) interacts with PG and sensory neuropeptides in the regulation of mucosal integrity influencing such factors as mucus secretion, mucosal blood flow and ulcer repair.[10] It has the capacity to down-regulate inflammatory responses in the GI tract, to scavenge various free radical species and to protect the mucosa from injury induced by topical irritants.[11] NO is synthesized from L-ARG, essential amino acid in the fetus and neonate, which plays versatile key roles in nutrition and metabolism.[12] In the last years, its beneficial properties in improving reproductive, cardiovascular, pulmonary, renal, liver and immune functions, and in facilitating wound healing have been shown.[13]

However, the role of this aminoacid in gastric protection has been little studied. Recently, it has been demonstrated that pretreatment with L-ARG, caused a dose-dependent reduction in 0.6 N HCl,[14] 100% ethanol,[15] acidified ASA or water-immersion and restraint stress-induced lesions in rats,[16,17] and this reduction was accompanied by a gradual increase in the gastric blood flow.

Ibuprofen (IB) is an arylpropionic acid derivative NSAID that exhibits a high analgesic and antipyretic activity associated with less GI toxicity. It has been formulated with equimolar doses of L-ARG in order to improve its pharmacokinetic parameters and to obtain quicker and more potent analgesic action. In previous studies, we found that this formulation protects the gastric mucosa against IB-induced gastric lesions, and this protective effect was comparable to the one exerted by ranitidine or misoprostol.[18] We also observed that pretreatment with NOS-inhibitors, reversed this effect[19] and recently, we showed that both COX/PGs and NO/guanylyl cyclase (GC) dependent mechanisms are involved in the gastroprotection.[20]

From the above-mentioned findings, we have designed the present study in order to investigate the role of formulation IB/L-ARG versus IB alone on oxidative stress in gastric mucosa. We assessed the effect of the aminoacid on leukocyte activation and LP levels. Moreover, changes in superoxide dismutase (SOD) and xanthine oxidase (XO) activity, as well as mucosal glutathione (GSH) metabolism, were also evaluated.

MATERIAL AND METHODS

Animal Groups and Drug Preparation

Male Wistar rats, (supplied by Animal Services, University of Seville), 180–200 g, were used throughout this study. The animals were deprived of food for 18 h before the experiments but had free access to water and they were placed in single cages which had wire-net floors to prevent coprophagy. The temperature was maintained at 22–24°C and humidity at 70–75% in a controlled room.

Groups of 9–10 rats were treated with IB (100 mg kg^{-1} b.w., Sigma Chemical Co., MO), L-ARG (100 mg kg^{-1} b.w., Sigma Chemical Co., MO), and IB/L-ARG (100/100 mg kg^{-1} b.w., Zambón S.A., Barcelona, Spain). The drugs were prepared in distilled water and were administered orally by gavage, in a dose of 1 ml/100 g body weight. Control groups received vehicle in comparable volume. The selection of doses was supported by previously published findings[18,20] and also by clinical data.

In order to study the evolution of gastric lesions, L-ARG (100 mg kg^{-1}) and the association IB/L-ARG (100/100 mg kg^{-1}) were assayed at different times, 30 and 90 min, 6 and 12 h.

In addition, in an attempt to demonstrate that the protection afforded by L-ARG is not only a topical, direct effect on gastric mucosa, new groups of animals received a similar dose of this agent by parenteral route (i.p.).

All experimental protocols were performed following the guidelines approved by the local Ethics Committee for Experimental Reseach of the University of Seville.

Experimental Protocol

Animals from different treated groups were killed using an overdose of ethyl ether and the stomachs were removed and cut along the smaller curvature. The gastric lesions were immediately evaluated and different parameters in relationship with damage were assessed:[18] (a) area of gastric damage: the product of ulcer length and width was calculated and expressed in terms of the ulcer index (UI, mm^2); (b) mucosal damage (%): reduction of damaged area of the different groups compared with the respective equimolar dose of IB alone (100%) and (c) presence of haemorrhage (score): 0-absence, 1-slight haemorrhage, 2-marked haemorrhage. The lesions were assessed by a person unaware of the type of treatment received by the animals. Following the analysis, the mucosa layer was blotted dry, and scraped off the underlying muscularis externa and serosa. Homogenous mixture of mucosa, damaged and macroscopically healthy tissue, was snap-frozen in liquid nitrogen, and stored at −70°C before biochemical studies.

