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Gastroprotective activity of solidagenone on experimentally-induced gastric lesions in rats

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Abstract

The gastroprotective effect of the labdane diterpene solidagenone was assessed on gastric ulcer in rats. The effect of a single oral dose of the compound was evaluated at 50, 100 and 200 mg kg^{-1} in the following test systems: pylorus ligature (Shay), aspirin- and ethanol-induced gastric ulcers. In pylorus-ligated rats (Shay model), the ulcerative index decreased by 37 % with solidagenone pre-treatment at the three assayed doses. The effect of a single oral dose of 50 mg kg^{-1} solidagenone was comparable with ranitidine at the same concentration and similar to higher doses of the compound. A significant effect ($P < 0.001$) at 100 and 200 mg kg^{-1} was observed in the aspirin-induced ulcer model. At both doses, reduction in the number of lesions was approximately 50 % compared with controls. The effect was comparable with the reference compound ranitidine (50 mg kg^{-1}). With the ethanol-induced gastric ulcers, the effect of solidagenone at 100 and 200 mg kg^{-1} was similar to a single oral dose of 20 mg kg^{-1} omeprazole with a 50 % reduction of the mean number of lesions compared with controls. In acute toxicity tests on mice, intraperitoneal administration of solidagenone showed no toxicity at doses up to 600 mg kg^{-1}. This is the first report on the gastroprotective activity of a labdane diterpene.

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Introduction

Traditional medicine uses plants to treat gastrointestinal disorders, including peptic ulcers. In the last few years efforts have been directed to identify new anti-ulcer drugs from natural sources. Plants are the source of known anti-ulcer drugs such as carbenoxolone from *Glycyrrhiza glabra*, solon from sophoradin and gefarnate from cabbage (Lewis & Hanson 1991). Other plants have been reported to show anti-ulcer effect (Lewis & Hanson 1991). Some terpenes or terpene derivatives display gastroprotective activity in different models of induced gastric lesions in animals (Giordano et al 1990; Lewis & Hanson 1991; Matsuda et al 1998). A powerful anti-ulcerogenic effect has been demonstrated for some clerodane diterpenes (Souza-Brito et al 1998). Solidagenone is a labdane diterpene occurring in the rhizomes of *Solidago chilensis*, a plant used to treat symptomatologies related to inflammation (Razmilic & Schmeda-Hirschmann 2000).

We now report the gastroprotective activity of solidagenone on three different models of experimentally-induced gastric ulcer in rats: pylorus ligature, ethanol and aspirin. Ethanol- and aspirin-induced gastric lesion models were investigated because they represent some of the most common causes of gastric ulcer in man.

Materials and Methods

Animals

Fasted male Sprague–Dawley rats (200–250 g) from the Central Animal House of the Universidad de Talca were used. The rats underwent fasting before ulcerogenic assay because the reference compounds (ranitidine and omeprazole) or solidagenone were administered orally. Swiss albino mice (30 ± 3 g) were used to investigate acute toxicological effects. The animals were fed on certified Champion diet with free access to water under standard humidity and temperature and a 12-h dark–light period.

The Universidad de Talca Institutional Animal Care and Use Committee approved the study protocols, which followed the recommendations of the Canadian Council on Animal Care (Olfert et al 1993).

Drugs

The following drugs were used: omeprazole (Losec, Merrell Lepetit), ranitidine, Tween 80 and aspirin (Sigma Chemical Co, St Louis, MO). Omeprazole and ranitidine were used as standard anti-ulcer drugs. Solidagenone (Figure 1) was isolated from *S. chilensis* rhizomes as reported previously (Schmeda-Hirschmann 1988; Razmilic & Schmeda-Hirschmann 2000) and recrystallized in hexane–CH_2Cl_2 as colourless crystals, m.p. 133°C; $[\alpha]^{20}$: −14.7 (c = 1.029, $CHCl_3$). The w/w yield from the starting dry material was 0.55%.

