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Protective Effect of L-Arginine Against Ibuprofen-induced Gastric Injury in Rats

M. J. MARTÍN CALERO, M. D. JIMÉNEZ, C. ALARCÓN DE LA LASTRA, C. LA CASA, J. M. HERRERÍAS*, L.BRUSEGHINI†, A. ESTERAS† AND V. MOTILVA

*Department of Pharmacology, Faculty of Pharmacy, University of Seville, *Department of Gastroenterology, Virgen Macarena Hospital, University of Seville, and †Zambon Group, Barcelona, Spain*

Abstract

This study has been designed to confirm the protective effect of different single oral doses of L-arginine in the presence of equimolar doses of ibuprofen, and to compare the results with those obtained after treatment with ibuprofen alone. Different parameters were assessed in rats: gastric damage (mm^2 and score), ratio of lesionated stomachs/total stomachs evaluated, and presence of haemorrhage.

Six hours after dosing, oral administration of ibuprofen (0·3, 0·6 and 1·2 mmol kg^{-1}) produced a progressive dose-dependent increase in damage to the gastric mucosa. All treatments with equimolar doses of L-arginine considerably reduced lesions (mm^2 and score) and the same tendency was observed with the other parameters examined. We also evaluated the gastroprotective effect of L-arginine against anti-ulcer reference drugs, ranitidine and roxatidine (two antisecretory agents) and misoprostol (a cytoprotective drug). The degree of inhibition of damage provided by L-arginine was similar to those obtained with the other drugs.

Thus, we conclude that the simultaneous administration of equimolar doses of ibuprofen and L-arginine offers significant protection compared with gastrolesive doses of ibuprofen alone, with an important decrease in the lesionated areas and improvement of the vascular state. The extent of this protective action is comparable with that observed with anti-ulcer reference drugs.

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most commonly used drugs in the world, but their use in the treatment of pain, fever and inflammation, and in particular, in the treatment of arthritis, is associated with significant untoward effects on the gastrointestinal tract. The bleeding and ulceration induced in the stomach by NSAIDs is the most common adverse reaction to medication and has a significant financial impact on health care. Their gastric toxicity is related to suppression of prostaglandin synthesis, through inhibition of cyclooxygenase activity, and in addition, several NSAIDs have topical irritant properties on gastric mucosa.

In the last few years, a number of different strategies for developing NSAIDs with reduced gastric toxicity have been used. These include formulating the agents with an enteric coating to prevent absorption in the stomach, the development of prodrugs, which are not active until they have undergone metabolism by the liver, and co-administration of either suppressors of acid (anti-H_2 or proton pump inhibitors) or exogenous prostaglandins.

Recently, a new strategy related to the role of nitric oxide (NO) in the gastrointestinal tract has been proposed. NO is a membrane-permeable gas that serves as a mediator of many physiological events (Moncada et al 1991). It is produced from L-arginine by a calcium-dependent NO synthase. Numerous studies have implicated NO as a critical mediator of mucosal defence, similar to prostaglandins (Whittle et al 1990). NO, or drugs that generate NO (e.g. sodium nitroprusside, glyceryl trinitrate) have been shown to reduce the severity of gastric mucosal injury in experimental models (Whittle et al 1990; Brown et al 1993) and some

Correspondence: M. J. Martín Calero, Laboratorio de Farmacología, Facultad de Farmacia, Prof. García González s/n, 41012-Sevilla, Spain.

authors have suggested that the gastroprotective effect of L-arginine could be through an NO-pathway (Kitagawa et al 1990; Konturek et al 1993) Ibuprofen is a potent NSAID that recently has been formulated with equimolar doses of L-arginine in order to improve pharmacokinetic parameters (higher and more rapid absorption) with quicker and more potent analgesic effect. In a previous report we found that administration of L-arginine before ibuprofen treatment induced significant inhibition of gastric lesions (Motilva et al 1996). Thus, we designed this study to confirm the protective effect of the ibuprofen and L-arginine formulation vs ibuprofen alone, and to compare this gastroprotection with other traditional therapies such as misoprostol (PGE_1 analogue), ranitidine (a classic antisecretory agent) or roxatidine (a new anti-H_2 drug).

Material and Methods

Animal groups and compounds

Male Wistar rats (supplied by Animal Services, Faculty of Medicine, Seville), 180–250 g, were used. The animals were fed a normal laboratory diet in a controlled room (temperature 22–24°C and humidity at 70–75%). They were placed in single cages with wire-net floors to prevent coprophagy, and were deprived of food for 18 h before the experiments but had free access to water. Groups of 9–10 rats were treated with different doses of ibuprofen (Sigma Chemical Co., MO), ibuprofen and L-arginine (Zambon S.A., Barcelona, Spain), ranitidine (Sigma Chemical Co., MO), roxatidine (Zambon S.A., Barcelona, Spain) and misoprostol (CECOFAR, Seville, Spain). The drugs were suspended in distilled water and were administered by the intragastric route, in a dose of 1 mL/100 g body weight. Control groups received vehicle in comparable volume. Each experiment was performed at least twice and results were pooled.