With the aim of detecting possible changes on intragastric pH following the administration of drugs, we used new groups of rats. Ten minutes after treatments, the animals were anesthetised by i.p. injection of sodium pentobarbital at a dose of 50 mg kg^{-1} b.w. The stomach was harvested and opened by incision along the greater curvature and its content was collected for pH measurement[21] with a conventional pH electrode (micro pH CRISON 2002).

LP Levels

The levels of thiobarbituric acid (TBA) reactants in the gastric mucosa as index of LP production, were measured according to the modified method of Ohkawa *et al.*[22] Samples of mucosa were weighed and homogenized in 10 ml ClK (10%). The homogenate was supplemented with 8.1% sodium lauryl sulphate, 20% acetic acid and 0.8% TBA, and boiled at 100°C for 1 h. After cooling, the reactants were supplemented with 2.5 ml *n*-butanol, shaken vigorously for 1 min and centrifuged for 10 min at 2600*g*. Absorbance was measured in a Perkin-Elmer Lambda 3 spectrophotometer at 532 nm, and the results were expressed as nmol thiobarbituric acid-reactive substances (TBARS)/mg protein. Protein concentration was calculated following the Bradford assay.[23]

Myeloperoxidase (MPO) Activity

MPO activity was assessed as a marker of neutrophil infiltration (PMN) in tissue.[24] Samples of gastric mucosa were weighed and homogenized in 10 volumes of 50 mM phosphate-buffer saline (PBS), pH 7.4. The homogenate was centrifuged at 20,000*g*, 20 min at 4°C. The pellet was again homogenized in 10 volumes of 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 a brief period of sonication. MPO activity was assayed spectrophotometrically using a minor modification of the method, which makes use of 3,3′,5,5′-tetramethylbenzidine (TMB) as substrate. In this method 0.5 μl of homogenate were added to a 0.5 ml reaction volume containing 80 mM PBS, pH 5.4, 0.5% HETAB and 1.6 mM 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 cathalase (20 μg/ml) and 2 ml of 0.2 mM Na–acetate, pH 3.0. One unit of MPO activity was defined as the amount of enzyme present that produced a change in absorbance at 655 nm of 1.0 Unit/min at 37°C in the final reaction volume containing the acetate.

XO Activity

XO is the enzyme responsible for the conversion of xanthine and hypoxanthine into 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 method described by Devenyi *et al.*[25] has been followed.

The tissue (100–150 mg) was homogenized in 2.5 ml buffer consisting of 100 mM Tris–HCl, ethylendiamintetraacetic (EDTA) 10 mM, phenyilmethylsulfonylfluoride (PMSF) 1 mM, 1,4-dithiothreitol (DTT) 1 mM, and leupeptine, pH 8.1. The homogenate was centrifuged at 4000*g*, 30 min, 4°C. The supernatant (500μl) was separated by Sephadex (G-25) column with 5 ml buffer and the eluate was collected. The mixture obtained by the addition of 200 μl eluate and 2.8 ml xanthine 60 μM, was used as substrate for XO activity studies and 0.67 mM NAD^+ were used for total XO activity determination. XO, and total XO activities were 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.

SOD Activity

The enzymatic activity of SOD is based on the inhibition of the reduction of cytochrome c according to the method of McCord and Fridovich.[26] Samples of gastric mucosa were homogenized (1:150) in a mixture of 50 mM PBS and 100 μM EDTA (pH 7.8). The homogenate was supplemented with 0.1% triton. The assay method used 10 μM ferricytochrome c, 50 μM xanthine, as source of O_2^-, and sufficient milk XO (5 nM) to give a rate of increase in absorbance of 0.025/min at pH 7.8 and 25°C at 550 nm at a rate of 0–60 s.