^1H and ^{13}C NMR spectral data of solidagenone

^{1}H NMR (400 MHz; $CDCl_3$, δ H in ppm; *j* in Hz): 1.5–1.6 m (H-1); 1.56 m (H-2); 1.38 m (H-3); 1.15 m (H-3′); 2.68 s (H-5); 5.72 d br (1.5) (H-7); 1.8–2.0 m (H-11); 2.65 m (H-12); 6.31 d br (1) (H-14); 7.38 dd (1.5, 1) (H-15); 7.26 br s (H-16); 2.02 d (1.5) (H-17); 1.16 s (H-18); 1.19 s (H-19); 1.01 s (H-20).

^{13}C NMR (50 MHz; $CDCl_3$, δ C in ppm): 31.61 t (C-1); 17.91 t (C-2); 42.63 t (C-3); 32.24 s (C-4); 55.83 d (C-5); 200.18 s (C-6); 129.25 d (C-7); 155.71 s (C-8); 76.56 s (C-9); 46.50 s (C-10); 33.32 t (C-11); 21.30 t (C-12); 125.13 s (C-13); 110.75 d (C-14); 143.10 d (C-15); 138.60 d (C-16); 20.09 q (C-17); 33.84 q (C-18); 21.72 q (C-19); 18.32 q (C-20).

In-vivo toxicity

Acute toxicity of solidagenone was assessed in 12-h fasted male Swiss albino mice using a protocol described by Souza-Brito et al (1998). Increasing doses of compound were administered intraperitoneally (i.p.) to groups of 10 mice for each dose level (100, 200, 400 or 600 mg kg^{-1}). Animals receiving the vehicle (12% Tween 80, 10 mL kg^{-1}) served as control. The groups were observed at 0, 30, 60, 120, 180 and 240 min after solidagenone administration and then twice a day for the next 14 days. At the end of this period the number of survivors was recorded and the acute toxicological effect was inferred on the basis of mortality, expressed as LD50 according to Litchfield & Wilcoxon (1949). The intraperitoneal route was selected due to the large amount of the compound to be administered and the low solubility of solidagenone in the vehicle.

Acute gastric lesions

The gastroprotective activity of solidagenone was assessed on three different experimentally-induced gastric ulcer models.

Shay ulcer

A total of 30 rats were randomly distributed into five groups and fasted for 48 h with free access to water. A pylorus ligature was performed (Shay et al 1945) 1 h after oral administration of solidagenone (50, 100 or 200 mg kg^{-1}), ranitidine (50 mg kg^{-1}) as a positive control or vehicle (a 12% solution of Tween 80; 10 mL kg^{-1}). Animals were killed 4 h after drug administration, the abdomen was opened and another ligature was placed around the oesophagus close to the diaphragm. The stomach was removed, inspected externally and its content drained into a graduated centrifuge tube and centrifuged at 2000 rev min^{-1} for 10 min. The supernatant volume and pH were recorded. Gastric lesions were evaluated examining the inner gastric surface with a dissecting binocular microscope. Mucosal lesions were counted and the ulcerative index (UI) was determined (Souza-Brito et al 1998). Briefly, the ulcerative index was calculated using the following formula:

$$UI = (A \times 3) + (B \times 2) + C$$

where A corresponded to ulcers > 3 mm, B to lesions between 1 and 3 mm, and C < 1 mm.

Aspirin ulcer

A total of 30 animals were randomly distributed into five groups and fasted for 24 h with free access to water before the experiment. One hour after oral administration of solidagenone (50, 100 or 200 mg kg^{-1}), ranitidine (50 mg kg^{-1}) or 12% Tween 80 (10 mL kg^{-1}), 200 mg kg^{-1} aspirin was orally administered to unanaes-

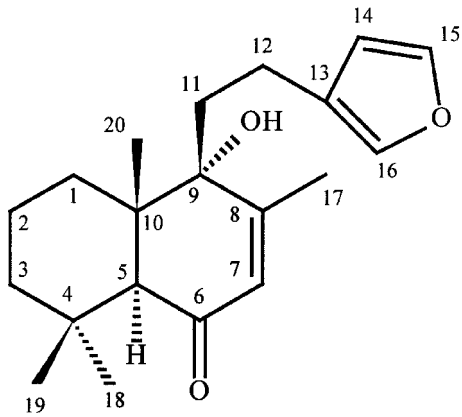

Figure 1 Structure of solidagenone.

thetized rats of each group according to Williamson et al (1996). Animals were killed 4 h later. Stomachs were removed, opened and gastric lesions determined as described above.

