

## Steamed American Ginseng Berry: Ginsenoside Analyses and Anticancer Activities

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This study was designed to determine the changes in saponin content in American ginseng berries after treatment by heating and to assess the anticancer effects of the extracts. After steaming treatment (100–120 °C for 1 h, and 120 °C for 0.5–4 h), the content of seven ginsenosides, Rg1, Re, Rb1, Rc, Rb2, Rb3, and Rd, decreased; the content of five ginsenosides, Rh1, Rg2, 20R-Rg2, Rg3, and Rh2, increased. Rg3, a previously identified anticancer ginsenoside, increased significantly. Two hours of steaming at 120 °C increased the content of ginsenoside Rg3 to a greater degree than other tested ginsenosides. When human colorectal cancer cells were treated with 0.5 mg/mL steamed berry extract (120 °C 2 h), the antiproliferation effects were 97.8% for HCT-116 and 99.6% for SW-480 cells. At the same treatment concentration, the effects of unsteamed berry extract were 34.1% for HCT-116 and 4.9% for SW-480 cells. After staining with Hoechst 33258, apoptotic cells increased significantly by treatment with steamed berry extract compared with unheated extracts. Induction of apoptosis activity was confirmed by flow cytometry after staining with annexin V/PI. The steaming of American ginseng berries augments ginsenoside Rg3 content and increases the antiproliferative effects on two human colorectal cancer cell lines.

**KEYWORDS:** *Panax quinquefolius* L.; berry; heating treatment; HPLC analysis; ginsenoside Rg3; antiproliferation; human colorectal cancer cells; apoptosis

### INTRODUCTION

American ginseng (*Panax quinquefolius* L., Araliaceae) is a commonly used herbal medicine in the U.S. (1). Asian ginseng (*Panax ginseng* C. A. Meyer), another plant in the genus *Panax*, has a long history worldwide as a medicinal herb (2). Asian ginseng may improve psychological function, immune activities, cardiovascular conditions, and prevent certain cancers (3–5). The major active components of ginseng are ginsenosides, a diverse group of steroidal saponins (6). Ginsenosides are distributed in many parts of the ginseng plant, including the root, leaf, and berry.

In Asia, ginseng root is air-dried into white ginseng or steamed at 100 °C to give red ginseng. It is believed that red ginseng is more pharmacologically effective than white ginseng (7). The differences in the biological effects of white and red

Asian ginseng are attributed to the significant changes in ginsenosides from the steaming treatment (8). No attempt at steaming any part of American ginseng has been reported. Asian ginseng and its chemical constituents have been tested since the 1970s for their inhibiting effect on human cancers (9). A number of investigators have found antitumor properties and other pharmacological activities of ginseng, and ginsenosides Rg3 and Rh2 are recognized as active anticancer compounds (5). Compared with white ginseng, red ginseng has stronger anticancer activities (10). Recently, American ginseng extracts were found to inhibit the growth of breast cancer cells (11, 12). Because the heat process was not applied to American ginseng, the chemical constituents and the activities of steamed American ginseng on cancer cells have not been studied.

As a perennial herb, American ginseng flowers and fruits in its third or fourth year (13). American ginseng root is normally harvested between its fifth to seventh year. As a byproduct, American ginseng berry can be harvested more than once before harvesting the root. The pharmacological effects of ginseng berries have been evaluated (14–16), and the berry has a ginsenoside profile distinct from that of the root (14, 17). In previous studies, we observed that American ginseng berries,

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in addition to attenuating chemotherapy-induced nausea and vomiting in an animal model (18), exerted antiproliferative activity against breast cancer cells (12). In this study, we treated American ginseng berry at various temperatures and heating times to observe the changes in ginsenoside content and anticancer activities in two human colorectal cancer cell lines.

## MATERIALS AND METHODS

**Chemicals.** All solvents were of high-performance liquid chromatography (HPLC) grade from Fisher Scientific (Norcross, GA). Milli Q water was supplied by a water purification system (US Filter, Palm Desert, CA). Standards for ginsenosides Rb<sub>1</sub>, Rb<sub>2</sub>, Rc, Rd, Re, and Rg<sub>1</sub> were obtained from Indofine Chemical Company (Somerville, NJ); ginsenosides Rb<sub>3</sub>, Rg<sub>2</sub>, Rg<sub>3</sub>, Rh<sub>1</sub>, Rh<sub>2</sub>, and 20(R)-ginsenoside Rg<sub>2</sub> (20R-Rg<sub>2</sub>) were obtained from the Delta Information Center for Natural Organic Compounds (Xuancheng, Anhui, China). All standards were of biochemical-reagent grade and at least 95% pure as confirmed by HPLC. All the plastic materials were purchased from Falcon Labware (Franklin Lakes, NJ). Trypsin, McCoy's 5A, Leibovitz's L-15 medium, fetal bovine serum (FBS), and penicillin/streptomycin solution (200×) were obtained from Mediatech, Inc. (Herndon, VA). Hoechst 33258, formaldehyde, and NP40 were obtained from Sigma (St. Louis, MO). A CellTiter 96 Aqueous One Solution Cell Proliferation Assay kit was obtained from Promega (Madison, WI).

