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Ginsenoside Rb_1: the Anti-Ulcer Constituent from the Head of *Panax ginseng*

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We previously reported that the butanol (BuOH) fraction of the head of *Panax ginseng* exhibited gastroprotective activity in peptic and chronic ulcer models. In order to identify the active constituent, an activity-guided isolation of the BuOH faction was conducted with a HCl·ethanol-induced gastric lesion model. The BuOH fraction was passed through a silica-gel column using a chloroform-methanol gradient solvent system, and six fractions (frs. **1**-**6**) were obtained. The active fr. **5** was further separated by silica-gel column, to yield **6** subfractions (subfrs. **a**-**f**). Subfr. d was composed of ginsenosides Re, Rc and Rb_1. The most active constituent was ginsenoside Rb_1 (GRb_1), a protopanaxadiol glycoside, which was investigated for its anti-ulcer effect. Gastric injury induced by HCl·ethanol, indomethacin and pyloric ligation (Shay ulcer) was apparently reduced with oral GRb_1 doses of 150 and 300 mg/kg. GRb_1 at these dosage significantly increased the amount of mucus secretion in an ethanol-induced model. The anti-ulcer effects were consistent with the result of histological examination. These results suggest that the major active constituent in the head of *Panax ginseng* is GRb_1, and that anti-ulcer effect is produced through an increase in mucus secretion.

Key words: *Panax ginseng* heac, Ginsenoside Rb_1, Anti-ulcer, Mucus secretion

INTRODUCTION

Stress-related gastric mucosal damage, nonsteroidal anti-inflammatory drug (NSAIDs)-induced gastric lesion and *Helicobacter pylori*-mediated ulcers are quite common. Gastritis and gastric ulcers are pathological conditions caused by an imbalance between aggressive factors, such as gastric acid, pepsin, stimulation of the vagus nerves, secretion of gastrin, and increase in the number of parietal cells, and protective factors, such as bicarbonate ion, mucus productivity, mucus secretion, and prostaglandins (Shay *et al.*, 1945). With the exception of the damaging role of gastric acid, reactive oxygen species (ROS), particularly hydroxyl radicals (· OH), are a major cause of the oxidative damage of the mucosa in most types of gastric ulcers (Phull *et al.*, 1995).

Panax ginseng C. A. Meyer (Araliaceae) has been used for treating diabetes, hepatitis and as a tonic for elevating the mood and relieving fatigue in East Asia for a long time (Jiansu New College Medicine Eds., 1977; Chung *et al.*, 2001). In experimental ulcer research, a few studies have investigated the effect of *Panax ginseng* on the stomach (Omar *et al.*, 2001; Sun *et al.*, 1992). The head of *Panax ginseng*, which is usually discarded in the process of making the general formula containing *Panax ginseng*, has only recently been used as an emetic (Hur, 1989). We previously reported that the butanol (BuOH) fraction of the *Panax ginseng* head had an anti-ulcer activity in rats based on the premise that the emetics and stomach function are related (Jeong, 2002).

Therefore, this study investigated the activity-guided isolation of the anti-ulcer constituents from the BuOH fraction of the *Panax ginseng* head. Since the major component showing gastroprotective activity was ginsenoside Rb_1 (GRb_1), which was isolated from activity-guided isolation, the anti-ulcer effect of GRb_1 was evaluated using HCl·ethanol-induced gastritis, an indomethacin-induced gastric ulcer, a Shay ulcer, a gastric juice secretion and mucus secretion.

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MATERIALS AND METHODS

Herbal material

The heads of *Panax ginseng* (10 Kg), purchased at a herbal market in Seoul, Korea, were washed and dried. A voucher specimen was authenticated by Prof. Eun Bang Lee in the Natural Products Research Institute, Seoul National University, and was deposited at the Research Institute of Pharmaceutical Sciences of Duksung Women's University. The ethanol (EtOH) extract was prepared by reflux using 95% EtOH for 4 h three times at 70°C in a water bath. The EtOH extract (350 g) was systematically fractionated with hexane, chloroform and BuOH.

