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Inhibitory Effect of Ginseng Polyacetylenes on Infection and Vacuolation of *Helicobacter pylori*

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Abstract – Polyacetylenes were isolated from *Panax ginseng* C.A. Meyer (Family Araliaceae), and their inhibitory effects on growth, infection and VacA vacuolation of *Helicobacter pylori* (HP) were investigated. Ginseng polyacetylenes did not inhibit the infection of HP into KATO cells. However, polyacetylenes inhibited HP growth and vacuolation of HeLa by VacA toxin. Panaxytriol showed the most potent inhibition with IC_{50} values of 0.05 and 0.046 mg/ml, respectively.
Keywords – *Panax ginseng*, polyacetylenes, panaxytriol, *Helicobacter pylori*, VacA toxin.

Introduction

Ginseng (the root of *Panax ginseng* C.A. Meyer, Family Araliaceae) is frequently used as a crude substance and taken orally in Asian countries as a traditional medicine. The major components of ginseng are ginsenosides, polyacetylenes, and polysaccharides (Tanaka *et al.*, 1972; Kim *et al.*, 1990). Ginsenosides and polyacetylenes have been reported to show various biological activities including anti-inflammatory activity, anti-*Helicobacter pylori* and anti-tumor effects (inhibition of tumor-induced angiogenesis and the prevention of tumor invasion and metastasis) (Ahn and Kim, 1988; Bae *et al.*, 2002; Lee *et al.*, 1999; Wakabayashi *et al.*, 1998; Wu *et al.*, 1992). Ginseng polysaccharides have been reported to exhibit various biological activities including anti-tumor effects and anti-hemagglutination induced by HP (Belogortseva *et al.*, 2000; Kim *et al.*, 1990). However, the effect of ginseng polyacetylenes on infection and vacuolation of *Helicobacter pylori* (HP) have not been studied.

Therefore, we examined *in vitro* inhibitory effects of ginseng polyacetylenes on the growth, infection and cell vacuolation of HP

Experimentals

Materials – Bacto Agar and Brucella broth were purchased from Difco Laboratories (USA). Fetal bovine serum (FBS) and Antibiotic-antimycological solution were obtained from Gibco BRL. Cell culture medium, neutral red and horse serum were acquired from Sigma Chemical Co. (USA). AnaeroPack Campylo was from Mitsubishi Gas Chemical Co., Inc. (Japan). Ginseng polyacetylenes were isolated according to our previous methods (Bae *et al.*, 2001).

Bacterial strains and isolation of VacA cytotoxin from HP – *H. pylori* strain ATCC 49503 and ATCC 43504 was purchased from American Type Culture Collection (USA). They were inoculated into Brucella agar plates supplemented with 7% horse serum and transferred into Brucella broth containing 10% FBS after 3 days. The bacteria were cultured for further 3 days at 37°C in a thermostatic rotary shaker under microaerophilic conditions (AnaeroPack Campylo: 85% N_2, 10% CO_2 and 5% O_2).

Vacuolation assay was purified according to the modified method of Cover and Blaser(1992). HP (ATCC 49503) was used as the source for toxin purification. HP was cultured for 48 h at 37°C in Brucella broth containing 5% FBS in an ambient atmosphere containing 5% oxygen. The culture was centrifuged at 16000×g for 20 min, and protein present in the supernatant were precipitated with a 50% saturated ammonium sulfate. After centrifugation at 16000×g for 15 min, the pellet was resuspended in 60 mM Tris-HCl (pH 7.5). Hydrophobic interactive chromatography was performed on Butyl-toyoperal column (1×5 cm) with the same buffer containing 0.6 M ammonium sulfate and eluted with the same buffer containing 0.4 M ammonium

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sulfate. The vacuolation-active fractions were dialyzed against the same buffer.

Growth inhibition assay of HP – Growth inhibition assay of HP ATCC 43504 was performed according to the previous method (Bae *et al.*, 2001). Ampicillin was used as a standard compound.

HP urease and H^+/K^+ ATPase Inhibition Assay – HP urease- and H^+/K^+-ATPase-inhibitory activities were assayed according to the previous method (Bae *et al.*, 2002). Acetohydroxamic acid and omeprazole were used as standard compounds, respectively.

Assay of HP vacuolation-inhibitory activity (neutral red uptake assay) – HeLa cells were cultured as a monolayer in plastic flasks in Dulbeccos modified Eagle's medium (DMEM) containing 10% FBS, 1% antibiotic-antimycological solution and 3.5 g/L sodium bicarbonate under 5% CO_2 at 37°C. Attached cells were released with trypsin/EDTA and seeded at a density of 7.0×10^3 cells / well in 96-well tissue culture plates one day before experiments.

Inhibitory effect of ginseng polyacetylenes on VacA vacuolation in HeLa cells was measured by neutral red uptake assay (Cover *et al.*, 1991). Briefly, seeded HeLa cells were incubated for 16 h with VacA toxin (0.05 mg) and serial dilutions of samples in a microtiter assay. To detect the vacuoles, cells were incubated for 8 min at a room temperature with 100 μl of 0.05% neutral red in PBS and washed twice with 0.9% NaCl containing 0.1% BSA. After the addition of 100 μl of acidified ethanol solution (70% ethanol, 0.36% HCl), the optical density of extracted neutral red was measured at 540 nm using a microtiter plate reader (Molecular Devices). All assays were performed in triplicate.

