

Research Article



Effects of D-002 on Non-steroidal Anti-Inflammatory Drugs-Induced Gastric Ulcer in Rats

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ABSTRACT

Non-steroidal anti-inflammatory drugs represent a pivotal cause of gastric ulceration. Gastroprotective effects of D-002 have been documented in different experimental models of gastric ulcer in rats, but its effects on ibuprofen and naproxen-induced ulcer remained unexplored. This study investigated the effects of orally administering D-002 (5, 25, 100 and 200 mg/kg) as single doses on ibuprofen and naproxen-induced gastric ulcer, respectively, in rats. D-002 (5-200 mg/kg) significantly, markedly and dose-dependently ($r = 0.986$; $p < 0.05$) reduced the ibuprofen-induced ulcer indexes by 63.4, 70.9, 74.5 and 76 %, respectively, in rats. Similarly, D-002 reduced significantly, markedly (43.97, 52.13, 61.68 and 67.62 % of inhibition, respectively) and in a dose dependent manner ($r = 0.995$; $p < 0.01$) the gastric ulcer index. Omeprazole (10 mg/kg), reference substance, significantly and markedly inhibited the gastric ulcer index induced by ibuprofen and naproxen-induced ulcer with 96.7 and 75.2 % of inhibition, respectively, which supports the validity of these models in our conditions and then the results here described. Concluding, D-002 (5, 25, 100 and 200 mg/kg) significantly, markedly and dose-dependently reduced the gastric ulcer index induced by ibuprofen and naproxen, respectively, in rats.

Keywords: D-002, ibuprofen, naproxen, gastric ulcer.

INTRODUCTION

Peptic ulcer, common gastrointestinal pathological condition, can occur as gastric or duodenal ulcer and it represents a chronic recidivant disease that affects the quality of life of the suffers.¹ Gastric ulcer, defined as a discontinuity in the gastric mucosa penetrating through the muscularis mucosa,² results from the imbalance between aggressive (acid, pepsin, *Helicobacter pylori*, nonsteroidal anti-inflammatory drugs –NSAIDs–, ethanol) and defensive (bicarbonate, mucus secretion, blood flow, cellular regeneration, endogenous protective agents like epidermal growth factors and prostaglandins –PG–) factors acting on the gastric mucosa.³⁻⁶

NSAIDs treatment constitutes one of the most important aggressive factors on the gastric mucosa, due to the high frequency of the gastrointestinal adverse effects (AE) associated to their use (peptic ulcer, stomach and intestinal bleeding)^{7,8} and to their elevated facultative prescription for treating osteoarthritis, (a very common chronic diseases in adulthood) as well as the use of aspirin for preventing the cardio and cerebrovascular diseases.^{9,10}

The inhibitory action of NSAIDs on ciclooxigenase (COX), fundamental mechanism of their anti-inflammatory effects, represents at the same time the principal mechanism of their ulcerogenic action since it curtails cytoprotective prostaglandins (PG) production, and then triggers the metabolism of arachidonic acid towards the 5-ipooxigenase (5-LOX) via with the consequent overproduction of leukotrienes (LTs), mainly of LTB₄,

gastrotoxic mediators that enhance the gastric damage induced by PGs-deficit.^{11,12}

For these reasons, at the present day it is very common the consumption of the gastro-protective drugs, as pump bum inhibitors (PBI), H₂-histamine receptors antagonist (H₂RA) and the muco-protective agents, although none of them is exempt of producing AE.¹³⁻¹⁶

Bearing in mind these facts, the search for new strategies that ameliorate NSAIDs-induced gastric damaged, being secure and well tolerated, is updated.