Reagents

Indomethacin and dioctyl sodium sulfosuccinate were purchased from the Sigma Chem. Co. Alcian blue (Janssen Chimica), sucrose (Shinyo Pure Chem. Co.), carboxymethyl cellulose (Junsei Chem. Co.) and ranitidine (Choongwea Pharm. Co.) were also used. All other chemicals were of analytical grade.

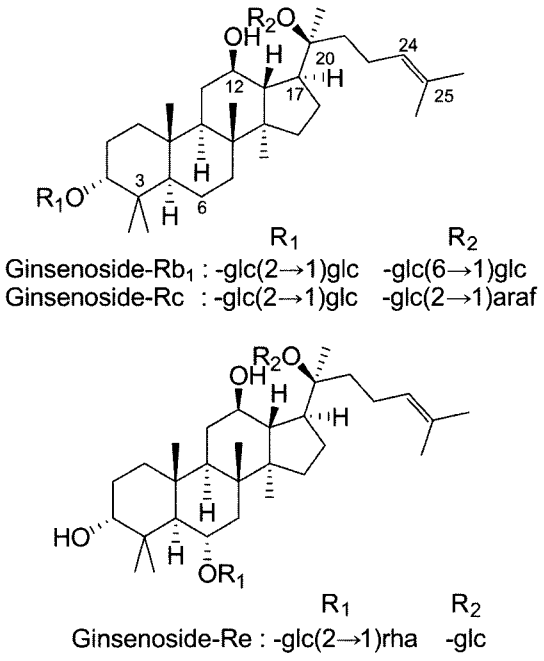

Fig. 1. Structure of GRb_1, Rc, and Re

Animals

Male Sprague-Dawley rats weighing 200±20 g were supplied from Samyook Animal Laboratories, Kyunggi-do. Solid food and water were supplied *ad libitum*. All rats were housed in a temperature-controlled (22-25°C) room on a 12 h light/dark cycle. The samples dissolved in saline were administered in a volume of 0.5 mL/100 g (body weight). The control group was given saline only.

Isolation of active constituents from the butanol fraction

A part (100 g) of the BuOH fraction was passed through a silica-gel column using a chloroform/ methanol gradient solvent system ($CHCl_3$: MeOH = 100 : 0~0 : 100), and 6 fractions (frs. **1**-**6**) were obtained. Among them, frs. **2**, **3**, and **5** significantly inhibited the HCl·ethanol-induced gastric lesion. One of the active fractions (fr. **5**) was separated by silica gel column using a chloroform/ methanol solvent system ($CHCl_3$: MeOH : H_2O = 20.3 : 10.7 : 2.3), and six subfractions (subfrs. **a**-**f**) were obtained. The most effective subfraction (subfr. **d**) was separated by a silica gel column using an ethylacetate/methanol solvent system (EtOAc : MeOH : H_2O = 9 : 2 : 1). Three components from subfr. d were identified as ginsenosides Re, Rc and Rb_1 by physicochemical and spectral methods (Fig. 1).

HCl·ethanol-induced gastric lesion

Briefly, the rats which were denied food for 24 h with free access to water prior to the experiment, were orally administered with 0.5 mL/100 g of a HCl·ethanol (60% EtOH with 150 mM HCl) solution. One hour later, the rats were anesthetized with ether, and their stomachs were removed and fixed with 2% formalin for 30 min. The amount of hemorrhage on the glandular portion was measured by summing the total length (mm) of each lesion and expressing it as a lesion index. GRb_1 and ranitidine were given orally 30 min prior to administration of the HCl·ethanol solution (Mizui and Dodeuchi, 1983).