**Plant Materials and Sample Preparation.** Fresh berries of American ginseng (*Panax quinquefolius* L.) were obtained from Roland Ginseng, LLC (Wausau, WI). All berries were gathered from 4-year-old plants. The voucher samples were deposited at the Tang Center for Herbal Medicine Research at University of Chicago (Chicago, IL). The seeds of the berry were removed. For the steaming of American ginseng berry, the pulps were steamed at 100 °C and 120 °C for 1 h or at 120 °C for 0.5, 1, 2, 3, and 4 h. Fresh and steamed pulps were lyophilized to obtain dried pulp samples.

For the HPLC analysis, dried American ginseng berry pulps were ground, and the ground sample (0.5 g) was extracted with methanol in a Soxhlet extractor for 8 h. The extract was concentrated, transferred into a 25-mL volumetric flask, and diluted to the desired volume with methanol. One milliliter of the solution was purified by solid-phase extraction (17). Purified solutions were stored at 4 °C until HPLC analysis.

For the *in vitro* anticancer studies, the process for the extraction of American ginseng berry samples was as follows: A pulp sample, unsteamed or steamed at 120 °C for 1 or 2 h, was ground to powder and passed through a 40 mesh screen. Then 25 g of powder was extracted with 500 mL of 70% ethanol for 4 h; the water bath was maintained at 90 °C. When cooled, the solution was filtered with P8 filter paper (Fisher Scientific, Pittsburgh, PA) and the filtrate was collected. The residue was extracted with 500 mL of 70% ethanol once more and then filtered while the solution was cooled. The filtrates were combined, and the solvent was evaporated under vacuum. The dried extract was dissolved in 100 mL of water and then extracted with water-saturated *n*-butanol. The *n*-butanol phase was evaporated under vacuum and then lyophilized.

**HPLC Instrumentation and Analysis.** The HPLC system was a Waters 2960 instrument (Milford, MA) with a quaternary pump, an automatic injector, a photodiode array detector (Model 996), and Waters Millennium 32 software for peak identification and integration. The separation was carried out on a 250 × 3.2 mm i.d., 5 μm, Ultrasphere C18 column (Alltech, Deerfield, IL) with a 7.5 × 3.2 mm i.d. guard column. For HPLC analysis, a 20-μL sample was injected into the column and eluted at room temperature with a constant flow rate of 1.0 mL/min. For the mobile phase, acetonitrile (solvent A) and water (solvent B) were used. Gradient elution started with 18% solvent A and 82% solvent B. Elution was changed to 21% A for 20 min and then to 26% A for 3 min and held for 19 min. It was then changed to 36% A for 13 min, to 50% A for 9 min, and to 95% A for 2 min and held for 3 min. Last, elution was changed to 18% A for 3 min and held for 8 min. The detection wavelength was set to 202 nm. All tested solutions were filtered through Millex 0.2-μm nylon membrane syringe filters (Millipore Co., Bedford, MA) before use. The linearity of this

method was assayed by analyzing standard solutions in the range of 2–400 μg/mL for the 12 ginsenosides. Calibration curves were constructed from the measured peak areas and the related amount of ginsenosides. Ginsenosides Rb<sub>1</sub>, Rb<sub>2</sub>, Rb<sub>3</sub>, Rc, Rd, Re, Rg<sub>1</sub>, Rg<sub>2</sub>, 20R-Rg<sub>2</sub>, Rg<sub>3</sub>, Rh<sub>1</sub>, and Rh<sub>2</sub> in extract samples were identified by comparison of their retention times with those obtained from the chromatograms of mixed ginsenoside standards. The contents of saponins in each sample were calculated using standard curves of ginsenosides.

