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Effects of D-002 (Beeswax Alcohols) on Concentrations of Prostaglandin E2 in Rat Gastric Mucosa

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Abstract

Background: Peptic ulcer is very common diseases in the adult population affecting the patient's life quality. Prostaglandin E2 (PGE2) play an important role in the gastric cytoprotection and the gastroprotective effects of some substances that increase the gastric mucus secretion is associated with increased concentrations of PGE2 in gastric mucosa. D-002 is a gastroprotective substance, but its effects on gastric mucosa PGE2 concentrations remained unexplored. This study investigated the effects of D-002 on PGE2 concentrations in gastric mucosa with ethanol induced gastric ulcer in rats.

Methods and Findings: Rats were randomized into six groups: a negative control that only received the vehicle and five groups with ethanol induced gastric ulcer: a positive control (vehicle-treated), three treated with D-002 (25, 100 and 200 mg/kg) and one with omeprazole (20 mg/kg) as a reference substance. Gastric ulcer index, gastric mucus amount and concentrations of PGE2 in gastric mucosa were quantified. D-002 (25, 100 and 200 mg/kg) significantly and markedly inhibited ulcer index (44.4; 47.8 and 75.2%, respectively), significantly prevented the reduction of gastric mucus content (89.8, 100 and 100%, respectively), and completely restored the concentrations of PGE2 depleted in gastric mucosa compared to the positive control group and significantly increased their levels compared to the negative control (60.3; 70.5 and 136.1%) group. No dose/effect relationship was found on any of these variables. Omeprazole was effective on all variables studied.

Conclusions: D-002 (25, 100 and 200 mg/kg) significantly and markedly increased PGE2 concentrations in gastric mucosa of rats with ethanol induced gastric ulcer, which supports at least in part its gastroprotector multifactorial mechanism.

Keywords: D-002; Ethanol induced-gastric ulcer; Prostaglandin E2

Introduction

Peptic ulcer is a chronic relapsing disease of the gastrointestinal tract which can be gastric or duodenal [1]. It is very common in the adult population and its major complications (bleeding, perforation, penetration and obstruction) lead to a significant impact on quality of life of sufferers [2]. The ulcer is defined as an interruption of the extended mucosa through the muscularis mucosae until inside the submucosa [3] and it occurs due to imbalance between aggressive (nonsteroidal acid secretion, pepsin, H. pylori, anti-inflammatory -AINEs-) and defensive factors (mucus secretion and bicarbonate, microcirculation, -PG- prostaglandins, growth factors) acting on the mucosa gástrica [4-7]. Thus, when a weakening of defense factors or the action of aggressive factors exceeds the defensive capacity of the gastric mucosa peptic ulcer occurs [8]. In this context, it has been documented the important role of prostaglandin E2 (PGE2), the most abundant PG of the gastrointestinal tract, in gastric cytoprotection through a multifactorial mechanism that involves regulation of the secretion of gastric mucus, acid secretion and gastric motility [9-12], and inhibition of release of free radicals from the neutrophils [13]. Another mechanism involved in gastric cytoprotection exerted by PGE2 is the inhibition of apoptosis induced by irritants agents in gastric mucosal cells, through activation of the enzyme protein kinase A and the subsequent increase in cAMP levels [14].

Therefore, the removal of PGE2 in the gastric mucosa represents an important etiological factor in the development and progression of peptic ulcer. This effect is produced among other causes by eating anti-inflammatory drugs (NSAIDs) widely used to treat acute and chronic inflammatory disorders [15], as their mode of action is based on inhibition of the activity of the enzyme cyclooxygenase (COX) which leads to the inhibition of PGs synthesis [16]. Consequently, the high prescription of NSAIDs for managing the osteoarthritis, one of the most common chronic diseases of the elderly [17], in the prevention of cardiovascular and cerebrovascular diseases [18] and in the chemoprevention of certain cancers [19] it has become in the

most common etiological factor for the development of peptic ulcer [15].

On the other hand, it has been described that the gastroprotective effects of some substances that increase the gastric mucus secretion is associated with increased concentrations of PGE2 in gastric mucosa [20,21].

Therefore, the search for new substances with potential stimulatory effect of PGE2 formation to strengthen the defense of the gastric mucosa against the action of NSAIDs is a topic of interest.

D-002 is a mixture of six aliphatic primary alcohols of higher molecular weight, isolated and purified from beeswax (Apis mellifera), whose principal component is triacontanol followed by hexacosanol, octacosanol, tetracosanol, dotraicontanol and tetratriacontanol [22].

