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ORIGINAL ARTICLE

Cisplatin's tumoricidal effect on human breast carcinoma MCF-7 cells was not attenuated by American ginseng

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Abstract
Purpose We previously observed that American ginseng berry and ginsenoside Re attenuated cisplatin-induced emesis in a rat model, suggesting that the herb may have a value in treating chemotherapy-induced nausea/vomiting. However, it is not clear whether consuming ginseng concurrently with chemotherapy affects the efficacy of chemotherapeutic agents. In this study, we explored if the ginseng extract and its constituents, ginsenosides Rb_1, Rb_3, and Re, alter tumoricidal activity of cisplatin in human cancer cells.
Methods Tumoricidal effects of cisplatin, and/or American ginseng berry extract (AGBE) and ginsenosides Rb_1, Rb_3, and Re, on human breast carcinoma MCF-7 cells were measured as cell proliferation in vitro. Cell counts were performed in MCF-7 cells pretreated with test agents for 72 h.
Results Cisplatin decreased MCF-7 cell proliferation significantly in a concentration-dependent manner. Compared to control group, cisplatin reduced the cell proliferations to 56.5±3.3% at 1 μg/ml, to 36.6±2.4% at 5 μg/ml, and to 26.9±2.4% at 15 μg/ml ($P<0.01$). AGBE also inhibited the cell proliferation significantly, although in a less extended manner. When the berry extract at 0.5 mg/ml was used with cisplatin at 1 μg/ml, a significant enhancement of cisplatin's activity was observed (35.8±2.5%; $P<0.05$). We also observed that Rb_1 did not change cisplatin's activity; Rb_3, at a higher concentration, increased cisplatin's anti-proliferation activity (48.0±1.2%; $P<0.05$); Re increased cisplatin's activity (Re 0.1 mg/ml, 48.0±2.8%; Re 0.3 mg/ml, 31.9±2.2%, $P<0.01$).
Conclusion Our data suggest that AGBE and the tested ginsenosides do not attenuate cisplatin's tumoricidal activity in MCF-7 cells, but in fact may actually enhance it. Additionally, the ginseng extract and ginsenoside Re by themselves exerted anti-proliferative activity against MCF-7 cells. The herb *might potentially* serve a complementary role with the chemotherapeutic agents in treating cancer, in addition to decreasing chemotherapy-induced nausea/vomiting.

Keywords American ginseng berry · Ginsenoside Rb_1 · Ginsenoside Rb_3 · Ginsenoside Re · Cisplatin · Herbal–drug interaction · Breast carcinoma MCF-7 cell

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Introduction

Treatment of cancer with chemotherapeutic agents such as cisplatin is associated with severe adverse effects. The drug-related adverse events not only worsen patients' quality of life but can also lead to their refusal to continue the potentially curative chemotherapy [20, 27]. Nausea/vomiting is one of the most significant adverse effects of chemotherapy [27]. It is vital to adequately control this drug-induced symptom and the anticipatory emesis of subsequent chemotherapy cycles [27]. The currently available anti-emetic drugs can relieve the symptoms especially when used in combinations, but lead to the possibility of additional adverse events and potential drug–drug interactions causing a reduction in the efficacy of chemotherapeutic agents [12, 32, 33]. Recently, we reported that the berry extract of American ginseng (*Panax quinquefolius* L.) and its major constituent, ginsenoside Re, attenuated cisplatin-induced emesis in a rat model, and demonstrated the potential value of the herb in treating chemotherapy-induced nausea/vomiting [21]. Patients with cancer often resort to complementary and alternative medical means to treat the side effects of chemotherapy [24]. It therefore appears that American ginseng berry extract (AGBE) may have a clinical utility in this setting.

As an anti-oxidant herb, ginseng's effect on cisplatin-induced adverse effects could be linked to its anti-oxidant activity [13, 21, 30]. However, to date, it is not clear if consuming anti-oxidants concurrently with chemotherapy hampers or helps the tumoricidal activity of chemotherapeutic agents [14]. In this study, we evaluated whether American ginseng extract and its active constituents, ginsenosides Rb_1, Rb_3, and Re, alter tumoricidal activity of cisplatin in human breast carcinoma MCF-7 cells [25], which are sensitive to cisplatin.