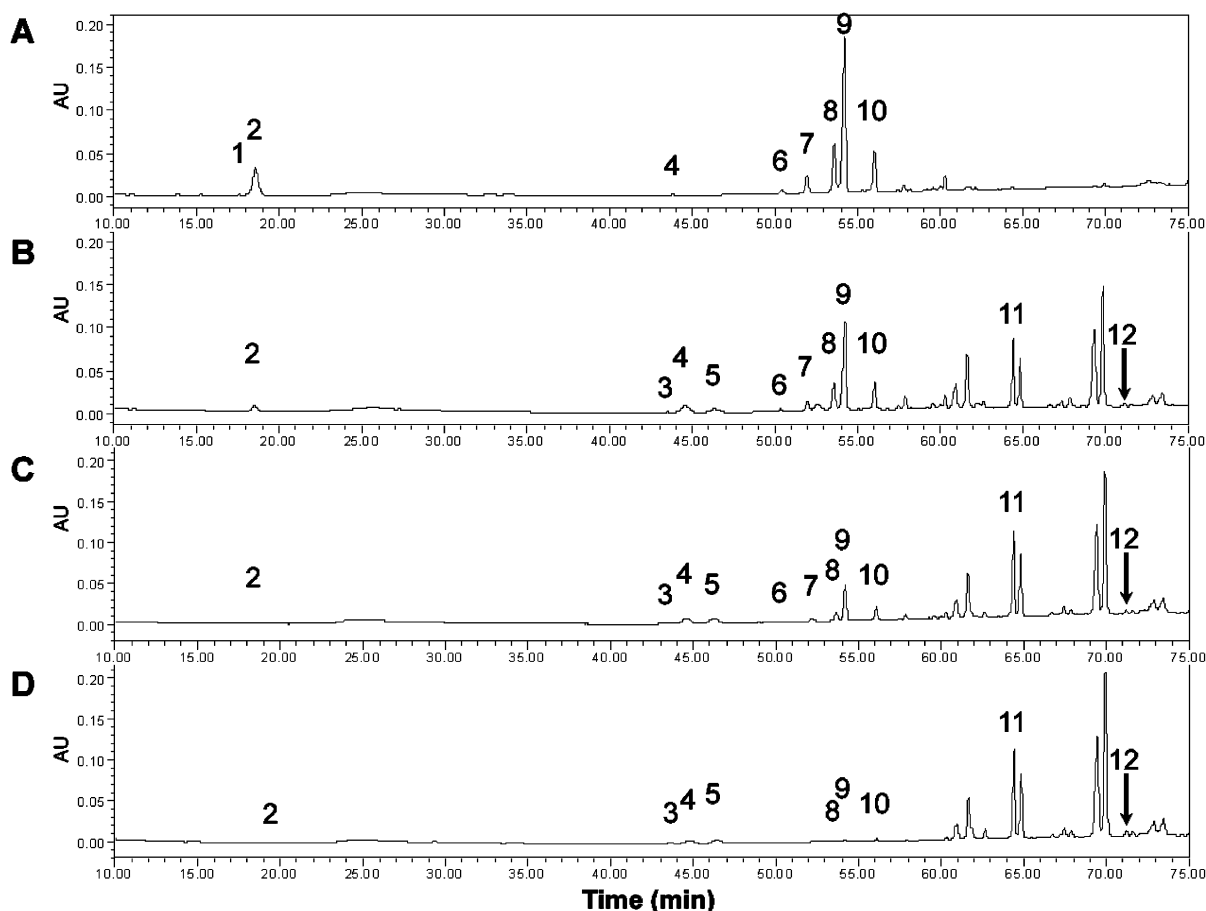
**Cell Culture.** The human colorectal cancer cell lines HCT-116 (McCoy's 5A) and SW480 (Leibovitz's L-15) were purchased from American Type Culture Collection (Manassas, VA) and grown in the indicated media supplemented with 10% FBS and 50 IU penicillin/streptomycin in a humidified atmosphere of 5% CO<sub>2</sub> at 37 °C. When the cells were in the late log/early plateau phase (with about 90% of the surface area covered) and healthy and free of contamination, the cell culture medium was removed. After the cells were washed with PBS to remove any trace of serum that would inactivate trypsin, the PBS was discarded. One milliliter of trypsin was added to a 25-mL flask to break the cell–cell and cell–substrate links. Fresh culture medium containing serum (5 mL) was then added to inactivate the trypsin in the cell suspension. After pipetting this suspension, a single-cell suspension was prepared. The cell suspension was then counted for accurate cell density. An aliquot of the cell suspension (1/4 for SW-480, 1/6 for HCT-116) was placed into a new 25 mL-flask with the full amount of cell culture medium (10 mL) required for the flask size. The medium was then changed as necessary until the next subculture.

**Cell Proliferation Analysis.** Unsteamed or steamed American ginseng berry extracts, and ginsenosides, were dissolved in 50% ethanol and were stored at 4 °C before use. Cells were seeded in a flat-bottomed 96-well plate with a multichannel pipet (2 × 10<sup>4</sup> cells/well). After 24 h, the medium was removed and 200 μL of fresh culture medium was added to each well. Various concentrations of extracts were added to the wells. The final concentration of ethanol in tested groups was 0.5%. Controls were exposed to culture medium containing the same quantity of ethanol without drugs. All experiments were performed at least in triplicate.

At the end of the drug exposure period (48 h), the medium was removed from all wells and 100 μL of fresh medium and 20 μL of CellTiter 96 aqueous solution were added to each well. CellTiter 96 aqueous solution is composed of a tetrazolium compound, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, an electron-coupling reagent (phenazine methosulfate), and buffer. When the solution contacts viable cells, it is bio-reduced by dehydrogenase enzymes in metabolically active cells into a formazan product. The quantity of formazan product, measured by the amount of absorbance at 490 nm, is directly proportional to the number of living cells in culture. The plate was then incubated for 1 h in a humidified atmosphere at 37 °C; 60 μL of medium from each well was transferred to an ELISA 96-well plate, and the absorbance of the formazan product at 490 nm was measured. The blank was recorded by measuring the absorbance at 490 nm with wells containing medium but no cells. Results were expressed as percent of control (ethanol vehicle set at 100%).

