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Effects of Ginsenosides Rg_3 and Rh_2 on the Proliferation of Prostate Cancer Cells

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Ginseng has an anti-cancer effect in several cancer models. This study was to characterize active constituents of ginseng and their effects on proliferation of prostate cancer cell lines, LNCaP and PC3. Cell proliferation was measured by [^{3}H]thymidine incorporation, the intracellular calcium concentration by a dual-wavelength spectrophotometer system, effects on mitogen-activated protein (MAP) kinases by Western blotting, and cell attachment and morphologic changes were observed under a microscope. Among 11 ginsenosides tested, ginsenosides Rg_3 and Rh_2 inhibited the proliferation of prostate cancer cells. EC_{50}s of Rg_3 and Rh_2 on PC3 cells were 8.4 μM and 5.5 μM, respectively, and 14.1 μM and 4.4 μM on LNCaP cells, respectively. Both ginsenosides induced cell detachment and modulated three modules of MAP kinases activities differently in LNCaP and PC3 cells. These results suggest that ginsenosides Rg_3 and Rh_2-induced cell detachment and inhibition of the proliferation of prostate cancer cells may be associated with modulation of three modules of MAP kinases.

Key words: Prostatic neoplasm, Ginseng, MAP kinases, Cell growth

INTRODUCTION

Prostate carcinoma is the most common malignancy and age-related cause of cancer death worldwide. Furthermore, its prevalence has progressively increased in recent decades (Rhim and Kung, 1997). Localized prostate carcinoma is potentially curable by a radical operation or definitive radiotherapy. However, metastatic prostate carcinoma can only be effectively controlled by hormone manipulation, since the prostate is an androgen-dependent organ. Although many therapeutic protocols have been proposed, none currently available has proved to be dramatically effective (See, 2003; Sandler *et al.*, 2003). An extensive search of other potential managements is now under way, however, the negative aspect of the newly developed managements is drug resistance or toxic side effects (Teicher *et al.*, 1997; Theyer *et al.*, 1993).

Ginseng, the root of *Panax ginseng* C.A. Meyer, is a medicinal plant consumed worldwide and has been reported to have various biological effects including anticarcinogenesis (Yun *et al.*, 2001; Nah *et al.*, 1995; Liao *et al.*, 2002). Ginsenosides, ginseng saponins, have been suggested to be the major effective ingredients in ginseng (Yun *et al.*, 2001; Nah *et al.*, 1995; Liao *et al.*, 2002). Among them, ginsenoside Rh_2 with a dammarane skeleton is shown to have some biological effects on cell differentiation, such as stimulation of melanogenesis in melanoma cells (Odashima *et al.*, 1985), glucocorticoid-like action in growth suppressive effect on various cancer cells (Lee *et al.*, 1996), and modulation of protein kinase C activity in HL-60 cells (Kim *et al.*, 1998). Ginsenoside Rg_3 has inhibitory effects on Ca^{2+} channel in sensory neurons and ventricular myocytes, cancer cells growth and platelet aggregation (Lee *et al.*, 1997; Rhim *et al.*, 2002; Bai *et al.*, 2003).

To clarify effects of ginseng on the prostate, we investigated the effect of ginsenosides on cell proliferation of prostate cancer cell lines. Among 11 ginsenosides (Rb_1, Rb_2, Rc, Rd, Re, Rf, Rg_1, Rg_2, Rg_3, Rh_2, and Ro) tested, ginsenosides Rg_3 and Rh_2 inhibited DNA synthesis in androgen dependent and independent prostate cancer cells.

