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Gastroprotective activity of a new semi-synthetic solidagenone derivative in mice

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Abstract

The gastroprotective activity of the new semi-synthetic solidagenone derivative 15,16-epoxy-8(9),13(16),14-labdatrien-7β-methoxy-6β-ol (ELMO) has been assessed on the model of HCl/EtOH-induced gastric lesions in mice. Human gastric epithelial cells (AGS) and fibroblasts (MRC-5) were used to determine its mode of action. The effect of ELMO on the prostaglandin E_2 content, cellular reduced glutathione (GSH) and protection against damage induced by sodium taurocholate was assessed against AGS cells. The effect of ELMO on the growth of AGS and fibroblast cultures was evaluated. The superoxide anion scavenging capacity of the compound was studied also. The cytotoxicity of ELMO, expressed as cell viability, was assessed using two independent endpoints: neutral red uptake (NRU) and the reduction of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) for MRC-5 fibroblasts and NRU for AGS cells. A single oral dose of ELMO (10 and 20 mg kg^{-1}) inhibited the appearance of gastric lesions in mice displaying similar values to lansoprazole at 20 mg kg^{-1}. At 40 μM ELMO increased the prostaglandin E_2 content but not GSH in AGS cells. The compound showed no effect on sodium taurocholate-induced damage and was devoid of superoxide anion scavenging activity. Concentrations of 0.5, 1, 2 and 4 μM stimulated fibroblast but not AGS cell proliferation. The compound showed weak cytotoxicity with values (IC50) of 411 (NRU) and 418 μM (MTT) for fibroblasts and 261 μM (NRU) for AGS cells. The results support further pharmacological study of this compound as a potential new anti-ulcerogenic drug.

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Introduction

A number of naturally occurring terpenes isolated from higher plants and several of their derivatives show preventive activity against the gastric lesions induced by different ulcerogenic procedures in animals (Lewis & Hanson 1991).

A powerful anti-ulcerogenic effect has been demonstrated for some clerodane diterpenes such as *trans*-dehydrocrotonin (Souza-Brito et al 1998) and more recently for crotonin (Albino de Almeida et al 2003). Semi-synthetic dehydrocrotonin derivatives have been shown to be more potent as gastroprotectives than the parent compound (Melo et al 2003).

Solidago chilensis Meyen (Asteraceae) is a medicinal plant used in South America to treat symptoms associated with inflammatory processes (Razmilic & Schmeda-Hirschmann 2000). We have reported the gastroprotective activity of solidagenone, a labdane diterpene occurring in the rhizomes of *S. chilensis*, and of its semi-synthetic and biotransformation derivatives in different experimentally-induced gastric lesions in animals (Rodríguez et al 2002; Schmeda-Hirschmann et al 2002). Although the gastroprotective effect of solidagenone and its derivatives has been determined, the mechanism involved in this protective activity remains poorly understood.

Following our studies on the anti-ulcerogenic effect of terpenes and in an attempt to elucidate the mechanism involved in the gastroprotection elicited by these compounds, we report the gastroprotective activity of the semi-synthetic solidagenone derivative 15,16-epoxy-8(9),13(16),14-labdatrien-7β-methoxy-6β-ol (ELMO), the assessment of its mode of action and the cytotoxicity towards selected cell line cultures.

Materials and Methods

Animals

Swiss albino mice (30 ± 3 g) were used. The animals were fasted for 24 h before the ulcerogenic assays because the reference compound (lansoprazole) and ELMO were administered orally. Animals were purchased from the animal house, Instituto de Salud Pública de Chile. Mice were fed on a certified Champion diet (23.4% protein, 12.3% amino acids, 4.5% fat, 270 ppm cholesterol, 5.8% crude fibre, 16% neutral detergent fibre, 8.2% acid detergent fibre, minerals and vitamins) with free access to water, under standard conditions: a 12-h dark–light period, 50 ± 10% relative humidity and 22 ± 1°C room temperature. The protocols were approved by the Universidad de Talca Institutional Animal Care and Use Committee that follows the recommendations of the Canadian Council on Animal Care (Olfert et al 1993).

Drugs

The following drugs were used: culture media, fetal bovine serum (FBS), antibiotics (Invitrogen Corp., USA). Lansoprazole, Tween 80, L-glutamine, *N*-acetyl-L-cysteine, neutral red, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), indometacin, xanthine oxidase, hypoxanthine, nitro blue tetrazolium, and 5,5-dithiobis-2-nitrobenzoic acid were from Sigma Chemical Co., USA.