**Hoechst 33258 Staining Assay.** The Hoechst 33258 {4-[5-(4-methyl-1-piperazinyl)[2,5'-bi-1H-benzimidazol]-2'-yl]phenol, trihydrochloride} is one compound in a family of fluorescent stains for labeling DNA in fluorescence microscopy. It is commonly used to visualize the structure of the nucleus. Hoechst 33258 is excited by ultraviolet light at around 350 nm and emits blue/cyan fluorescent light around an emission maximum at 461 nm. Cells (1 × 10<sup>5</sup> cells/well in a 24-well plate) were treated with extracts for 48 h. Then the cells were separated with trypsin and transferred into a 1.5 mL tube. After being centrifuged for 5 min at 2000 rpm, the supernatant was removed. The cells were stained for 10 min in the dark with the solution containing 0.01 mg/mL Hoechst 33258, 33 mg/mL formaldehyde, and 5 mg/mL NP-40 in PBS. The cells were then examined using a fluorescence microscope with an excitation wavelength of 365 nm.

**Apoptosis Assay Using Flow Cytometry.** Annexin V is a Ca<sup>++</sup>-dependent phospholipid-binding protein that binds strongly to phos-



**Figure 1.** Typical chromatograms of (A) unsteamed and (B–D) steamed American ginseng berries recorded at 202 nm. American ginseng berries were steamed at 120 °C for 1 h (B), 2 h (C), and 4 h (D). Ginsenoside peaks: (1) Rg1, (2) Re, (3) Rh1, (4) Rg2, (5) 20R-Rg2, (6) Rb1, (7) Rc, (8) Rb2, (9) Rb3, (10) Rd, (11) Rg3, (12) Rh2. Peak numbers are not shown if saponins were not detected or less than 0.01%.

phatidylserine residues on the cell membrane. In a normal cell, these residues are on the inner surface of the membrane and therefore inaccessible to annexin V. At an early stage of apoptosis, the phosphatidylserine residues are translocated to the outside of the cell at which point the cells die. Cell apoptosis can be assayed by flow cytometry after staining with annexin V (19). Cells were seeded in 24-well tissue culture plates ( $2 \times 10^5$ ). After culture for 2 days, the medium was changed and American ginseng berry extracts were added. After treatment for 8 h, cells floating in the medium were collected. The wells were washed with PBS to remove serum. The adherent cells were detached with trypsin. Then culture medium containing 10% FBS (and floating cells) was added to inactivate trypsin. After being pipetted gently, the cells were centrifuged for 5 min at 600g. The supernatant was removed, and cells were stained with annexin-V FITC and propidium iodide (PI) according to the manufacturer's instructions. Untreated cells were used as control for double staining. Cells were analyzed immediately after staining using a FACScan flow cytometer (Becton Dickinson, Mountain View, CA) and FlowJo software (Tree Star, Ashland, OR). For each measurement, at least 50,000 cells were counted.

**Statistical Analysis.** Data are presented as mean  $\pm$  standard deviation (SD). A one-way ANOVA was employed to determine whether the results had statistical significance. In some cases, Student's *t*-test was used for comparing two groups. The level of statistical significance was set at  $p < 0.05$ .

## RESULTS AND DISCUSSION

**Effects of Temperature on the Ginseng Constituents.** Compared with unsteamed American ginseng berries, in berries steamed at 100 °C for 1 h total, ginsenoside contents decreased slightly from 10.23% to 9.49%. For the main saponin contents,

ginsenoside Rb3 decreased from 5.28% to 4.88%, and Re decreased from 1.69% to 1.10%; ginsenoside Rg3 increased from 0.004% to 0.19%. At 120 °C for 1 h, total ginsenoside content decreased to 5.75%. Rb3 was 2.38%; Re, 0.28%; and Rg3, 0.88%. Although total ginsenoside content was reduced significantly, Rg3, a recognized active anticancer reagent (20), increased significantly.

The chemical and pharmacological properties of steamed Asian ginseng have been studied thoroughly. Heat processing of red ginseng inactivates catabolic enzymes, thereby preventing deterioration of antioxidant-like substances, which inhibit lipid peroxide formation (21). Although 100 °C is usually used in steaming red ginseng, steaming at higher temperatures enhanced biological activity (22). In our study total ginsenoside content decreased at 120 °C, but ginsenoside Rg3 increased, compared with normal steaming at 100 °C.

**Effects of the Steaming Time on Ginseng Constituents.** Typical HPLC chromatograms of unsteamed and steamed American ginseng berries (1, 2, and 4 h at 120 °C) are shown in **Figure 1**. The peak area of saponins in unsteamed berries, ginsenosides Re, Rb2, Rb3, and Rd decreased during the steaming process. After 4 h of steaming, these four ginsenosides were difficult to identify in the chromatogram (**Figure 1D**). On the other hand, ginsenoside Rg3, which is a trace saponin in unsteamed berries, increased during the steaming process (**Figures 1B–D**). The content of 12 ginsenosides in steamed American ginseng berries is shown in **Table 1**. During the steaming process, seven ginsenosides (Rg1, Re, Rb1, Rc, Rb2, Rb3, and Rd) decreased, while five ginsenosides (Rh1, Rg2,

