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Anti-ulcer Activity of Higher Primary Alcohols of Beeswax

DAISY CARBAJAL, VIVIAN MOLINA, SURIA VALDÉS, LOURDES ARRUZAZABALA AND ROSA MÁS

National Center for Scientific Research, Havana, Cuba

Abstract

The anti-ulcer effects of a natural mixture of higher aliphatic primary alcohols, designated D-002, isolated from beeswax, were compared with those of cimetidine on indomethacin-, ethanol-, water-immersion-induced ulcers and on gastric secretion in rats.

D-002 (25–50 mg kg^{-1} p.o.) was similar to cimetidine in dose-dependently reducing the duration of indomethacin-induced ulcers while also being effective in preventing ethanol-induced ulcers, which are not affected by cimetidine. On the other hand, D-002 (100 mg kg^{-1}) moderately decreased the volume of gastric basal secretion in pylorus-ligated rats, but not the acidity. Nevertheless, it inhibited gastric ulcer induced by pylorus-ligation at doses (50 mg kg^{-1}) that were ineffective in decreasing the volume. In addition, 100 mg kg^{-1} of D-002 prevented the formation of acute gastric ulcers induced in rats by water-immersion stress.

The results demonstrate the anti-ulcer activity of the preparation in different experimental models suggesting its potential value for ulcer therapy.

A natural mixture of higher aliphatic primary alcohols (D-002) isolated and purified from beeswax, contains triacontanol, octacosanol, dotriacontanol, hexacosanol and tetracosanol with tetratriacontanol as a minor component. When administered orally D-002 induces a mild anti-inflammatory effect. It reduces the weight of the cotton-pellet granuloma and reduces the levels of leukotriene B_4 (LTB_4) in the exudate of carrageenan-induced pleurisy (Carbajal et al 1995).

Nevertheless, no gastric mucosal damage was induced by its oral administration up to doses of 1000 mg kg^{-1}, and its action is considered to differ from that of non-steroidal anti-inflammatory drugs, the basis of its anti-inflammatory activity being related to the reduction of LTB_4 levels; leukotrienes play a role in the etiology of gastric mucosal damage (Guslandi 1987).

This study was designed to investigate the effect of D-002 on experimental models used for the screening of anti-ulcer agents such as indomethacin- and ethanol-induced gastric ulcers, on gastric secretion and ulcers in pylorus-ligated rats and on ulcers induced by restraint water-immersion stress.

Materials and Methods

Animals

Sprague Dawley rats, 200–220 g, obtained from the Centro Nacional para la Producción de Animales de Laboratorio (Cenpalab, Cuba) were housed in environmentally controlled rooms (25 ± 2°C, 12 h light, 12 h dark cycle) with free access to standard chow produced by Cenpalab and tap water. The animals were randomly allocated to different experimental groups.

Correspondence: D. Carbajal, Center of Natural Products, National Center for Scientific Research, Post Box 6990, La Habana, Cuba.

Administration and dosage

D-002 was supplied by Laboratorios Dalmer (Havana City, Cuba). The batch used had a 92% purity according to the quality criteria specifications checked by gas chromatography. The batch composition was as follows: triacontanol 26·63%, octacosanol 17·49%, dotriacontanol 16·95%, hexacosanol 15·34%, tetracosanol 13·24% and tetratriacontanol 2·23%. The material was suspended in a 2% Tween 20/water vehicle. Indomethacin (Sigma, USA) was dissolved in 5% sodium bicarbonate and cimetidine (Sigma, USA) was dissolved in distilled water. D-002, indomethacin and cimetidine were orally administered by gastric gavage (5 mL kg^{-1}). A control group received only the same volume of Tween 20/water vehicle.

Indomethacin-induced gastric ulcer

Rats were allocated to five experimental groups (10 animals per group), comprising a control group (vehicle) and groups treated with 5, 25, 50 mg kg^{-1} D-002, or 25 mg kg^{-1} cimetidine.

One hour after D-002 and cimetidine treatment, indomethacin was administered in a single oral dose at 30 mg kg^{-1}. The rats were killed 4 h after indomethacin dosing (Yamasaki et al 1989).

Ethanol-induced gastric ulcer

The rats were allocated to five experimental groups as above. One hour after treatment, animals received 60% ethanol (5 mL kg^{-1}), intragastrically. The animals were killed 1 h later.