Compounds

The melting point was determined on a Koffler hot stage apparatus (Electrothermal 9100) and was uncorrected. Optical rotation was obtained for a solution in $CHCl_3$ (concentration expressed in g/100 mL) on a Jasco DIP 370 polarimeter. The IR spectrum was recorded on a Bruker FT-IR instrument. The ^{1}H NMR spectrum was recorded at 400 MHz and ^{13}C NMR data were obtained at 100 MHz on a Bruker spectrometer (δ scale). The mass spectrum was measured using a Varian unit at 70 eV and was presented as m/z (% rel. int.). TLC spots were visualized by spraying the chromatograms with *p*-anisaldehyde-ethanol and heating at 110°C for 3 min. Column chromatography was performed over Merck Kieselgel 60, particle size 0.063–0.200 mm.

Solidagenone was isolated from the rhizomes of *Solidago chilensis* as reported by Schmeda-Hirschmann et al (2002). THF was distilled from sodium under a N_2 atmosphere. Treatment of solidagenone (2.04 g) with diisobutylaluminum hydride (DIBALH) in THF yielded, after column chromatography in silica gel, some 1.17 g unreacted solidagenone, 148 mg solidagen-6β-ol (17.1%) and a complex mixture (165 mg) which was further purified by preparative HPLC. The HPLC conditions were as follows. Column: LiChrosorb RP-18 (7 μm) pre-packed Hibar column RT 250-25; mobile phase: water:MeOH 25:75 to 100% MeOH in 40 min followed by 10 min 100% MeOH. Detection: UV, 225 nm. The following compounds were obtained: 15,16-epoxy-8(9),13(16),14-labdatrien-6β,7β-diol (8 mg, 0.9%, Rt 36.5 min) and 35.4 mg 15,16-epoxy-8(9),13(16),14-labdatrien-7β-methoxy-6β-ol (ELMO, 3.8%, Rt 40.38 min). The compounds obtained were identified by their spectroscopic data and physical constants. Signal assignation was performed using ^{1}H-^{13}C COSY data and comparison with similar compounds (Schmeda-Hirschmann et al 2002). The aim of this work was to prepare different solidagenone derivatives, looking for structure–activity relationships as gastroprotective compounds. Therefore, the obtention of the derivatives was more important than the reaction yields that could be optimized if a compound proved to be promising. The low yield in this reduction reaction was reported by Razmilic & Schmeda-Hirschmann (2000).

The ^{1}H NMR spectrum of ELMO (Table 1) presented the typical signals for the furan ring at δ 6.34, 7.28 and 7.39. Instead of the br d at δ 5.72 ($J = 1.5$ Hz) coupled with the methyl group at δ 2.02 of solidagenone, the compound presented two broad singlets at δ 4.41 and 3.26 and a methyl singlet at δ 3.52, indicating methoxylation of one of the hydroxy functions. This assumption was supported by the ^{13}C NMR spectrum which presented two doublets at δ 66.93 and 85.63, and the OMe group at δ 58.01. Heteronuclear multiple-bond correlation (HMBC) experiments indicated clear correlations of the C at δ 66.93 with the proton signals at δ 3.26 and 1.44, and between the C at δ 85.63 with the H signals at δ 4.41, OMe, 1.84 and 1.44, respectively. The stereochemistry at C-6 and C-7 followed from the small coupling constants J 5,6 and 6,7 (signals appear as br s), which indicated that the functions at C-6 and C-7 were in the β-configuration.

ELMO was isolated by preparative HPLC chromatography from a complex fraction obtained after reduction

Table 1 ^{1}H and ^{13}C NMR spectral data of 15,16-epoxy-8(9),13(16),14-labdatrien-7β-methoxy-6β-ol in $CDCl_3$, δ C in ppm