Results were expressed as U/mg protein. One unit of SOD (U) is defined as the amount of enzyme that causes 50% inhibition of cytochrome c reduction.

Effect on GSH Metabolism

Total GSH Determination

GSH is an important constituent of intracellular protective mechanisms against a number of noxious stimuli, and it is known to be the most important low molecular weight scavenger of free radicals in citoplasm. GSH and GSSG occur in tissues, and GSH is by far the predominant form. More than 99.5% of tissue total glutathione tGSH is in form of GSH.[27]

The tissue was homogenized in trichloroacetic acid (TCA), 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 (GSSG-Rd) and NADPH. The rate of TNB formation is followed at 412 nm and is proportional to the sum of GSH and GSSG (tGSH) present in the sample.

Glutathione Peroxidase (GSH-Px) Activity

GSH-Px activity was quantified by the method of Yoshikawa *et al.*[28] The reaction mixture consisted of 50 mM PBS pH 7, 1 mM EDTA, 1 mM NaN_3, 0.2 mM NADPH, 1 U/ml oxidized GSSG-Rd in PBS buffer, pH 7.8, 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. The absorbance at 340 nm was recorded for 5 min. The activity was the slope of the lines as μmol of NADPH oxidized per minute. The blank datum (the enzyme was replaced with distilled water) was subtracted from each value. Results were expressed as nmol/min/mg protein.

Analysis of Results

Values are given as arithmetic means ± SEM. The significance of differences between means was evaluated by one-way analysis of variance (ANOVA's test) followed by the Fisher test or the Mann–Whitney *U*-test.

RESULTS

Effect of L-ARG on IB-induced Gastric Lesions

IB induced linear or dotted shaped lesions which were often located on the upper part of the mucosal folds (Fig. 1). Time-dependent action following oral administration of the NSAID was evaluated (Fig. 2). The highest damaging response was after 6 h of treatment (17.9 ± 2.7 mm^2), and at the same time the maximum protection of oral L-ARG was also observed (6.3 ± 1.3 mm^2, $p < 0.001$). Although the effect of both treatments declined along the following 12 h, the gastroprotection afforded by L-ARG continued to be effective ($p < 0.05$).

Six hours after dosing IB (100 mg kg^{-1} b.w., p.o.) the NSAID produced an important damage on the gastric mucosa (Table I). Oral treatment of the animals with equimolar doses of L-ARG (IB100/L-ARG 100 mg kg^{-1}), considerably reduced the gastric lesions (mm^2) versus IB alone ($p < 0.001$), and also the percentage of mucosal damage (35.2%).

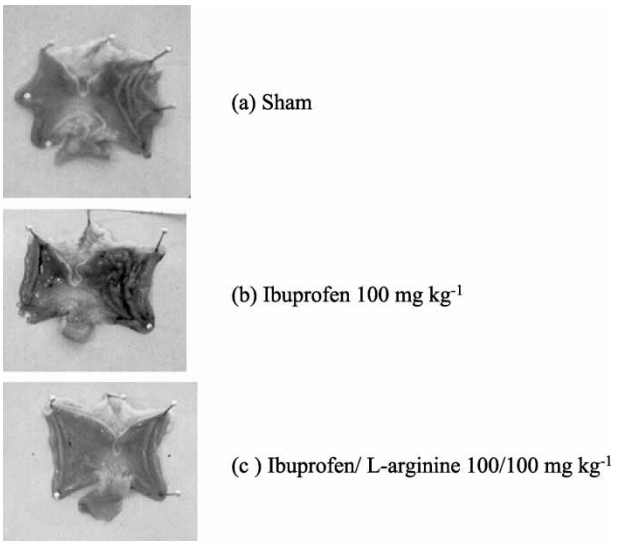

FIGURE 1 Macroscopic aspect of rat gastric mucosa 6 h after drug administration: (a) sham, (b) IB 100 mg kg^{-1}, (c) IB 100/L-ARG 100 mg kg^{-1}. The haemorrhagic lesions were observed along the crests and folds of the gastric corpus and their shape was either dotted or longitudinal (b). Following the simultaneous administration of L-ARG, an important protection was detected (c).