**Table 1.** Saponin Content in American Ginseng Berries Steamed at 120 °C (mean ± SD)

| ginsenoside | unsteamed   | steaming time |             |             |             |             |
|-------------|-------------|---------------|-------------|-------------|-------------|-------------|
|             |             | 0.5 h         | 1 h         | 2 h         | 3 h         | 4 h         |
| Rg1         | 0.04 ± 0.01 | 0.03 ± 0.01   | TR          | TR          | N.D.        | N.D.        |
| Re          | 1.69 ± 0.03 | 0.78 ± 0.02   | 0.28 ± 0.01 | 0.04 ± 0.01 | 0.04 ± 0.01 | 0.03 ± 0.01 |
| Rh1         | TR          | TR            | 0.03 ± 0.01 | 0.04 ± 0.01 | 0.04 ± 0.01 | 0.05 ± 0.01 |
| Rg2         | 0.07 ± 0.01 | 0.28 ± 0.01   | 0.40 ± 0.01 | 0.46 ± 0.01 | 0.34 ± 0.01 | 0.32 ± 0.01 |
| 20R-Rg2     | N.D.        | 0.15 ± 0.01   | 0.26 ± 0.01 | 0.33 ± 0.01 | 0.35 ± 0.01 | 0.37 ± 0.01 |
| Rb1         | 0.18 ± 0.01 | 0.11 ± 0.01   | 0.07 ± 0.01 | 0.02 ± 0.01 | TR          | N.D.        |
| Rc          | 0.67 ± 0.03 | 0.46 ± 0.01   | 0.27 ± 0.01 | 0.09 ± 0.01 | 0.02 ± 0.01 | N.D.        |
| Rb2         | 1.40 ± 0.03 | 1.09 ± 0.03   | 0.65 ± 0.02 | 0.29 ± 0.01 | 0.07 ± 0.01 | 0.03 ± 0.01 |
| Rb3         | 5.28 ± 0.10 | 3.93 ± 0.10   | 2.38 ± 0.05 | 1.02 ± 0.01 | 0.21 ± 0.01 | 0.07 ± 0.01 |
| Rd          | 0.90 ± 0.01 | 0.75 ± 0.01   | 0.54 ± 0.01 | 0.34 ± 0.01 | 0.14 ± 0.01 | 0.07 ± 0.01 |
| Rg3         | TR          | 0.41 ± 0.01   | 0.82 ± 0.02 | 1.49 ± 0.04 | 1.69 ± 0.04 | 1.80 ± 0.02 |
| Rh2         | N.D.        | 0.03 ± 0.01   | 0.05 ± 0.01 | 0.08 ± 0.01 | 0.09 ± 0.01 | 0.10 ± 0.01 |
| total       | 10.23       | 8.01          | 5.75        | 4.20        | 2.98        | 2.84        |

<sup>a</sup> *n* = 3; N.D., not detected; TR, trace (less than 0.01%); values are expressed as percentage of dry weight.

20R-Rg2, Rg3, and Rh2) increased. As others have indicated, 20R-Rg2 was not detected in any parts of American ginseng (17, 23). The presence of this saponin can be a marker compound for the heating treatment during processing.

The trend of the main saponins during the heating process (120 °C) is shown in **Figure 2B**. Ginsenoside Rb3 (**Figure 2A**), the main constituent in the American ginseng berry, decreased significantly during steaming from 0.5 to 3 h. Ginsenoside Rg3 (**Figure 2A**) increased significantly from 0.5 to 2 h. At 2 h steaming, ginsenoside Rg3 (1.49%) was greater than ginsenoside Rb3 (1.02%), and Rg3 became the main constituent in the steamed berries. The total ginsenoside content decreased during the heating process (1 h, 5.75%; 2 h, 4.20%; 4 h, 2.84%) compared with that of unsteamed berries (10.23%). Total saponin contents and proportions of ginsenosides Rb2, Rb3, Re, and Rg3 in the total content are shown in **Figure 2C**. Although total saponin contents and the proportion of ginsenoside Rb3 decreased during the heating process, the proportion of ginsenoside Rg3 in the detected ginsenosides increased significantly (unsteamed, <0.1%; 1 h, 10.2%; 2 h, 35.5%; 4 h, 63.4%).

The chemical structure of ginsenoside Rg3, which results from the mild acidic hydrolysis of ginsenosides Rb1, Rb2, and Rc, was confirmed by Shibata et al. (24). Ginsenoside Rg3 was then isolated from white ginseng (25) and red ginseng (26). Since ginsenoside Rg3 was found to inhibit the growth of tumor cells (20), studies of the sources of ginsenoside Rg3 were emphasized. Although this saponin can be obtained by biological transformation (27) and chemical synthesis (28), the process is complicated and the yield is limited. Red ginseng, although a source of ginsenoside Rg3, has low yields at the typical steaming temperature of 100 °C. When we increased the steaming temperature to 120 °C, ginsenoside Rg3 was increased significantly compared with that in berries treated at 100 °C, and the proportion of Rg3 in total ginsenoside content increased during the steaming process.