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MATERIALS AND METHODS

Agents

Ginsenosides (Rb_1, Rb_2, Rc, Rd, Re, Rf, Rg_1, Rg_2, Rg_3, Rh_2, and Ro) were purified in Choi's laboratory, the Korea Ginseng and Tobacco Research Institute, and the purities were more than 99.9%. They were dissolved in absolute methanol and stored at - 20°C. 1-Oleoyl-lysophosphatidic acid (LPA) was purchased from Avanti Pola Lipid (Alabaster, AL), [^{3}H]thymidine was from Amersham Bioscience (Seoul, Korea), and antibodies for phospho-p44/42 MAP kinase(Thr202/Tyr204), phospho-p38 MAP kinase(Thr180 /Tyr182), and phospho-SAPK/JNK(Thr183/Tyr185) were from Cell Signaling (Beverly, MA). All the rest of chemicals were purchased from Sigma Chemical Company (St. Louis, MO).

Prostate cancer cell line culture

LNCaP and PC3 cells were purchased from Korean Cell Line Bank (Seoul, Korea) and grown in monolayers at 37°C in a 5% CO_2/95% air atmosphere in growth medium which consisted of: 90% Minimum Eagles Medium (MEM, Gibco Laboratories, Grand Island, NY), 10% fetal bovine serum, 2 mM glutamine, 1 mM sodium pyruvate, 100 units/mL penicillin, and 50 μg/mL streptomycin.

Assessment of cell proliferation

The cells were seeded in 48 well plates at 1×10^5 cells per well and incubated for 24 h. The cells were further cultured in 0.5% FBS containing MEM for 24 h. Ginsenosides were added to monolayer cultures, and [^{3}H]thymidine (1 μCi/well) was added to the cultures after 18 h incubation. After 4 h incubation, cells were washed once with cold phosphate-buffered saline (PBS) and three times with 10% trichloroacetic acid. Cells were solubilized with 0.1 N NaOH, 2% Na_2CO_3, and 0.1% SDS, and the radioactivity in the alkaline extract was measured in a liquid scintillation counter (Ohta *et al.*, 2003).

Measurement of MAP kinase activaties by Western blotting

The cells starved in 0.5% FBS-containing media for 48 h were trypsinized and collected by centrifugation at 1000 rpm for 5 min. After washing twice with PBS, cells were resuspended in Hepes-buffered medium (HBM), which consisted of 20 mM Hepes, pH 7.4, 103 mM NaCl, 4.8 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 0.5 mM $CaCl_2$, 25 mM $NaHCO_3$, 15 mM glucose and 0.1% BSA, were incubated for 10 min at 37°C, and further 10 min with ginsenosides, followed by the addition of serum (10%) or lysophosphatidic acid (10 μM). The cells were cooled down by transferring to conical tubes containing 10 times volume of ice-cold PBS. After centrifugation at 2000 rpm for 10 min, the cell pellets were dissolved and boiled in 150 μL of sample buffer containing 62.5 mM Tris-HCl (pH 6.8), 10% glycerol, 2% SDS, 5% 2-mercaptoethanol, and 0.05% bromophenol blue. Proteins were resolved by 12% SDS-polyacrylamide gel electrophoresis and electrophorectically transferred to a nitrocellulose paper. The nitrocellulose paper was incubated with the specific rabbit antibody, which recognized the active-phosphorylated forms of p44/42 MAP kinase (ERK), p38 MAP kinase, or JNK. Goat anti-rabbit horseradish-linked IgG was used as the secondary antibody. Signals were developed using an enhanced chemiluminescence system (Pierce Biotechnology Inc., Rockford, IL) (Hooks *et al.*, 2001).

Microscopic observation

LNCaP and PC3 cells were grown on cover glasses for 24 h and treated with vehicle or ginsenosides. After incubation for the indicated times, cells were washed with PBS once at room temperature and fixed with 3.7% formaldehyde at room temperature for 1 h. Coverslips were then mounted, and images were obtained using an inverted microscope (TS-100, Nikon, Japan) and a digital camera (Coolpix 4500, Nikon, Japan).

Measuring intracellular calcium concentration

The cells were collected by trypsin-EDTA treatment and centrifugation at 1000 rpm for 5 min. The cell pellet was resuspended in HBM and then incubated for 40 min with 5 μM fura-2/acetoxymethyl ester (Calbiochem, Damstadt, Germany). Fluorescence emission at 510 nM was monitored at 37°C by a dual-wavelength spectrometer (F-4500, Hitachi, Japan) with excitation at 340 and 380 nm, and $[Ca^{2+}]_i$ was estimated from the change in the fluorescence ratio (340/380) (Im *et al.*, 2000).