Assay of HP Infection-inhibitory activity – KATO III cells were cultured in RPMI 1640 medium supplemented with 10% FBS, 1% antibiotic-antimycological solution and 2.2 g/L sodium bicarbonate under 5% CO_2 at 37°C. The cells were harvested with trypsin/EDTA for bacterial adhesion experiment (Kamisago *et al.*, 1996). Serial dilutions of polyacetylenes were incubated with the equal volume of *H. pylori* suspension in phosphate-buffered saline (PBS) for 30 min in a 37°C water bath and mixed with KATO III cells (5.0×10^6 cells/ml). After further 1 h incubation, incubation mixture was loaded on 15% sucrose, centrifuged and washed once in PBS. Subsequently, urease activity of the precipitated cells was determined by measuring the amount of ammonia released from urea in the phenol-hypochlorite urease assay as previously described (Weatherburn, 1967).

Results and Discussion

Ginsengs are classified into ginseng, red ginseng and steamed ginseng according to the temperature pretreated to make ginseng manufactures. These ginsengs contain the different kinds and contents of saponins. Therefore, the biological activities are different depending on the contents of saponins and polyacetylenes. For example, heated ginseng contains higher contents of ginsenoside Rg3, which is vasorelaxant (Kim *et al.*, 2000; Kim *et al.*, 1999). Red contains higher contents of panaxytriol (Ahn and Kim, 1988). To evaluate anti-*Helicobacter pylori* activity of the processed ginsengs, the effects of ginseng polyacetylenes isolated from ginseng on growth, infection and vacuolation of HP were investigated (Table 1). Poylacetylenes did not inhibit the infection of HP into KATO cells. However, these compounds inhibited HP growth and vacuolation of HeLa cells by VacA toxin. Panaxytriol, which is a major polyacetylene of red ginseng, exhibited the most potent inhibition with IC_{50} values of 0.05 and 0.046 mg/ml (Fig. 1). However, polyacetylenes did not inhibited H^+/K^+ ATPase of rat stomach as well as HP urease (Table 2).

Ginseng contains polysaccharides and protopanaxadiol except polyacetylenes. These components inhibited HP growth, infection into Kato III cells or VacA vacuolation of HeLa cells, although polysaccharides did not exhibit all HP-inhibitory activities. We previously reported that 20(*S*)-ginsenosides Rg3 and Rh2 inhibited H^+/K^+ ATPase of rat stomach and 20(S)-protopanaxadiol isolated from ginseng inhibited HP growth (Bae *et al.*, 2001; 2002). Therefore, we believe that combined components of ginseng could cure synergistically gastritis induced by *H. pylori* and prevent the relapse of duodenal ulcer as a folk medicine.

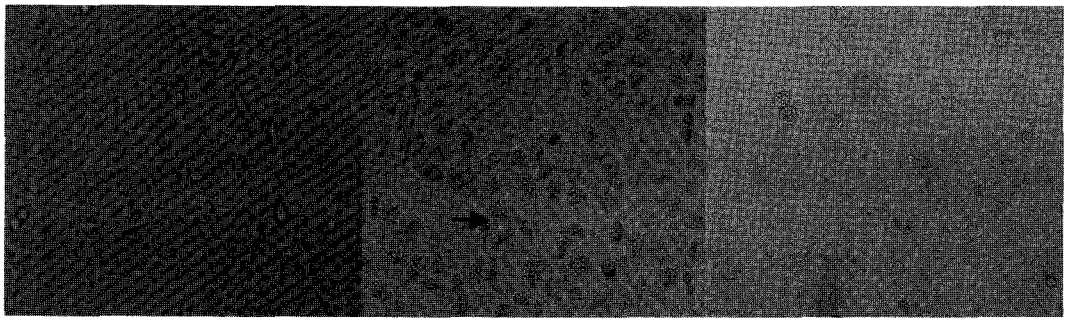
Table I. Inhibitory Effects of Polyacetylenes on Growth, Infection and Vacuolation of *Helicobacter pylori*

Agent	MIC[a] (μg/ml)	IC_{50} (mg/ml)	
		Infection	VacA Vacuolation
Panaxynol	>100	>0.2	0.098
Panaxydol	>100	>02	0.088
Panaxytriol	50	>0.2	0.046
Ampicillin	1	–[b]	–

[a]HP ATCC 43504 was used fro MIC assay.
[b]not determined

Table 2. Inhibitory Effects of Polyacetylenes on HP Urease and Rat Stomach H^+/K^+ ATPase

Compound	IC_{50} (mg/ml)	
	HP urease	Stomach H^+/K^+ ATPase
Panaxynol	>1	>1
Panaxydol	>1	>1
Panaxytriol	>1	>1
Acetohydroxamic acid	0.18	–
Omeprazole	–	0.21



(A) (B) (C)

Fig. 1. Inhibition of Panaxytriol on VacA-induced Vacuolation in HeLa cells. HeLa cells were cultured in DMEM containing 10% FBS. VacA toxin (0.05 mg) and sample (0.05 mg/ml) were treated, and then incubated 16 h. (A), treated with saline alone; (B), treated with VacA toxin alone; (C), treated with panxaytriol and VacA toxin. Arrow indicates vacuolation in HeLa cells.

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