An important haemorrhagic score was also detected in the group treated with IB which was significantly reduced by L-ARG ($p < 0.001$) (Table I, Fig. 1b and c).

No lesions were observed after parenteral administration of the aminoacid (100 mg kg^{-1}, i.p.), without changes in parameters analysed related to damage of gastric mucosa.

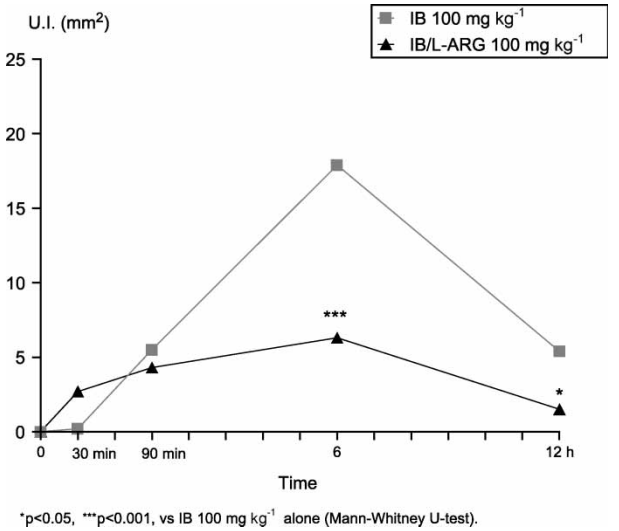

FIGURE 2 Mean of gastric lesions in rats exposed to different times (30 and 90 min, 6 and 12 h) following oral administration of IB (100 mg $^{-1}$ p.o.) and IB/L-ARG (100/100 mg kg^{-1} both p.o.). The highest damaging response occurred after 6 h of treatment, and at the same time the maximum protection of L-ARG was observed. Values are means ± SEM for 10 rats. *Significant changes compared with IB alone.

TABLE I Gastric protection of oral (p.o) or parenteral (i.p.) L-arginine (L-ARG 100 mg kg^{-1} b.w.) in presence of equimolecular doses of ibuprofen (IB 100 mg kg^{-1} b.w.), after 6 h of treatment

Treatment (mg kg^{-1} b.w.)	Gastric damage (mm^2)	Mucosal damage (%)	Haemorrhage (score)
Sham	–	–	–
L-ARG 100 (p.o.)	–	–	–
IB 100 (p.o)	17.9 ± 2.7	100	1.01 ± 0.1
IB 100 /L-ARG 100 (both p.o)	6.3 ± 1.3***	35.2	0.3 ± 0.1***
L-ARG 100 (i.p.)	–	–	–
IB 100 (p.o.) /L-ARG 100 (i.p)	7.7 ± 1.2***	39.4	0.3 ± 0.1***

Comparative data are shown as means ± SEM (***$p < 0.001$, IB/L-ARG versus the same dose of IB alone, Mann–Whitney *U*-test).

Regarding pH levels, no significant changes from control group were found 10 min after treatments (Table II).

Effects of L-ARG on IB-induced Neutrophil Infiltration, MPO Activity

Figure 3 compares MPO activity as index of neutrophil infiltration in control mucosa samples with those obtained after oral administration of IB (100 mg kg^{-1}) versus IB/L-ARG (100/100 mg kg^{-1}) 6 h after treatment. L-ARG *per se* did not induce any changes in MPO activity (20.0 ± 2.7 U/mg protein $\times 10^{-2}$). However, our data show that this parameter was significantly increased with IB treatment (29.0 ± 2.3 U/mg protein $\times 10^{-2}$, $p < 0.05$) from control (19.0 ± 1.2 U/mg protein $\times 10^{-2}$), and this result did not change by simultaneous administration of the aminoacid.