Data presentation

All experiments were performed in duplicate or triplicate. The results of multiple observations are presented as representative or means ± SE of at least three separate experiments, unless otherwise stated.

RESULTS

Effects of ginsenosides Rg_3 and Rh_2 on proliferation of prostate cancer cells

Among the ginsenosides tested (Rb_1, Rb_2, Rc, Rd, Re, Rf, Rg_1, Rg_2, Rg_3, Rh_2, and Ro), ginsenosides Rg_3 and Rh_2 at 20 μM and 200 μM showed significant inhibition on DNA synthesis in androgen dependent (LNCaP) and independent (PC3) prostate cancer cells (data not shown). Structures of both ginsenosides are shown in Fig. 1. Figure 2 shows the effect of Rg_3 or Rh_2 on the proliferation of both prostate cell lines. Both ginsenosides inhibited

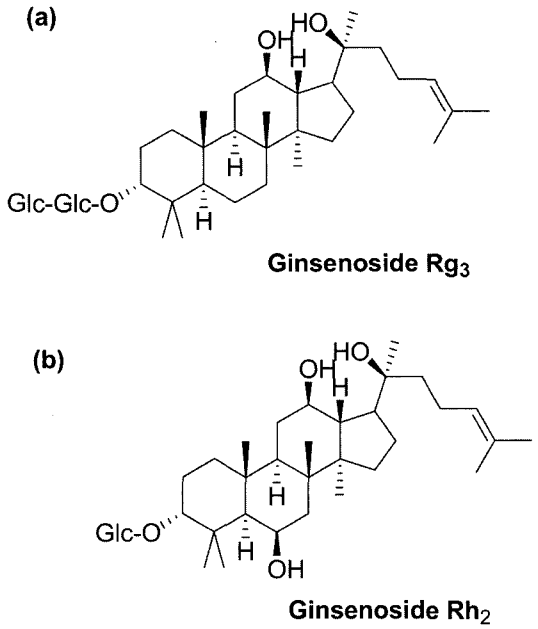

Fig. 1. Structures of ginsenosides Rg_3 (a) and Rh_2 (b).

[^{3}H]thymidine incorporation in a dose-dependent manner. EC_{50}s of Rg_3 and Rh_2 on PC3 cells were 8.4 μM and 5.5 μM, respectively (Fig. 2a and b), and EC_{50}s of Rg_3 and Rh_2 on LNCaP cells were 14.1 μM and 4.4 μM, respectively (Fig. 2c and d).

Effects of ginsenosides Rg_3 and Rh_2 on cell attachment of prostate cancer cells

Ginsenoside-induced inhibition of cell proliferation was found to be related to detachment of the cells from culture dishes during the experiment, and this was further comfirmed through microscopic observation. Treatment of the cells with ginsenosides for 24 h induced detachment of the prostate cells (Fig. 3a and b). In androgen-independent PC3 cell line, 74.4% and 88.3% of cells treated with 150 μM ginsenoside Rg_3 were detached 1 and 24 h after the treatment, respectively, whereas 60.9% and 95.1% of the cells treated with 150 μM ginsenoside Rh_2 were detached 1 and 24 h after the treatment (Fig. 4a). In androgen dependent LNCaP prostate cells, cell detachment was more severe after 1 h treatment: 90.4% in 150 μM ginsenoside Rg_3-treated cells and 79.9% in 150 μM Rh_2-treated cells (Fig. 4b). Since cell rounding was observed only in the Rg_3-treated cells after 30 min of treatment, but not in Rh_2-treated cells, we speculate different mechanisms might be involved between ginsenoside Rg_3- and Rh_2-induced cell detachment (Fig. 3). Furthermore, cell-detachment was much faster in the Rg_3-treated cells than the Rh_2-treated cells at 1 h after the treatment (Fig. 4), whereas more cells in the Rh_2-treated group than those in

