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Effects of *Panax notoginseng*, ginsenoside Rb1, and notoginsenoside R1 on proliferation of human breast carcinoma MCF-7 cells

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SUMMARY

In this study, we evaluated the antiproliferative effects of *Panax notoginseng*, ginsenoside Rb1, and notoginsenoside R1 in the human breast carcinoma MCF-7 cell line. Our results indicated that both *Panax notoginseng* radix extract (NRE) and *Panax notoginseng* rhizoma extract (NRhE) possess significant antiproliferative activities in MCF-7 cells. Compared to control group (100%), at the concentrations of 0.05, 0.5, and 1.0 mg/ml NRE, cell growth was concentration-dependently reduced to 81.0 ± 6.1 ($P < 0.01$), 34.2 ± 4.8 ($P < 0.001$), and 19.3 ± 1.9 ($P < 0.001$), respectively. Similar results with NRhE at concentrations of 0.5 and 1.0 mg/ml were obtained in these MCF-7 cells. To identify the responsible chemical constituent, we tested the antiproliferation effects of two representative saponins, ginsenoside Rb1 and notoginsenoside R1, on the MCF-7 cells. The data showed that ginsenoside Rb1 was endowed with antiproliferative properties, while notoginsenoside R1 did not have an inhibitory effect in the concentrations tested. Our studies provided evidence that *Panax notoginseng* extracts and ginsenoside Rb1 may be beneficial, as adjuvants, in the treatment of human breast carcinoma.

Key words: Panax notoginseng; Ginsenoside Rb1; Notoginsenoside R1; Antiproliferation effect; Breast carcinoma MCF-7 cells

INTRODUCTION

Breast carcinoma is the most prevalent malignancy among women in the industrialized world and an important cause of both morbidity and mortality. Breast carcinoma accounts for 30% of all cancer in women and it remains the second leading cause of death in western women (Sezgin *et al.*, 2002; Raobaikady *et al.*, 2003; Akbas *et al.*, 2005). One out of every nine women will ultimately be diagnosed with breast cancer in the U.S. before the age of 85 (Society, 2000). Although many active chemotherapeutic agents are used in the treatment of this disease, there is no curative therapy for breast carcinoma (Akbas *et al.*, 2005).

Panax notoginseng (Burk) F. H. Chen (Araliaceae) or notoginseng is a commonly used traditional Chinese medicinal herb indigenous to the mountains of Yunnan and Guangxi provinces in China (Ng *et al.*, 2004; Zhu *et al.*, 2005). Historical study has indicated that notoginseng was first applied as a botanical by the minority ethnic groups in southwestern China during the Ming Dynasty

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(Zhang *et al.*, 2005). Notoginseng possesses multiple constituents with multifaceted pharmacological functions (Lam *et al.*, 2002; Chung *et al.*, 2004; Park *et al.*, 2005). It reportedly exerts beneficial effects on the treatment of trauma and bleeding due to internal and external injury, inflammation, cardiovascular diseases, and carcinoma (Yoshikawa *et al.*, 1997; Yoshikawa *et al.*, 2001; Lam *et al.*, 2002; Wei *et al.*, 2002; Chen *et al.*, 2004; Chung *et al.*, 2004; Sun *et al.*, 2004; Li *et al.*, 2005; Park *et al.*, 2005; Wang *et al.*, 2005), while cancer prevention by notoginseng has received increasing attention recently (Zhong *et al.*, 1996; Chung *et al.*, 2004; Li *et al.*, 2005; Park *et al.*, 2005; Wang *et al.*, 2005). However, the effects of notoginseng extracts and their constituents have not been reported in respect to inhibitory effects in human breast carcinoma MCF-7 cells. The aim of this study was to evaluate whether notoginseng extracts and their representative constituents, ginsenoside Rb1 and notoginsenoside R1, could reduce the growth of MCF-7 breast cells to open a window for developing a potential adjuvant for treating human breast carcinoma.

MATERIALS AND METHODS

Preparation of notoginseng radix extract (NRE) and notoginseng rhizoma extract (NRhE)

Radix and rhizome of notoginseng, from single lots, were obtained from the Yunnan Chinese Herbal Medicine Company, China. Dried notoginseng radix was ground to powder. Twenty-five grams of the powder was extracted with 500 ml methanol for 4 h, and the temperature of water bath was 80°C. After the solution was cooled, it was filtered with filter paper and filtrate was collected. The residue was extracted further with 500 ml methanol for 4 h and then filtered while the solution was cooled. The solvent of the mixed filtrate was evaporated under vacuum. This extract was dissolved in 100 ml water and then was extracted with water-saturated n-butanol. The n-butanol phase was evaporated under vacuum and the extract was lyophilized (Park *et al.*, 1996; Woo *et al.*, 2004). NRhE was prepared in the same manner.