D-002 produces anti-inflammatory, antioxidant and gastroprotective effects, demonstrated in experimental and clinical studies [23-35]. The gastroprotective effects of D-002 have been evaluated in models of gastric ulcers induced in rats by ethanol [24,25], stress[24], aspirin [26,27], indomethacin [28,29], ibuprofen, naproxen [30], pylorus ligation [31], acetic acid [32], and ischemia reperfusión [28] and have been associated with a cytoprotective multifactorial mechanism that involves improving the quality and increasing of the gastric mucus secretion, its antioxidant action on gastric mucosa and dependent effects of prostaglandins (PG) [28,29,31,33,34].

The antioxidant action of D-002 on gastric mucosa is supported by a mechanism that involves sequestering hydroxyl radical and stimulating enzymes activities of endogenous antioxidant system in gastric mucosa (catalase, superoxide dismutase and glutathione peroxidase) [29,34].

Moreover, although it is known that the gastroprotective action of D-002 is dependent on PGs [31] still is unknown whether D-002 modifies the values of PGE2 in gastric mucosa. Therefore, this study was undertaken for investigating the effects of D-002 on PGE2 concentrations in gastric mucosa with ethanol induced ulcer in rats.

Materials and Methods

Animals

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

The experiments were performed in accordance with the care and use of experimental animals prescribed by the Cuban Guidelines for the care of laboratory animals and the Cuban Code of Good Laboratory Practice (GLP), after obtaining the approval of the Institutional Board for animal use.

Administration and dosage

The batch of D-002 (030151211), supplied by the Plants of Natural Products (National Center for Scientific Research, Havana City, Cuba) was used after corroborate its quality criteria. Batch composition, assessed with a validated gas chromatographic method [35], was as follows: 1-tetracosanol (5%), 1-hexacosanol (10.2%), 1-octacosanol (14%), 1-triacontanol (34.21%), 1-dotriacontanol (24.24%) and 1-tetratriacontanol (3.03%). Purity (total content of these alcohols) was 90.7%.

D-002 was prepared as a suspension in Tween 20/H2O vehicle (2%) and omeprazole (batch: 0912027, Pharmaceutical Cuban, Havana, Cuba) in a suspension in an acacia/water vehicle (1%).

Rats were randomized into six groups (10 rats/group): a negative control that only received the vehicle and five groups with ethanol induced gastric ulcer: a positive control (vehicle-treated), three treated with D-002 (25, 100 and 200 mg/kg) and one with omeprazole (20 mg/kg) as a reference substance.

All treatments (vehicle, D-002 and Omeprazol) were administered orally by gastric gavage (5 ml/kg) as single doses one hour before gastric ulcer induction.

Ethanol induced gastric ulcer induction in rats

The rats were fasted 24 hours before the experiment with free access to water. We proceeded according to Zengil et al. [36]. One hour after administration of single dose of the vehicle, D-002 (25, 100 and 200 mg/kg) and omeprazole (20 mg/kg), each rat received 60% ethanol (1 mL/200 g) by intubation gastric. One hour later the rats were sacrificed under an overdose of thiopental anesthesia and the stomachs were extracted, weighed in rapid Mettler Toledo balance, opened by greater curvature and the mucus was obtained by careful scraping with a scalpel. Subsequently the stomachs were washed in saline and ulcer index was measured.

Determination of the gastric mucus amount

Gastric mucus was collected, weighed and the amount of mucus was expressed as the ratio of the weight of mucus (mg)/ stomach weight (g).

Quantitation of concentrations of PGE2 in gastric mucosa

For quantification of PGE2 concentrations gastric mucus was homogenized in saline phosphate buffer (PBS) at 4oC (125 mg tissue/ml PBS). The homogenate was centrifuged at 4500 rpm for 15 minutes at 4oC and the supernatant was used for determination of PGE2 levels by ELISA (R&D Systems) commercial kit and total protein concentrations by the method of Lowry modified [37]. PGE2 values were expressed as PGE2 ng/mg protein.

Evaluation of gastric ulceration

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 [38].

Statistical Analyses

Comparisons among groups were done with the Kruskal Wallis test; paired comparisons between each treated and control groups with the Mann-Whitney U test. Statistical significance was chosen for α=0.05. Data were processed with the Statistics Software for Windows (Release 6.1 Stat Soft Inc, Tulsa OK, USA). The study of dose-effect relationship was performed by the method of linear regression and correlation using the Primer of Biostatistics (Stanton A Glantz, version 3.01) program.

