Anti-*Helicobacter pylori*, Cytotoxic, and Anti-inflammatory Activities of White Ginseng Extract

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The anti-*Helicobacter pylori* activity, cytotoxicity, and anti-inflammatory activity of white ginseng extract (WGE) were investigated in vitro in this study. The antimicrobial effects of WGE toward *H. pylori* strains S2, J99, SSI, and 51 were tested using the disk diffusion method. Among these *H. pylori* strains, *H. pylori* S2 was the most sensitive, having the largest inhibition zone (19 mm), followed by J99, SSI, and 51. The zone of inhibition due to WGE increased significantly with increasing dosage. The cytotoxicity of WGE toward the human cancer cell lines A-549 (human lung carcinoma), HEC-1-B (human endometrial adenocarcinoma), HeLa (human uterine adenocarcinoma), and SW-136 (human kidney carcinoma) was measured using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. WGE exhibited an inhibitory effect on cell growth at 2.0 mg/mL for all tumor cell lines. An analysis of anti-inflammatory activity using the RAW 264.7 cell line showed that the inhibition of nitric oxide (NO) production increased as the WGE content increased. These results demonstrate the potential of WGE to be used as a health-promoting substance.

Keywords: white ginseng extract, *Helicobacter pylori*, anti-*Helicobacter pylori* activity, cytotoxicity, anti-inflammatory activity

Introduction

Ginseng (*Panax* spp.; Araliaceae) has been widely used in traditional oriental medicine for its wide spectrum of medicinal effects including anti-aging (1), anti-diabetic (2), anti-tumor (3), anti-stress (4), anti-fatigue (5), and memory enhancing (6) effects, as well as the promotion of some types of protein synthesis (7). Commercially available ginseng is classified into fresh, white, and red ginseng. White ginseng is made by peeling the fresh ginseng roots and drying them without steaming. Many of its medicinal effects are attributed to the triterpene glycosides known as ginsenosides (saponin) (8). Ginseng saponin (ginsenosides) is one of the most important secondary metabolites in ginseng and has various pharmacological activities. Beginning in the late 1960's, a variety of studies has been conducted concerning its constituents. Pharmacological studies show that the bioactivities of the different ginsenosides vary, depending on the extraction method. Most extraction procedures utilize methanol or ethanol (9) as a solvent in pure form or as an aqueous solution (10), and often couple with heat, refluxing, or sonication. So far, approximately 200 substances have been isolated and characterized from *Panax ginseng* including ginsenosides, polysaccharide, alkaloid, polysaccharides, oligosaccharides, oligopeptides, flavonoids, lipids, vitamins, and minerals (11).

Previous studies have found that various components of ginseng extract can inhibit microbial growth. For example, acidic polysaccharide from *P. ginseng*, PG-F2, were shown to have anti-adhesive effects toward *Actinobacillus actinomycetemcomitans*, *Propionibacterium acnes*, and *Staphylococcus aureus* but not toward *Lactobacillus acidophilus*, *Escherichia coli*, or *Staphylococcus epidermidis* (12). Acidic polysaccharides have also been shown to inhibit *Helicobacter pylori*. Other proteins isolated from the *Panax* family (*panaxagin* and *quinqueginsin*) also exhibit antifungal activity (13).

*H. pylori* is a motile, microaerophilic Gram-negative bacterium that is a pathogenic agent which colonizes the gastric intestinal tract. For many years, natural compounds such as propolis, herbs, and other plants have been tested for inhibitory effects on *H. pylori* (14). These include the protective roles of red ginseng extract (RGE) from *H. pylori*-associated cytotoxicity through the inhibitory actions of both mitogen-activated-protein kinase (MAPK) and redox sensitive transcriptional activity (15). The influence of *H. pylori* infection on the lipooxygenase (LOX) pathway and the efficacy of RGE modulating the arachidonate-5-LOX-inflammatory link in gastric epithelium after *H. pylori* infection have also been reported (16).

The purpose of this study was to test the anti-*H. pylori* activity, cytotoxicity toward human cancer cells, and anti-inflammatory activity of white ginseng extract (WGE).

Materials and Methods

Materials White ginseng extract (WGE) was provided by the Dongwon Korean Ginseng Co., Ltd. (Seoul, Korea). Dried white ginseng was extracted 4 times using 70% aqueous ethanol for 4 h at 95°C. The 4 extracts were combined, and the solvent was evaporated until 60% solid contents remained by rotary evaporator in vacuum. The WGE was then precipitated 3 times with 80% alcohol. And supernatant was obtained that 3 times ethyl alcohol added to former residue. Purified ginseng extracts were...
obtained by the filtration and vacuum evaporation of supernatants. Acetonitrile and distilled water for high performance liquid chromatography (HPLC) were purchased from J. T. Baker SOLUSORB (Phillipsburg, NJ, USA). 20(S)- and 20(R)-ginsenoside Rg2 standards from R. ginseng were purchased from LKT Laboratories, Inc. (St. Paul, MN, USA). The composition of WGE is as follows: water solid contents 89.1%, crude protein 13.16%, crude lipid 2.98%, crude fiber 6.47%, ash 4.65%, total saponin contents 12.14%, total ginsenosides 47.9 g/ g, ginsenoside Rb1 12.3 mg/g, Rb2 10.5 mg/g, Re 11.29 mg/g, Rd 4.23 mg/g, Re 5.6 mg/g, Rg1 3.8 mg/g, and Rg2 3.7 mg/g.