Ginsenoside Rb1 and notoginsenoside R1 (Fig. 1), with purity of > 95%, were obtained from National Institute for the Control of Pharmaceutical and Biological Products, China.

Cell and culture conditions

The human breast carcinoma MCF-7 cells (ATCC, Manassas, VA) were routinely grown in Dulbecoo's modification of Eagle's minimal essential medium (DMEM), supplemented with 5% fetal bovine serum and penicillin-streptomycin (50 unit/ml; Invitrogen, Carlsbad, CA). 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 70 - 80% confluence, they were trypsinized, harvested, and seeded into a new tissue culture dish (Choi *et al.*, 2005; Mahmud *et al.*, 2005; Marin *et al.*, 2005).

Cell proliferation assay

On selected days after removal of incubation medium, the cell monolayer was washed twice with phosphate buffered saline (PBS). To examine the antiproliferative effect of the notoginseng, MCF-7 cells were seeded in a 24-well plate at about 10,000 cells/well with regular DMEM medium and allowed to adhere for 24 h. After adhesion 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

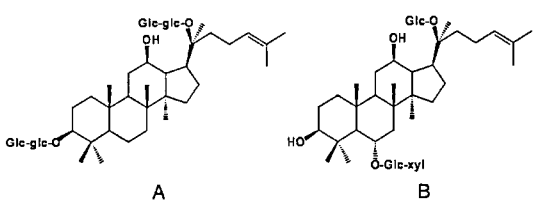

Fig. 1. Chemical structure of ginsenoside Rb1 (A) and notoginsenoside R1 (B).

incubated in medium alone. At the end of pretreatments, MCF-7 cells were detached using trypsin and counted using a Coulter Counter (Counlter Electronics, Hialeah, FL) (Colston *et al.*, 1998; Sezgin *et al.*, 2002; Xie *et al.*, 2006). All assays were performed at least two or three times. The percentage of MCF-7 carcinoma cell proliferation was calculated (Ye *et al.*, 2005 at www.sciencedirect.com) as follows: Cell proliferation (%) = 100 × (each cell number in experimental well/ total cell number in the control well).

Influence of DMSO on cell proliferation

DMSO was used to dissolve ginsenoside Rb1 and notoginsenoside R1. To ensure the cell growth changes were not induced by the dissolvent DMSO, we assayed the effect of DMSO alone on the proliferation of the cells as a vehicle group. After cells were pretreated with DMSO (5 μl/ml) alone for 72 h, cells were tested again (Ye *et al.*, 2005 at www.sciencedirect.com).

Morphological observation

To observe cell morphological changes (Raobaikady *et al.*, 2003; Choi *et al.*, 2005; Marin *et al.*, 2005), the MCF-7 cells were seeded in 24-well plates with regular DMEM and allowed to adhere for 24 h. After the MCF-7 cells incubated with NRE at various concentrations for 72 h, the cellular morphological changes were observed under phase contrast microscopy (Nikon, Eclipse, TE2000-U, Japan). Control cultures were incubated in medium alone.

Statistical analysis

Data are expressed as mean ± standard error (S.E.). Statistical analysis was performed using one-way ANOVA in combination with Student's *t*-test. Differences were considered significant if $P < 0.05$.

RESULTS

Inhibitory effect of NRE on MCF-7 carcinoma cells

The inhibitory activity of NRE on MCF-7 carcinoma cells is shown in Fig. 2. NRE decreased MCF-7 carcinoma cell proliferation significantly in a concentration dependent manner. Compared to control group, NRE reduced the cell proliferations to 81.0 ± 6.1% at 0.05 mg/ml ($P < 0.01$), to 34.2 ± 4.8% at 0.5 mg/ml ($P < 0.001$), and to 19.3 ± 1.9 at 1.0 mg/ml ($P < 0.001$).

Inhibitory effect of NRhE on MCF-7 carcinoma cells

Fig. 3 shows the antiproliferative effects of NRhE on MCF-7 cells. NRhE suppressed the growth of MCF-7 cells significantly compared to the control. At 0.5 mg/ml and 1.0 mg/ml, it inhibited growth to 42 ± 7.0% and 14 ± 1.7%, respectively (both $P < 0.001$). However, there was no significant effect on the cells at 0.05 mg/ml (96 ± 4.5%).