**Steamed American Ginseng and the Proliferation of Human Colorectal Cancer Cells.** The active principals of ginseng include saponins, polysaccharides, flavonoids, and volatile oils. In cancer therapeutics the saponins have engendered the greatest investigation (5). The major active components are ginsenoside Rg3 and Rh2, which are components of red ginseng (29). Extracts from American ginseng berries steamed at 1 or 2 h were used to test their effects on HCT-116 and SW-480 human colorectal cancer cells (**Figure 3**). Although unsteamed American ginseng berry extract had antiproliferative effects on both types of colon cancer cells, the effects were not strong enough even at high doses. After treatment with 0.5 mg/mL of

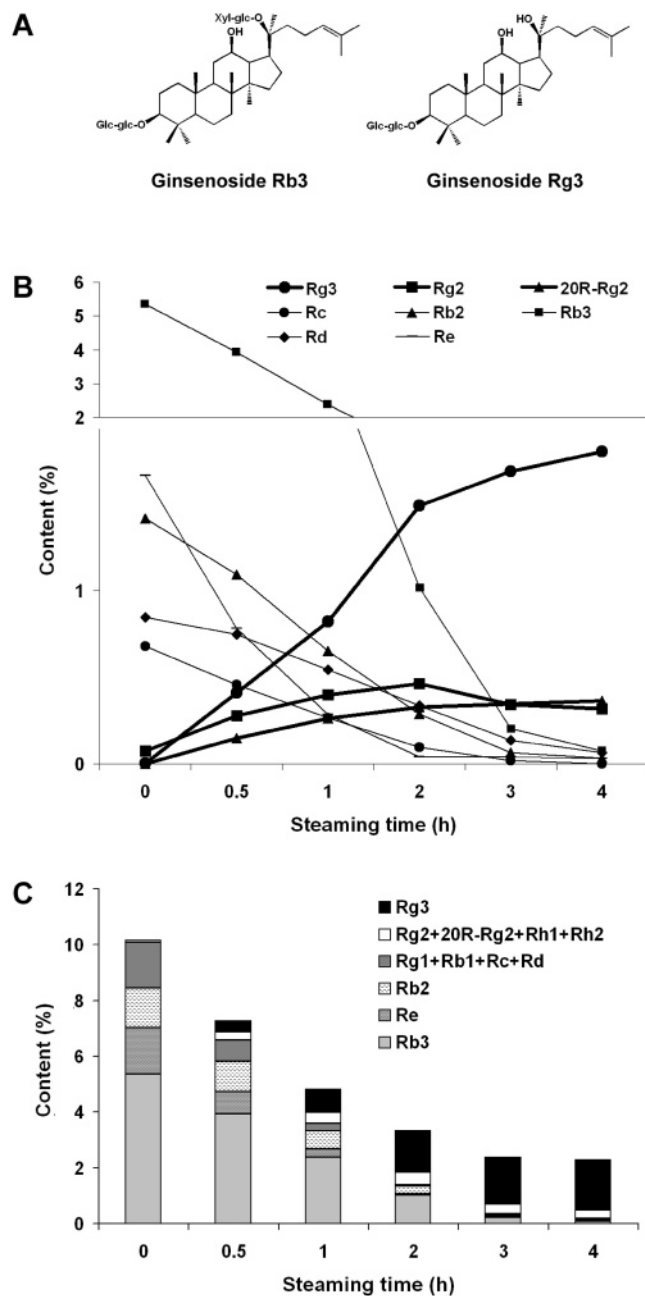
unsteamed American ginseng berry extracts for 48 h, the antiproliferative effects were 34.1% for HCT-116 and 4.9% for SW-480 cells. At the same dose of 0.5 mg/mL with berry extract steamed for 2 h, antiproliferation was 97.8% for HCT-116 and 99.6% for SW-480 cells. At 0.25 mg/mL, the antiproliferative effect of steamed berry extracts was 45.8% (steamed for 1 h) and 89.9% (2 h) on HCT-116 cells, and 31.3% (1 h) and 47.0% (2 h) on SW-480 cells (**Figure 3**). Steaming increased the anticancer effect of American ginseng berries significantly.

Four representative ginsenosides, two of which are major constituents in unsteamed berries (Rb3 and Re), and the other two of which are major constituents in steamed berries (Rg2 and Rg3), were used to test the antiproliferative effect on human colon cancer cells. In the results shown in **Figure 4**, 30–1000 μM ginsenoside Rb3, Re, and Rg2 had antiproliferation effects of <30%. Ginsenoside Rg3, the main constituent in steamed American ginseng berries, showed a strong antiproliferation effect on HCT-116 cells: 36.8% at 100 μM, and 97.7% at 300 μM.

#### Effects of Steamed Extracts on Cancer Cell Apoptosis.

There are two types of cell death in biological systems, namely necrosis and apoptosis. In apoptosis the cell shrinks and then deforms. After detaching from its neighbors it undergoes chromatin condensation and internucleosomal cleavage of the DNA before fragmenting into compact membrane-enclosed structures termed “apoptotic bodies.” Apoptotic bodies can be observed with a fluorescent microscope after they are stained with Hoechst 33258. To test whether the decrease in cell viability observed after treatment with unsteamed and steamed American ginseng extracts was due to apoptosis or not, HCT-116 and SW-480 cells were stained with Hoechst 33258 dye after exposure to the treatments for 48 h. The dye stains condensed the chromatin of apoptotic cells more brightly than the chromatin of normal cells (**Figure 5**).

