J. Microbiol. Biotechnol. (2003), **13**(5), 706–709

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Inhibitory Effect of Ginseng Saponins and Polysaccharides on Infection and Vacuolation of *Helicobacter pylori*

KIM, JONG-MI[1], JI-EUN SHIN[2], MYUNG JOO HAN[2], SUNG-HWAN PARK[1], AND DONG-HYUN KIM[1]*

[1]College of Pharmacy, and [2]Department of Food and Nutrition, Kyung Hee University, Seoul 130-701, Korea

Received: January 28, 2003
Accepted: July 15, 2003

Abstract Ginsenosides and polysaccharides were isolated from *Panax ginseng* C.A. Meyer (Family Araliaceae) by treating at low (60°C, LT), mild (100°C, MT), and high (120°C, HT) temperatures, and their inhibitory effects on growth, infection, and VacA vacuolation of *Helicobacter pylori* (HP) were investigated. The molecular weights of polysaccharides decreased as the processing temperature increased. Ginseng polysaccharides inhibited the HP infection into KATO III cells, but did not inhibit growth of HP and VacA vacuolation of HeLa cells. HT polysaccharides showed the most potent inhibition with IC_{50} value of 6.8 mg/ml. Ginseng saponins did not inhibit the infection of HP into KATO cells. However, 20(s)-protopanaxadiol showed the most potent inhibition of HP growth and vacuolation of HeLa by VacA toxin with IC_{50} values of 0.05 and 0.067 mg/ml, respectively.

Key words: *Panax ginseng*, polysaccharide, ginsenoside, *Helicobacter pylori*, infection, VacA toxin

Helicobacter pylori (HP) was isolated from the gastric antrum of chronic gastritis patients by Warren and Marshall in 1983 [22]. Pathogenic HP produces urease and strongly vacuolating toxin. HP urease hydrolyzes urea to CO_2 and ammonia. This ammonia generated by HP protects itself from the environment of gastric acid in the stomach, and directly damages the gastric mucosal cell. HP vacuolating toxin (VacA toxin) may be potentiated by the urease-mediated ammonia production [8, 10, 20]. Therefore, the inhibitions of HP growth, infection, urease, and VacA toxin vacuolation are important for treating patients with gastritis and peptic ulcer.

Ginseng (the root of *Panax ginseng* C.A. Meyer, Family Araliaceae) is frequently used as a traditional medicine taken orally in Asian countries. The major components of ginseng are ginsenosides, which contain an aglycone with a dammarane skeleton and polysaccharides [14, 18, 19]. Ginsenosides have been reported to show various biological activities, including anti-inflammatory activity, and anti-*Helicobacter pylori* and antitumor effects (inhibition of tumor-induced angiogenesis and prevention of tumor invasion and metastasis) [4, 5, 17, 21, 23]. Ginseng polysaccharides have been reported to exhibit various biological activities, including antitumor effects and anti-hemagglutination induced by HP [7, 14]. However, studies conducted on the effect of ginseng saponins together with polysaccharides and ginsenosides on infection and vacuolation of *Helicobacter pylori* (HP) have yet to be completed. Therefore, we examined *in vitro* inhibitory effects of ginseng saponins and polysaccharides on the growth, infection and vacuolation of HP.

*Corresponding author
Phone: 82-2-961-0374; Fax: 82-2-966-3885;
E-mail: dhkim@khu.ac.kr

Materials and Methods

Materials

Bacto Agar and Brucella broth were purchased from Difco Laboratories (U.S.A.). Fetal bovine serum (FBS) and antibiotic-antimycological solution were obtained from Gibco BRL. Cell culture medium, neutral red, and horse serum were from Sigma Chemical Co. (U.S.A.), and AnaeroPak Campylo was from Mitsubishi Gas Chemical Co., Inc. (Japan). Ginsenosides were isolated according to the methods described previously [2, 4, 6].

Extraction of Polysaccharide

The powdered white ginseng (1 kg) prepared from the roots of *Panax ginseng* C.A. Meyer (Family Araliaceae) was treated at low (60°C, LT), mild (100°C, MT), and high (120°C, HT) temperatures for 2 h, and extracted three times with aqueous 85% methanol. The remaining residues were extracted twice with 5 l of water at 60°C for 2 h. Each extract was concentrated to a suitable volume and then

dialyzed against water for 7 days. It was centrifuged to remove insoluble materials and precipitated with 5 volumes of ethanol. The resulting precipitates were freeze-dried and named LT, MT, and HT polysaccharides, respectively. Their average molecular weights were determined by Sephadex G-75 to be >100-70, 100-70, and 80-60 kDa, respectively.