Effects of L-ARG on IB-induced Changes in LP Levels and XO-activity

The determination of TBA-reactive substances in the gastric mucosa as index of LP, reflected that IB (100 mg kg^{-1}) induced an increase in the levels from control group, which was detectable at 30 and 90 min, and was significant after 6 h of administration (sham 0.8 ± 0.2 nmol/mg protein, IB 2.2 ± 0.6 nmol/mg protein, $p < 0.05$) (Fig. 4). The treatment of IB/L-ARG (100 mg/100 kg^{-1}), significantly decreased the levels of TBARS in gastric mucosa versus IB alone at the same period (0.4 ± 0.2 nmol/mg protein, $p < 0.05$).

IB also induced an increase in uric acid concentration, final product formed after XO activity (3.3 ± 0.4 μmol/min/mg protein $\times 10^{-4}$, $p < 0.05$) versus untreated group (2.1 ± 0.2 μmol/min/mg protein $\times 10^{-4}$). Simultaneous treatment with L-ARG significantly inhibited this enzymatic action (2.2 ± 0.2 μmol/min/mg protein $\times 10^{-4}$, $p < 0.05$) (Fig. 5).

Effects of IB/L-ARG on Mucosal Antioxidant Substances: tGSH Levels, GSH-Px and SOD Activity

None of the treatments induced relevant changes in tGSH nor SOD activity (Table III). Although IB (100 mg kg^{-1}) induced an increase in GSH-Px activity (155.8 ± 18.0 versus 134.9 ± 15.2 of sham group) it was not significant.

DISCUSSION

The data obtained in this experimentation show that haemorrhagic lesions induced by IB on gastric mucosa of rat were already evident 30 min after its

TABLE II Basal pH of solutions and intraluminal pH following 10 min of its administration

Treatment (mg/kg)	pH of solutions before its administration	Intragastric pH
Sham (distilled water)	7.20	5.21 ± 0.28
L-ARG 100	6.08	6.55 ± 0.61
IB 100	6.77	6.09 ± 0.32
IB 100/L-ARG 100	6.80	6.05 ± 0.63

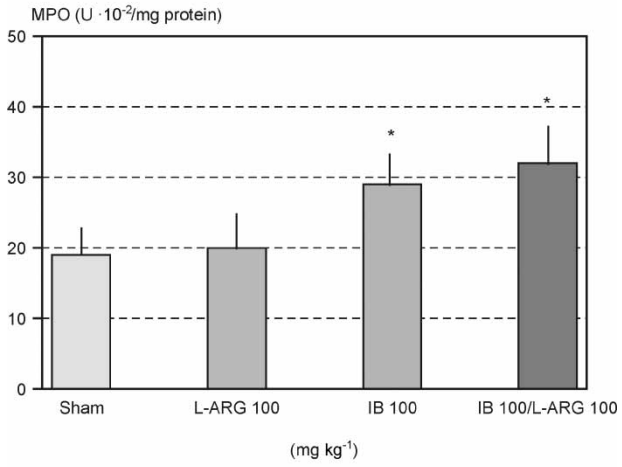

FIGURE 3 MPO activity obtained 6 h after oral administration of L-ARG (100 mg kg^{-1}), IB (100 mg kg^{-1}) and IB/L-ARG (100/100 mg kg^{-1}). Values means ± SEM for 10 rats. The simultaneous treatment with L-ARG does not modify the IB-induced neutrophil infiltrate in gastric mucosa. *Significant change compared with sham.

