Gastroprotective and ulcer healing effect of ferruginol in mice and rats: Assessment of its mechanism of action using in vitro models

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Abstract

The gastroprotective activity of the diterpene ferruginol isolated from Prumnopitys andina wood and bark was determined on HCl/EtOH-induced gastric lesions in mice. The effect of the compound on the healing of subacute gastric lesions in rats was also studied. The mode of action of the diterpene was assessed using human gastric epithelial cells (AGS) and MRC-5 fibroblasts. The effect of ferruginol on the prostaglandin E2 content, protection against sodium taurocholate induced-damage and reduced glutathione content was evaluated on AGS cells as well as on the growth of AGS and fibroblast cultures. The free radical scavenging effect of ferruginol was assessed by the 1,1-diphenyl-2-picryl-hydrazil radical and superoxide anion assays. The effect of ferruginol on human erythrocyte membrane lipoperoxidation was determined. The cytotoxicity of the compound was assessed by means of the neutral red uptake.

At 25 mg/kg, ferruginol inhibited the appearance of gastric lesions by 60% showing similar effects than lansoprazole at 20 mg/kg. Additionally, the compound displayed a significant ulcer healing activity in rats at 25 and 50 mg/kg with curative ratios of 36.0% and 92.5%, respectively, while the reference compound ranitidine at 50 mg/kg showed a curative ratio of 79.6%. At 6 and 12 μM, ferruginol increased significantly the prostaglandin E2 content. A strong inhibition of lipoperoxidation was found (IC50: 1.4 μM), but no effect was observed on the sodium taurocholate induced-damage or reduced glutathione content. Ferruginol stimulated cell proliferation at 1–2 μM in AGS cells and at 4–8 μM in fibroblasts, with cytotoxicities (IC50) of 24 and 26 μM, respectively. Our results support that ferruginol acts as gastroprotective increasing the PGs content, protecting the cells against lipid peroxidation and improving the gastric ulcer healing by a stimulating effect on the cell proliferation. These findings encourage further pharmacological studies of ferruginol as a potential new anti-ulcerogenic drug.

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Keywords: Gastroprotective; Diterpene; Ferruginol; AGS cells; Cytotoxicity

Introduction

Traditional medicine uses plants and herbs since ancient times to treat different gastrointestinal illnesses, including peptic ulcers. Recently, many efforts have been done in order to identify new anti-ulcer drugs from natural resources. Plants have originated some anti-ulcer drugs such as carbinoxolone from Glycyrrhiza glabra, solon from sophoradin and gefarnate from cabbage (Lewis and Hanson, 1991). Many other plants including Quassia amara L. (Toma et al., 2002), Turnera ulmifolia L. (Gracioso et al., 2002), Syngonanthus arthrotrichus (Batista et al., 2004) have been also reported as displaying anti-ulcerogenic activity.

Several terpenes or their derivatives have been shown to posses gastroprotective activity in different models of induced gastric lesions in animals (Lewis and Hanson, 1991; Souza-Brito et al., 1998; Rodríguez et al., 2001; Matsuda et al., 2002; Melo et al., 2003) and promoting healing of subacute gastric lesions in rats (Rodríguez et al., 2003).

Ferruginol is an abietane diterpene occurring in plants belonging to the Podocarpaceae (Cambie et al., 1984), Cupressaceae (Sharp et al., 2001), Lamiaceae (Ulubelen and Topcu, 1992) and Verbenaceae (Ono et al., 1999) families among others.
Several biological activities have been reported for ferruginol including antifungal and antibacterial (Becerra et al., 2002), miticidal (Chang et al., 2001), cardioactive (Ulubelen et al., 2002), antioxidative (Ono et al., 1999), antiplasmodial (Clarkson et al., 2003) and antitumoral effect (Iwamoto et al., 2003).

The aim of this work was to assess the gastroprotective activity of ferruginol from the Chilean tree Prumnopitys andina (Poepp. Ex Endl.) de Laub (Podocarpaceae) on the model of experimentally HCl/EtOH-induced gastric lesions in mice. This model was used because the induced gastric damage includes the generation of free radicals. The effect of ferruginol on the healing of subacute gastric lesions in rats was also studied. The assessment of the mechanism of action of this diterpene was carried out using different in vitro models in order to reduce the number of animals due to ethical and economical considerations. The effect of ferruginol on lipoperoxidation of human erythrocyte membranes, free radical scavenger activity and reduced glutathione (GSH) content was studied. Additionally, other effects of the compound were investigated, namely: prostaglandin E$_2$ (PGE$_2$) levels in AGS cells, its protection against sodium taurocholate-induced damage, its capacity to stimulate the proliferation of human epithelial gastric cells and human fibroblasts in culture and its cytotoxicity.