Bacterial Strains and Isolation of VacA Cytotoxin from HP

H. pylori strain ATCC 49503 and ATCC 43504 were purchased from the American Type Culture Collection (U.S.A.). 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 further for 3 days at 37°C in a thermostatic rotary shaker under microaerophilic conditions (AnaeroPak Campylo: 85% N_2, 10% CO_2, and 5% O_2).

Vacuolating cytotoxin (VacA) was purified according to the modified method of Cover and Blaser [9]. *H. pylori* 60190 (ATCC 49503) was used as the source of toxin purification. HP was cultured for 72 h at 37°C in Brucella broth, containing 10% FBS, at an ambient atmosphere with 5% oxygen. The culture was centrifuged at 6,000 ×g for 30 min, and proteins in the supernatant were precipitated with 50% saturated ammonium sulfate solution. After centrifugation at 10,000 ×g for 40 min, the pellet was resuspended in 60 mM Tris (pH 7.5). Hydrophobic interactive chromatography was performed on butyl-toyopearl column (7×5 cm) with the same buffer containing 0.6 M ammonium sulfate and eluted with the same buffer containing 0.4 M ammonium 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 method described previously [1].

Assay of HP Vacuolation-Inhibitory Activity (Neutral Red Uptake Assay)

HeLa cells were cultured as a monolayer in plastic flasks in Dulbecco's 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 saponins and polysaccharides on VacA vacuolation in HeLa cells was measured by neutral red uptake assay [8]. Briefly, seeded HeLa cells were incubated for 16 h with VacA (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 phosphate-buffered saline (PBS) and washed twice with 0.9% NaCl containing 0.1% BSA. After adding 100 µl of acidified ethanol solution (70% ethanol, 0.36% HCl), the optical density of extracted neutral red was measured at 540 nm by 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 [13]. Serially diluted samples were incubated with an equal volume of *H. pylori* suspension in PBS for 30 min in a 37°C water bath and mixed with KATO III cells (5.0×10^6 cells/ml). After incubation for one hour, the 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 [9].

RESULTS AND DISCUSSION

Ginsengs are classified into ginseng, red ginseng, and heated ginseng according to the temperature pretreatment to manufacture ginseng. These ginsengs contain different kinds and contents of saponins. Therefore, the biological activities are different depending on the contents of saponins. For example, heated ginseng contains higher contents of ginsenoside R_{g3}, which is vasorelaxant [15, 16]. On the other hand, the difference of molecular weight and biological activity of polysaccharides due to processing temperatures has not been studied. Therefore, we isolated polysaccharides from ginsengs treated at 60°C (LT), 100°C (MT), and 120°C (HT), and measured the molecular weight of polysaccharides by applying the gel filtration process. The molecular weight of polysaccharides was decreased as the processing temperature increased; however, the differences were not

Table 1. Inhibitory effects of ginseng polysaccharides on the growth, infection, and vacuolation of *Helicobacter pylori*.

Agent	MIC[a] (mg/ml)	IC_{50} (mg/ml)	
		Infection	VacA vacuolation
LT polysaccharide	>0.4	17.6	>0.4
MT polysaccharide	>0.4	9.1	>0.4
HT polysaccharide	>0.4	6.8	>0.4
Ampicillin	0.001	-[b]	-
Heparin	-	4.4	-

[a]HP ATCC 43504 was used for MIC assay.
[b]Not determined.

