J. Microbiol. Biotechnol. (2004), **14**(1), 202–204

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Inhibitory Effect of Ginseng Polysaccharides on Rotavirus Infection

BAE, EUN-AH, JI-EUN SHIN, SUNG-HWAN PARK, AND DONG-HYUN KIM*

College of Pharmacy, Kyung Hee University, Seoul 130-701, Korea

Received: July 15, 2003
Accepted: October 21, 2003

Abstract Polysaccharides and saponins were isolated from the root of *Panax ginseng* C.A. Meyer (Family Araliaceae), treated at various temperatures, and their inhibitory effects on rotavirus were investigated. As the temperature of processing increased, the molecular weight of the polysaccharides decreased, but the yields of water extracted increased. These polysaccharides inhibited rotavirus infection in MA104 cells, but there were no significant differences in rotavirus infection-inhibitory potency. However, ginseng saponins did not exhibit rotavirus infection-inhibitory activity.

Key words: *Panax ginseng*, polysaccharide, ginsenoside, rotavirus

Rotavirus is the most important etiological cause of severe diarrhea in infants and young children in developed as well as developing countries, being responsible for approximately 35–50% of such illness [9, 11, 16, 22]. To prevent rotaviral diarrhea, many kinds of orally administered vaccines against each of the epidemiologically important serotypes have been developed [8, 10]. However, the vaccines developed were not completely effective in preventing rotaviral diarrhea until now. Recent therapeutic approaches in the treatment of rotavirus diarrhea include the introduction of oral rehydration solutions within 4–6 h of infection. Alternative therapies have not been developed.

Ginseng (the root of *Panax ginseng* C.A. Meyer, Araliaceae) is frequently used as a crude substance taken orally in Asian countries as a traditional medicine. The major components of ginseng are ginsenosides, which contain an aglycone with a dammarane skeleton and polysaccharides [12, 20]. Ginsenosides have been reported to show various biological activities including antiallergic [5], anti-*Helicobacter pylori* [2], and antitumor effects (inhibition of tumor-induced angiogenesis and the prevention of tumor invasion and metastasis) [6, 17, 21]. Ginseng polysaccharides have been reported to exhibit various biological activities including bifidogenic [18], antitumor [2], and anti-HP-induced hemagglutination effects [7]. However, the effect of ginseng polysaccharides on infection by rotavirus has not been studied. Therefore, this study was undertaken to examine the *in vitro* inhibitory effects of ginseng polysaccharides and ginsenosides on the infection of MA104 cells by rotavirus.

The SA11 virus (simian rotavirus) was purchased from Korea National Institute of Health, Korea. Macaccus Rhesus monkey kidney cells (MA-104) and Wa virus (a wild-type of human rotavirus) were kindly donated by Toyama Institute of Health, Japan. Dulbecco's modified Eagle's medium (DMEM) and phosphate buffered saline (PBS) were purchased from Sigma Co. (U.S.A.). Antibiotics-antimycotics, trypsin-EDTA (×10), trypsin (1:250), and fetal bovine serum (FBS) were from Gibco Co. (U.S.A.). Ginsenosides were isolated according to our previously published methods [4–6].

Polysaccharides were isolated as follows. One-kilogram of dried white ginseng roots (5-year-old *Panax ginseng* C.A. Meyer) was powdered, the fat removed by extracting with petroleum ether, and saponins were excluded by extracting them three times with aqueous 85% methanol [12, 18]. The remaining residues were extracted twice with 5 l of water at 60°C, 100°C, or 120°C for 2 h. Each extract was concentrated to a suitable volume and dialyzed against water for 7 days. It was then centrifuged to remove insoluble materials, and precipitated with 5 volumes of ethanol. The resulting precipitates were freeze-dried and named LT (fraction extracted at 60°C), MT (fraction extracted at 100°C), and HT polysaccharides (fraction extracted at 120°C), respectively.

To measure the rotavirus infection-inhibitory activity of ginseng saponins and polysaccharides, MA104 cells were cultured in DMEM containing 10% FBS, 1% antibiotic-antimycological solution, and 3.5 g/l sodium bicarbonate under 5% CO_2 at 37°C according to the previously published methods [1, 3, 19]. To prepare the active rotavirus, rotavirus strains Wa and SA11 (400 μl) were activated with

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

20 μl of 0.1 mg of trypsin per ml at 37°C for 0.5 h. A monolayer of washed MA104 cells (2.0×10^6 cells/25 cm^2 flask) was inoculated with the activated rotavirus, incubated for 1 h, and then aspirated. After the inoculum was removed and serum-free medium containing 5 μg/ml trypsin was added, the cells were grown until the cytopathic effect (CPE) was visible, usually within 3-4 days. Quantifying the rotavirus in a given sample was performed according to an end-point dilution assay. The assay of the inhibitory activity of ginseng polysaccharides was based on the inhibition of rotavirus-induced cytopathogenicity. In short, 50 μl of 10^{-3}-diluted Wa (10^{-4}-diluted for SA11) virus (1×10^3 pfu) were inoculated into 100 μl of MA104 cells (2×10^5 cells/ml) containing 50 μl of the sample. The cells were then grown until a cytopathic effect was visible, and the inhibitory effect on rotavirus infectivity was measured.

