Ginseng, the root of *Panax ginseng* C. A. MEYER (Araliaceae), is one of the most widely used herbal medicines in the Orient. Thousands of papers have reported its chemical constituents and biological activities. Recently, our group reported that steaming ginseng at high temperature enhances its biological activity.\(^1\) For example, this processed ginseng (SG) exhibited greatly enhanced vasorelaxation activity\(^1\) and cancer chemoprevention activity.\(^2\) In the course of study on the biological activity of SG, we found that the cytotoxicity is greatly increased compared with that of raw ginseng. Figure 1A demonstrates the cytotoxicity of SG, red ginseng (RG), and white ginseng (WG) analyzed by MTT assay using SK-Hep-1 hepatoma cancer cells. The GI\(_{50}\) (50% growth inhibition concentration) of each type of ginseng was 70, 410, and 500 \(\mu\)g/ml, respectively. To search for the active principles, SG was fractionated by solvent partition between water and \(-\)butanol. The butanol-soluble fraction (SG-BuOH) showed strong cytotoxicity, while the aqueous layer (SG-Aqueous) showed none (Fig. 1B).

The butanol layer (SG-BuOH) was further fractionated by HPLC using an analytical C\(_{18}\) bonded silica column.\(^3\) The HPLC eluate was collected in a 96-well microplate, which was subjected to the MTT assay. The upper part of Fig. 2 shows the HPLC profile of SG-BuOH detected using an evaporative light scattering detector (ELSD), while the lower part shows the activitygram of SG-BuOH analyzed using the MTT assay method. Cytotoxicity occurred near the peaks of ginsenosides Rg3, Rk1, Rg5, Rs5, and Rs4 (Fig. 2, lower part).

Each ginsenoside was isolated from SG extract in our laboratory.\(^3\) Ginsenosides Rg3, Rg5, and Rk1 were the most abundant ginsenosides in SG. SG was refluxed with MeOH for 6 h. The organic solvent was removed and the residue was dissolved in MeOH, which was subjected to HPLC determination. Each ginsenoside was separated and purified using a semipreparative HPLC system. Semipreparative separation was performed on a Mightysil RP-18 (5 \(\mu\)m, 250 \(\times\) 10 mm I.D., Kanto Chemical, Tokyo, Japan) column. A isocratic elution system of CH\(_3\)CN:H\(_2\)O (50 : 50) was used. The solvent flow rate was 4.0 ml/min.

### Experimental

WG (4 years old) was the product of Keumsan Ginseng Cooperative Federation. RG (6 years old) was the product of Korea Ginseng Corporation. WG and RG were commercial products widely available in Korean markets. SG was produced by steaming (120 °C, 3 h) and drying the rootlet ginseng as in the previous report.\(^1\) Each 10 g of WG, RG, and SG were extracted with 100 ml of MeOH under reflux for 6 h. Solvent was removed at reduced pressure below 40 °C to yield WG-MeOH (2.2 g), RG-MeOH (2.4 g), and SG-MeOH (2.9 g), respectively.

Ginsenosides Rg3 (6.1%), Rk1 (2.9%), Rg5 (3.3%), Rs5 (0.02%), and Rs4 (0.03%) were isolated from SG extract in our laboratory.\(^3\) The GI\(_{50}\) of each type of ginseng was 70, 410, and 500 \(\mu\)g/ml, respectively. To search for the active principles, SG was fractionated by solvent partition between water and \(-\)butanol. The butanol-soluble fraction (SG-BuOH) showed strong cytotoxicity, while the aqueous layer (SG-Aqueous) showed none (Fig. 1B).

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Each ginsenoside was isolated from SG\(^4\) and its cytotoxicity was evaluated. Ginsenosides Rg3, Rk1, Rg5, Rs5, and Rs4 exhibited GI\(_{50}\) values of 41, 11, 13, 37, and 13 \(\mu\)M, respectively (Fig. 3). Recently, ginsenoside Rg3 was developed as an anticancer drug in China.\(^9\) However, it is interesting that the dehydrated compounds at the C-20 position, i.e., ginsenosides Rk1 and Rg5, have more potent activity than the hydroxylated derivative ginsenoside Rg3. Cisplatin, a potent anticancer platinum complex, had a GI\(_{50}\) value of 84 \(\mu\)M in the same assay conditions.

### Key words

Panax ginseng; cytotoxicity; ginsenoside

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* To whom correspondence should be addressed.  e-mail: hillpark@snu.ac.kr  © 2002 Pharmaceutical Society of Japan
MTT Assay  Cytotoxicity was evaluated using SK-Hep-1 cells according to the published method. Cells were suspended in medium supplemented with 5% calf serum, and 200 μl of a single-cell suspension containing 4×10^4 cells was added to the individual wells of 96-well microplates. The plates were incubated at 37 °C in a CO_2 incubator for 24 h. Samples (200 μl) were added to each well to give the final concentration, and the plates were incubated at 37 °C for 24 h. MTT 20 μl in 7.2 mM phosphate buffer solution, pH 6.5 (5 mg/ml), was added to each well, and the plates were incubated for an additional 2 h. After the removal of solutions in the well, 100 μl of dimethyl sulfoxide were added to dissolve formazan products, and the plates were shaken for 5 min on a plate shaker (Seoulin Bioscience, Seoul, Korea). The absorbance of each well was recorded on a microplate spectrophotometer (Molecular Devices Spectra Max 340 pc, Global Medical Instrumentation, Minnesota, U.S.A.) at 570 nm. Concentration–absorbance curves were plotted for samples, and the GI50 values were calculated.

On-line HPLC/ELSD/Fraction Collection/MTT Assay  HPLC separation of SG-BuOH was carried out by the previously reported method with a small modification in solvent programming. A gradient elution system of A (CH_3CN:H_2O=15:80) and B (CH_3CN:H_2O=80:20) was used [0% B (0 min); 30% B (10 min); 50% B (25 min); 100% B (40 min); 100% B (50 min)]. Fifty microliters of SG-BuOH 35 mg/ml were injected into the HPLC system. Column effluent was split into two parts, one for the ELSD and the other for the fraction collector with the ratio of 1 : 2. A 96-well microplate was used for fraction collection. The HPLC effluent was collected into each well at an interval of 30 s. The solvent was removed in a UV-sanitized oven at 40 °C. The on-line MTT assay procedure was similar to the above MTT assay method with minor modifications. Samples in 96-well microplates were dissolved with 10 μl of dimethyl sulfoxide. Cell suspension (190 μl) containing 3.8×10^4 cells was added to the individual sample wells on 96-well microplates. The plates were incubated for 24 h. MTT (20 μl) in
phosphate-buffered saline was added to each well, and plates were incubated for an additional 2 h. Solutions were removed and 100 μl of dimethyl sulfoxide were added, and the plates were shaken for 5 min on a plate shaker. The absorbance of the well was recorded on a microplate spectrophotometer at 570 nm.

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References