	H	C	HMBC
1	1.5–1.7 m	39.37 t	1.36, 1.44
2	1.5–1.7 m	19.09 t	1.4, 1.7
3	1.2–1.4 m	42.75 t	1.06, 1.27
4	–	33.68 s	4.41, 1.44
5	1.44 s	49.63 d	4.41, 3.26, 1.27, 1.06
6	4.41 s	66.93 d	3.26, 1.44
7	3.26 s	85.63 d	4.41, OMe, 1.84, 1.44
8	–	123.72 s	4.41, 3.26, 2.26, 2.36, 1.84
9	–	145.98 s	3.26, 2.54, 2.36, 2.26, 1.84, 1.36
10	–	39.15 s	4.41, 2.36, 2.26, 1.36
11	2.26 m, 2.36 m	28.95 t	2.26, 2.36
12	2.54 t (8.6, 8.3)	25.18 t	2.20–2.40
13	–	125.39 s	7.39, 7.28, 6.34, 2.54
14	6.34 tbr (1.3)	110.77 d	7.28, 2.54
15	7.39 dd (1.3, 1)	142.75 d	7.28, 6.34
16	7.28 s	138.46 d	7.39, 6.34, 2.54
17	1.84 s	17.90 q	3.26
18	1.27 s	24.20 q	1.06
19	1.06 s	33.29 q	1.27
20	1.36 s	21.66 q	1.84
OMe	3.52 s	58.01 q	3.26

of solidagenone with DIBALH in anhydrous THF. The low yield in the reaction suggested that ELMO was formed from solidagen 6β-ol during work-up. To confirm this assumption, solidagen 6β-ol (80 mg) was treated with methanol/H^+ at 40°C over 1 h followed by 23 h at room temperature. A complex mixture was obtained, affording 14 mg ELMO after column chromatography. This suggested that reduction of the α,β unsaturated ketone function of solidagenone to solidagen-6β-ol was followed by methanol addition and migration of the 7,8-double bond to 8,9. The product was also obtained by treating solidagen-6β-ol with diluted HCl/MeOH (Figure 1). The loss of the tertiary OH group in solidagenone to afford the 8,9-en derivative was reported by Anthonsen et al (1970). Those authors proposed a close related reaction intermediate derived from the protonated form of solidagenone via an allylic addition–elimination.

HMBC experiments confirmed the proposed structure, which was in agreement with the NMR data and the MS, indicating the molecular formula $C_{21}H_{32}O_3$. Thus, ELMO was 15,16-epoxy-8(9),13(16),14-labdatrien-7β-methoxy-6β-ol (Figure 2). Table 1 shows the ^{1}H and ^{13}C NMR spectral data of ELMO.

15,16-Epoxy-8(9),13(16),14-labdatrien-7β-methoxy-6β-ol: colorless crystals, mp 182°C. EIMS m/z (rel. int.%) 332.235 (calcd. for $C_{21}H_{32}O_3$: 332.235) (3), 316 (10), 300 (16), 219 (100), 205 (41), 149 (55), 135 (60), 121 (24), 109 (41). FT-IR (KBr, cm^{-1}): $[\alpha]^{20}$: 3.33 (c = 1.71, $CHCl_3$).

HCl/EtOH-induced lesions

The gastroprotective activity of ELMO was assessed in the HCl/EtOH-induced lesion model in mice (Yesilada et al 1997). Mice were randomly divided into groups of eight animals each and fasted for 24 h with free access to water

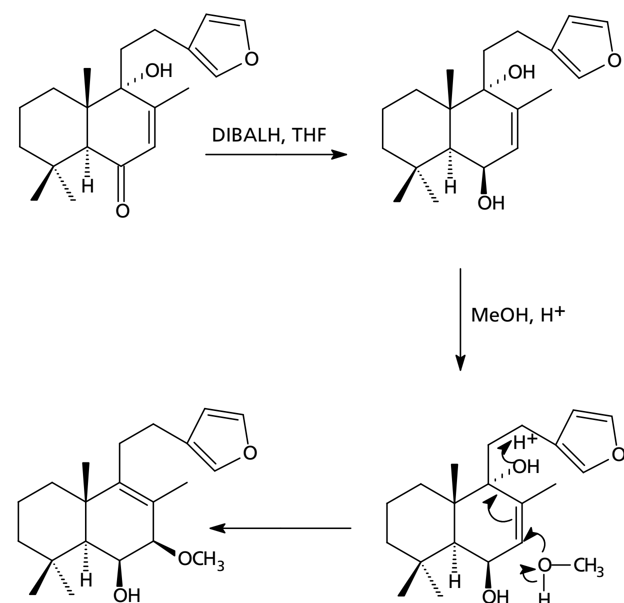

Figure 1 Scheme of the obtention of ELMO from solidagenone.