Material and methods

Chemicals

All solvents used were of analytical grade. Chloroform, ethanol and methanol were obtained from J.T. Baker (Phillipsburg, NJ, USA). TLC analysis was carried out on aluminum coated Silicagel (Sigma-Aldrich, St. Louis, MO, USA), 1,1-diphenyl-2-picrylhydrazyl (DPPH), nitroblue tetrazolium (NBT), thiobarbituric acid, Tween 80®, lansoprazole, DMSO, N-acetyl-L-cysteine (NAC), L-glutamine, indomethacin, sodium taurocholate (NaT), carbenoxolone disodium salt, quercetin, catechin, glutathione, and neutral red were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Culture media, antibiotics and fetal bovine serum were obtained from Invitrogen Corp. (Carlsbad, CA, USA).

Compound

Ferruginol was isolated from wood and bark of Prumnopitys andina (Poepp. ex Endl.) de Laub. (Podocarpaceae) as described by Becerra et al. (2002). Plant material was collected in Termas de Chillán, VIII Region, Chile, in March 2000 and was identified at the Departamento de Botánica, Universidad de Concepción, Chile. Voucher specimens are deposited at the herbarium of the Facultad de Ciencias Biológicas y Oceanográficas, Universidad de Concepción. The purity of the compound was controlled by TLC and HPLC. The melting point, infrared, $^1$H- and $^{13}$C NMR spectra are in full agreement with the literature data. The molecular weight of ferruginol calculated for C$_{20}$H$_{30}$O is 286.2297. The percentage of pure ferruginol from extractables was 14.2% and 16.5% for wood and bark, respectively. The structure of ferruginol is presented in the Fig. 1.

Animals

Male Sprague–Dawley rats weighing 200–250 g and fasted Swiss albino mice weighing 30±3 g were used. Fasting (24 h) prior to ulcerogenic assays was used because reference compound (lansoprazole) or ferruginol were administered orally. The animals were fed on certified Champion diet with free access to water under standard conditions of 12 h dark–light period, humidity and 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). The number of animals was determined on the basis of statistical power calculations as reported in the methodology by other authors (Souza-Brito et al., 1998; Hiruma-Lima et al., 2001).

HCl/EtOH-induced gastric lesions

This experiment was performed as we previously reported (Schmeda-Hirschmann et al., 2002). A total of 40 mice were randomly distributed into 5 groups and fasted for 24 h with free access to water prior to the experiment. The compounds were orally administered as follows: ferruginol (25, 50 and 100 mg/kg), lansoprazole (20 mg/kg) or 12% Tween 80® (10 ml/kg). Fifty min after compounds administration, all groups were orally treated with 0.2 ml of a solution containing 0.3 M HCl/60% ethanol (HCl/EtOH) for gastric ulcer induction. Animals were sacrificed 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 lesions 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.

Acetic acid-induced subacute gastric ulcers

Subacute gastric ulcers were induced in male Sprague–Dawley rats with acetic acid as described by Takagi et al.
A total of 28 rats were randomly distributed into four groups. Animals were anaesthetized with ether, the abdomen was exposed and 0.05 ml of 30% acetic acid (v/v) were injected into the submucosal layer at the junction of the fundus with the antrum, about 1 cm proximal to the pylorus. Ferruginol (25 and 50 mg/kg), ranitidine (50 mg/kg) and 12% Tween 80 (10 ml/kg) were orally administered once a day during 14 consecutive days, beginning 2 days after surgery. Animals were sacrificed one day after the last administration. The stomach was removed, fixed with formalin and incised along the greater curvature. Subsequently, the ulcer area (mm²) and the curative ratio (%) were measured as described by Schmeda-Hirschman et al. (1969) using the following formula:

$$\text{Curative ratio} = \frac{\text{Control} (\text{ulcer index}) - \text{Test} (\text{ulcer index})}{\text{Control} (\text{ulcer index})} \times 100.$$  

**Histological preparation**

Fixed stomach specimens from subacute gastric ulcers experiment were used to determine the effect of ferruginol on the recovery of the gastric mucosal thickness. A sample obtained from the middle of the gastric lesion was processed according to conventional procedure and stained with hematoxylin-eosin.