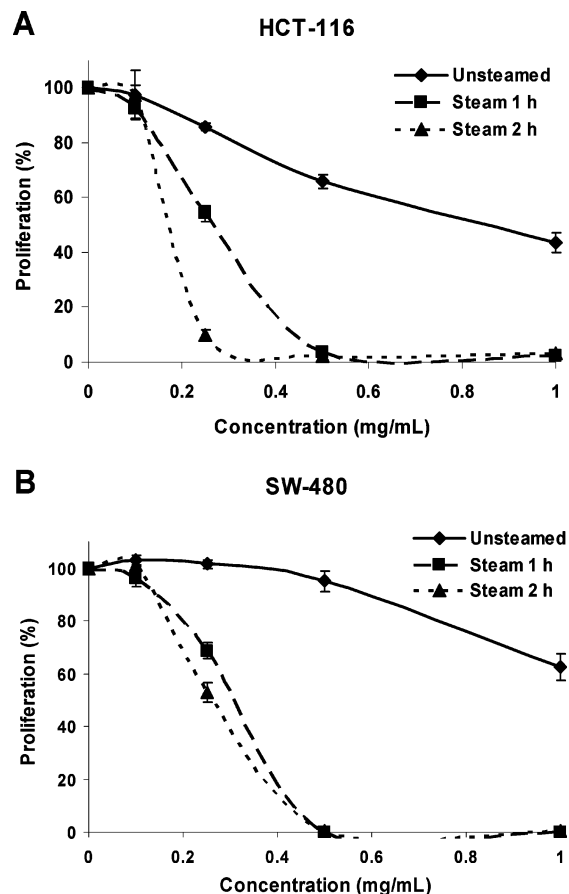
Induction of apoptosis also was confirmed by flow cytometry after staining with annexin V/PI. After treatment with unsteamed and steamed berry extracts for 8 h, cells were stained with annexin V/PI. Viable cells were negative for both annexin V and PI; early apoptotic cells were positive for annexin V and negative for PI. Late apoptotic cells displayed both positive for annexin V and PI; nonviable cells which underwent necrosis were positive for PI and negative for annexin V. The percentage of apoptotic cells (early and late) induced by unsteamed and steamed berry extracts (0.25 and 0.5 mg/mL) is shown in **Figure 6**. Steamed berry extracts induced a higher percentage of apoptotic cells than did unsteamed berry extracts. At 0.5 mg/mL, the percentage of apoptotic cells was 8.7% (unsteamed)



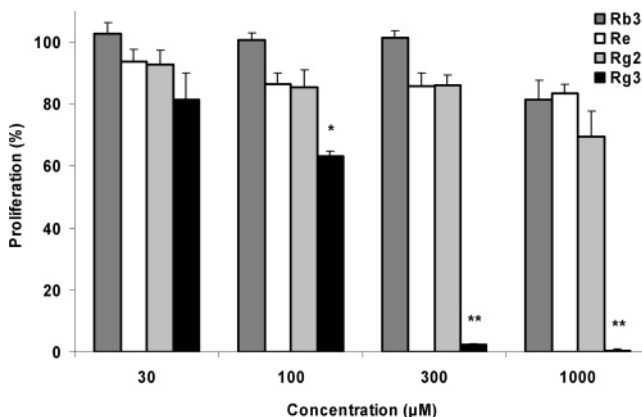
**Figure 2.** Saponin content in American ginseng berries steamed at 120 °C for 0–4 h. (A) structure of ginsenosides Rb3 and Rg3. The content of (B) individual ginsenosides was changed during the steaming process, and (C) the total ginsenoside content was also changed.

and 29.7% (steamed) for HCT-116 cells, and 8.6% (unsteamed) and 21.5% (steamed) for SW-480 cells. After steaming, ginseng berry extracts enhanced apoptosis in colorectal cancer cells significantly.

Human colorectal cancer is a significant public health problem in the Western world. In the United States, this cancer is the second-leading cause of cancer-related deaths and the second most prevalent cancer worldwide (30, 31). For *in vitro* studies, there are several human colorectal cancer cell lines, of which HCT-116 is widely used in laboratory cancer research (32) and as a model for cellular pathway studies of chemotherapy on cancer cells (33). SW-480, another cell line that has been established for more than 30 years, also is commonly used in colon cancer research (34, 35).

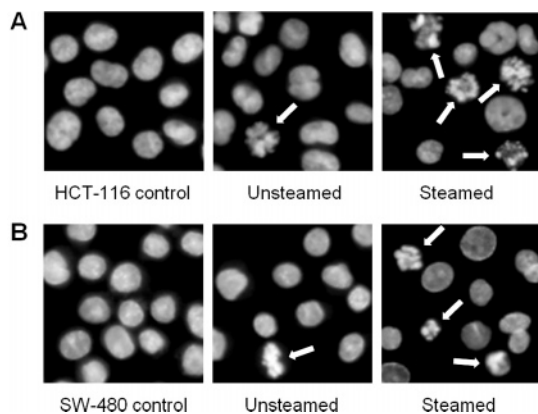


**Figure 3.** Effects of American ginseng berry extracts on (A) proliferation of HCT-116 and (B) SW-480 human colorectal cancer cells assayed by the MTS method. Extracts were taken from American ginseng berries steamed at 120 °C for 1 h or 2 h or unsteamed berries.