**Bacterial strains**  
*H. pylori* strains 51, 52, J99, and SS1 were kindly provided by the H. pylori Korean Type Culture Collection (HpKTC; Gyeongang National University, Jinju, Korea). The 4 strains were incubated on Brucella agar (Difco Laboratories, Detroit, MI, USA) supplemented with 5% horse serum at 37°C for 3 days under aerobic conditions (10% CO2 atmosphere).

**Anti-*H. pylori* activity**  
The anti-*H. pylori* activity of WGE toward 4 different strains was tested by the disk diffusion method. Briefly, 10^5 CFU/mL of *H. pylori* were inoculated on Brucella agar in petri dishes. Sterilized filter paper disks were placed on the surface of Brucella agar. Test samples were diluted in sterilized distilled water at concentrations of 2.5, 5.0, 10.0, 20.0, and 40.0 mg/mL, and 50 µL of each dilution was added to the filter paper disks. The lowest concentration of WGE tested for which an inhibitory zone was observed at the points of inoculation was then treated with the test drug at 37°C for 1 hr before stimulation with 1 µg/mL lipopolysaccharides (LPS, Sigma-Aldrich, St. Louis, MO, USA) for 24 hr in a final volume of 200 µL. Fifty µL of each culture supernatant was mixed with 50 µL of Griess reagent (1% sulfanilamide, 0.1% naphthylethylene diamide dihydrochloride, and 2% phosphoric acid) at room temperature. After 15 min, the absorbance was determined at 540 nm using a microplate reader.

**MTT/mL of phosphate buffered saline, PBS) was added to each cell suspension, followed by incubation at 37°C for 44 hr. At the end of the incubation, MTT solution (2.5 mg MTT/mL of phosphate buffered saline, PBS) was added and the plate was further incubated for 4 hr. The supernatant was then removed from each cell suspension and 100 µL of dimethyl sulfoxide was added to dissolve the colored formazan crystals produced from reaction with MTT. The optical density (OD) values of each solution were then measured with an microplate reader at 540 nm.

**Nitrile assay**  
The accumulation of nitrite (NO2- ) in the culture media, an indicator of NO synthase activity, was measured using the Griess reaction. RAW 264.7 cells at a density of 1 × 10^6 cells/well were distributed into 96-well microtiter plates and allowed to adhere overnight. Cells were then treated with the test drug at 37°C for 1 hr before stimulation with 1 µg/mL lipopolysaccharides (LPS, Sigma-Aldrich, St. Louis, MO, USA) for 24 hr in a final volume of 200 µL. Fifty µL of each culture supernatant was mixed with 50 µL of Griess reagent (1% sulfanilamide, 0.1% naphthylethylene diamide dihydrochloride, and 2% phosphoric acid) at room temperature. After 15 min, the absorbance was determined at 540 nm using a microplate reader.

**Results and Discussion**

**Anti-*H. pylori* activity**  
The inhibitory effects of WGE on growth of several strains of *H. pylori* were measured using the disk diffusion method as presented in Table 1. The data obtained indicate that *H. pylori* 52 was the most sensitive strain tested, having the largest zone of inhibition (19 mm), followed by J99, SS1, and 51, all with large zones of inhibition (16-17.5 mm). The zones of inhibition were in the range of 11-17.5 mm, and the WGE inhibition zone increased in a dose-dependent manner. The negative control used in this study, did not effect growth of any strains tested.

Bae et al. (23) reported that the ginseng BuOH fraction, and ginsenoside Rb1, Rb2, Rc, Rg1, and Rh2 did not inhibit the growth of *H. pylori* (43504, 82548). However, ginsenoside Rg3-enriched ginseng and fermented ginsenoside Rh2-enriched ginseng did inhibit *H. pylori* growth. The minimum inhibitory concentration (MIC) for each were from 500-1,000 µg/mL. 20(S)- and 20(R)-protopanaxadioloids also inhibited *H. pylori* growth with MIC of 50-100 µg/mL. The inhibitory effects of these compounds on the activities of *H. pylori* urease and H+/K+ ATPase were also measured. Most of the tested compounds did not inhibit these enzymes. However, 20(S)-ginsenoside Rg2 and Rh2 weakly inhibited H+/K+ ATPase from rat stomach, with IC50 values of 0.6 and 0.48 µg/mL, respectively. Park (16) reported that RGE at levels of 1 to 100 µg selectively suppressed *H. pylori*-stimulated C-5(S)-hydroxyeicosatetraenoic acid (HETE) production implying the attenuation of 5-lipoxygenase activity, which is similar to the known LOX inhibitor nordihydroguaiaretic acid (NDGA). In this paper, it has shown that WGE has an antimicrobial effect on *H. pylori* to add RGE and some ginsenoside Rg3 and Rh2.