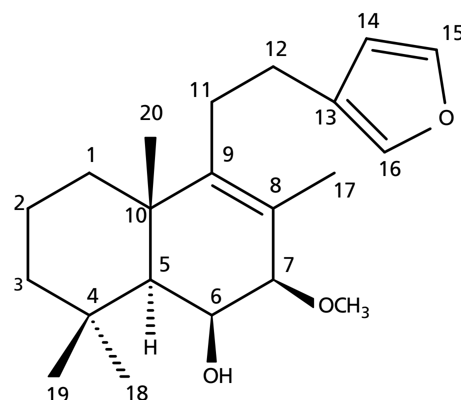

Figure 2 Chemical structure of 15,16-epoxy-8(9),13(16),14-labdatrien-7β-methoxy-6β-ol (ELMO).

before the experiment. Fifty minutes after oral administration of ELMO (10, 20 or 40 mg kg^{-1}), lansoprazole (20 mg kg^{-1}) or 12% Tween 80 (10 mL kg^{-1}), all groups were orally treated with 0.2 mL of a solution containing 0.3 M HCl/60% ethanol (HCl/EtOH) for gastric lesion induction. Animals were killed 1 h after the administration of HCl/EtOH, and the stomachs were excised and inflated by injection of saline (1 mL). The ulcerated stomachs were fixed in 5% formalin for 30 min and opened along the greater curvature. Gastric damage visible to the naked eye was observed in the gastric mucosa as elongated black-red lines, parallel to the long axis of the stomach similar to the HCl/EtOH-induced lesion in rats. The length (mm) of each lesion was measured, and the lesion index was expressed as the sum of the length of all lesions.

MRC-5 cell culture

Human lung fibroblasts MRC-5 (ATCC CCL-171) were grown as monolayers in minimum essential Eagle medium, with Earle's salts, 2 mM L-glutamine and 2.2 g L^{-1} sodium bicarbonate, supplemented with 10% heat-inactivated FBS, 100 IU mL^{-1} penicillin and 100 μg mL^{-1} streptomycin in a humidified incubator with 5% CO_2 in air at 37°C. Cell passage was maintained between 10–16, and medium was changed every two days.

AGS cell culture

Human epithelial gastric cell AGS (ATCC CRL-1739) were grown as monolayers in Ham F-12 medium containing 1 mM L-glutamine and 1.5 g L^{-1} sodium bicarbonate, supplemented with 10% heat-inactivated FBS, 100 IU mL^{-1} penicillin and 100 μg mL^{-1} streptomycin in a humidified incubator with 5% CO_2 in air at 37°C. Cell passage was maintained between 42–48, and medium was changed every two days.

Determination of prostaglandin (PGE_2)

One day after confluence AGS cells were treated for 1 h with the compound at 10, 20 or 40 μM. ELMO was

dissolved in dimethyl sulfoxide (DMSO; 1% final concentration) and volume completed with medium only. A control without compound was included. Indometacin (100 μM) was used as a standard inhibitor of prostaglandin synthesis. After incubation, prostaglandins were determined by means of a specific enzyme immunoassay kit (RPN 222, Amersham, UK). A calibration curve was performed using standards provided with the kit (2.5–320 pg/well).

Cellular glutathione content (GSH)

Reduced glutathione (GSH) levels in cells accounts for the majority of soluble reduced sulfhydryls in cells (Mutoh et al 1990). Soluble reduced sulfhydryl content was determined according to Romano et al (1990) with slight modifications. AGS cells were cultured in Petri dishes (3.5 cm diameter). One day after confluence, cells were incubated for 4 h with or without ELMO at 25 or 50 μM in separate experiments. Compound was dissolved first in DMSO (1% final concentration) and then in medium only. After the treatment, monolayers were washed three times with 1 mL phosphate-buffered saline (PBS) containing 0.02% EDTA. Subsequently, 1.4 mL 0.2% triton X-100 and 2.5% sulfosalicylic acid in PBS-EDTA buffer were added to the Petri dish. Cells were harvested with a cell scraper and sonicated for 1 min on ice. Cell suspensions were cleared by centrifugation. Supernatant (1 mL) was added to a test tube containing 2 mL 0.3 M Na_2HPO_4. Optical density was determined at 412 nm 0.5 min after the addition of 0.25 mL 5,5-dithiobis-2-nitrobenzoic acid (0.4 mg mL^{-1} in 1% sodium citrate). *N*-acetyl-L-cysteine (0.75 mM) was used as the positive control. A standard curve using known concentrations of GSH (1–50 nmol mL^{-1}) was carried out with each assay. Results were expressed as nmol soluble reduced sulfhydryls/10^6 cells.