Ginsengs are classified into white ginseng (Baeksam), red ginseng (Hongsam), and heated ginseng (Sunsam) according to the treatment at different temperatures. They were pretreated to make different ginseng products. These ginsengs contain different kinds and compositions of saponins. Therefore, the biological activities are different depending on the kinds and compositions of saponins. For example, heated ginseng contains higher amounts of ginsenoside Rg3, which is a vasorelaxant [13, 14]. However, the differences in molecular weight and biological activity of polysaccharides due to different processing temperatures have not been studied. Therefore, polysaccharides from ginsengs treated at various temperatures were isolated, and the molecular weights of polysaccharides were measured by gel filtration (Table 1). The molecular weight of the polysaccharides decreased in proportion to the increase in processing temperature. However, the yield of soluble polysaccharides extracted with water increased in proportion to the increase of processing temperature. The yield extracted at 120°C was 7.0±2.42%.

The antirotaviral activity of these polysaccharides was measured (Table 2). All tested polysaccharides exhibited antirotaviral activities with 50% inhibitory concentration values of 6.3-16.6 μg/ml. There were no significant differences in the rotavirus infection-inhibitory action of these polysaccharides. Their inhibitory potency was comparable to that of hesperidin, which is the most potent agent for rotavirus infection into MA104 cells [15]. The antirotaviral activity of saponins was also measured. Not all ginsenosides tested showed antirotaviral activity.

Many kinds of vaccines and medicines for rotaviral diarrhea have been developed [8, 10]. However, the vaccines developed are not completely effective in preventing rotaviral infection. In addition, ginseng has been used for a long time to prevent or cure many kinds of diseases such as tumors and diarrhea. Ginseng shows no serious toxicity, and has been considered as one of the most beneficial herbal medicines. Therefore, this study concludes that these ginseng polysaccharides can contribute to the prevention of rotaviral illnesses.

Table 1. Molecular weight and yields of ginseng polysaccharides extracted with water.

	Treated temperature (°C)	Molecular weight[a] (kDa)	Yield (%)
LT polysaccharide	60	>100-70	0.2±0.11
MT polysaccharide	100	100-70	2.5±0.65
HT polysaccharide	120	80-60	7.0±2.42

[a]Their average molecular weights were determined to be >100-70, 100-70, and 80-60 kdaltons by Sephadex G-75, respectively.

Table 2. Inhibitory effects of ginseng polysaccharides and ginsenosides on the infection of rotavirus.

Agent	IC_{50} (μg/ml)	
	Wa virus	SA11 virus
LT polysaccharide	10.4	12.5
MT polysaccharide	6.3	6.3
HT polysaccharide	16.6	9.4
Ginsenoside Rb1	>30	>30
Ginsenoside Rb2	>30	>30
Ginsenoside Rc	>30	>30
Ginsenoside Rd	>30	>30
Ginsenoside Re	>30	>30
Ginsenoside Rg3	>30	>30
Ginsenoside Rh1	>30	>30
Ginsenoside Rh2	>30	>30
Compound K	>30	>30
Hesperidin	6.1	-[a]

10^{-3}-diluted Wa and 10^{-4}-diluted SA11 virus were used. CPE was observed for 3-5 days after virus infection into cells.
[a]Not determined.

REFERENCES

1. Albert, M. J. and R. F. Bishop. 1984. Cultivation of human rotavirus in cell culture. *J. Med. Virol.* **13:** 377-383.
2. 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.
3. Bae, E.-A., M. J. Han, M.-J. Song, and D.-H. Kim. 2002. Purification of rotavirus infection-inhibitory protein from *Bifidobacterium breve* K-110. *J. Microbiol. Biotechnol.* **12:** 553-556.
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., 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.

6. 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.
7. Belogortseva, N. I., J. Y. Yoon, and K. H. Kim. 2000. Inhibition of *Helicobacter pylori* hemagglutination by polysaccharide fractions from roots of *Panax ginseng*. *Planta Med.* **66:** 217-220.
8. Bishop, R. F. 1993. Development of candidate rotavirus vaccines. *Vaccine* **11:** 247-254.
9. Cukor, G. and N. R. Blacklow. 1984. Human viral gastroenteritis. *Microbiol. Res.* **48:** 157-179.
10. Flores, J. and A. Z. Kapokian. 1992. Rotavirus, pp. 255-288. *In* R. W. Ellis (ed.), *Vaccines: New Approaches to Immunological Problems*. Butterworth-Heinemann press, Boston.
11. Kim, K. H., I. S. Suh, J. M. Kim, C. W. Kim, and Y. J. Cho. 1989. Etiology of childhood diarrhea in Korea. *J. Clin. Microbiol.* **27:** 1192-1196.
12. 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.
13. 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.
14. 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.
15. Kim, D.-H., M.-J. Song, E.-A. Bae, and M. J. Han. 2000. Inhibitory effect of herbal medicines on rotavirus infectivity. *Biol. Pharm. Bull.* **23:** 356-358.
16. Konno, T., H. Suzuki, A. Imai, T. Kutsuzawa, N. Ishida, N. Katsushima, M. Sakamoto, S. Kitaoka, R. Tsuboi, and M. Adachi. 1978. A long term survey of rotavirus infection in Japanese children with acute gastroenteritis. *J. Infec. Dis.* **138:** 569-576.
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. Smith, E. M., M. K. Esters, D. Y. Graham, and C. P. Gerba. 1979. A plaque assay for the simian rotavirus SAII. *J. Gen. Virol.* **3:** 513-519.
20. 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.
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. Woods, P. A., J. Gentsch, V. Gouvea, L. Mata, A. Simhon, M. Santosham, Z. S. Bai, and S. Urasawa. 1992. Distribution of serotype of human rotavirus in different populations. *J. Clin. Microbiol.* **30:** 781-785.