**Erythrocyte membranes lipoperoxidation**

Lipid peroxidation was determined according to the method described by Schmeda-Hirschman et al. (2003) using human erythrocyte membranes. Ferruginol was tested at concentrations of 0.35, 0.7, 1.4, 2.7, 5.5, 10.9 and 21.8 μM. The compound was dissolved in DMSO, as previously reported (Schmeda-Hirschman et al., 2003). Ferruginol was dissolved in methanol and several methanolic dilutions of DPPH served as controls. Quercetin was used as the reference compound. Each determination was repeated four times. The IC₅₀ was graphically obtained from the dose–response curve of ferruginol.

**DPPH assay**

The free-radical scavenging effect of ferruginol was assessed at 35, 175 and 350 μM by the decoloration of a methanolic solution of DPPH, as previously reported (Schmeda-Hirschman et al., 2003). Ferruginol was dissolved in methanol and several methanolic dilutions of DPPH served as controls. Quercetin was used as the reference compound. Experiments were carried out in quadruplicate.

**Superoxide anion scavenging**

The superoxide anion scavenging capacity of ferruginol was determined as reported previously (Schmeda-Hirschman et al., 2003). The compound was evaluated at 175 μM. Quercetin was used as the reference compound.

**MRC-5 cell culture**

MRC-5 fibroblasts (MRC-5) (ATCC CCL-171) were grown as monolayers in minimum essential Eagle medium (MEM), with Earle’s salts (Invitrogen Corp. Cat. Nr. 61100-053), 2 mM L-glutamine and 2.2 g/l sodium bicarbonate, supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Invitrogen Corp. Cat. Nr. 16000-044), 100 IU/ml penicillin and 100 μg/ml streptomycin (Invitrogen Corp. Cat. Nr. 15140-148) in a humidified incubator with 5% CO₂ in air at 37 °C. Cell passage was maintained between 10 and 16, and medium was changed every two days. For the subsequent experiments, cells were plated at a density of 2.5 x 10⁵ cells/ml per well in 96-well plates.

**AGS cell culture**

AGS cells (ATCC CRL-1739) were grown as monolayers in Ham F-12 medium (Invitrogen Corp. Cat. Nr. 21700-026) containing 1 mM L-glutamine and 1.5 g/l sodium bicarbonate, supplemented with 10% heat-inactivated FBS (Invitrogen Corp. Cat. Nr. 16000-044), 100 IU/ml penicillin and 100 μg/ml streptomycin (Invitrogen Corp. Cat. Nr. 15140-148) in a humidified incubator with 5% CO₂ in air at 37 °C. Cell passage was maintained between 42 and 56, and medium was changed every two days. For the subsequent experiments, cells were plated at a density of 2.5 x 10⁵ cells/ml per well in 96-well plates.

**Determination of cellular reduced glutathione content**

One day after confluence, AGS cells were incubated during 4 h with or without ferruginol at 5 or 10 μM in separate experiments. Compound was dissolved first in DMSO (1% final concentration) and then in medium only. N-acetyl-L-cysteine (NAC) was used as the positive control at 0.75 mM. A standard curve using known concentrations (1–50 nmol/ml) of GSH was carried out with each assay. Results are expressed as nanomol of soluble reduced sulphydryls/10⁶ cells (Romano et al., 1990).

**Determination of prostaglandin E₂ content**

One day after confluence, AGS cells were treated for 1 h with the compound at 3, 6 and 12 μM. Ferruginol was dissolved in DMSO (1% final concentration) and volume completed with medium only. A control without compound was included. Indomethacin (100 μM) was used as a standard inhibitor of prostaglandin synthesis. In addition, AGS cells were treated for 1 h with 100 μM indomethacin and then incubated with 12 μM ferruginol during 1 h. After incubation, PGE₂ content was determined by means of a specific enzymeimmunooassay kit (RPN 222, Amersham, UK). A calibration curve was performed using standards provided with the kit (2.5–320 pg/well).
Sodium taurocholate-induced damage to AGS cells

The effect of sodium taurocholate (NaT) on cell viability was determined according to Romano et al. (1990). One day after confluence, AGS cells were incubated with the compound at 0.1, 0.5, 1, 4 and 8 μM for 60 min. Then, 10 mM NaT was added to all wells for 30 min. Untreated cells were used as controls. After incubation, cell viability was determined by means of the neutral red uptake (NRU) assay (Rodrı́guez and Haun, 1999).