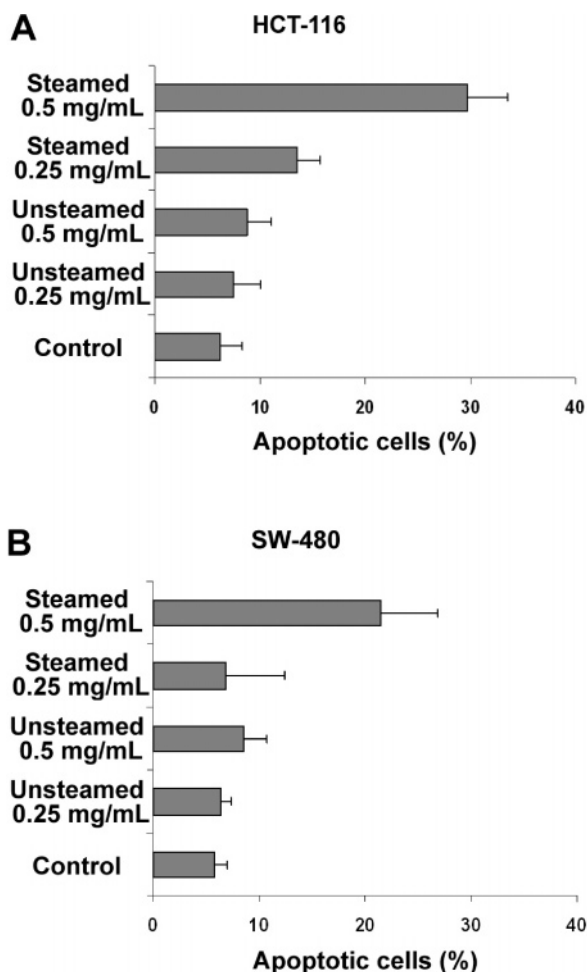


**Figure 4.** Effects of ginsenosides Rb3, Re, Rg2, and Rg3 on the proliferation of HCT-116 human colorectal cancer cells. The treatment concentrations of ginsenosides were 30, 100, 300, and 1000 μM. \* $p < 0.05$ ; and \*\* $p < 0.01$  vs control.

Although many early stage colorectal cancers are cured by surgical resection alone, most often surgery is combined with adjuvant chemotherapy. However, chemotherapies have adverse effects and dose-limiting toxicity. Newer chemotherapeutic agents continue to be investigated, including those derived from botanical sources (36). The use of complementary and alternative medicine such as botanical extracts is becoming increasingly popular among cancer patients. Botanical products are used to boost the immune system, decrease chemotherapy-induced side effects, and improve quality of life (37, 38). Using a rat model,



**Figure 5.** Effects of steamed and unsteamed American ginseng berry extract on apoptosis in HCT-116 and SW-480 human colorectal cancer cells. (A) HCT-116 and (B) SW-480 cells were treated with 0.25 mg/mL extracts of unsteamed and steamed berry extract (120 °C 2 h) for 48 h. Cells were stained with a DNA specific dye, Hoechst 33258. Apoptotic cells are marked with arrows.



**Figure 6.** Flow cytometric analysis of apoptotic cells after annexin-V FITC/propidium iodide (PI) staining. Percentage of apoptotic cells (including early and late apoptotic cells) is shown. (A) HCT-116 cells and (B) SW-480 cells were treated with extracts of unsteamed or steamed (120 °C 2 h) American ginseng berries with the concentration of 0.25 and 0.5 mg/mL for 8 h.

we evaluated the preventative effect of different botanical extracts on chemotherapy-induced nausea and vomiting (39–41). American ginseng berry extract reduced gastric side effects induced by cisplatin (18) and had an antiproliferative effect on

human breast cancer cells (12). In our study using two colon cancer cell lines, we found that American ginseng berry extract can inhibit cell growth to a limited degree. After steaming at 120 °C for 2 h, the berry extracts had a strong antiproliferative effect on colon cancer cells. Compared with three other representative ginsenosides, Rg3 was the most potent for the antiproliferative effect on colorectal cancer cells.

Steamed American ginseng berry extracts also induced cancer cell apoptosis. Studies found that cell apoptosis is mediated by many factors. P53 is a transcription factor placed at the nexus of a number of pathways that mediate apoptosis in response to a wide range of cellular stresses. HCT-116 cells are p53 wild-type, while SW-480 cells are mutant in the p53 tumor suppressor gene. The differences of chemosensitivity of the two types of cell lines may provide some information such as whether or not the induction of apoptosis of steamed American ginseng berry extract is dependent on p53. The mechanism of apoptosis induction will be the focus of future studies. Since the root of American ginseng is a commonly used herbal medicine, in the future we will also study the anticancer properties of steamed American ginseng roots.

In summary, analytical and pharmacological data obtained from this study suggest that steamed American ginseng berries significantly augment ginsenoside Rg3 content, which is responsible for the increase in anticancer activity.

#### NOTE ADDED AFTER ASAP PUBLICATION

The original posting of December 8, 2006, contained a minor error in Table 1. This has been corrected with the posting of December 12, 2006.

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