Indomethacin-induced gastric lesion

The rats, which were denied food for 24 h with free access to water, were given GRb_1 orally. Thirty minutes later, 35 mg/kg indomethacin suspended in 0.5% CMC was injected subcutaneously. The rats were sacrificed 7 h after the indomethacin injection, and the excised stomach was treated and the lesion index was measured as described above (Suzuki *et al.*, 2000).

Shay ulcer and gastric secretion

The rats, which were fasted for 24 h with free access to water, were immediately administered GRb_1 and ranitidine intraduodenally after pyloric ligation (Shay *et al.*, 1945). The procedure was then divided as described below.

Shay ulcer

Seventeen hours later, the rats were sacrificed and the excised stomachs were treated as described above. Gastric ulcers in the fore-stomach, which were induced by pyloric ligature, were assessed by the following ulcer index according to the severity of the ulcer: **1**, no lesion; **2**, bleeding or light; **3**, moderate; **4**, severe; **5**, perforation.

Gastric secretion

Four hours after pyloric ligation, the rats were sacrificed and the contents of the stomach were collected and centrifuged at 500 g for 10 min. The total gastric juice volume and pH were measured, and the acidity was determined by titration of the gastric juice with 0.05 N NaOH using phenolphthalein as the indicator.

Mucus secretion

After the rats were fasted for 24 h before the experiment with free access to water, absolute EtOH (1 mL/100 g) was given orally 30 min after the oral administration of GRb_1. The rats were sacrificed 1 h later, and the secreted mucus was determined (Kitagawa *et al.* 1986). The glandular portion separated from the excised stomach was opened along the lesser curvature and everted. The stomach was soaked for 2 h in 0.1% alcian blue 8GX dissolved in 0.16 M sucrose buffered with 0.05 M CH_3COONa (adjusted to pH 5.8 with HCl). The mucus combined with the alcian blue was extracted with 20 mL of 70% EtOH containing 30% dioctyl sodium sulfosuccinate and centrifuged for 10 min at 500 g. The optical density of the supernatant was measured at 620 nm.

Histological examination

The stomachs fixed for 48 h with 10% formalin were dehydrated by passage successively through a different alcohol-water mixture (gradually with 50, 80, 95 and finally 100% alcohol), cleared in xylene and embedded in paraffin. Sections (4-5 μm thick) were prepared and then stained with hematoxylin-eosin dye for microscopic observations (×100).

Statistical analysis

All data is presented as mean±S.E. Statistical analyses of the data were performed using a one-way of analysis of variance followed by Student's *t*-test. A $p<0.05$ level was considered significant.

RESULTS

Isolation of active constituents from the butanol fraction

The activity-guided isolation was performed with the animal model of HCl·ethanol-induced gastric lesion in order to isolate the anti-ulcer constituent(s) from the head of *Panax ginseng* (Fig. 2). The EtOH extract (350 g) was then systematically fractionated with hexane (15.0 g), chloroform (27.5 g) and BuOH (115.4 g). The BuOH fraction (100 g) was separated into frs. **1** (0.4 g), **2** (3.1 g), **3** (6.4 g), **4** (12.7 g), **5** (49.9 g) and **6** (27.7 g). Fr. **5** was separated into subfrs. **a** (1.7 g), **b** (6.9 g), **c** (4.5 g), **d** (15.7 g), **e** (3.1 g) and **f** (11.9 g). Subfr. **d** was further separated and the constituents were identified as ginsenosides Re (GRe, 0.52 g), Rc (GRc, 0.91 g) and Rb_1 (GRb_1, 8.06 g) using physicochemical and spectroscopic methods including ^{13}C-NMR, ^1H-NMR and mass analyses. These spectral data were compared to the standards and the data from the literature (Yoshikawa *et al.*, 1994; Meng *et al.*, 2000; Qin *et al.*, 2000).

