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In Vitro Anti-*Helicobacter pylori* Activity of Panaxytriol Isolated from Ginseng

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This study investigated the effect that some polyacetylenes and protopanaxatriol, which were isolated from heated ginseng (family Araliaceae), have on inhibiting *Helicobacter pylori* (HP) growth. Among the compounds tested, panaxytriol was quite effective in inhibiting HP growth with an MIC of 50 μg/ml. Ginsenoside Rh1 and protopanaxatriol weakly inhibited H^+/K^+-ATPase from a rat stomach.

Key words: *Panax ginseng*, Panaxytriol, *Helicobacter pylori*

INTRODUCTION

Helicobacter pylori (HP) was first isolated from the gastric antrum of chronic gastritis patients by Warren and Marshall in 1983 (Warren and Marshall, 1983; Marshall *et al.*, 1990). HP is a risk factor for gastric cancer. Pathogenic HP produces urease, which hydrolyzes urea to ammonia and carbamate. HP urease is considered to play an important role in the pathogenesis of gastritis and peptic ulcers. Therefore, eradicating this bacteria, inhibiting the urease and stomach H^+/K^+ ATPase is important for treating patients with gastroduodenal diseases (Eaton *et al.*, 1991). Some compounds, such as ponciretin, capsaicin, magnolol and decursin, have been isolated from herbal sources and identified as having anti-bacterial properties against HP (Bae *et al.*, 1998; Jones *et al.*, 1997; Bae *et al.*, 1999). However, the inhibitory effect of polyacetylenes and protopanaxatriol isolated from heated ginseng (family Araliaceae) has not been investigated.

Therefore, in this study, the inhibitory effects of some polyacetylenes and protopanaxatriol on the growth and urease activity of HP *in vitro* and on H^+/K^+-ATPase of rat stomach were examined.

MATERIALS AND METHODS

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Materials

The Brucella agar and Brucella broth were purchased from Difco, Co. (U.S.A.). The horse serum and acetohydroxamic acid were purchased from Sigma Chem. Co. (U.S.A.). AnaeroPak Campylo was obtained from Mitsubishi Gas Chemical Co., Inc. (Japan). Panaxytriol, panaxydol, panaxynol, ginsenoside Rh1 and protopanaxatriol were isolated from the HP growth inhibitory fractions of heated ginseng (*Panax ginseng*, Araliaceae) according to previously reported methods (Ahn and Kim, 1988; Baek *et al.*, 1995).

Bacterial strains

The HP ATCC43504 strain was purchased from ATCC. The NCTC11637 and NCTC11638 strains were obtained from NCTC and the HP82516, HP82548 and HP4 strains were clinically isolated from Korean gastroscopic samples. These strains were inoculated into Brucella agar plates supplemented with 7% horse serum and cultured for 3 days at 37°C under microaerophilic conditions (AnaeroPak Campylo: 85% N_2, 10% CO_2 and 5% O_2).

Growth inhibition assay of HP

The HP growth inhibition assay was performed according to a previously described method (Bae *et al.*, 1998). One milliliter of each isolated compound was added to a petri dish containing unsolidified 7 ml bucella agar supplemented with 7% horse serum. Each com-

pound was dissolved at 10 mg/ml with dimethylsulfoxide (DMSO) and diluted with water. The final concentrations of each compound in the medium were 100, 50, 25, 12.5, 6.25, 3.125 and 1.56 μg/ml. The final DMSO concentration in the medium was 1%. Approximately 5×10^5 CFU of HP were inoculated into the agar plates and cultured microaerobically for 3 days at 37°C in an anaerobic jar. The MIC was determined by visually judging the HP growth. Ampicillin was used as a positive control and its final concentrations were 8, 4, 2, 1, 0.5, 0.25 and 0.125 μg/ml. All experiments were conducted in triplicate.

Preparation and assay of HP urease

The HP was inoculated into 30 ml of brucella broth supplemented with 10% fetal bovine serum in a 100 ml flask, which was cultured at 37°C under microaerophilic conditions with constant shaking. The harvested cells were washed with 10 ml of 20 mM phosphate buffer (pH 7.0), sonicated and centrifuged at 5000 × g for 30 min. The supernatant fraction was used for the activity measurement.

The urease activity was determined by the indophenol method (Odake *et al.*, 1994). Acetohydroxamic acid was used as a positive control.