Superoxide anion scavenging

The enzyme xanthine oxidase (XO) is able to generate superoxide anion by oxidation of reduced products from intracellular ATP metabolism. In this reaction, the XO oxidizes the substrate hypoxanthine generating superoxide anion which reduces the nitro blue tetrazolium dye (NBT), leading to a chromophore with absorption maxima at 560 nm. Superoxide anion scavengers reduce the speed of generation of the chromophore. The activity of the compound was measured spectrophotometrically and the percentage of superoxide anion scavenging was calculated as reported by Schmeda-Hirschmann et al (2003). ELMO was evaluated at 50 μm L^{-1}. Quercetin was used as a reference compound.

Sodium taurocholate-induced damage to AGS cells

The effect of sodium taurocholate on cell viability was determined according to Romano et al (1990). One day post confluence, AGS cells were incubated with the compound at 50, 100 or 200 μM for 60 min. Sodium taurocholate (10 mM) was then added to all the wells and incubated for 30 min. Untreated cells were used as controls. Each concentration was tested in quadruplicate. After incubation, the neutral red uptake (NRU) assay was performed (Rodríguez & Haun 1999).

Proliferation assay of MRC-5 and AGS cells

MRC-5 or AGS cells were seeded at a density of 2.5×10^4 cells mL^{-1} in 96-well plates. One day after seeding, cells were treated with medium containing ELMO, at concentrations ranging from 0.5 to 64 μM, over four days and finally, the NRU assay was performed. Untreated cells were used as controls. Compounds were dissolved in DMSO (1% final concentration) and medium supplemented with 10% FBS.

Cytotoxicity assay

Confluent cultures of MRC-5 and of AGS cells were treated over 24 h with medium containing ELMO at concentrations ranging from 0 to 500 μM. The substance was first dissolved in DMSO (1% final concentration) and then in the corresponding culture medium supplemented with 2% FBS. Untreated cells were used as controls. At the end of incubation, the NRU and the MTT reduction assays were carried out as described by Rodríguez & Haun (1999).

Statistical analysis

Results were expressed as the mean ± s.d. Experiments with cells were performed three times using different preparations. Each concentration was tested in quadruplicate. Statistical differences between several treatments and their respective control were determined by Kruskal-Wallis test followed by the nonparametric multiple comparison Nemenyi's test. Regarding the gastroprotection assay, statistical differences between the treated and the control group were determined by one-way analysis of variance after the Bartlett's test for homogeneity of variance. Analysis of variance was followed by the Dunnett's multiple comparison test. The level of significance was set at $P < 0.05$.

Results

The effects of oral administration of ELMO on the HCl/EtOH-induced gastric lesions in mice are presented in Table 2. Oral doses of 10, 20 or 40 mg kg^{-1} significantly inhibited ulceration ($P < 0.01$). The inhibition displayed by ELMO at 20 mg kg^{-1} (55%) was similar to that observed with lansoprazole at the same dose (62%), while the strongest effect was observed at 40 mg kg^{-1} (70%).

Table 2 shows the effect of ELMO on the PGE_2 content of AGS cell cultures treated with the compound for 1 h. A significant increase of PGE_2 content was observed at 40 μM but not at 10 or 20 μM.

Table 2 Effects of ELMO on HCl/EtOH-induced gastric lesions in mice and on the PGE_2 content of postconfluent AGS cell cultures pretreated over 1 h with the compound

Treatment	Dose (mg kg^{-1})	Lesion index (mm)	Concn (μM)	PGE_2 (pg/well)
Control	–	38.6 ± 5.9		
Lansoprazole	20	17.1 ± 3.6**		
ELMO	10	20.1 ± 6.2**		
	20	14.4 ± 6.4**		
	40	11.8 ± 5.6**		
Control			–	15.8 ± 2.8
Indometacin			100	7.8 ± 2.4*
ELMO			10	16.4 ± 2.5
			20	19.2 ± 3.1
			40	57.2 ± 3.3**

*$P < 0.05$, **$P < 0.01$ compared with the corresponding control.