HCl·ethanol-induced gastric lesion

The effects of the fractions and subfractions on the HCl·ethanol-induced lesion are shown in Table I. Fr. **5** (300 mg/kg), which showed 84.3% inhibition, was further separated by a silica gel column, and six subfractions, **a-f**, were obtained. Subfr. **d** (350 mg/kg), which significantly inhibited the lesion by 89.9%, was then separated into ginsenosides GRe, GRc and GRb_1. Among them, GRb_1 showed 60.6% inhibition of the HCl·ethanol-induced gastric lesion at a dose of 300 mg/kg (b.w.), which was also confirmed by histological examination.

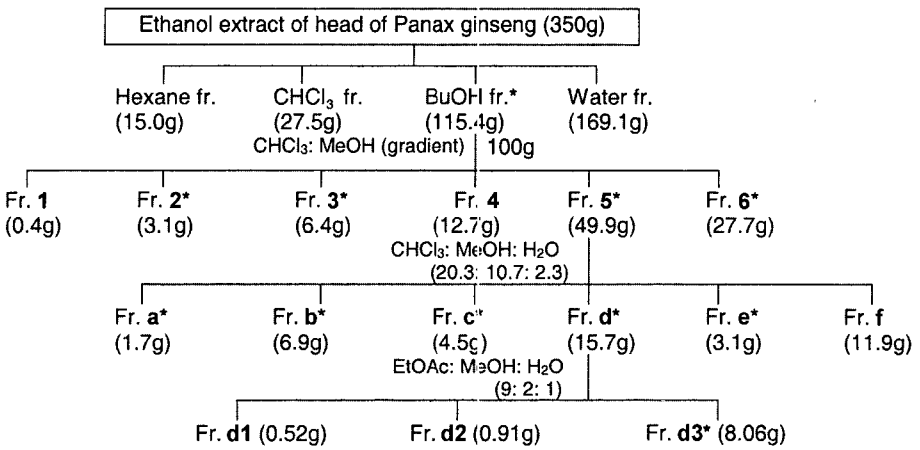

Fig. 2. Isolation scheme of the anti-ulcer constituents from the *Panax ginseng* head. *Significant inhibition ($p<0.05$) of the HCl·ethanol-induced gastric lesion.

Table I. Effects of fractions and subfractions of the butanol fraction of *Panax ginseng* head on HCl·ethanol-induced gastric lesion in rats

Treatment	Fraction No.	Dose (mg/kg)	Lesion index (Mean±S.E.)	Inhibition (%)
Control	–	0	44.7 ±19.8	–
BHPG fraction	**1**	0.03	23.5 ±10.8	47.4
	2	2.7	10.0 ± 2.0*	77.6
	3	5.5	9.3 ± 2.3*	79.2
	4	100	24.8 ± 6.0	44.5
	5	300	7.0 ± 1.3*	84.3
	6	200	9.6 ± 1.0*	78.5
Control	–	0	8.53± 2.66	–
BHPG subfraction	**a**	40	1.65± 0.53*	80.7
	b	150	1.50± 0.37*	82.4
	c	100	1.10± 0.39*	87.1
	d	350	0.86± 0.43*	89.9
	e	70	1.73± 0.70*	79.7
	f	250	5.60± 1.02	34.3
Control	–	–	11.4 ± 1.4	–
ginsenoside Re	**d1**	20	6.2 ± 2.1	45.9
ginsenoside Rc	**d3**	30	9.5 ± 1.7	16.7
ginsenoside Rb_1	**d5**	300	4.5 ± 0.5**	60.6
Ranitidine	–	150	2.5 ± 0.7**	78.1

*p<0.05, **p<0.01, significantly different from the control group. n=6
BHPG : butanol fraction of *Panax ginseng* head.

Shay ulcer and indomethacin-induced gastric lesion

The effect of GRb_1 on the Shay ulcer and the indomethacin-induced models is shown in Table II. In the pylorus-ligated rats, GRb_1 at intraduodenal doses of 150 and 300 mg/kg significantly inhibited the Shay ulcer in the muscular portion by 49.1 and 68.6%, respectively. GRb_1 at a dose of 300 mg/kg also significantly inhibited the indomethacin-nduced gastric lesion in the glandular portion by 54.2%.