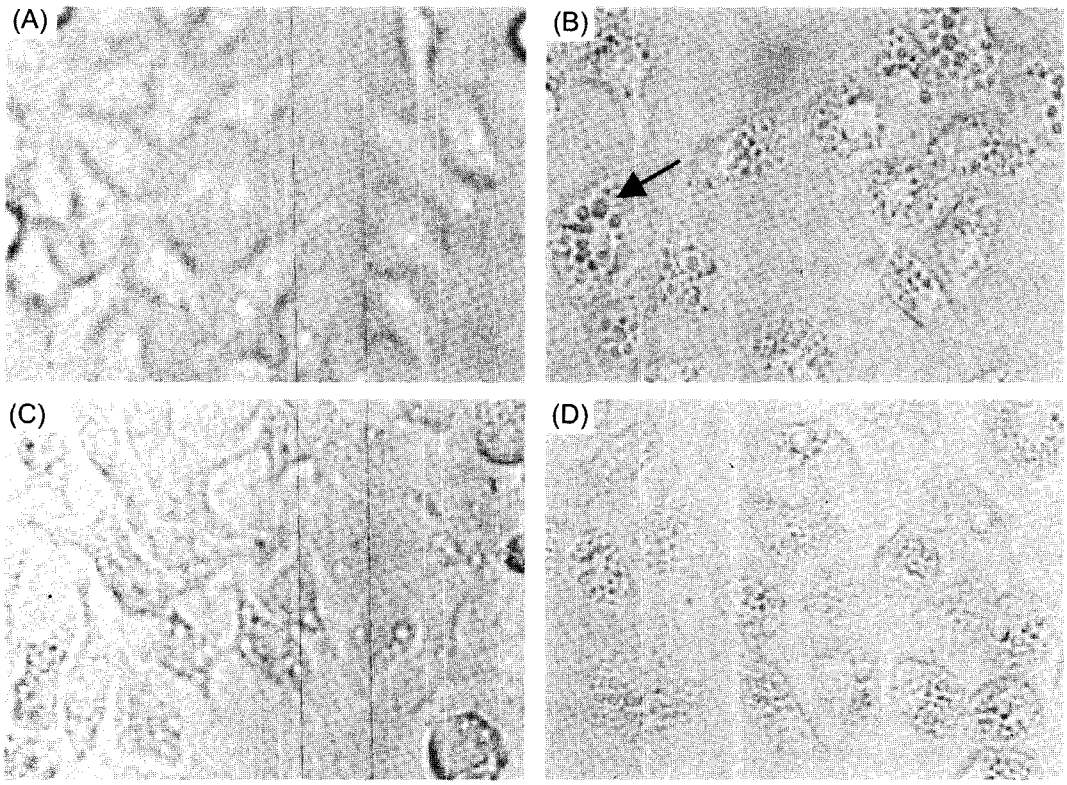

Fig. 1. Inhibition of 20(*S*)-protopanaxadiol and MT polysaccharide on VacA-induced vacuolation in HeLa cells.
HeLa cells were cultured in DMEM containing 10% FBS. VacA toxin (0.05 mg) and sample (0.25 mg/ml) were treated, and then incubated for 16 h. (A), Treated with medium alone; (B) treated with VacA toxin alone; (C), treated with 20(*S*)-protopanaxadiol and VacA toxin; (D), treated with ginseng polysaccharide (MT) and VacA toxin. Arrow indicates vacuolations in HeLa cells.

significant. The average molecular weights of LT, MT, and HT were determined to be >100-70, 100-70, and 80-60 kDa by Sephadex G-75, respectively (data not shown). Therefore, we investigated the effects of polysaccharides isolated from various ginsengs on HP growth, infection, and vacuolation (Table 1). All LT, MT, and HT polysaccharides did not inhibit HP growth or vacuolation of HeLa cells by VacA toxin. However, these polysaccharides inhibited the HP infection into KATO III cells. HT polysaccharides showed the most potent inhibition with an IC_{50} value of 6.8 mg/ml.

The effects of ginseng saponins isolated from various ginsengs on HP growth, infection, and vacuolation were investigated (Table 2). Most ginseng saponins did not

Table 2. Inhibitory effects of ginseng saponins on the growth, infection, and vacuolation of *Helicobacter pylori*.

Agent	MIC[a] (mg/ml)	IC_{50} (mg/ml)	
		Infection	VacA vacuolation
Ginsenoside R_{b1}	>0.1	>0.2	>0.1
20(*S*)-Protopanaxatriol	>0.1	>0.2	0.075
Ginsenoside R_{b1}	>0.1	>0.2	>0.1
Ginsenoside R_d	>0.1	>0.2	>0.1
20(*S*)-Ginsenoside R_{g3}	>0.1	>0.2	>0.1
20(*S*)-Ginsenoside R_{b2}	>0.1	>0.2	>0.1
Compound K	>0.1	>0.2	>0.1
20(*S*)-Protopanaxadiol	0.05	>0.2	0.067
Ampicillin	0.001	-[b]	-

[a]HP ATCC 43504 was used for MIC assay.
[b]Not determined.