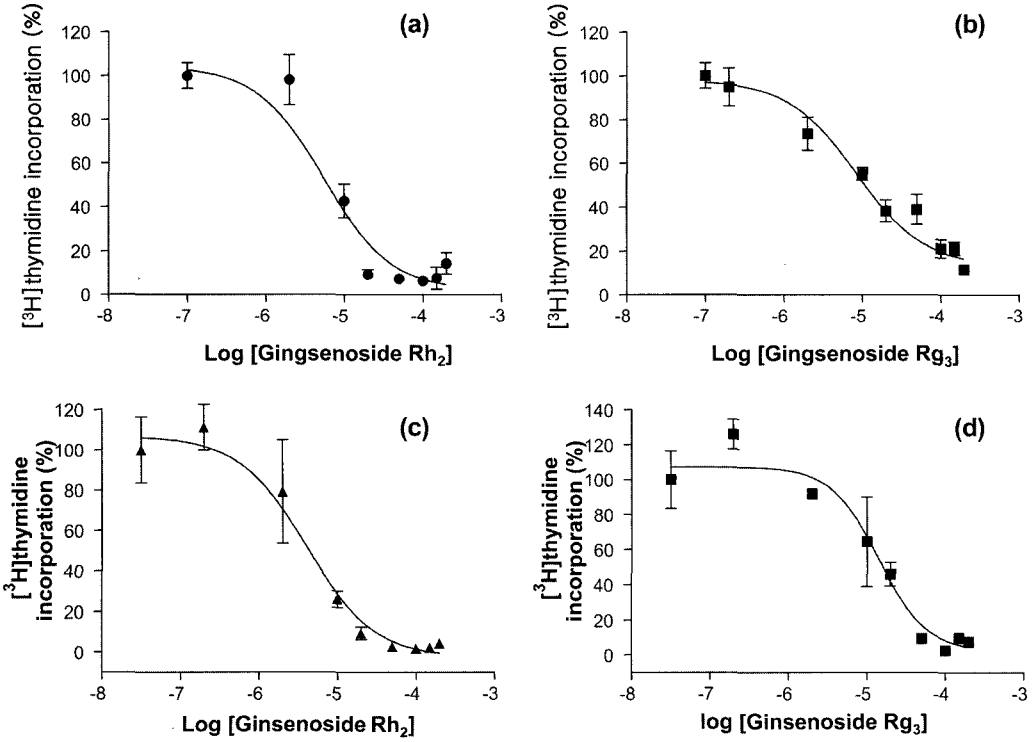

Fig. 2. Dose-response curves of ginsenosides on DNA synthesis. PC3 (a and b) or LNCaP (c and d) cells starved in 0.5% FBS for 24 h were stimulated with vehicle or different concentrations of ginsenoside Rg_3 (b and d) or Rh_2 (a and c). Then, the cells were labeled with 1 μCi/mL of [^{3}H]thymidine for 4 h, and radioactivity incorporated was measured for DNA synthesis.