Gastric secretion

The effect of GRb_1 on gastric secretion is shown in Table III. In the 4 h pylorus-ligated rats, GRb_1 at doses of 150 and 300 mg/kg slightly increased the pH and reduced the total acid output in a dose-dependent manner. In contrast, ranitidine significantly reduced both the gastric volume and the total acid output.

Table II. Effect of GRb_1 on Shay ulcer and indomethacin-induced ulcer in rats

Treatment	Dose (mg/kg)	Ulcer index (Mean±S.E)	
		Shay ulcer	Indomethacin ulcer
Control	–	5.5± 0.8	4.9± 0.8
GRb_1	150	2.8±0.9* (49.1)	3.2±0.7 (34.2)
	300	1.8±0.4**(68.6)	2.2±0.9*(54.2)
Ranitidine	150	1.3±0.7**(76.4)	3.6±0.8 (26.5)

*P<0.05, **P<0.01, significantly different from the control. n=8
Numbers in parentheses indicate inhibition rate (%).

Mucus secretion

As shown in Table IV, GRb_1 at oral doses of 150 and 300 mg/kg significantly increased the mucus content to 324.0 and 388.4 μg, respectively, compared to 180.2 μg for the control group. In the ranitidine group, the mucus content at 204.5 μg was almost the same as the control group.

Gross observation and histological examination

In the gross observations (Fig. 3), the amount of hemorrhage induced by HCl·ethanol was remarkably reduced,

Table III. Effect of GRb_1 on gastric secretion in pylorus-ligated rats

Treatment	Dose (mg/kg, i.d.)	Volume (mL)	pH	Titratable acidity (mEq/L)	Total Acid Output (mEq)
Control	–	4.72±0.39	1.51±0.03	129.1±11.3	0.60± 0.64
GRb_1	150	4.23±0.51	1.62±0.05	91.4± 4.8	0.39± 0.06
	300	4.78±0.44	1.56±0.02	97.8± 4.9	0.47± 0.05
Ranitidine	150	3.22±0.26	1.71±0.03*	89.6± 4.2*	0.36± 0.07*

The values are expressed as mean±S.E.
*P<0.01, significantly different from the control group. n=8

Table IV. The effect of GRb_1 on mucus secretion in rats

Treatment	Dose (mg/kg, i.d.)	Mucus contents (μg as alcian blue)
Control	–	180.2±14.4
GRb_1	150	324.0±17.2*
	300	388.4±24.6**
Ranitidine	150	204.5±19.2

The values are means±S.E.
*P<0.05, significantly different from the control group. n=8.

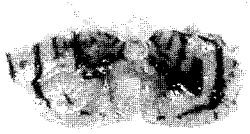

Fig. 3. The protective effect of GRb_1 on HCl·ethanol-induced gastric lesion in rats (macrography). The amount of hemorrhage in the glandular portion of the gastric tissue obtained from the rats treated with 300 mg/kg GRb_1 (right) was dramatically reduced compared to the control (left).

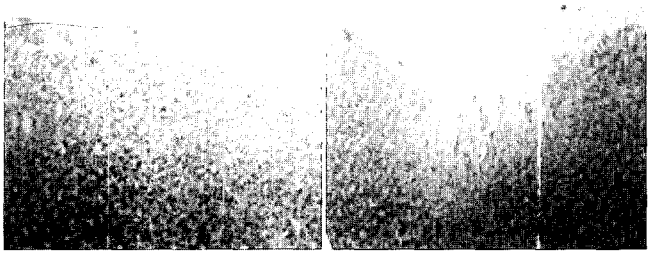

Fig. 4. The protective effect of GRb_1 on the HCl·ethanol-induced gastric lesion in rats (micrography, ×100). The mucosal layer injury of the gastric tissue obtained from the rats treated with 300 mg/kg GRb_1 (right) was almost completely protected compared to the control (left).

and in the GRb_1 treated group the glandular portion which had shown lesions had recovered to almost that of the normal conditions. The histological examination (×100), showed that the mucosal layer of the GRb_1 treated gastric tissue had recovered while the gastric tissue of the control group was severely injured (Fig. 4).