Results

Table 1 followed by the **Figure 1** show the results of the effects of single oral doses of D-002 on the gastric ulcers index induced by ethanol in rats. Oral administration of ethanol produced a significant gastric ulceration in animals of the positive control group compared with the negative control group, which did not present any ulceration. Oral administration with D-002 (25, 100 and 200 mg/kg) markedly, significantly and non-dose dependently prevented the ulcer index formation (44.4; 47.8 and 75.2% of inhibition, respectively) compared with the positive control group. The omeprazole reference drug (20 mg/kg), significantly and markedly inhibited the gastric ulcer index (95.8%) respect to the positive control group, corroborating the validity of these results in our experimental conditions.

Figures 2 and **3** shows the results of the effects of D-002 on the amount of gastric mucus and PGE2 concentrations in gastric mucosa, respectively **(Table 2).** Oral administration of ethanol significantly reduced the gastric mucus content (expressed as mucus weight/stomach weight) and concentrations of PGE2 in gastric mucosa (expressed as ng of PgE2/mg of protein) in animals of the positive control group compared with the negative control group.

Table 1 Effects of D-002 on ethanol induced ulcer in rats.

Groups	Doses (mg/kg)	Ulcer Index (mm)	I (%)
Negative control	__	0 ± 0 ****	__
Positive Control	__	65.7 ± 9.09	__
D-002	25	36.5 ± 2.67 *	44.4
D-002	100	34.3 ± 3.03 *	47.8
D-002	200	16.26 ± 4.48 ***	75.2
Omeprazole	20	2.77 ± 1.15 ***	95.8

I (%): Percent Inhibition, *p<0,05; ***p<0,001;****p<0,001 Comparison with positive control, (Mann Whitney U test)

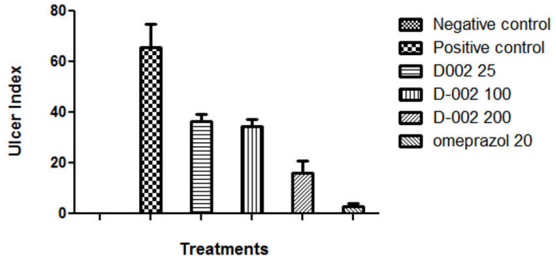

Figure 1 Effects of D-002 on ethanol induced ulcer in rats.

Table 2 Effects of D-002 on the amount of gastric mucus and concentrations of PGE2 in gastric mucosa.

Groups	Doses (mg/kg)	MW/SW (mg/g)	I (%)	PGE2 (ng/mg de Pt)	I (%)
Negative control	__	85.95 ± 4.45 **	__	0.647 ± 0.07 *	__
Positive control	__	64.36 ± 3.89	__	0.491 ± 0.02	__
D-002	25	83.75 ± 3.28 **	89.8	1.037 ± 0.16 **+	100
D-002	100	86.68 ± 4.34 **	100	1.103 ± 0.20 **+	100
D-002	200	101.74 ± 6.71***	100	1.528 ± 0.46***++	100
Omeprazole	20	74.87 ± 3.31*	48.7	0.96 ± 0.08 ***+	100

I (%): Percent Inhibition, MW/SW (mg/g): mucus weight (mg)/stomach weight (g).
*p<0,05; **p<0,01; ***p<0,001 Comparison with positive control
+ p<0,05; ++ p<0,01; Comparison with negative control
(Mann Whitney U test)

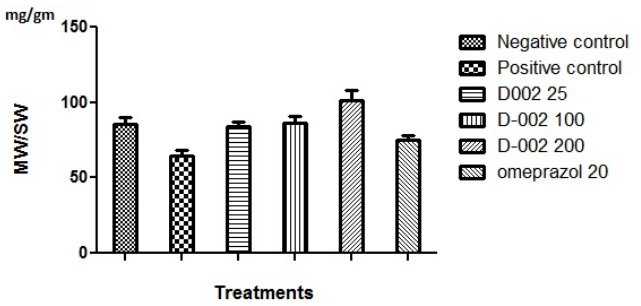

Figure 2 Effects of D-002 on the gastric mucus amount in gastric mucosa with ethanol induced ulcer in rats.