Experimental protocol

Different groups of animals were treated with ibuprofen (0·3, 0·6 and 1·20 mmol kg^{-1}) and equimolar doses of ibuprofen and L-arginine (0·3/0·3, 0·6/0·6 and 1·20/1·20 mmol kg^{-1}). Six hours after treatment the animals 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 the following parameters related to damage were assessed. Gastric damage (score): 0, absence of lesions; 1, haemorrhagic or necrotic mucosa; 2, pointed lesions; 3, pointed lesions with lesions < 3 mm; 4, mainly lesions > 3 mm. Area of gastric damage (mm^2): the product of ulcer length and width was calculated. Mucosal damage (%): reduction of damaged area of the different groups compared with the respective equimolar dose of ibuprofen alone (100%). Ratio lesioned stomachs/total stomachs evaluated. Presence of haemorrhage (score): 0, absence; 1, slight haemorrhage; 2, marked haemorrhage.

The intermediate gastrolesive dose of ibuprofen (0·6 mmol kg^{-1}) noted in these experiments was selected to examine the protective effect of misoprostol, ranitidine and roxatidine. New groups of animals received the following treatments: ibuprofen alone; equimolar dose of ibuprofen and L-arginine (0·6/0·6 mmol kg^{-1}); ibuprofen and misoprostol ($0{\cdot}6/0{\cdot}05 \times 10^{-3}$ mmol kg^{-1}); ibuprofen and ranitidine (0·6/0·05 mmol kg^{-1}); and ibuprofen and roxatidine (0·6/0·05 mmol kg^{-1}). After the experimental period, the animals were killed and the stomachs removed, opened and assessed as in the previous experiments. The same parameters were evaluated.

Analysis of results

Statistical analysis was performed using conventional methods (arithmetic mean ± s.e.). One-way analysis of variance was used to test for significant differences of means of treatment groups. Multiple comparison between means was performed using the Mann–Whitney U-test and χ^2-test at a protection level of 0·05.

Results

Six hours after dosing, ibuprofen given orally in various doses (0·3, 0·6 and 1·20 mmol kg^{-1}) produced a progressive dose-dependent increase of damage to the gastric mucosa ($4{\cdot}12 \pm 1{\cdot}01$, $17{\cdot}88 \pm 2{\cdot}74$ and $66{\cdot}41 \pm 9{\cdot}17$ mm^2, respectively). Oral treatment of the animals with equimolar doses of L-arginine considerably reduced the gastric lesions (0·6 and 1·20 mmol kg^{-1}, $P < 0{\cdot}001$ against ibuprofen alone, mm^2 and score). The same tendency was evaluated with the other parameters, degree of haemorrhage and percentage of mucosal damage (Table 1).

Significant decreases in the gastric lesion parameters were found with all the treatments shown in Table 2. The most marked reduction of the lesion (mm^2 and score), as well as haemorrhagic score and percentage of mucosal damage, was observed with ranitidine, roxatidine and misoprostol ($P < 0{\cdot}001$). However, L-arginine treatment also

Table 1. The anti-ulcer protection of different doses of L-arginine in the presence of equimolar doses of ibuprofen in rats.

Treatment	Haemorrhage (score)	Gastric damage (mm^2)	Mucosal damage (%)	Lesioned stomachs/ stomachs evaluated	Gastric damage (score)
None	0·00 ± 0·00	0·00 ± 0·00	0·0	0/20	0·00 ± 0·00
Ibuprofen (0·3 mmol kg^{-1})	0·20 ± 0·06	4·12 ± 1·01	100	19/20	2·15 ± 0·20
Ibuprofen and L-arginine (0·3 mmol kg^{-1})	0·08 ± 0·04	2·47 ± 0·68	59·8	19/20	1·95 ± 0·15
Ibuprofen (0·6 mmol kg^{-1})	1·03 ± 0·12	17·88 ± 2·74	100	19/19	3·21 ± 0·10
Ibuprofen and L-arginine (0·6 mmol kg^{-1})	0·32 ± 0·11*	6·29 ± 1·33*	35·2	19/19	2·31 ± 0·11
Ibuprofen (1·20 mmol kg^{-1})	1·82 ± 0·10	66·41 ± 9·17	100	19/19	4·0 ± 0·01
Ibuprofen and L-arginine (1·20 mmol kg^{-1})	0·23 ± 0·09*	17·97 ± 4·15*	27·1	19/20	2·45 ± 0·18*

Results are means ± s.e. (n = 19 or 20). *$P < 0·001$, ibuprofen and L-arginine vs ibuprofen alone (Mann–Whitney U-test).