Materials and methods

Preparation and analysis of AGBE

American ginseng berry (Wisconsin Ginseng Board, Wausau, WI, USA) was cultivated under standard conditions. The dried berry, obtained from a single batch, was ground into fine powder, dispersed in 75% ethanol, sonicated and refluxed for 1 h at 50°C [28]. The extraction procedure was repeated three times. The cooled mixture was filtered through Whatman No. 1 paper (Maidstone, England) and the filtrate was collected. The final residue was washed with ethanol and the solution was combined with the extract. The ethanol extract was dried under vacuum with rotary evaporator R-205 (Buchi Labortechnik AG, Flawil, Schweiz), dissolved in water, and extracted with water-saturated butanol (three times). The butanol layers were combined, washed with water, evaporated under vacuum, and freeze-dried.

High-performance liquid chromatography (HPLC) was conducted to confirm the ginsenoside profile of the AGBE using a Liquid Chromatography System of Waters model 2960 (Milford, MA, USA), with a quaternary pump, automatic injector, a 996 photodiode array detector, and Waters Millennium^{32} software for peak identification and integration. Ginsenosides were separated on a Phenomenex Prodigy ODS (2), 5 μm, 150×3.2 mm analytical column (Phenomenex, Torrance, CA, USA) [34, 35]. The separation of ginsenosides was obtained at room temperature by gradient elution using acetonitrile (A) and water (B) as eluents. Gradient elution started with 20% solvent A and 80% solvent B, changed to 20.3% A for 25 min, then changed to 26.8% A for 3 min and held for 26 min; changed to 35.6% A for 20 min; changed to 50% A for 11 min; changed to 68% A for 10 min; changed to 95% A for 1 min and held for 3 min; changed to 20% A for 3 min and held for 8 min. The absorbance was measured at 202 nm using the photodiode array detector. Figure 1 shows a chromatogram of AGBE assayed by HPLC, and three selected ginsenosides in AGBE.

Ginsenosides Rb_1 and Re, with purity of >98%, were obtained from Indofine Chemical Company (Somerville, NJ, USA). Ginsenoside Rb3, with purity of >95%, was obtained from Delta Information Center for Natural Organic Compounds (Xuancheng, China).

Cell and culture conditions

The human breast carcinoma MCF-7 cells (ATCC, Manassas, VA, USA) were routinely grown in Dulbecco's modification of Eagle's minimal essential medium (DMEM), supplemented with 10% fetal bovine serum and penicillin-streptomycin (50 unit/ml; Invitrogen, Carlsbad, CA, USA). Cells were maintained in a tissue culture dish (100 mm in diameter) and kept in a humidified incubator (5% CO_2 in air at 37°C). Medium was changed every 2–3 days. When the cells reached 80–90% confluence, they were trypsinized, harvested, and seeded into a new tissue culture dish [6, 15, 19].

Cell proliferation assay

To examine the anti-proliferative effect of the test agents, MCF-7 cells were seeded in a 24-well plate at approximately 10,000 cells/well with regular DMEM medium and allowed to adhere for 24 h. After adhesion