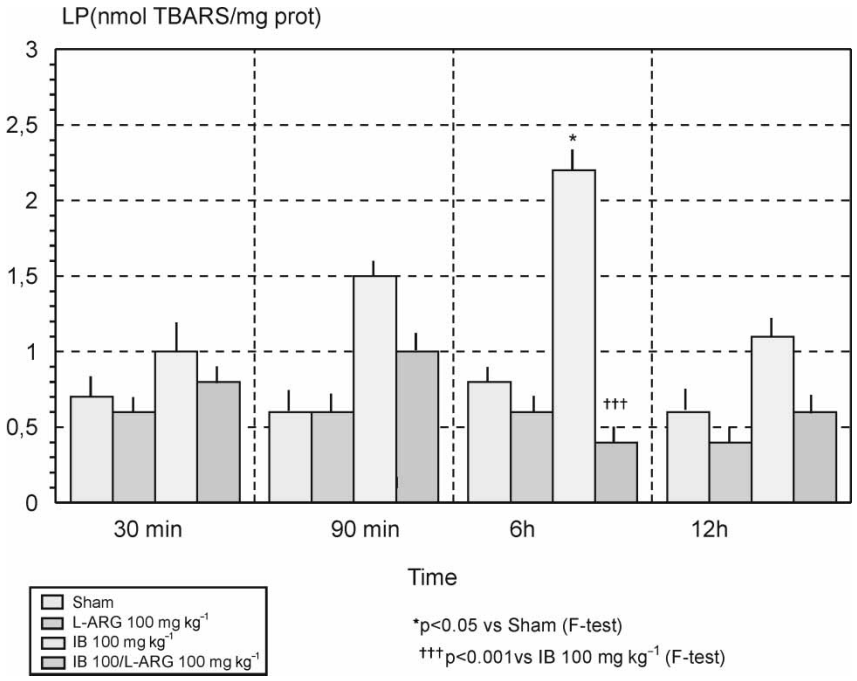

FIGURE 4 TBARS concentrations in rats exposed to 30 and 90 min, 6 and 12 h treatment with L-ARG (100 mg kg^{-1}), IB (100 mg kg^{-1}) and IB/L-ARG (100/100 mg kg^{-1}). Values means ± SEM for 10 rats. The maximum increase of IB-induced TBARS levels, at 6 h, is reversed by L-ARG. *Significant change compared with sham. †Significant change compared with IB alone.

administration and persisted along 12 h, but the maximum damage was induced during 6 h-test period and this was accompanied by an increase in neutrophil infiltration, XO activity and LP levels. These results are in agreement with the hypothesis that the oxy-radicals produced by different mechanisms play an important role in NSAID-induced gastropathy.

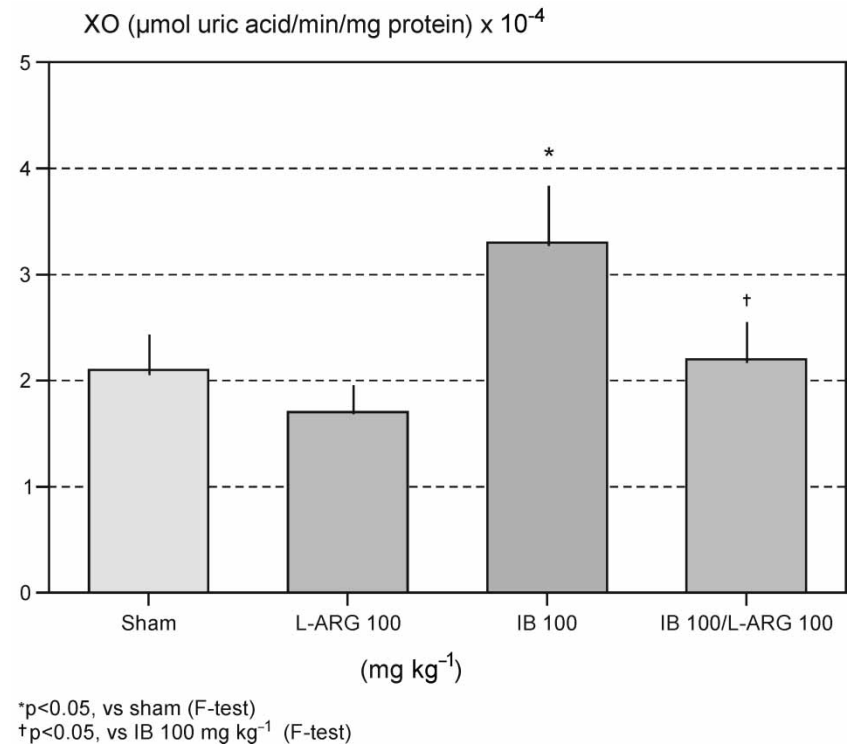

FIGURE 5 XO activity obtained 6 h after oral administration of L-ARG (100 mg kg^{-1}), IB (100 mg kg^{-1}) and IB/L-ARG (100/100 mg kg^{-1}). Values means ± SEM for 10 rats. The simultaneous administration of L-ARG reverses the increase of uric acid concentrations induced by IB. *Significant change compared with sham. †Significant change compared with IB alone.