**Cytotoxic effects of WGE**  
MTT is a tetrazolium salt used to assay cell proliferation activity, which is reduced to formazan by living cells via the reaction with MTT. The colorimetric MTT assay is used to assay cell proliferation...
To determine the cytotoxic effects of WGE on tumor cells, A-549, HEC-1-B, HeLa, and SW-156 cells were treated with a range of 0.5 to 4.0 mg/mL WGE for 48 hr. The inhibition of tumor cell proliferation by WGE at various concentrations is shown in Fig. 2. WGE demonstrated cytotoxic activity that increased steadily with increasing concentration. In particular, proliferation of the SW-156 cell line was inhibited more than 93% at a WGE concentration of 2.0 mg/mL. However, WGE did not inhibit tumor cells growth at a concentration of 0.5 mg/mL.

Jung et al. (18) reported that RGE and WGE had cytotoxicity toward cancer cells, various mouse cancer cells and peritoneal macrophages, in vitro, and that red ginseng was more cytotoxic toward cancer cells than white ginseng. Our result shows that cytotoxicity of WGE was very effective on various human cancer cells when compared to mouse cancer cells. Keum et al. (19) reported that ginsenoside Rg3 inhibits phorbol ester-induced cyclooxygenase-2 expression, NF-k activation, and tumor promotion. Yun et al. (20) reported that Rg3 causes a statistically significant reduction of lung tumor incidence, as does. These results strongly demonstrate that the anticancer effect of ginseng is due to the ginsenosides Rg3, Rg5, and Rh2 present in Korean red ginseng.

**Anti-inflammatory activity of WGE** The over-production of NO by iNOS has been implicated in the pathogenesis of cancer via reactive nitrogen oxide species-mediated reactions such as the deamination of DNA bases and DNA strand breaks (21). To analyze the potential anti-inflammatory properties of WGE, we used the murine macrophage cell line RAW 264.7, which can produce NO upon stimulation with LPS, thus providing a suitable model for studying inflammatory responses in culture cells. As a first step, the cytotoxic activity of WGE on RAW 264.7 macrophages in the absence and presence of LPS was assessed using the MTT assay. Cell viability was not affected by WGE at concentrations up to 200 µg/mL. The results are expressed as percentages with the LPS-only values considered as 100%. Values represent the mean±SE of 3 independent determinations.

**Table 1. Anti-*Helicobacter pylori* activity of white ginseng extract (WGE)**

<table>
<thead>
<tr>
<th>Sample (mg/mL)</th>
<th>H. pylori 51</th>
<th>H. pylori 52</th>
<th>H. pylori 99</th>
<th>H. pylori SS1</th>
</tr>
</thead>
<tbody>
<tr>
<td>WGE 40</td>
<td>16.0 ± 0.000</td>
<td>16.5 ± 0.707</td>
<td>17.5 ± 1.414</td>
<td>16.5 ± 0.707</td>
</tr>
<tr>
<td>20</td>
<td>13.0 ± 0.000</td>
<td>14.0 ± 0.000</td>
<td>13.5 ± 0.707</td>
<td>13.0 ± 0.000</td>
</tr>
<tr>
<td>10</td>
<td>12.5 ± 0.707</td>
<td>13.0 ± 0.000</td>
<td>13.0 ± 0.000</td>
<td>13.0 ± 0.000</td>
</tr>
<tr>
<td>5</td>
<td>11.0 ± 0.000</td>
<td>11.0 ± 0.000</td>
<td>12.0 ± 1.414</td>
<td>11.5 ± 0.707</td>
</tr>
</tbody>
</table>

Values represent the mean±SD (n=2).
subsequent experiments. When RAW 264.7 macrophages were treated with these concentrations of WGE together with LPS (1 µg/mL) for 24 hr, a significant concentration-dependent inhibition of nitrite production was detected with more than 200 µg/mL WGE. Wu et al. (22) reported that ginsenoside Rg2 (0.01-10 µM) and -Re (10, 100 µM), which belong to the protopanaxatriol group, decreased NO2- production. However, ginsenoside Rb2 and -Rd, which belong to protopanaxadiol group, did not show any effects.

Acknowledgments
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References