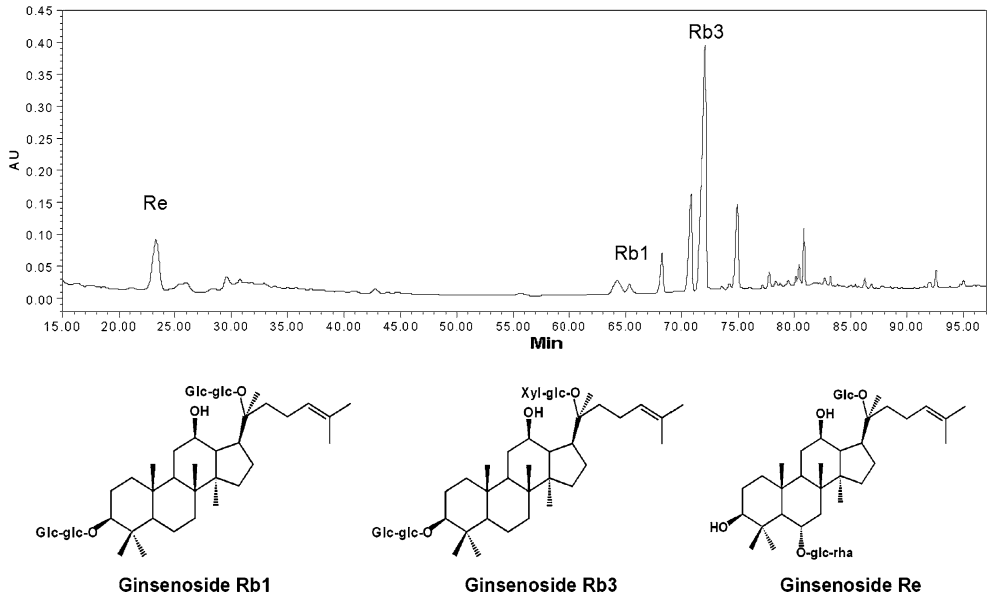

Fig. 1 Chromatogram of American ginseng berry extract using HPLC analysis. Chromatographic conditions were described in Sect. 'Materials and methods'. The peaks of ginsenoside Rb1, Rb3, and Re are indicated. Chemical structures of these three ginsenosides are also shown

of MCF-7 cells, the culture medium was changed prior to the addition of drugs. The MCF-7 cells were incubated with testing material at various concentrations for 72 h. Control cultures were incubated in medium alone. At the end of treatments, MCF-7 cells were detached using trypsin and counted using a Coulter Counter (Coulter Electronics, Hialeah, FL, USA) [5, 7]. On selected days after the removal of the incubation medium, the cell monolayer was washed twice with phosphate buffered saline. All assays were performed at least three times. The percentage of MCF-7 carcinoma cell proliferation was calculated as follows: cell proliferation (%) = 100 × (cell number in experimental well/cell number in the control well).

Statistical analysis

Data are expressed as mean ± standard error. Statistical analysis was performed using analysis of variance followed by a post hoc test for comparison of means. Differences were considered significant if $P<0.05$.

Results

Tumoricidal effect of cisplatin on MCF-7 carcinoma cells

The tumoricidal activity of cisplatin on MCF-7 carcinoma cells is shown in Fig. 2. Cisplatin decreased MCF-7 carcinoma cell proliferation significantly in a concentration-dependent manner. Compared to control group (100%), cisplatin reduced the cell proliferations to 56.5±3.3% at 1 μg/ml, to 36.6±2.4% at 5 μg/ml, and to 26.9±2.4% at 15 μg/ml ($P<0.01$). Cisplatin at a dose of 1 μg/ml was selected for studying the interaction between cisplatin and ginseng.

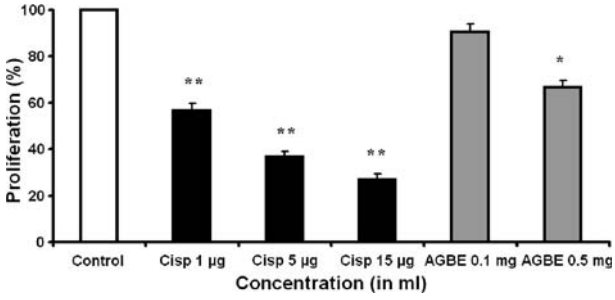

Fig. 2 Anti-proliferation effects of cisplatin and American ginseng berry extract on MCF-7 carcinoma cells after 72 h treatment. Control is normalized to 100%. **, $P<0.01$, compared to control. *Cisp* cisplatin, *AGBE* American ginseng berry extract

Figure 2 also shows the effects of AGBE on MCF-7 cells. AGBE inhibited the cell proliferation significantly as well, although in a less extended manner. Compared to control group, AGBE reduced the cell proliferations to 90.4±3.6% at 0.1 mg/ml, and to 66.7±3.0% at 0.5 mg/ml ($P<0.01$).