TABLE III Effect of oral administration of IB (100 mg kg^{-1} b.w.), L-ARG (100 mg kg^{-1} b.w.) and IB/L-ARG (100/100 mg kg^{-1} b.w.), after 6 h of treatments, on neutralizing systems of free radicals: total glutathione (tGSH), glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD) activity

Treatment (mg kg^{-1} b.w.)	GSH-Px (nmol NADPH/min/mg prot)	tGSH (nmol/mg prot)	SOD (U/mg prot)
Sham	134.9 ± 15.2	6.9 ± 1.9	6.0 ± 1.0
L-ARG 100	126.6 ± 15.7	10.2 ± 1.5	4.1 ± 0.3
IB 100	155.8 ± 18.0	8.6 ± 2.5	5.4 ± 0.9
IB 100/L-ARG 100	132.9 ± 10.6	7.5 ± 1.1	6.2 ± 0.4

Ischaemia or reduced gastric perfusion is known to be associated with mucosal injury in several different clinical settings including trauma, major surgery and NSAID administration. It constitutes the earliest event in the pathogenesis of NSAID-induced ulceration.[28,29] Focal reduction of mucosal blood flow would impair the ability of the mucosa to withstand back-diffusion of acid, thereby leading to tissue necrosis. Increasing evidence indicates that the greatest injury occurs during reperfusion through oxygen radical species, such as superoxide anion (O_2) and hydroxylradical ($^{\cdot}OH$) generated via xanthine-XO system and activated neutrophils.[30] LP mediated by oxygen free radicals is an important cause of destruction and damage to cell membranes through perturbation of its functions, including transport processes, maintenance of ion and metabolite gradients or receptor mediated signal transduction leading to mucosal damage.[31] In this experimentation, soon after IB administration we observed an enhancement of TBARS concentration as index of LP. Although this augmentation was significant only at 6 h of IB treatment, it is possible that it might be related to the beginning of mucosal damage. Moreover, the simultaneous increase in XO activity following IB administration suggests that free radicals derived via xanthine-XO might also be involved in NSAID-induced gastric lesions. These results are in agreement with those obtained by some authors which found that XO activity and lipoperoxides content in gastric mucosa were also increased after administration of indomethacine, piroxicam or diclofenac.[7,8,32,33]

The circulating neutrophils have been implicated in the pathogenesis of many forms of GI injury including NSAID-induced ulceration.[3,8,34] Activation of leukocytes subsequent to their adherence to the vascular endothelium appears to be the earliest detectable event after NSAID administration. They clog the microvasculature and lead to the generation and release of a number of tissue damaging factors, including reactive oxygen metabolites and proteolitic enzymes that affect vascular tone and permeability exacerbating tissue ischaemia.[35] After IB treatment, our results show an important increase in MPO activity, which indicates that the neutrophils are also involved in the gastrolesive effect of this drug.

By contrast, sulphydryl compounds such as reduced GSH, have been shown to protect the gastric mucosa because they bind free radicals generated following tissue injury by noxious agents, including ethanol[36] or NSAID,[37] and also after water immersion-restraint stress.[38] GSH is a major endogenous antioxidant in the organism and it is present in high concentrations in the stomach and bowel of both, rodents and man. GSH-Px is an important enzyme which plays a significant role in the elimination of H_2O_2 and lipid hydroperoxides in the gastric mucosal cells. Although depletion of endogenous glutathione as well as GSH-Px and SOD, other strong cellular antioxidants, are involved in NSAID-gastropathy,[7,37] in our experimental conditions IB did not induce any changes on their levels versus control group, indicating that the ulcerogenic effect of this drug could not be associated with inhibition of the glutathione metabolism or decrease in SOD activity.