DISCUSSION

This study demonstrated that GRb_1 isolated from the BuOH fraction of *Panax ginseng* head exhibited gastroprotective activity against gastric damages in rats.

It was reported that a HCl·ethanol-induced lesion is produced by the direct irritation of a gastric mucosal barrier (Seiki *et al.*, 1990). A Shay ulcer is generated from the oversecretion of the gastric juices. Indomethacin, a nonsteroidal anti-inflammatory drug, demonstrates its ulcerogenic action by inhibiting the synthesis of PGE_2 which has cyto-protective effect of gastric membrane (Suzuki *et al.*, 2000; Konturek, 1981). The fractions and subfractions from the BuOH fraction of the *Panax ginseng* head, as well as the final ginsenoside product GRb_1, inhibited the ulcerogenesis induced by HCl·ethanol, and the inhibitory effect of GRb_1 was consistent with the gross and histological examinations. Hence, the anti-ulcer effect of the fractions and GRb_1 on the HCl·ethanol-induced gastric lesion might be related to the protection from direct irritation. In contrast, GRb_1 showed better inhibition of the gastric damage induced by the HCl·ethanol and pyloric ligation (Shay ulcer) than that observed in the indomethacin model. The positive control drug, ranitidine, did not show any inhibition of the indomethacin-induced gastric ulcer because ranitidine only antagonizes the H_2 receptor to reduce acid secretion, and is not related to the endogenous PGE_2. This observation is consistent with the findings that have previously been reported (Paiva *et al.*, 1998).

In the gastric secretion study, the effect of GRb_1 differed from that of ranitidine. It has been established that the inhibition of acid secretion is the most important factor for treating gastric ulcers. While ranitidine decreased both the gastric fluid volume and the total acidity in the stomach, GRb_1 enhanced the former but decreased the latter. This suggests that GRb_1 decreased gastric acidity by promoting mucus or bicarbonate secretion. Furthermore, GRb_1 significantly increased the gastric mucus in the EtOH-induced mucus secretion model in rats. In the mucus secretion model, even though EtOH was induced in the rats to reduce the secretion, GRb_1 enhanced it. This finding confirmed that the gastroprotective activity of GRb_1 originated from the stimulation of mucus secretion. However, in order to demonstrate that GRb_1 stimulates the mucus secretion, the degree of mucus secretion was examined to determine if it is stimulated in the absence of EtOH.

Cytoprotection denotes the ability of the prostaglandins, when administered in non-antisecretory doses, to prevent gastric mucosal damage, including the mucosal necrosis evoked in the rat stomach by the various necrotizing agents (Robert *et al.*, 1979). However, it has been shown that cytoprotection is not limited to the role of prostaglandins, but is also shared by other compounds such as sucralfate (Hollander and Tarnawski, 1987). The changes in the lesion-induced stomach tissues are consistent with the reported results of the gastroprotective effect. The anti-ulcer effect of GRb_1 is independent of the changes in the secretory response of gastric acid. Instead, an increase in mucus secretion might be one of the protective actions of the anti-ulcer activity of GRb_1. This study has provided evidence for the cytoprotective action of orally administered GRb_1 on gastric mucosal injuries in rats.

In conclusion, a major gastroprotective component, GRb_1, was isolated by activity-guided isolation, and the gastroprotective effect of this ginsenoside might be attributable to the stimulation of mucus secretion as an elevated protective factor rather than as an inhibition of the various aggressive factors. The intragastrically administered GRb_1 demonstrated beneficial effects on gastric damage. Such cytoprotective activities of GRb_1 on the injured gastric tissue might be used to prevent or treat gastritis and gastric ulcers.

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