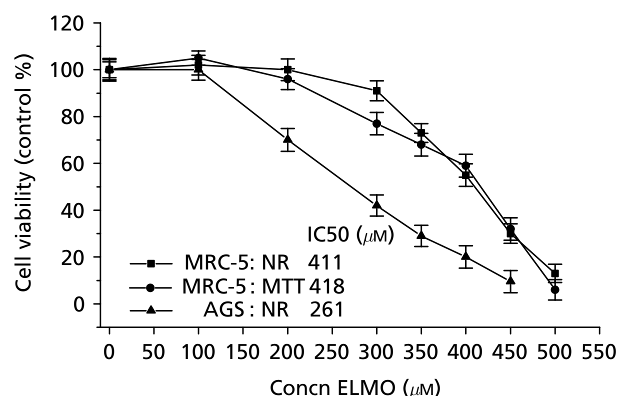

Figure 4 Viability of MRC-5 fibroblasts and AGS cells after treatment with ELMO for 24 h. Endpoints evaluated for cytotoxicity MRC-5: NR uptake and MTT reduction, AGS: NR uptake.

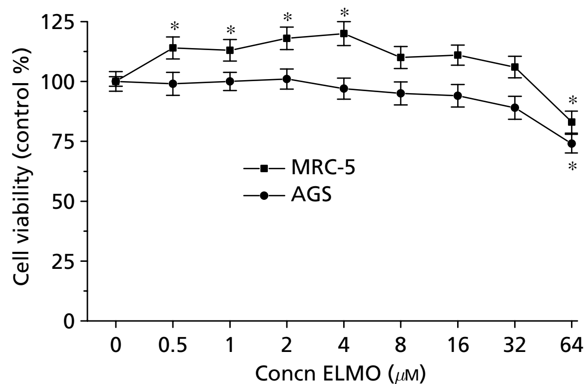

Figure 3 Effects of ELMO on the proliferation of MRC-5 fibroblasts and AGS cells. *$P < 0.01$ compared with the control group.

Treatment with ELMO did not affect the GSH content of AGS cells. Additionally, the compound did not exhibit superoxide anion scavenging activity nor a protective effect against damage induced by sodium taurocholate on AGS cells (data not shown).

The effects of ELMO on the proliferation of MRC-5 fibroblasts and AGS cells are presented in Figure 3. The compound at 0.5, 1, 2 and 4 μM stimulated the MRC-5 fibroblasts but not AGS cell proliferation ($P < 0.01$).

Figure 4 shows the cytotoxicity of ELMO towards MRC-5 fibroblasts and AGS cells. The IC50 values for fibroblasts were 411 and 418 μM in the NRU and MTT reduction assays, respectively, while for AGS cells this value was 261 μM using the NRU assay.

Discussion

The impairment of the balance between aggressive and defensive factors in the gastric mucosa could lead to gastric ulceration (Lewis & Hanson 1991). Different and complementary mechanisms including restitution of gastric epithelium, bicarbonate, blood flow, prostaglandins, GSH, NO, protection against damage induced by free radicals, among others, are involved in the gastroprotective activity of many compounds. Several terpenoids have been shown to protect the gastric mucosa against the damage caused by different ulcerogens in animal models of induced gastric lesions. Gastroprotection by virtue of terpenoids seems to be related to stimulation of the defensive factors in the gastric mucosa rather than inhibition of the aggressive factors such as gastric acid or pepsin (Lewis & Hanson 1991; Matsuda et al 2002).

The reported gastroprotective terpenoids are active at different oral doses in animal models of induced gastric lesions. The diterpene *trans*-dehydrocrotonin (DHC) at 100 mg kg^{-1} reduced the occurrence of lesions by nearly 50% in rats and mice (Souza-Brito et al 1998; Rodríguez et al 2004). At the same dose, *trans*-crotonin prevented gastric lesions by 51% (Hiruma-Lima et al 2002), while crotonin and its derivatives inhibited them by 80% (Albino de Almeida et al 2003). The diterpene aparisthman at 100 mg kg^{-1} reduced lesions by 59% (Hiruma-Lima et al 2001). At 50 mg kg^{-1} the diterpenes centipedic acid and 12-acetoxy-hawtriwaic acid lactone prevented gastric damage by 53 and 63%, respectively (Guedes et al 2002). The diterpene solidagenone and two semi-synthetic derivatives at 100 mg kg^{-1} reduced gastric lesions by 50–76% (Schmeda-Hirschmann et al 2002). Some triterpenes or their derivatives have been reported as gastroprotectors. Carbenoloxone at 100 mg kg^{-1} reduced gastric lesions in rats by 60% (Farina et al 1998). Oleanolic acid at 200 mg kg^{-1} inhibited induced gastric lesions in mice by 53% as well as several oleanolic acid derivatives (Astudillo et al 2002).