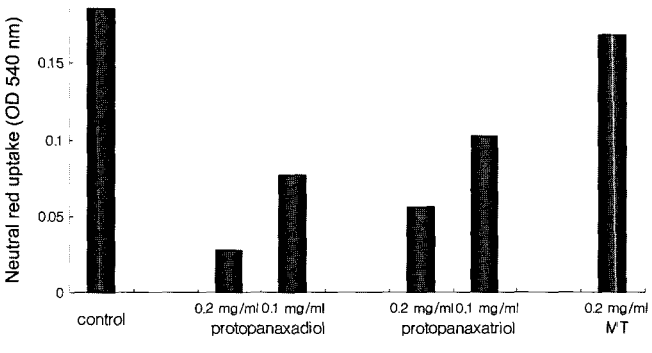

Fig. 2. Inhibitory effects of 20(*S*)-protopanaxadiol and 20(*S*)-protopanaxatriol on neutral red dye uptake by VacA-treated HeLa cells.
HeLa cells were incubated with VacA toxin and test compounds, and dyed with 0.05% neutral red solution. Sample materials were treated at the concentration of 0.2 and 0.1 mg/ml and performed in triplicate.

inhibit the infection of HP into KATO cells. However, 20(S)-protopanaxadiol inhibited HP growth and vacuolation of HeLa cells by VacA toxin with IC_{50} values of 0.05 and 0.067 mg/ml, respectively (Table 2). Furthermore, cell vacuolation was also inhibited by 20(S)-protopanaxatriol (Table 2, Fig. 1).

Ginseng contains polysaccharides and protopanaxadiol. Some components inhibited HP growth, infection into Kato III cells, or VacA vacuolation of HeLa cells, although a single type of ginsenosides, polyacetylenes, or polysaccharides did not exhibit all HP-inhibitory activities. We previously reported that 20(*S*)-ginsenosides R_{g3} and R_{b2} inhibited H^+/K^+ ATPase of rat stomach, and polyacetylenes isolated from ginseng inhibited HP growth [3, 4]. Therefore, we suggest that a combination of ginseng components could synergistically cure gastritis induced by *H. pylori* and prevent the relapse of duodenal ulcer.

Acknowledgment

This work was supported by financial support received from the Korea Science and Engineering Foundation (R01-2002-00220).