Preparation and assay of rat stomach H^+/K^+-ATPase

Gastric H^+/K^+-ATPase was partially purified from the parietal cell-rich fraction of the male Sprague-Dawley rat (200-250 g) stomach as described by Saccomani and Mukidjam (1987). The rat gastric H^+/K^+-ATPase activity was also determined according to a modification of the method reported by Saccomani and Mukidjam (1987). The enzyme protein (0.2 mg/ml) was preincubated at 37 °C for 30 min in a medium consisting of 10 mM imidazole buffer (pH 7.4) and 1 mM of the isolated compounds (final volume of 0.5 ml). The enzyme reaction was started by adding 0.5 ml of a mixture containing 4 mM $MgCl_2$, 10 mM ATP, 80 mM imidazole buffer (pH 7.4) and 10 mM KCl. The reaction was stopped after 15 min by the addition of 1 ml of ice-cold 24% trichloroacetic acid. The inorganic phosphate formed from ATP hydrolysis was determined by a colorimetric method based on the complex formation of malachite green with phosphomolybdate (van Veldhoven and Mannaerts, 1987).

RESULTS AND DISCUSSION

Ginseng was heated at 121°C for 2 h, extracted with MeOH, then evaporated under a rotary vacuum evaporator. The extract was suspended in water and fractionated stepwisely with ether, ethylacetate and n-butanol. The inhibitory activity of each extracted fraction on HP growth was measured. The ether and ethylacetate fractions exhibited the inhibitory activity on HP growth. Therefore, from ether and ethylacetate fractions, compounds, such as panaxytriol, panaxydol and panaxynol, and ginsenoside Rh1 and protopanaxatriol were isolated, and the inhibitory effect of the polyacetylenes and protopanaxtriol on HP growth was assessed (Table I). Ginsenoside Rh1 and protopanaxatriol did not inhibit HP growth. However, the polyacetylenes inhibited HP growth. Among them, panaxytriol was best with an MIC of 50 μg/ml.

The inhibitory effects of the isolated compounds on HP urease activity and H^+/K^+ ATPase activity were measured (Table II). However, all the tested compounds except ginsenoside Rh1 and 20(S)-protopanaxadiol did not inhibit these enzymes. Ginsenoside Rh1 and 20(S)-protopanaxadiol only weakly inhibited H^+/K^+ ATPase at 1 mM. The eradication of HP has been known to cure gastritis and prevent a relapse of a duodenal ulcer. Therefore, antibiotics, such as ampicillin and tetracyclines, have normally been used. However, the occurrence of pathogens resistant to these antibiotics and their side effects, means that there is a need for other effective drugs (Goodwin, 1993). Compared to these antibiotics (MIC of ampicillin, 0.5 - 2 μg/ml), panaxytriol isolated from heated ginseng showed weak inhibitory effects on HP growth at a one-order higher concentration. In addition, ginseng is

Table I. Inhibitory effects of the polyacetylenes and protopanaxatriols on the growth of *Helicobacter pylori*

Compound	MIC (μg/ml)			
	HP ATCC43504	HP NCTC11638	HP 82516	HP 4
Panaxynol	>100	>100	>100	100
Panaxydol	>100	>100	>100	>100
Panaxytriol	50	50	50	50
Ginsenoside Rh1	>100	>100	>100	>100
Protopanaxatriol	>100	>100	>100	>100
Ampicillin	1	0.5	1	2

Table II. Inhibitory effects of the polyacetylenes and protopanaxatriols on HP Urease and rat stomach H^+/K^+ ATPase

Compound[a]	Inhibition (%)	
	HP urease	Stomach H^+/K^+ATPase
Panaxynol	0	0
Panaxydol	0	0
Panaxytriol	0	0
Ginsenoside Rh1	5	15
Protopanaxatriol	2	21
Acetohydroxamic acid	96	-
Omeprazole	-[b]	94

[a]Final concentration was 1 mM.
[b]Was not determined.

not toxic and is used frequently as a food. The concentration of active panaxytriol against HP is higher in red ginseng (heated ginseng) than in the white variety (Kim, 1988). Furthermore, Kim *et al.* (1998) reported that red ginseng protects against stomach uclers. Based on these findings, red ginseng (heated ginseng) may cure gastritis and prevent the relapse of a duodenal ulcer, even if they are not potent H^+/K^+-ATPase inhibitors in a rat stomach.

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