Oral treatment with D-002 (25, 100 and 200 mg/kg) significantly and markedly prevented the reduction of gastric mucus content (89.8, 100 and 100% inhibition, respectively) compared to the positive control group. All doses of D-002 caused a 100% restoration of concentrations of PGE2 in gastric mucosa compared to the positive control group and significantly increased their levels compared to the negative control group (60.3; 70.5 and 136.1%). The analysis of the dose/effect relationship showed no dependence with dose on any of these variables.

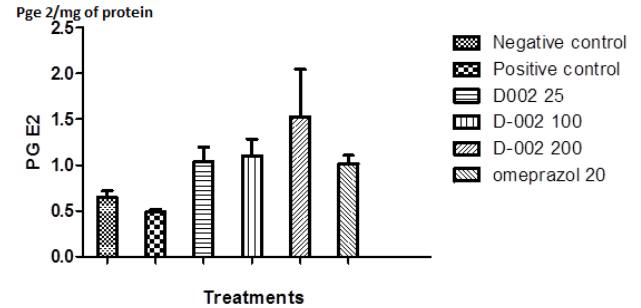

Figure 3 Effects of D-002 on concentrations of PGE2 in gastric mucosa.

Oral treatment with omeprazole (20 mg/kg) restored depletion mucus and gastric content concentrations PGE2 in 48.7 and 100%, respectively, compared with the positive control group, while it increased levels of the latter over the negative control in 48.4%.

Discussion

The present study demonstrated that increased gastric mucus secretion, as an important gastroprotective mechanism of D-002, is supported by a stimulating effect on the generation of PGE2 in gastric mucosa.

Ethanol gastric ulcer induction in rats significantly reduced gastric mucus content and PGE2 concentrations in gastric mucosa of the positive control group animals compared to the negative control group.

Omeprazole treatment (20 mg/kg), reference substance, prevented the formation of gastric ulcers associated with a restoration of the content of mucus and of PGE2 levels in gastric mucosa, which demonstrates the validity of the model in our experimental conditions.

Treatment with single oral doses of D-002 (25, 100 and 200 mg/kg) produced a significant and marked inhibition of gastric ulcer index induced by ethanol in rats, and completely restored the gastric mucus content, while it stimulated PGE2 generation in gastric mucosa, reaching levels that not only allowed to restore their depletion, but exceeded the baseline values corresponding to the negative control.

In addition, the results of this study are consistent with those obtained by other authors that have shown that the efficacy of some gastroprotective substances involves stimulation in gastric PGE2 levels corroborating the efficacy of omeprazole as reference substance [21].

Given that PGE2 is a cytoprotective prostaglandin of gastric mucosa whose depletion is associated with the formation of gastric ulcers [39] the fact that the D-002 stimulates its formation is a promising result not only for treatment but for prevention of peptic ulcerative disease of the gastrointestinal tract in terms of improving gastric health.

Previous studies have shown that the gastroprotective effect of D-002 was prostaglandins-dependent [29], so the present study confirms this hypothesis and evidence for the first time that its mechanism involves increased stimulation of gastric concentrations of PGE2 as important defense factor of the gastric mucosa.

On the other hand, it has been reported that D-002, additionally to its gastroprotective effects, presents an anti-inflammatory profile [40-44] associated to dual inhibition on ciclooxigenase (COX) and 5-lipooxigenase (5-LOX) activities demonstrated *in vitro*, with greater affinity for 5-LOX, acting as a dual COX/5-LOX inhibitor [45].

Then, the stimulation on PGE2 formation in gastric mucosa exerted by D-002, demonstrated in the present study, may seem contradictory respect to the fact that D-002 inhibits COX activity. However, if we consider that the COX enzyme involves two main isoforms: COX-1 and COX-2, of which constitutive COX-1 contributes more than inducible COX-2 to gastric PGE2 production mediating the prostaglandin dependent gastric protection [46], it is logical to assume that inhibition of D-002 on COX can be specified on COX-2, unmodified COX-1. Therefore, further studies should elucidate it.

Taking into account that the gastroprotective effect of D-002 is associated with a stimulation of defensive factors of gastric mucosa including the increased secretion of gastric mucus, improvement of the quality of the gastric mucosa and antioxidant effects [28,29,31,33,34], the efficacy of D-002 for stimulating the PGE2 formation in gastric mucosa here found constitutes another gastric mucosa defensive factor involved in this gastroprotective multifactorial mechanism of D-002.

Conclusions

Oral treatment with single oral doses of D-002 (25, 100 and 200 mg/kg) significantly and markedly increased PGE2 concentrations in gastric mucosa of rats with ethanol induced gastric ulcer, which supports at least in part its gastroprotector multifactorial mechanism.

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