REFERENCES

1. Bae, E. A., M. J. Han, N. J. Kim, and D. H. Kim. 1998. Anti-*Helicobacter pylori* activity of herbal medicines. *Biol. Pharm. Bull.* **21:** 990-992.
2. Bae, E. A., S. Y. Park, and D. H. Kim. 2000. Constitutive β-glucosidases hydrolyzing ginsenoside R_{b1} and R_{b2} from human intestinal bacteria. *Biol. Pharm. Bull.* **23:** 1481-1485.
3. Bae, E. A., M. J. Han, N. I. Baek, and D. H. Kim. 2001. *In vitro* anti-*Helicobacter pylori* activity of panaxytriol isolated from ginseng. *Arch. Pharm. Res.* **24:** 297-299.
4. Bae, E. A., M. J. Han, M. K. Choo, S. Y. Park, and D. H. Kim. 2002. Metabolism of 20(*S*)- and 20(*R*)-ginsenoside R_{g3} by human intestinal bacteria and its relation to *in vitro* biological activities. *Biol. Pharm. Bull.* **25:** 58-63.
5. Bae, E. A., N. Y. Kim, M. J. Han, and D.-H. Kim. 2003. Transformation of ginsenosides to compound K (IH-901) by lactic acid bacteria of human intestine. *J. Microbiol. Biotechnol.* **13:** 9-14.
6. Bae, E. A., M. K. Choo, E. K. Park, H. Y. Shin, and D. H. Kim. 2002. Metabolism of ginsenoside R_c by human intestinal bacteria and its related antiallergic activity. *Biol. Pharm. Bull.* **25:** 743-747.
7. Belogortseva, N. I., J. Y. Yoon, and K. H. Kim. 2002. Inhibition of *Helicobacter pylori* hemagglutination by polysaccharide fractions from roots of *Panax ginseng*. *Planta Med.* **66:** 217-220.
8. Cover, T. L., W. Puryear, G. I. Perez-Perez, and M. J. Blaser. 1991. Effect of urease on HeLa cell vacuolation induced by *Helicobacter pylori* cytotoxin. *Infect. Immun.* **59:** 1264-1270.
9. Cover, T. L. and M. J. Blaser. 1992. Purification and characterization of the vacuolating toxin from *Helicobacter pylori*. *J. Biol. Chem.* **267:** 10570-10575.
10. Eaton, K. A., C. L. Brooks, D. R. Morgan, and S. Krakowka. 1991. Essential role of urease in pathogenesis of gastritis induced by *Helicobacter pylori* in gnotobiotic piglets. *Infect. Immun.* **59:** 2470-2475.
11. Gang, J. G., S. K. Yun, K. M. Choi, W. J. Lim, J. K. Park, and S. Y. Whang. 2001. Significance of urease distribution across *Helicobacter pylori* membrane. *J. Microbiol. Biotechnol.* **11:** 317-325.
12. Gutmann, I. and H. U. Bergmeyer. 1974. Urea, pp. 1791-1794. *In* H. U. Mergmeyer (ed.), *Methods of Enzymatic Analysis*: vol. 4. Academic Press, Inc., New York, U.S.A.
13. Kamisago, S., M. Iwamori, T. Tai, K. Mitamura, Y. Yazaki, and K. Sugano. 1996. Role of sulfatides in adhesion of *Helicobacter pylori* to gastric cancer cells. *Infect. Immun.* **64:** 624-628.
14. Kim, Y. S., K. S. Kang, and S. I. Kim. 1990. Study on antitumor and immunomodulating activities of polysaccharide fractions from *Panax ginseng*: Comparison of effects of neutral and acidic polysaccharide fraction. *Arch. Pharm. Res.* **13:** 330-337
15. Kim, N. D., S. Y. Kang, J. H. Park, and V. B. Schini-Kirth. 1999. Ginsenoside R_{g3} mediates endothelium-dependent relaxation in response to ginsenosides in rat aorta: Role of K^+ channels. *Eur. J. Pharmacol.* **367:** 41-49.
16. Kim, W. Y., J. M. Kim, S. B. Han, S. K. Lee, N. D. Kim, M. K. Park, J. K. Kim, and J. H. Park. 2000. Steaming of ginseng at high temperature enhances biological activity. *J. Nat. Prod.* **63:** 1702-1704.
17. Lee, S. J., J. H. Sung, S. J. Lee, C. K. Moon, and B. H. Lee. 1999. Antitumor activity of a novel ginseng saponin metabolite in human pulmonary adenocarcinoma cells resistant to cisplatin. *Cancer Lett.* **144:** 39-43.
18. Lee, J.-C., K. Na, J.-M. Yun, and J.-K. Hwang. 2001. *In vitro* Bifidodogenic effect of nondigestible oligosaccharides isolated from red ginseng Marc. *J. Microbiol. Biotechnol.* **11:** 858-862.
19. Tanaka, N., O. Tanaka, and S. Shibata. 1972. Chemical studies on the oriental plant drugs. XXVIII. Saponins and sapogenins of ginseng; Stereochemistry of sapogenin of ginsenoside Rb1, Rb2 and Rc. *Chem. Pharm. Bull.* **20:** 1212-1216.
20. Tsujii, M., N. Kawano, S. Tsujii, H. Fusamoto, T. Kamada, and N. Sato. 1992. Mechanism of gastric mucosal damage induced by ammonia. *Gastroenterology* **102:** 1881-1888.
21. Wakabayashi, C., H. Hasegawa, J. Murata, and I. Saiki. 1998. *In vivo* antimetastatic action of ginseng protopanaxadiol saponins is based on their intestinal bacterial metabolites after oral administration. *Oncol. Res.* **9:** 411-417.
22. Warren, J. R. and B. J. Marshall. 1983. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. *Lancet* **1:** 1273-1275.
23. Wu, J. Y., B. H. Gardner, C. I. Murphy, J. R. Seals, C. R. Kensil, J. Recchia, G. A. Beltz, G. W. Newman, and M. J. Newman. 1992. Saponin adjuvant enhancement of antigen-specific immune responses to an experimental HIV-1 vaccine. *J. Immunol.* **148:** 1519-1525.