Some different experimental models of NSAIDs-gastric induced ulcer (aspirin, ibuprofen and naproxen) have been used in order to evaluate the potential gastro-protective effect of new substances.¹⁷⁻²⁰

D-002 is a mixture of six high molecular weight fatty alcohols purified from the beeswax, (*Apis mellifera*, L) with the following composition: tetracosanol (6 – 15%), hexacosanol (7 – 20%), octacosanol (12 – 20%), triacontanol (25 – 35%), dotriacontanol (18 – 25%), tetratriacontanol ($\leq 7.5\%$) (purity $\geq 85\%$).²¹

D-002 has been shown to produce anti-inflammatory, antioxidant and gastroprotective effects in experimental and clinical studies.²²⁻³⁴

The gastroprotective effects of D-002 have been demonstrated in different experimental models of gastric ulcer in rats. Briefly, D-002 treatment prevented ethanol,²³ stress,²³ aspirin,^{24,25} indomethacin,^{26,27} pylorus ligation,²⁸ acetic acid,²⁹ and ischemia-reperfusion-induced gastric ulceration in rats.²⁶ In particular, the gastroprotective effects of D-002 comprise a multiple mechanism that involves the increased secretion and



improved quality of the gastric mucus, as well as antioxidant effects and reduction of neutrophil infiltration in the gastric mucosa.²⁷⁻³¹

Up today the NSAIDs induced- gastric ulcer models utilized for evaluating the gastroprotective effects of D-002 only have included the aspirin and indomethacin-induced gastric ulcer models.

Single oral doses of D-002 (50 and 100 mg/kg) significantly reduced the gastric ulcer induced by aspirin in rats,^{24, 25} which was associated to a reduction of neutrophil infiltration. Similarly, D-002 (25-100 mg/kg) administered to rats as single oral doses significantly reduced the indomethacin-induced gastric ulcer, which was associated to a reduction in gastric mucosa of the lipid peroxidation,^{26, 27} hydroxyl radical (*OH) generation, protein oxidation and myeloperoxidase (MPO) activity.³¹

However, up to now the effects of D-002 on gastric ulcer induced by NSAIDs others as ibuprofen and naproxen (widely used in the clinical practice) have not been evaluated.

In light of these issues, this study was undertaken to investigate the effects of D-002 on ibuprofen and naproxen-induced gastric ulcer, respectively, in rats.

MATERIALS AND METHODS

Animals

Male Sprague Dawley rats (250-300g) were purchased from the National Center for Laboratory Animal Production (CENPALAB, Havana, Cuba) and adapted for 7 days to the following conditions: temperature (22-23 °C), relative humidity (55-60%) and 12 hours dark/light cycles. Food and water were freely supplied. The animals fasted for 24 hours prior to the experiments.

The experiments were performed after obtaining the approval of the Institutional Board for animal use, and were conducted to the Cuban Guidelines for the Care of Laboratory Animals and the Cuban Code of Good Laboratory Practice (GLP).

Administration and dosage

The batch of D-002 (030151211), supplied by the Plants of Natural Products (National Center for Scientific Research, Havana, Cuba) was used after corroborating its quality criteria. Batch composition, assessed with a validated gas chromatographic method,³² was as follows: 1-tetracosanol (5 %), 1-hexacosanol (10.2 %), 1-octacosanol (14 %), 1-triacontanol (34.21 %), 1-dotriacontanol (24.24 %) and 1-tetracontanol (3.03 %). Purity (total content of these alcohols) was 90.7%. Omeprazole (DOMER, México D.F, México), was used as a reference substance in the experiments. Both D-002 and Omeprazole were suspended in 1% Arabic gum/water.

Two experiments were performed for evaluating the effects of different doses of D-002 on ibuprofen and naproxen-induced gastric ulcer, respectively, in rats.

Ibuprofen (Nanjing Baijingyu Pharmaceutical, Nanjing, China) and naproxen (MEDSOL, LaHabana, Cuba), inductors substances of gastric ulcer were prepared in suspensions of acacia gum/water (1%)

In both experimental series the rats were distributed in six groups (10 rats each) in which gastric ulcer by ibuprofen or naproxen, respectively, was induced: a control group treated with the vehicle, four with D-002 (5, 25, 100 and 200 mg/kg) and one with omeprazole (10 mg/kg), reference substance.

First experiment: Effects of D-002 on ibuprofen-induced gastric ulcers

All treatments (vehicle, D-002 and Omeprazole) were administered as single doses by gastric gavage (5mL/kg) one hour before the ulcer induction. Two oral doses of ibuprofen (300 mg/kg) were administered by gastric intubation with 15 hours of time interval between them.¹⁸ Six hours after the second ibuprofen administration the rats were sacrificed under an overdose of thiopental anesthesia. Their stomachs were washed with cold saline and removed for examining the ulcer index macroscopically.