The results of the present study show that the simultaneous administration of L-ARG by oral but also by i.p., significantly reduces the number and intensity of lesions, and also the LP levels and XO activity. However, it does not induce changes in MPO activity and cellular antioxidant reactive substances (tGSH, GSSG-Px, and SOD). L-ARG exhibits multiple biological properties including improvement of nitrogen balance, restoration of depressed immunity, and acceleration of wound healing.[13] At gut level, it has been demonstrated that preadministration of L-ARG dose-dependently attenuated the mucosal lesions developed in rats subjected to water immersion restraint stress[17] or after ischaemia-reperfusion injury, possibly through a NO-dependent mechanism.[39] On isolated rabbit gastric cells, pretreatment with L-ARG also prevented the increase in lipid peroxides production, as well as the synthesis and secretion of mucus induced by hypoxia-reoxygenation.[40] In our experimentation, L-ARG reversed the IB-induced gastric damage, showing the gastroprotective effect of the aminoacid against IB-induced gastric injury.

Recently, L-ARG has been shown to be partially protective in some pathologic conditions associated with oxidative stress. Following severe tissue injury and during the subsequent repair period, the demand form L-ARG increases at the local site.[41,42]

Our results are in agreement with these findings since that the simultaneous administration of the aminoacid reverses the IB-increases XO-activity. Although the mechanism of this effect remains unknown, it is possible that it may occur through the inhibition of microvasculature disturbance and ischaemic situation developed after NSAID administration. In this way, it has been found that L-ARG in the presence of IB augments significantly the tissular concentration of cGMP,[43] and also induces an enhancement of gastric mucosal blood flow.[44] Furthermore, in a recent study,[20] we suggested the involvement of NO in the L-ARG protective effect on the rat stomach against IB, which could be explained in different ways: the early phase, is possibly mediated by COX/PGs, although NO liberated by a cNOS/cGMP pathway could be also decisive. In addition, clearly we found the expression of iNOS mRNA in the gastric mucosa 6 h after the administration of IB, however, the signal-level corresponding to oral IB/L-ARG treated rats was decreased. In contrast, no differences in the gastric cNOS expression were found between the different groups. Since it has been proposed that endogenous NO has dual action in the GI tract: protective by cNOS/NO and proulcerogenic by iNOS/NO,[10,45,46] these findings suggest that enhanced iNOS activity could be responsible for gastric mucosal overproduction in IB administration, and L-ARG may favor activation of mechanisms capable of counteracting efficiently the damaging effect of the NSAID, and previously to iNOS expression.

Brzozowski *et al.*[16] studied the effects of L-ARG on gastric secretion and acute gastric lesions provoked in rats by different experimental models, absolute ethanol, stress and acidified ASA. The aminoacid caused a dose-dependent reduction of the lesions, which was accompanied by a gradual increase in the gastric blood flow. The authors indicate that L-ARG acts on the gastric mucosa locally as a mild irritant inducing adaptative cytoprotection, because when it was administered by routes different from the intragastric administration, e.g. systemic pretreatment (i.v.), the aminoacid failed to affect the lesions caused by 100% ethanol. Our results indicate that both oral and i.p. administration of L-ARG protect the gastric mucosa against IB-induced mucosal lesions, suggesting the systemic effect of the aminoacid, in addition to topical protection.

In conclusion, the findings of this study indicate that L-ARG confers an important protection against IB-induced gastric injury. We confirm that the oxygen reactive metabolites derived both from XO pathway and activated neutrophils, are associated with IB-induced gastropathy, since this drug produced a significant enhancement of LP levels as well as XO and MPO activity. The decrease in LP and XO activity by simultaneous administration of L-ARG, suggests that the gastrosparing effect of the aminoacid could be partially due to the inhibition of the oxidative stress derived via xanthine-XO, probably because it reverses the ischaemic event through its hyperaemic and vasodilator properties, although this supposition requires more profound studies.

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