Proliferation assay of MRC-5 and AGS cells

One day after seeding, cells were treated with medium containing ferruginol at concentrations ranging from 0.5 up to 32 μM during 4 days and finally, the NRU assay was carried out (Rodrı́guez and Haun, 1999). The compound was dissolved in DMSO (1% final concentration) and medium supplemented with 10% FBS. Untreated cells were used as controls.

Cytotoxicity assay

Confluent cultures of MRC-5 as well as AGS cells were treated with medium containing ferruginol at concentrations ranging from 1 up to 40 μM. The terpenoid carbenoxolone disodium salt was used as reference compound for cytotoxicity. The substance was first dissolved in DMSO and then in the corresponding culture medium supplemented with 2% FBS. The final concentration of DMSO in the test medium and controls was 1%. Cells were exposed for 24 h to test medium with or without the compound (control). At the end of the incubation, the NRU assay was performed (Rodrı́guez and Haun, 1999).

Statistical analysis

Results were expressed as the mean ± SEM. Experiments with cells were done 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 (ANOVA) after the Bartlett’s test for homogeneity of variance. ANOVA was followed by the Dunnett’s multiple comparison test. The level of significance was set at \( p < 0.05 \).

Results

Ferruginol displayed a strong gastroprotective effect at 25 mg/kg comparable to lansoprazole at 20 mg/kg in the gastric lesions induced by HCl/EtOH in mice (Fig. 2). The effect of ferruginol on the prevention of induced lipoperoxidation of human erythrocyte membranes and its free radical scavenging capacity was assessed, because the mechanism involved in the ulceration induced by HCl/EtOH includes the generation of free radicals. No effect on the decoloration of DPPH at different concentrations of ferruginol was observed, neither on superoxide anion scavenging and GSH content (not shown). Interestingly, a significant inhibition of lipoperoxidation on erythrocyte membranes was observed with an IC50 value as low as 1.4 μM (Fig. 3). In the same assay the reference compound, catechin, showed an IC50 value of 260 μM. Since some terpenoids act as gastro-

<table>
<thead>
<tr>
<th>Concentration (μM)</th>
<th>PGE2 (pg/well)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control –</td>
<td>19.5 ± 2.5</td>
</tr>
<tr>
<td>Indomethacin 100</td>
<td>8.6 ± 2.2*</td>
</tr>
<tr>
<td>Ferruginol 3</td>
<td>18.1 ± 2.9</td>
</tr>
<tr>
<td></td>
<td>25.3 ± 2.7*</td>
</tr>
<tr>
<td></td>
<td>27.7 ± 3.1**</td>
</tr>
<tr>
<td>Indomethacin+Ferruginol 100+12</td>
<td>22.4 ± 2.2</td>
</tr>
</tbody>
</table>

Table 1

Effects of ferruginol on the PGE2 content of postconfluent AGS cell cultures pretreated during 1 h with the compound

Fig. 2. Effects of lansoprazole (20 mg/kg) and ferruginol on HCl/EtOH-induced gastric lesions in mice. The columns represent the mean ± SEM (n = 8). ANOVA followed by Dunnett’s multiple comparison test. * \( p < 0.01 \) compared to control group.

Fig. 3. Effect of ferruginol on the inhibition of the lipoperoxidation induced by tert-butylhydroperoxide in human erythrocyte membranes. Data are expressed as the mean of three different experiments in quadruplicate ± SEM.
protectives increasing the gastric prostaglandin content, we studied the effect of ferruginol on the PGE2 content of AGS cell cultures. The compound at concentrations of 6 and 12 µM induced a strong increase on the PGE2 levels in the cell cultures. This stimulating effect of ferruginol on the PGE2 content was attenuated when the cells were pretreated with indomethacin (Table 1). Ferruginol did not protect AGS cells against the damage induced by NaT (not shown). Ferruginol proved to have a strong ulcer healing activity in rats at 25 and 50 mg/kg with curative ratios of 36.0% and 92.5%, respectively, while the reference compound ranitidine at 50 mg/kg showed a curative ratio of 79.6%. Additionally, treatment with ferruginol at 50 mg/kg displayed a significant increase of the gastric mucosal thickness similar to ranitidine (Table 2). In order to determine the ability of ferruginol to stimulate the cell proliferation we studied its effect on the growth of MRC-5 fibroblasts and AGS cells. As presented in Fig. 4 a stimulating effect on the cell proliferation was observed at 1 and 2 µM for AGS cells and at 4 and 8 µM for MRC-5 fibroblasts. This fact might explain its ulcer healing effect observed in vivo. The cytotoxicity of ferruginol towards confluent cell cultures assessed by the neutral red uptake is presented in the Fig. 5. The compound showed almost the same toxicity value against AGS and MRC-5 cells with IC50 values of 24 and 26 µM, respectively. The reference terpenoid carbenoxolone showed *p<0.01 compared to control group.