Inhibitory effect of ginsenoside Rb1 on MCF-7 carcinoma cells

As shown in Fig. 4, ginsenoside Rb1 inhibited MCF-7 cell proliferation significantly in a concentration-dependent manner. Compared to control group, at 0.1 and 0.3 mM, Rb1 inhibited cell growth to 79.0

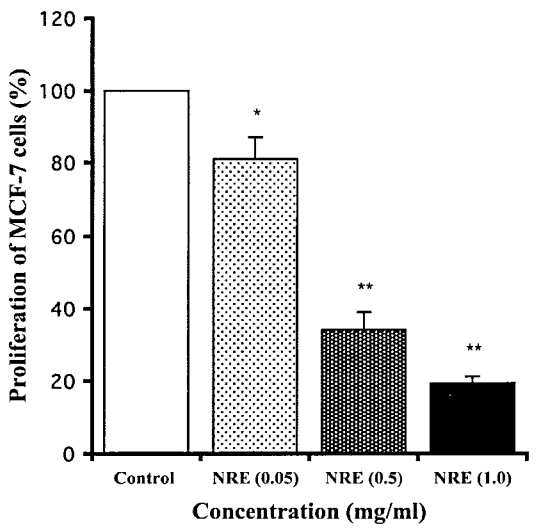

Fig. 2. Antiproliferation effects of *Panax notoginseng* radix extract (NRE) on MCF-7 carcinoma cells after 72 h pretreatment. $^{*}P < 0.01$; $^{**}P < 0.001$.

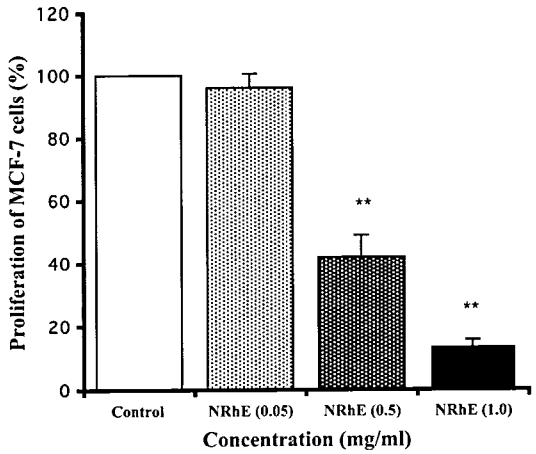

Fig. 3. Antiproliferation effects of *Panax notoginseng* rhizoma extract (NRhE) on MCF-7 carcinoma cells after 72 h pretreatment. $^{**}P < 0.001$.

± 0.9% and 65.0 ± 3.8%, respectively (both $P < 0.001$). In addition, Fig. 4 also shows that vehicle group (DMSO 5 ml/ml) did not influence the cell proliferation (101.0 ± 1.2%).

Effect of notoginsenoside R1 on MCF-7 carcinoma cells

In this study, we also observed that notoginsenoside R1, another single constituent of notogingeng, did not inhibit cell proliferation. Our data showed

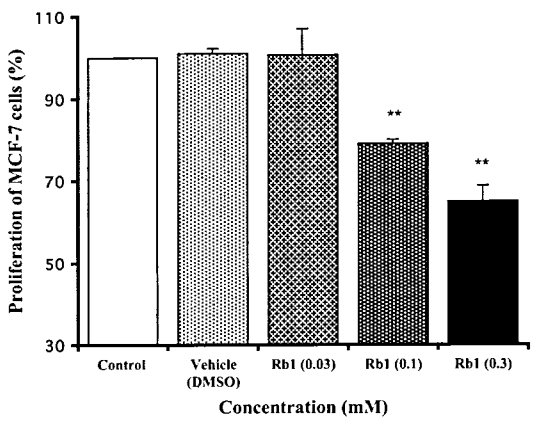

Fig. 4. Antiproliferation effects of ginsenoside Rb1 and DMSO on MCF-7 carcinoma cells after 72 h pretreatment. $^{**}P < 0.001$.

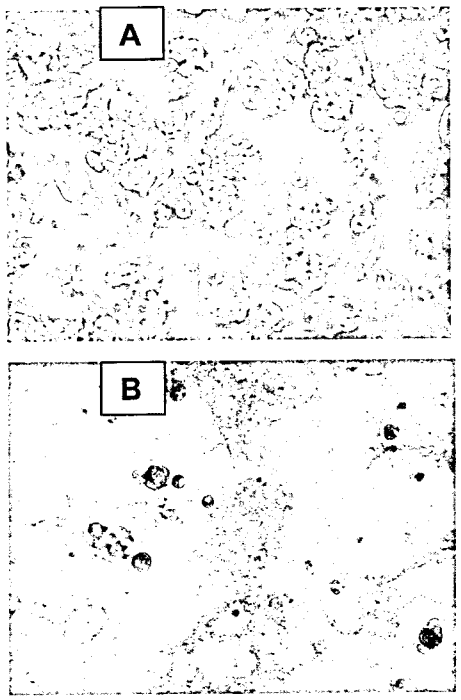

Fig. 5. Effects of *Panax notoginseng* radix extract (NRE) on MCF-7 carcinoma cell morphology. A: Untreated control cells. B: Cells treated with 1.0 mg/ml NRE (× 200 magnification).

that after treatment with R1 the percentage of proliferation of MCF-7 cells were 107.5 ± 3.2, 117.5 ± 4.7, and 106.7 ± 5.2 at concentrations of 0.03, 0.1, and 0.3 mM, respectively.