Ethanol-induced ulcer

A total of 30 animals were randomly distributed into five groups and fasted for 24 h with free access to water before the experiment. The ethanol-induced lesion assay was carried out according to the method of Morimoto et al (1991). Ethanol (99.5% ; 1 mL) was orally administered to the animals which 1 h previously had been treated with solidagenone (50, 100 or 200 mg kg^{-1}), omeprazole (20 mg kg^{-1}) or 12% Tween 80 (10 mL kg^{-1}). Animals were killed 1 h after ethanol administration, the stomachs were removed, opened and the ulcerative index determined as described above.

Statistical analysis

Results are presented as mean$\pm$s.d. Statistical significance was determined by one-way analysis of variance followed by Duncan's test, with the level of significance set at $P < 0.05$.

Results

Acute toxicity

Solidagenone at intraperitoneal doses of up to 600 mg kg^{-1} did not show any observable symptoms of toxicity or mortality in mice. Therefore, the intraperitoneal LD50 for solidagenone in mice was higher than 600 mgkg^{-1} and the compound could be regarded as "not harmful".

Acute gastric lesions

The effects of solidagenone on the three models of induced gastric lesions are shown in Figures 2–4. A

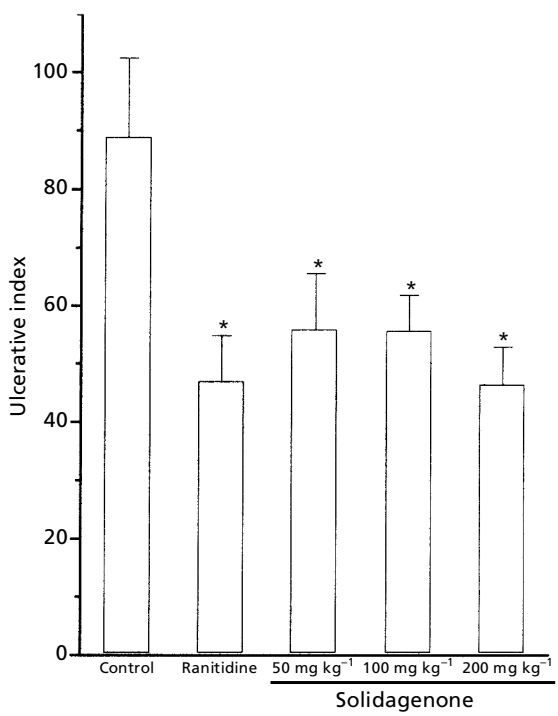

Figure 2 Effects of solidagenone (50, 100, 200 mg kg^{-1}) and ranitidine (50 mg kg^{-1}) on pylorus ligature-induced gastric ulcers in rats. Results are expressed as means$\pm$s.d. (n = 6). *$P < 0.001$, compared with corresponding control (analysis of variance followed by Duncan's test).

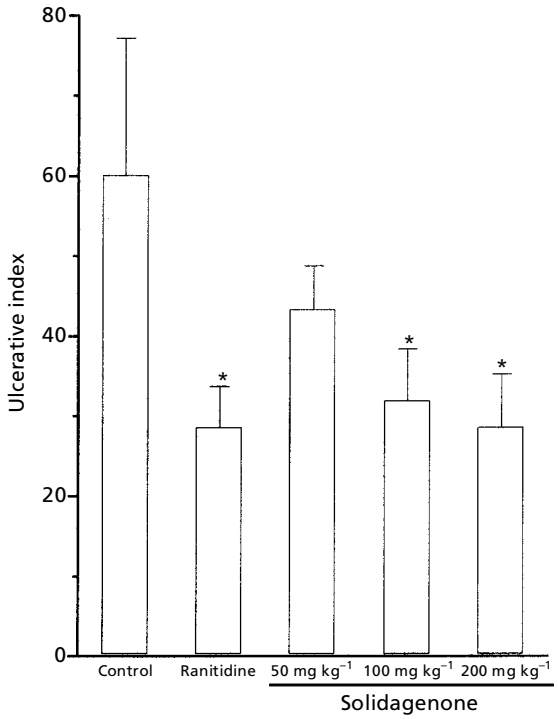

Figure 3 Effects of solidagenone (50, 100, 200 mg kg^{-1}) and ranitidine (50 mg kg^{-1}) on aspirin-induced gastric ulcers in rats. Results are expressed as means±s.d. (n = 6). *$P < 0.001$, compared with corresponding control (analysis of variance followed by Duncan's test).