**Table 2** Healing effect of oral administration during 14 days of ferruginol and ranitidine after an experimental gastric ulcer produced by injection of acetic acid solution into the rat stomach walls

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>n</th>
<th>Lesion area (mm²)</th>
<th>Curative ratio (%)</th>
<th>Mucosal thickness (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.5</td>
<td>7</td>
<td>33.3±3.6</td>
<td>–</td>
<td>312±48</td>
</tr>
<tr>
<td>Ranitidine</td>
<td>50</td>
<td>7</td>
<td>6.8±2.8*</td>
<td>79.6</td>
<td>759±67*</td>
</tr>
<tr>
<td>Ferruginol</td>
<td>25</td>
<td>7</td>
<td>21.3±2.5</td>
<td>36.0</td>
<td>422±86</td>
</tr>
</tbody>
</table>

Lesion area data are expressed as mean±SEM. ANOVA followed by Dunnett’s multiple comparison test. *p<0.01 compared to control group.

In the last years the gastroprotective activity of different diterpenes occurring in plants or their derivatives has been reported. The assessment of this activity is commonly carried out using several models of induced gastric lesions employing laboratory animals, evaluating the preventive capacity of the diterpenes on the appearance of such lesions. The majority of the reported gastroprotective diterpenes including clerodane (Almeida et al., 2003), labdane (Schmeda-Hirschmann et al., 2002) and abietane (Sepulveda et al., 2005) skeletons show significant effects at oral doses of 50 to 100 mg/kg. The abietane diterpene ferruginol at a single oral dose of 25 mg/kg showed a gastroprotective activity similar to the reference drug lansoprazole at 20 mg/kg in the model of gastric lesions induced by HCl/ EtOH in mice, reducing the appearance of lesions by 60%.

Although the mode of action of diterpenes has not been well established, these compounds seem to protect the gastric mucosa mainly through mechanisms that enhance the defensive factors of the stomach. The gastroprotective activity of diterpenes and their derivatives, observed in different models of induced gastric lesions in animals, has been explained by mechanisms that include stimulation of prostaglandin synthesis, increase of mucus production and suppression of gastric acid secretion (Hiruma-Lima et al., 1999, 2002). In previous studies we have observed that ferruginol was devoid of effect on the basal acid secretion nor on the gastric secretion volume in ligated pylorus model in mice.

Considering that the ulceration induced by HCl/EtOH includes the generation of toxic free radicals (Mutoh et al., 1990; Phull et al., 1995), we assessed the effect of ferruginol on the prevention of induced lipoperoxidation on human erythrocyte membranes, GSH content in AGS cells as well as its free radical scavenging capacity. The compound displayed a strong inhibition of the lipoperoxidation on erythrocyte membranes, being some 190-fold more active than the reference compound catechin. Ferruginol was devoid of effect as free radical scavengers.
scavenger and did not modify the GSH levels. These results may explain the gastroprotective activity of this diterpene observed in the gastric lesions induced by HCl/EtOH in mice.

It is well established that endogenous PGs play an important role in maintaining mucosal integrity (Miller, 1983) and protecting the gastric mucosa against various damaging agents (Chaudhry and Robert, 1980; Robert et al., 1983). The significant increase of the PGE2 content induced by ferruginol on AGS cell cultures contributes to explain the gastroprotection displayed by the compound in the in vivo gastric ulcer assay.

The gastroprotective effect of lansoprazole towards gastric lesions induced by HCl/EtOH in mice could be explained by the increase of the gastric levels displayed by the anti-secretory drugs (Hakanson and Sundler, 1990). In addition, recently it has been reported that lansoprazole protects the gastric mucosa against HCl/EtOH-induced injuries by a reduction of gastric oxidative damage (Natale et al., 2004).