Effects of NRE on MCF-7 cell morphology

The effects of NRE on MCF-7 cell morphology are shown in Fig. 5. In untreated cells (Fig. 5A), rounded cells were visible per field. After inoculated with 1.0 mg/ml NRE, cellular morphological changes were observed during the culture period. These alterations included shrinkage, rounding, detachment, and segregation of cellular structure. Fig. 5B shows that after treated with 1.0 mg/ml NRE for 72 h, many broken cell fragments can be observed, while no regular cancer cells can be found. At the lower concentrations (0.5 and 0.05 mg/ml), however, NRE induced less changes on cell morphology (data did not show).

DISCUSSION

Breast carcinoma is a major malignancy that affects one in every eight women in the US, Europe, Australia, and Latin America, and it accounts for 1 in 3 of all female cancers (Bawadi *et al.*, 2005). Breast carcinoma is a systemic disease, which originates in the breast but has more widespread manifestations (Raobaikady *et al.*, 2003; Jameel *et al.*, 2004). Successful treatment regimes for breast cancer require combination strategies, including chemotherapy and adjuvants. Although many active anticancer agents are used in the treatment of this disease, there is no curative therapy for breast carcinoma (Society, 2000; Akbas *et al.*, 2005). Clinically, the major obstacles of cancer chemotherapy are the development of drug resistance and the severe side effects (Efferth and Volm, 2005). Cancer chemotherapy is often limited by patient's toxicity and tumor drug resistance, indicating that modification of existing drugs and new drug development are critical for improving the therapeutic response (Efferth *et al.*, 2005). Traditional Chinese herbal medicine could be a source of possible anticancer agents. After screening 71 Chinese medicinal herbs, Campbell et al. showed that some of the herbs used in traditional Chinese medicine for the treatment of cancer might have inhibitory effects on the growth of breast cancer cells (Campbell *et al.*, 2002; Shoemaker *et al.*, 2005). Reportedly, notoginseng extract may be beneficial in the treatment of various illnesses (Yoshikawa *et al.*, 2001; Lam *et al.*, 2002; Wei *et al.*, 2002; Chen *et al.*, 2004; Sun *et al.*, 2004; Park *et al.*, 2005; Shi *et al.*, 2005; Wang *et al.*, 2005; Zhong *et al.*, 2005). However, existing data is limited in respect to its potential anti-cancer effects (Chung *et al.*, 2004; Wang *et al.*, 2004; Li *et al.*, 2005). In the present study, we investigated growth inhibition and morphological changes in MCF-7 human breast carcinoma cells using notoginseng extracts, ginsenoside Rb1 and notoginsenoside R1. The results showed that both NRE and NRhE are endowed with significant antiproliferation properties in the cells. Compared to NRhE, NRE at the lower concentration (0.05 mg/ml) still showed effectiveness on MCF-7 cells. Ginsenoside Rb1 and notoginsenoside R1 are two representative saponins in notoginseng. Rb1 is a major ginsenoside of the ginseng family. On the other hand, R1 can only be found in notoginseng. We observed that Rb1 inhibited MCF-7 cell growth significantly in a concentration-dependent manner. However, R1, with a similar chemical structure compared to Rb1, did not have significant antiproliferative activity in MCF-7 cells under our experimental conditions.

Our microscopic examination of cell gross morphology suggests that MCF-7 carcinoma cells died by apoptosis (Purohit *et al.*, 2001). Although the antiproliferation mechanisms involved are not completely understood, morphological observation suggests that the effects of notoginseng on MCF-7 cells are not due to a direct killing of cells. It has been reported that apoptosis results in loss of cellular integrity, nuclear condensation, and DNA fragmentation. Cancer cells were condensed with fragmented nuclear and disintegrated membranes (Losso *et al.*, 2004). In the final stage of apoptosis, the cell itself fragments into a number of membrane-bound vesicles called apoptotic bodies, which contain condensed chromatin and/or intact organelles (Simboli-Campbell *et al.*, 1996). It appears that the inhibitory effect of notoginseng on MCF-7 carcinoma cells is associated with apoptosis activity.

In summary, our studies provided evidence that *Panax notoginseng* extracts and ginsenoside Rb1 inhibited proliferation of human breast carcinoma MCF-7 cells. Our data may open a window for further study to understanding the anti-cancer effects of notoginseng, which has the potential to be developed as a new adjuvant for anti-neoplastic drugs.

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