Effect of AGBE on cisplatin's tumoricidal activity

As shown in Fig. 3, compared to cisplatin alone at 1 μg/ml (56.5±3.3%), cisplatin at 1 μg/ml plus AGBE at

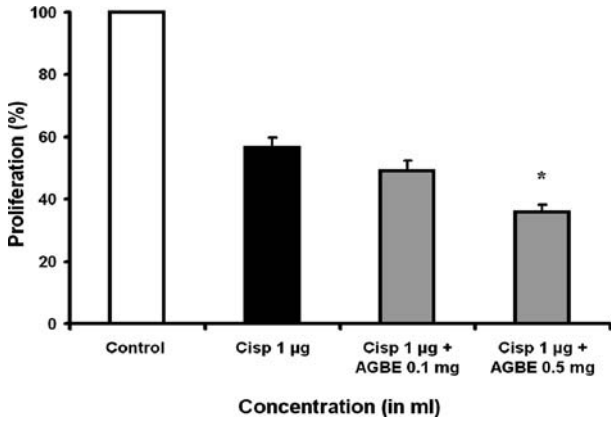

Fig. 3 Additive effects of American ginseng berry extract on cisplatin's anti-proliferation activity in MCF-7 carcinoma cells. *, $P<0.05$, compared to cisplatin alone. *Cisp* cisplatin, *AGBE* American ginseng berry extract

0.1 mg/ml elicited an increasing trend in anti-proliferation activity (49.1±3.2%). When AGBE at 0.5 mg/ml was used with the same concentration of cisplatin, a significant change was observed (35.8±2.5%; $P<0.05$ compared to cisplatin alone). These data suggest that AGBE does not attenuate cisplatin's tumoricidal activity. Rather, at a concentration of 0.5 mg/ml, AGBE enhanced the tumoricidal activity of cisplatin on MCF-7 cells.

Interaction between cisplatin and ginsenosides on MCF-7 carcinoma cells

To explore which ginsenosides in AGBE play a major role in the additive effects of cisplatin's tumoricidal activity, three ginsenosides were tested (Fig. 4). While, ginsenoside Rb_1 did not change cisplatin's activity significantly, ginsenoside Rb_3, at a higher concentration, increased cisplatin's activity (48.0±1.2; $P<0.05$). We also observed that ginsenoside Re increased cisplatin's activity in a concentration-related manner (Re 0.1 mg/ml, 48.0±2.8; Re 0.3 mg/ml, 31.9±2.2, $P<0.01$).

Discussion

Ginseng has been used for centuries in Oriental countries as a panacea that promotes longevity [2, 11, 17], and is considered reasonably safe [8]. There are mainly two different kinds of ginseng based on their geographical location of cultivation, i.e., Asian ginseng and American ginseng. Both of them show comparable properties of restoring and enhancing normal well-being and are thereby collectively known as adaptogens [2, 11, 17]. In this study, we used American ginseng, one of the most commonly consumed herbs in the United States [3]. The berry of ginseng, which was reported in our previous investigation as reducing chemotherapy-induced nausea/vomiting [21], was used in this study. We have also previously demonstrated that the berry has a significantly higher content of total ginsenosides than the root of ginseng [35].

In our previous study, an anti-emetic effect of AGBE was observed using a cisplatin-induced rat model, and our data suggest that this effect was mediated through the extract's anti-oxidant activity [21]. Clinically, specific beneficial effects such as anti-ischemic, anti-hypertensive and *immune modulating effects* have been attributed to the use of ginseng [16, 30]. These pharmacological effects are, to a significant extent, also considered to be due to the anti-oxidant properties of the herb [22]. Kitts et al. reported that American ginseng exhibited effective anti-oxidant activity in both lipid-soluble and water-soluble medium by chelating metal ions and through the direct scavenging of DPPH and hydroxyl radicals in an in vitro study [13]. In addition to anti-oxidant properties, American ginseng also stimulates NO (nitric oxide) release, which could modulate the anti-oxidant activity [11].