The capacity of re-epithelialization is crucial in the recovery of the gastric mucosa after ulceration. Ulcer healing is a complex and tightly regulated process of filling the mucosal defect with proliferating and migrating epithelial and connective tissue cells (Tarnawski et al., 2001). Growth factors, such as epidermal growth factor, basic fibroblast growth factor, trefoil peptides, platelet derived growth factor, and other cytokines produced locally by regenerating cells, control re-epithelialization and the reconstruction of glandular structures. In order to determine whether ferruginol could have an effect on the ulcer healing process, we assessed the activity of the compound on the healing of acetic acid-induced subacute gastric ulcers in rats. The strong ulcer healing effect observed in this model could be attributed to the stimulating effect of ferruginol on the PGs content and its protective activity against the lipid peroxidation. Cyclooxygenase-2 (COX-2) is a key enzyme in the PGs biosynthesis that mediates pathophysiological reactions such as inflammation. The expression of COX-2 is increased in experimental ulcers stimulating the PGE2 synthesis (Peskar et al., 2001). PGs accelerate ulcer healing, possibly via angiogenesis, epithelial cell proliferation, production of growth factors such as hepatocyte growth factor and transforming growth factor beta, reconstruction of extracellular matrices, and suppression of inflammatory cell infiltration, in addition to gastroprotective mechanisms (Arakawa et al., 1998). COX-2 inhibitors delay the healing of subacute gastric ulcers in experimental animals and decrease epithelial cell proliferation, angiogenesis and maturation of the granulation tissue (Peskar et al., 2001). It has been reported recently that the diterpene derivative ecabet sodium improves the wound repair in intestinal epithelial cells elicited by hydrogen peroxide by inducing the expression of COX-2 (Sasaki et al., 2005).

Additionally, during the inflammatory response induced by acetic acid in the injured gastric mucosa, polymorphonuclear (PMN) leukocytes synthesize superoxide anion that is further metabolized into other free radicals with pronounced cytotoxic activity. The increase in the amount of TBARs in gastric mucosa from activated PMNs may aggravate mucosal injury by lipid peroxidation (Tanaka and Yuda, 1996). Our results showing that ferruginol display a strong inhibition of the lipoperoxidation on erythrocyte membranes further suggest that this activity contributes to recover the ulcerated lesion. The acceleration of the ulcer healing observed in rats treated with ranitidine may be explained by the well established elevation of serum gastrin and its associated increase in growth factors induced by gastric anti-secretory agents (Hakanson and Sundler, 1990).

The assessment of ferruginol on cell proliferation showed a significant stimulation of the growth of MRC-5 fibroblast and AGS cell cultures. This stimulating activity found in both cell lines supports the fact that ferruginol may improve the healing of wounds after gastric mucosal damage stimulating the repair of the injured tissue.

Different cytotoxicities have been reported for gastroprotective diterpenes. The cytotoxicity of trans-dehydrocrotonin showed IC50 values ranging from 240 up to 360 μM, while crotonin presented IC20 values of 200–500 μM on V79 fibroblasts (Rodriguez and Haun, 1999; Almeida et al., 2003). Solidagone displayed IC50 values of 83–115 μM on human MRC-5 fibroblasts and AGS cells, respectively (Rodriguez et al., 2005a). The semisynthetic derivative of solidagone 15,16-epoxy-8(9),13(16),14-labdatrien-7β-methoxy-6β-ol showed IC50 values of 411 and 261 μM for MRC-5 fibroblasts and AGS cells, respectively (Rodriguez et al., 2005b). Ferruginol showed similar cytotoxicity values for AGS cells and fibroblasts, while the reference compound carbenoxolone proved to be less toxic.

In summary, the diterpene ferruginol protects against the gastric lesions induced by HCl/EtOH in mice. This gastroprotective activity seems to be related with a reduction of the damage in the cell membrane induced by free radicals and an increase of PGE2 levels in the gastric mucosal cells. In addition, the compound could improve the gastric ulcer healing process by stimulating the cell proliferation. The promising gastroprotective activity of ferruginol encourages the pharmacological study of this compound and the synthesis/isolation of less cytotoxic derivatives as potential new anti-ulcerogenic drugs.

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