Gastric secretion and ulcer quantification in pylorus ligated rats

Rats were allocated to five experimental groups (13 animals per group), comprising a group control (vehicle), and groups teated with 25, 50, or 100 mg kg^{-1} D-002 or 25 mg kg^{-1} cimetidine.

Laparotomy was followed by ligation of the pylorus under ether anaesthesia by the method of Shay et al (1945), and the abdomen was then sutured. The gastric content was collected 4 h after pylorus ligation; the samples were centrifuged at 3000 rev min^{-1} for 10 min and the volume of the supernatant was measured. The acidity was estimated by titration with 0·1 M NaOH to pH 7·0. The stomach was opened and ulcers quantified.

Induction of water immersion restraint stress-induced ulcer

Rats were allocated to five experimental groups (13 animals per group), comprising a control group (vehicle) and groups treated with 25, 50, or 100 mg kg^{-1} D-002 or 100 mg kg^{-1} cimetidine.

Water immersion restraint-stress ulcer was induced according to the method described previously by Takagi & Okabe (1968). Rats were placed individually in compartments of a metal stress-cage and immersed up to the level of the xiphoid process in a water-bath thermoregulated at 24 ± 1°C and the animals were killed 7 h later.

Evalation of gastric mucosal damage

The stomachs of animals were removed and cut along the greatest curvature. Lesions were assessed by the total sum of the lengths (mm) of the gastric lesions. Observation and measurement of the lengths of lesions were performed by two independent blinded observers (Ohara et al 1992).

Statistical analysis

Statistical comparisons between groups were performed using the Mann-Whitney U-test.

Results and Discussion

Oral pretreatment with D-002 in ulcers experimentally induced by indomethacin, a nonsteroidal anti-inflammatory drug, or ethanol, a necrotizing agent showed interesting results; rather than reinforcement of ulcerogenic activity, as usually occurs with other anti-inflammatory compounds, an antiulcerogenic activity of D-002 was observed in both models.

D-002 orally administered significantly inhibited indomethacin-induced ulcers. Effective doses were from 25 to 50 mg kg^{-1}, reaching a maximal inhibition of 50%. In this respect, D-002 showed similar efficacy to cimetidine (Table 1).

Table 1. Effect of D-002 on indomethacin-induced gastric ulcer in rats.

Treatment	Dose (mg kg^{-1})	Ulcer length (mm)	Inhibition (% control)
Control	–	23·7 ± 1·83	–
D-002	5	20·6 ± 3·98	13
	25	17·7 ± 4·30*	25·3
	50	11·8 ± 3·18**	50·2
Cimetidine	25	18·8 ± 0·65***	20·6

Each dose of D-002 and cimetidine was administered orally 30 min before indomethacin (30 mg kg^{-1}) administration. Rats were killed 4 h later. Values represent the mean ± s.e. (n = 10).
*$P < 0·05$, **$P < 0·01$, ***$P < 0·001$ (Mann-Whitney U-test).

Table 2. Effect of D-002 on ethanol-induced gastric ulceration in rats.

Treatment	Dose (mg kg^{-1})	Ulcer length (mm)	Inhibition (% control)
Control	–	61·5 ± 16·46	–
D-002	5	39·2 ± 7·99	36·2
	25	27·2 ± 8·69*	55·7
	50	20·3 ± 7·70**	66·9
Cimetidine	25	37·4 ± 8·32	39·1

Each dose of D-002 and cimetidine was administered orally 30 min before ethanol. Rats were killed 1 h after indomethacin treatment. Values represent the mean ± s.e. (n = 10).
*$P < 0·05$, **$P < 0·01$ (Mann-Whitney U-test).

Oral administration of D-002 was also effective in preventing ethanol-induced ulcer (Table 2). In this case, a significant and gentle dose-effect relationship was noted, achieving maximal inhibition at approximately 66·9%. In this model cimetidine administered at 25 mg kg^{-1} was ineffective.

The presence of gastric acid, the inhibition of prostaglandin (PG) synthesis and the consequent disruption of the gastric mucosa barrier are the main factors involved in the pathogenesis of indomethacin-induced ulcer (Miller 1983; Konturek et al 1981; Asano et al 1990). Likewise, for ethanol-induced ulcer, the necrotizing mechanism is acid-independent (Asano et al 1990).