Second experiment: Effects of D-002 on naproxen-induced gastric ulcers

A single oral dose of naproxen (80 mg/kg) was administered to rats by gastric intubation.¹⁹ Six hours after naproxen administration the rats were sacrificed, the stomach removed and the index ulcer determined similarly the first experiment above described.

Evaluation of gastric mucosal damage

The stomachs were opened along the greater curvature and washed with saline solution. The lesions in the gastric mucosa were examined macroscopically using magnification 3x. Ulcer indexes were determined as the sum of the lengths of the whole gastric lesions (in mm). Two independent, blinded observers performed the observations and measurements of lesion lengths.³³

Statistical analyses

Comparisons among groups were done with the Kruskal Wallis test; while the Mann-Whitney U test was used for paired comparisons between each treated and control groups. Statistical significance was chosen for $\alpha = 0.05$. Data were processed with the Statistics Software for Windows (Release 6.1 Stat Soft Inc, Tulsa OK, USA).

RESULTS

Table 1 shows the effects of D-002 on ibuprofen-induced gastric ulcer in rats. Oral acute administration of D-002 (5, 25, 100 and 200 mg/kg) significantly, markedly and dose-dependently ($r = 0.986$; $p < 0.05$) reduced the ibuprofen-induced ulcer indexes by 63.4, 70.9, 74.5 and 76 %, respectively, in rats. The omeprazole (10 mg/kg) oral administration marked and significantly reduced the ulcer



index (96.7 % of inhibition), corroborating the validity of this model in our experimental conditions.

Table 1: Effects of D-002 on ibuprofen-induced gastric ulcers in rats

Groups	Doses (mg/kg)	UI (mm)	I (%)
Control	—	41.65 ± 7	--
D-002	5	15.22 ± 3.7 **	63.45
D-002	25	12.12 ± 3.21 **	70.90
D-002	100	10.60 ± 2.78 ***	74.54
D-002	200	10.00 ± 1.77***	76.00
Omeprazol	10	1.38 ± 0.39 ***	96.7

I (%): Inhibition percentage, UI: Ulcer Index, ** $p < 0,01$; *** $p < 0, 0001$ Comparison vs Control (Mann Whitney U test).

Table 2 shows the effects of D-002 on naproxen-induced gastric ulcer in rats. Single oral administration with D-002 reduced significantly, markedly (43.97, 52.13, 61.68 and 67.62 % of inhibition, respectively) and in a dose dependent manner ($r = 0.995$; $p < 0.01$) the gastric ulcer index. The administration with unique doses of omeprazole (10 mg/kg) produced significant and marked inhibitions (75.2 %), which corroborate the validity of this model in our experimental conditions.

Table 2: Effects of D-002 on naproxen-induced gastric ulcers in rats

Groups	Doses (mg/kg)	IU (mm)	I (%)
Control	—	21.56 ± 2.75	--
D-002	5	12.08 ± 2.58 *	43.97
D-002	25	10.32 ± 2.99 *	52.13
D-002	100	8.26 ± 1.73 ***	61.68
D-002	200	6.98 ± 2.27 **	67.62
Omeprazol	10	5.35 ± 1.75 ***	75.18

I (%): Inhibition percentage, UI: Ulcer Index, * $p < 0,05$; ** $p < 0,01$; *** $p < 0, 0001$ Comparison vs Control (Mann Whitney U test)

DISCUSSION

The present study demonstrated that the oral acute administration of D-002 (5, 25, 100 and 200 mg/kg) significantly, markedly and dose dependently prevented against gastric ulceration induced with ibuprofen and naproxen, respectively, in rats. The oral administration with omeprazole (10 mg/kg), reference substance, inhibited marked and significantly the gastric ulcer induced by both NSAIDs, which supports the validity of these models in our conditions and then the results here described.

In both experimental models the anti-ulcer efficacy of D-002 was observed from the lowest dose assayed of 5 mg/kg. Then, we can't define the minimal effective dose because of lower doses were not evaluated. Similarly, the

mayor efficacy of D-002 on the two models was achieved with the highest dose evaluated of 200 mg/kg without that the maximal effective dose could be defined due to that superior doses were not investigated. For this reason, ulterior studies must enlarge the dose range for this evaluation in order to characterize the dose-effect relation in these two experimental models of gastric ulcer induction by NSAIDs which will permit define the minimal and maximal effective dose, respectively, of D-002.