Our results showed that ELMO exhibited significant gastroprotective effect at lower doses than those reported for the above mentioned terpenes including its parent compound solidagenone, reducing the HCl/EtOH induced gastric lesions by 48, 64 and 70% at 10, 20 and 40 mg kg^{-1}, respectively.

Different mechanisms have been proposed to explain the gastroprotective activity displayed by terpenes. DHC, *trans*-crotonin, polygodial and related compounds seemed to act by increasing the prostaglandin content of the gastric mucosa (Matsuda et al 2002). The same stimulating effect on the gastric prostaglandins has been reported for the semi-synthetic crotonin (Albino de Almeida et al 2003), derivatives of glycyrrhetic, oleanolic and ursolic acids (Farina et al 1998), aparisthman (Hiruma-Lima et al 2001) and cordatin (Hiruma-Lima et al 2000), among others. DHC ($100\,mg\,kg^{-1}$) increased the gastric PGE_2 content by 50% in rats (Hiruma-Lima et al 1999). ELMO increased the prostaglandin content in AGS cell cultures fourfold, proving that its gastroprotective activity could be explained by this effect.

Compounds that increase the intracellular GSH content can prevent gastric lesions in ethanol-induced gastric damage in rats (Matsuda et al 2002). The lack of significant effect on the GSH content of AGS cells and the absence of superoxide anion scavenging capacity suggested that the gastroprotective activity of ELMO was not related to protection against free radical generation in the gastric mucosa. Some gastroprotective compounds act by protecting the gastric mucosa against injury induced by biliary salts (Nagashima 1981; Graham et al 1984; Yu et al 2003). ELMO was not able to protect the AGS cells against the damage induced by sodium taurocholate, which proved that its mode of action was different.

Re-epithelialization is a crucial factor in gastrointestinal mucosal injury and ulcer healing. Tarnawski et al (2001) pointed out that ulcer healing was a complex and tightly regulated process of filling the mucosal defect with proliferating and migrating epithelial and connective tissue cells. Growth factors, such as epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), trefoil peptides (TP), platelet derived growth factor (PDGF) and other cytokines produced locally by regenerating cells control re-epithelialization and the reconstruction of glandular structures. Rebamipide, a mucosal protective and ulcer-healing drug, significantly accelerated ulcer healing and produced a significant increase in EGF and EGF-R expression in normal gastric mucosa and increased expression of EGF and EGF-R in regenerating glands of the ulcer scar (Tarnawski et al 1998). Recently, we reported that the triterpene oleanolic acid promoted healing of acetic acid-induced chronic gastric lesions in rats (Rodriguez et al 2003). In the assessment of the ability of ELMO to act as an ulcer-healing drug we found a significant stimulating activity on the fibroblast proliferation but not on AGS cells. Our results suggested that ELMO could improve the healing of wounds after mucosal damage. Mensah et al (2001) reported that the aqueous extract of *Buddleja globosa* leaves, a plant used traditionally in Chile for wound healing, increased fibroblast growth.

Gastroprotective terpenes showed cytotoxicity IC50 values ranging from 240 to 360 μM for DHC, while crotonin presented IC50 values of 200–500 μM on fibroblasts (Rodríguez & Haun 1999; Albino de Almeida et al 2003). Our results showed that ELMO exhibited IC50 values of 411–418 μM on fibroblasts. We observed that the parent compound solidagenone presented higher cytotoxic effects with IC50 values of 42–83 μM on human fibroblasts (data not shown).

Conclusions

The diterpene derivative ELMO exhibited gastroprotective activity in-vivo that might be explained by its ability to increase the prostaglandin content. Additionally, ELMO stimulated fibroblast growth proving that the compound might have accelerated the wound healing after gastric ulceration. The strong gastroprotective activity of ELMO and its low cytotoxicity should encourage pharmacological study of this compound as a potential new anti-ulcerogenic drug.

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