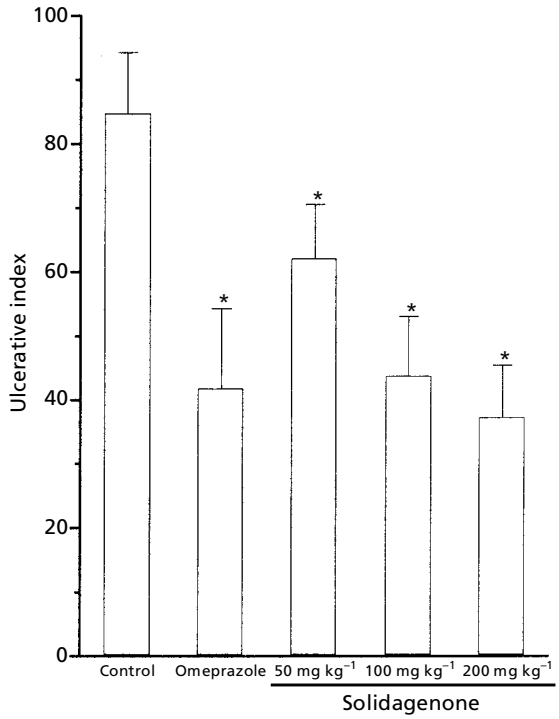

Figure 4 Effects of solidagenone (50, 100, 200 mg kg^{-1}) and omeprazole (20 mg kg^{-1}) on ethanol-induced gastric ulcers in rats. Results are expressed as means±s.d. (n = 6). *$P < 0.001$, compared with corresponding control (analysis of variance followed by Duncan's test).

single oral administration of solidagenone at 100 or 200 mg kg^{-1} inhibited the appearance of gastric lesions induced by ethanol, aspirin and pylorus ligature. No significant differences were observed between the gastroprotective effect of the compound in the three models at 100 or 200 mg kg^{-1}.

In pylorus-ligated rats (Shay model), the ulcerative index decreased by 37–47% with solidagenone pretreatment at the three doses tested. The effect of a single oral dose of 50 mg kg^{-1} solidagenone was comparable with ranitidine at the same concentration and similar to higher doses (100 or 200 mg kg^{-1}) of solidagenone. No significant modifications in gastric volume and gastric pH were observed (Table 1). A significant effect ($P < 0.001$) at 100 and 200 mg kg^{-1} was observed in the aspirin-induced ulcer model. At both doses, reduction in the number of lesions was approximately 50% compared with controls. The effect was comparable with 50 mg kg^{-1} ranitidine, the reference compound. For the ethanol-induced gastric ulcers, the effect of 100 or 200 mgkg^{-1} solidagenone was similar to a single oral dose of 20 mg kg^{-1} omeprazole with a 50% reduction of the mean number of lesions compared with controls.

Table 1 Effect of solidagenone on the pH and volume of gastric secretion in pylorus-ligated (Shay) rats.

Treatment (p.o.)	Dose (mg kg^{-1})	n	pH	Volume of secretion (mL)
Control	–	6	2.85±0.22	3.65±1.31
Ranitidine	50	6	5.47±0.15*	7.84±2.17*
Solidagenone	200	6	3.08±0.26	3.92±1.84

Results are expressed as means±s.d. *$P < 0.01$. Analysis of variance followed by Dunnett's test.

Discussion

Anti-ulcerogenic terpenoids include some triterpenes, diterpenes and terpenic derivatives isolated from plants (Giordano et al 1990; Lewis & Hanson 1991; Souza-Brito et al 1998). Although the anti-ulcerogenic properties of the reported active terpenoids are well established, some aspects of their mechanism of action remain poorly understood. The triterpenic derivative carbenoxolone

has been extensively investigated for its mode of action. Carbenoxolone is an excellent stimulant of mucus synthesis, maintains the prostaglandin content of gastric mucosa at high levels and has been reported to inhibit pepsin secretion (Lewis & Hanson 1991). Hiruma-Lima et al (1999) reported that the diterpene lactone dehydrocrotonin exhibited gastroprotective properties that could be due to synergistic effects including an increase in prostaglandin E_2 release and non-competitive antagonism of H_2-receptors and of muscarinic receptors. The sodium salt of 12-sulfodehydroabietic acid has been shown to enhance the mucin metabolism in rat gastric mucosa (Ichikawa et al 2000). Over the last ten years, several biological effects have been reported for labdane diterpenes but none have been related to a gastroprotective effect (Singh et al 1999). This is the first time that a compound with a labdane skeleton has been reported to show gastroprotective activity.