On the other hand, in pylorus-ligated rats, oral administration of 100 mg kg^{-1} D-002 significantly decreased the gastric volume, while ulcer induction was significantly inhibited at 50 and 100 mg kg^{-1}, reaching maximal inhibition by approximately 70%. Nevertheless, acidity was unchanged by D-002. Otherwise, cimetidine decreased gastric volume and acidity more effectively than D-002, but it was less effective in inhibiting ulcers induced in this model (Tables 3 and 4).

In restraint water-immersion stress, D-002 significantly reduced ulcer formation at doses of 100 mg kg^{-1} (Table 5).

The gastroprotective activity of D-002 seems to be independent of the acidity, since protection of the mucosa was observed at a dose of 50 mg kg^{-1}, which was ineffective in reducing the volume of gastric juice. These facts suggest that the mechanism of antiulcerogenic action of D-002 is different from that of cimetidine, a classical antagonist of the histamine H_2 receptors (Okabe et al 1977).

Table 3. Effect of D-002 on gastric volume and acidity in pylorus-ligated rats.

Treatment	Dose (mg kg^{-1})	Volume (mL)	Acidity (mEq H^+ mL^{-1})
Control	–	8·06 ± 0·42	0·11 ± 0·004
D-002	25	6·28 ± 0·81	0·09 ± 0·010
	50	9·07 ± 0·62	0·11 ± 0·005
	100	5·34 ± 0·84*	0·09 ± 0·007
Cimetidine	25	5·20 ± 0·67**	0·08 ± 0·006**

D-002 was administered orally 1 h before pylorus ligation. Four hours later, the animals were killed and gastric juice was collected. Values represent the mean ± s.e. (n = 10).
*$P < 0·05$, **$P < 0·01$ (Mann-Whitney U-test).

Table 4. Effect of D-002 on gastric ulcers induced by pylorus ligation in rats.

Treatment	Dose (mg kg^{-1})	Ulcer length (mm)	Inhibition (% control)
Control	–	19·9 ± 4·77	–
D-002	25	9·74 ± 3·03	51·2
	50	7·01 ± 2·77*	64·9
	100	5·13 ± 1·55**	74·3
Cimetidine	25	9·52 ± 2·86*	52·3

Each dose of D-002 or cimetidine was administered orally 1 h before pylorus ligation. Rats were killed 4 h later. Values represent the mean ± s.e (n = 13). *$P < 0.05$, **$P < 0.01$ (Mann-Whitney U-test).

Table 5. Effect of D-002 on gastric ulcers induced by restraint water-immersion stress in rats.

Treatment	Dose (mg kg^{-1})	n	Ulcer length (mm)	Inhibition (% control)
Control	–	26	23·91 ± 2·53	–
D-002	25	15	23·38 ± 3·62	2·2
	50	15	24·40 ± 3·86	0
	100	15	12·73 ± 2·72**	47·7
Cimetidine	100	13	4·73 ± 2·65**	80·2

Each dose of D-002 or cimetidine was administered orally immediately before water immersion restraint-stress in rats. Rats were killed 7 h later. Values represent the mean ± s.e. **$P < 0.01$ (Mann-Whitney U-test).

Increase of gastric acid secretion, reduction of gastric mucus and alteration in the microcirculation of the gastric mucosa plays a role in the pathogenesis of stress-induced ulcers (Guth & Hall 1966; Hakkinen et al 1966; Kitagawa et al 1979). The anti-ulcer activity of D-002 in this model appeared only at higher doses (100 mg kg^{-1}), similar to that which diminished gastric volume in pylorus-ligated rats.

Peptic ulceration is caused by a failure of the balance between gastric aggressive factors and gastric defensive factors (Shay & Sun 1964). Prostaglandins have been reported to prevent the formation of gastric mucosal lesions induced by various necrotizing agents (Robert et al 1979). This protective effect is unrelated to their anti-secretory properties and has been termed as cytoprotection (Robert 1979).

D-002 showed a moderate inhibitory effect in reducing gastric acid secretion in pylorus-ligated rats and on the stress observed at relatively high doses (100 mg kg^{-1}), being highly effective against ethanol- and indomethacin-induced ulcers. Therefore, the antiulcer activity of D-002 could be related to the stimulation of defensive mechanisms rather than to the suppression of aggressive mechanisms and the inhibition of LTB_4 levels induced by D-002. Nevertheless, a dual mechanism at different dose ranges cannot be ruled out.

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