The fact that D-002 was effective against gastric ulcer induced by the two NSAIDs, ibuprofen and naproxen, is agree with their efficacy for inhibiting the gastric ulcer induced by other agents appertaining to this therapeutic class as aspirin^{24,25} and indomethacin.^{26, 27} As can be observed the efficacy of D-002 for inhibiting the gastric ulcer by ibuprofen, aspirin and indomethacin have been very similar ($\cong 75\%$ of inhibition) while that their efficacy against the naproxen-induced ulcer was lightly inferior in a 67.6 % of inhibition.

Here, omeprazol (the reference substance) also resulted more effective against ibuprofen (96.7% of inhibition) than to prevent the gastric ulcer induced by naproxen (75.18% of inhibition). The fact that the omeprazole was effective on both models is according with the reported by other authors.^{19, 20}

The pathogenesis of NSAIDs-induced gastric ulceration mainly involves the non selective inhibition on the two isoformes of cyclooxygenase (COX-1 and COX-2) activities.^{34, 35} COX-1 is the constitutive isoforme that is present in gastric mucosa, responsible of the cytoprotective PG production, while COX-2 is the inducible isoforme that is present in the inflammatory cells.^{36, 37}

Some authors have reported the pivotal role of COX-1 inhibition in the ulcerogenic activity of NSAIDs.³⁸ However; more recent studies have evidenced the crucial importance of the inhibition on both isoformes since the COX-1 inhibition regulates the expression of the COX-2 activity for producing cytoprotective PG, as a compensatory response of the organism to such inhibition. Likewise, this process has been related with the gastric hipermotility, neutrophil infiltration and free radicals generation as additional pathogenic factors.⁵

On the other hand, the COX inhibition by NSAID conduces in parallel to triggers the metabolism of arachidonic acid towards overproduction of LTs, potent quimiotactic agents for neutrophils and source of free radicals generation, for what these metabolites are widely implicated in the NSAIDs pathogenesis.³⁹

Recent studies have documented that D-002 is a dual inhibitor of the enzymes 5-LOX and COX-2,⁴⁰ which support its anti-inflammatory effects previously reported and its relation with the reduction of LTB4 generation.²² Then, the 5-LOX inhibition by D-002 could be implicated in its gastroprotective action against both ibuprofen and naproxen anti-inflammatory agents.



Moreover, the ability of D-002 for inhibiting the neutrophils infiltration in gastric mucosa of rats with aspirin induced- ulcer^{24, 25} as well as for inhibiting the lipid peroxidation and protein oxidation in gastric mucosa of rats with indomethacin induced gastric ulcer^{26, 27} could be contributing to the gastroprotective mechanism of D-002 for counteracting the gastric ulcer induced by ibuprofen and naproxen observed in this study.

Thus, taking into account that the pathogenesis of the gastric ulcer induced by NSAIDs involve basically a common mechanism and that in a general way D-002 presents a gastroprotective efficacy very similar against the lesions induced by different NSAIDs, the gastroprotective mechanism of D-002 against NSAIDs could be supported by its ability for inhibiting 5-LOX and its antioxidant effects, but up to now if D-002 affects gastric motility induced by NSAIDs is unknown and ulterior studies must investigate it.

On the other hand, taking into account that the experimental toxicological studies have demonstrated absence of toxicity of D-002 associated to the treatment⁴¹⁻⁴⁵ and that the clinical studies corroborated a good security and tolerability profile,⁴⁶⁻⁴⁹ its concomitant use with the NSAIDs, classically utilized in the clinical practice, could represent an advantage in the prevention of gastrointestinal complications of this therapeutic class in relation to PBI, H2RA and the muco-protective agents due to the AE that they presents.¹³⁻¹⁶

CONCLUSION

Single oral doses of D-002 (5, 25, 100 and 200 mg/kg) significantly, markedly and dose-dependently reduced the gastric ulcer index induced by ibuprofen and naproxen, respectively, in rats.

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