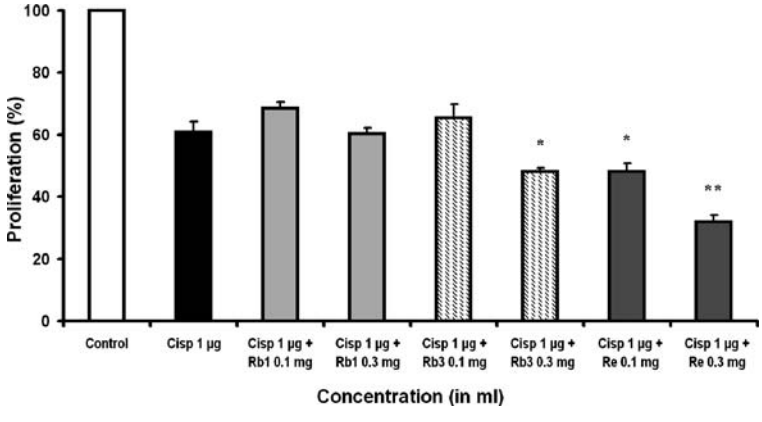

Fig. 4 Effects of ginsenosides Rb1, Rb3, and Re on cisplatin's anti-proliferation activity in MCF-7 carcinoma cells. *, $P<0.05$, and **, $P<0.01$, compared to cisplatin alone. *Cisp* cisplatin

The mechanism of tumoricidal action of cisplatin is similar to that of the alkylating group of drugs and is achieved through DNA cross-linking [37]. Thus, the chemotherapeutic activity of cisplatin is not mainly mediated through oxygen radical generation. However, oxygen radical production is a significant adverse event *that occurs* with cisplatin treatment and results in tissue injury [26, 29, 36]. Administering anti-oxidants could treat the oxidant-mediated side effects but might have possible interactions with the tumoricidal actions of cisplatin [14]. Interactions of cisplatin with the anti-oxidant, *N*-acetyl cysteine, demonstrated an inhibition of anti-tumor activity [14]. On the contrary, other anti-oxidants, such as quercetin and glutathione, showed a trend towards greater tumor response or anti-tumor activity [1, 14]. Other investigators have shown that anti-oxidant treatment could help to prevent gastrointestinal toxicity, nephrotoxicity, and ototoxicity, without affecting the anti-cancer activity [4, 36]. L-Carnitine prevented cisplatin-induced oxidant injury of the kidneys and intestines while preserving cisplatin's tumoricidal activity in a rat model [4]. It thus appears that the outcomes of interaction between cisplatin and anti-oxidants depend on the specific anti-oxidants. Since the anti-proliferative activity of ginseng in MCF-7 cells appears to be mediated by arresting cell cycle, it should not interfere with cisplatin's anti-tumor activity [23]. In the present study, we observed that the interaction between ginseng and cisplatin is complementary.

Ginsenosides are active constituents in ginseng [2], and some ginsenosides have individually exhibited anti-oxidant properties [10, 18]. Ginseng root has a relatively high content of Rb_1 compared to that of ginseng berry, while the berry has significantly higher contents of Rb_3 and Re [31]. Corbit et al. recently observed an anti-proliferative effect of American ginseng root on MCF-7 cells [9]. In this study, the effects of AGBE and selected ginsenosides on cisplatin's tumoricidal activity were individually tested in the MCF-7 cell line. Our data showed that Re has a significant additive effect on cisplatin's activity, and that Re could be responsible for the ginseng berry's positive interaction with cisplatin.

Cisplatin is used extensively as an effective chemotherapeutic agent in treating a variety of cancers. Our data demonstrated that the ginseng berry extract and Re possess tumoricidal activity and contribute an additive effect to cisplatin's activity. The anti-cancer effect of ginseng increases the likelihood that the anti-oxidant herb might serve a complementary role with the chemotherapeutic agents in treating cancer, in addition to potentially decreasing chemotherapy-induced nausea/vomiting. Cancer patients are known to use herbal products more often than the general populace [24], and thus, well-investigated herbal therapies can offer patients with a practical alternative.

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