Our study was based on three different experimental models of peptic ulcer which operate by distinct mechanisms of gastric ulcerogenesis (Desai & Parmar 1993). Ethanol is a necrotizing agent that produces gastric ulceration by causing direct damage to the mucosa independent of gastric acid secretion (Szabo 1987; Cho & Ogle 1992). The mechanism by which ethanol induces gastric damage involves the disruption of the defensive factors such as the gastric mucosal barrier, gastric mucus and mucosal circulation (Takase et al 1994). The involvement of ethanol-generated toxic oxygen radicals causing ethanol-induced gastric lesions has also been suggested (Mutoh et al 1990). This explains why ethanol-induced ulcers are not inhibited by anti-secretory agents such as cimetidine, but are greatly inhibited by agents that enhance mucosal defensive factors (Cho & Ogle 1992). Solidagenone at doses of 100 or 200 mg kg^{-1} markedly inhibited the formation of ethanol-induced gastric lesions with no significant changes on gastric volume and gastric pH. These results suggested that the gastroprotective effect of solidagenone was probably attributable to an enhancement of mucosal defensive factors and was not exerted by inhibition of gastric acid secretion.

Aspirin is regarded as a gastric ''mucosal breaker'' that induces the back diffusion of gastric H^+ and finally produces haemorrhagic mucosal lesions (Garner et al 1987). Non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin inhibit prostaglandin synthesis (Noreen et al 1998). The consequent loss of the gastroprotective effect of prostaglandins against acid and pepsin leads to mucosal ulceration within a few hours after aspirin administration. Aspirin-induced gastric lesions can be prevented by drugs inhibiting aggressive factors such as gastric acid secretion (Takase et al 1994). Thus drugs that enhance gastric mucosal defensive factors may also display gastroprotective activity against lesions induced by aspirin (Lewis & Hanson 1991; Morimoto et al 1994). The cytoprotective action of some anti-ulcer drugs and the ''counter-irritant'' action of mild irritants are mediated by the action of endogenous prostaglandins, which play an important role in maintaining mucosal integrity (Miller 1983) and protect the gastric mucosa against various damaging agents (Guth & Moler 1979; Chaudhury & Robert 1980; Konturek et al 1981; Robert et al 1983).

Our results and the absence of significant effects on gastric volume and pH in pylorus-ligated rats suggested that solidagenone could not protect against aspirin-induced ulcers by increasing prostaglandin release because the aspirin would prevent cyclooxygenase activity. Compounds having gastroprotective properties that may act by a mechanism independent of endogenous prostaglandins have been reported by Sun et al (1992) and Martin et al (1994).

The stomach digestive effect of accumulated gastric juice in the induction of gastric ulcers is well documented in the pylorus-ligation model (Brodie 1966). Solidagenone was able to inhibit the gastric lesions induced by pylorus ligation confirming their gastroprotective activity in rats. As discussed for the other models, an increase in gastric mucosal resistance might be a key factor in inhibiting the pylorus-ligature-induced ulcers.

Solidagenone was active at 50 mg kg^{-1} whereas terpenes belonging to different structural types have shown gastroprotective activity at doses ranging from 40 to 100 mg kg^{-1} (Giordano et al 1990; Hiruma-Lima et al 1999).

Our results show that a single oral dose of solidagenone protected the gastric mucosa in-vivo against the damage induced by pylorus ligature, ethanol and aspirin. The gastroprotective activity of solidagenone seemed to be exerted by an increase in mucosal defensive factors by means of a mechanism probably independent of endogenous prostaglandins. These results and the low toxicity observed support the value of further pharmacological study of solidagenone as a potential gastroprotective drug.

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