Table 2. The effects of different treatments in the presence of ibuprofen (0·06 mmol kg^{-1}): L-arginine (0·6 mmol kg^{-1}), ranitidine (0·05 mmol kg^{-1}), roxatidine (0·05 mmol kg^{-1}) and misoprostol (0·05 × 10^{-3} mmol kg^{-1}) on parameters of gastric damage in rats.

Treatment	Haemorrhage (score)	Gastric damage (mm^2)	Mucosal damage(%)	Lesioned stomachs/ stomachs evaluated (%)	Gastric damage (score)
Ibuprofen alone	1·03 ± 0·12	17·88 ± 2·74	100	100	3·21 ± 0·10
+ L-Arginine	0·32 ± 0·11*	6·29 ± 1·33*	35·2	100	2·31 ± 0·11*
+ Misoprostol	0·13 ± 0·06*	2·25 ± 0·76*	12·6	89·5	1·95 ± 0·21*
+ Ranitidine	0·10 ± 0·05*	1·43 ± 0·61*	8·0	68·4†	1·50 ± 0·29*
+ Roxatidine	0·10 ± 0·02*	0·08 ± 0·07	0·5	66·7†	1·00 ± 0·25

Results are means ± s.e. (n = 19 or 20). *$P < 0·001$, treatment groups vs ibuprofen alone, Mann–Whitney U test; †$P < 0·01$, χ^2 test.

induced very marked decreases in all parameters evaluated ($P < 0·001$).

Discussion

There is overwhelming evidence that NO is one of the most important mediators of mucosal defence, affecting factors such as mucus secretion, mucosal blood flow, ulcer repair and the activity of a variety of mucosal immunocytes. Endogenous NO has the capacity to down-regulate inflammatory responses in the gastrointestinal tract (Kubes & Wallace 1995), and it has been shown to be involved in certain types of gastroprotection such as that induced by capsaicin, papaverine (Brzozowski et al 1993), sucralfate and mild irritants (Konturek et al 1994). In this way, the ability of NO to reduce the severity of damage induced by NSAIDs has recently been exploited in an attempt to produce drugs which are not ulcerogenic. By attaching an NO-releasing moiety to standard NSAIDs (by incorporation of a nitroxybutyl moiety (e.g. flurbiprofen, ketoprofen and diclofenac nitroxy-butyl-ester), the gastrointestinal toxic effects of these compounds was greatly reduced but did not interfere with their ability to suppress inflammatory processes, inhibit prostaglandin synthesis or inhibit platelet aggregation (Wallace & Cirino 1994). Other recent strategies such as prophylactic therapy with the PGE_1 analogue misoprostol have been shown to reduce the incidence of NSAID-induced ulcers significantly, but the use of this drug is limited by its adverse effects in a considerable number of patients.

However, the effect of L-arginine, the precursor of NO, on the gastric mucosal integrity has not

been much studied. Our data show that simultaneous treatment with L-arginine significantly decreases ibuprofen-induced gastric damage in a dose-dependent way. This result is in agreement with Kitagawa et al (1990) who reported that L-arginine given intravenously prevented gastric lesions caused by 0·6 M HCl or water-immersion stress. Similar results were obtained by Takeuchi et al (1993) who found that oral treatment with L-arginine exhibits gastric cytoprotection against 0·6 M HCl-induced lesions in a dose dependent manner. This effect was mimicked by the enantiomer D-arginine and was not affected by previous administration of N^G-nitro-L-arginine methyl ester (L-NAME), a potent inhibitor of NO biosynthesis. Thus they suggested that the mucosal protective action of L-arginine (p.o.) is unrelated to the NO pathway, and its mechanism may appear through adaptative cytoprotection mediated by endogenous prostaglandins. In contrast, in a previous study, we did not observe any protective effect against diclofenac-induced gastric injury after D-arginine treatment. However, the protection induced by L-arginine was maintained after administration of L-NAME suggesting, at least in part, the involvement of NO in the protective mechanism of oral L-arginine (Motilva et al 1997).

Ferraz et al (1994) found that intra-arterial administration of L-arginine before the application of 20% ethanol produced a dose-dependent increase in the extent of damage. L-Arginine significantly reduced gastric blood flow relative to controls, and the authors suggested that the increase of gastric injury was partly NO dependent and partly NO independent. They suggested a paradoxical effect of L-arginine on gastric mucosal integrity. While small amounts of NO produced constitutively by endothelial cells appear to be critical for maintenance of mucosal perfusion, large amounts of NO can exacerbate mucosal injury, probably as a consequence of the cytotoxic properties of the NO radical or the peroxynitrite radical formed through the interaction of NO with superoxide.

We conclude that, under our experimental conditions, intragastric administration of L-arginine protects the gastric mucosa against ibuprofen-induced injury, and that this protective effect is comparable with those of antisecretory agents or misoprostol. However the mechanism involved in this beneficial action have not been studied. It is possible that L-arginine achieves direct cytoprotective effect on mucosal tissue and that this property could be related to the NO-pathway, but these suppositions require further exploration.

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