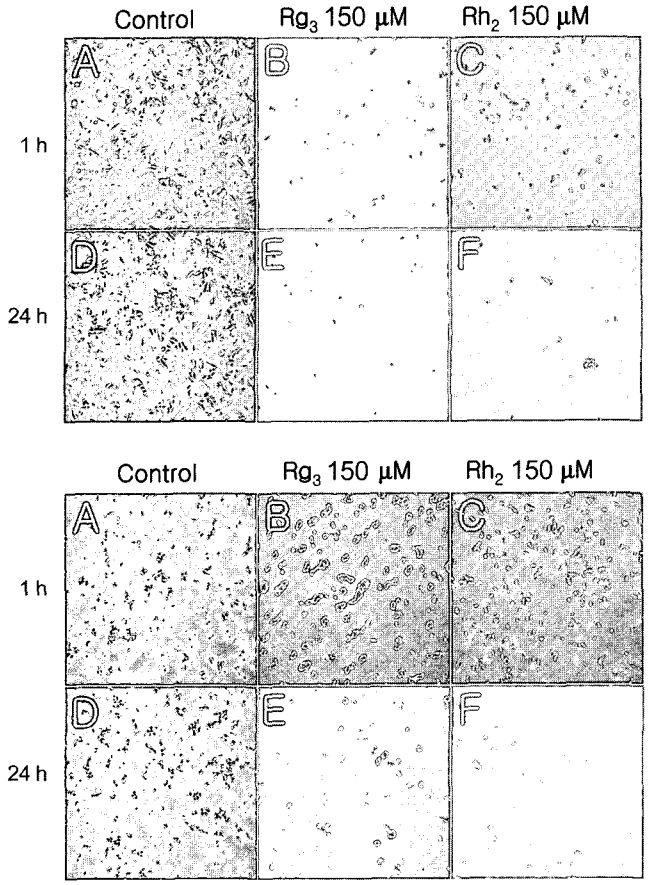

Fig. 3. Microscopic observation of cell detachment. PC3 (a) or LNCaP (b) cells grown on coverslips were treated with vehicle (A and D) or 150 μM ginsenoside Rg_3 (B and E) or Rh_2 (C and F) for 1 h (A, B, and C) or 24 h (D, E, and F). Then, the cells were fixed with 3.7% formaldehyde, and images were taken with a digital camera.

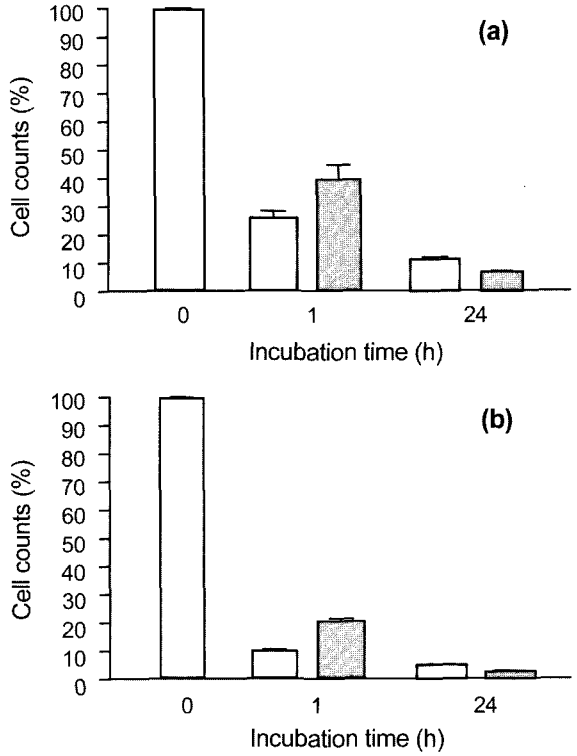

Fig. 4. Changes of attached cell numbers after treatment with ginsenosides. PC3 (a) or LNCaP (b) cells grown on coverslips were treated with vehicle or 150 μM ginsenoside Rg_3 (light-filled) or Rh_2 (dark-filled) for 1 h or 24 h. Then, the cells were fixed with 3.7% formaldehyde, and images were taken with a digital camera. Cell numbers are expressed as percentages to cell numbers of the vehicle-treated group.

the Rg_3-treated were detached after 24 h of the treatment, further suggesting different mechanisms involved in the cell detachment (Fig. 4).

Effects of ginsenosides Rg_3 and Rh_2 on mitogen-activated protein kinases in prostate cancer cells

Three distinct mammalian MAP kinase modules including extracellular signal-regulated kinase (ERKs, also known as p42/44 MAP kinases), p38 mitogen-activated protein (p38 MAP) kinase, and c-Jun *N*-terminal kinase (JNK), have been well characterized (Chang and Karin, 2001; Hommes *et al.*, 2003). ERKs are stimulated predominantly by mitogens and growth hormones, and the activation of ERKs induces proliferation or differentiation of cells (Chang and Karin, 2001; Hommes *et al.*, 2003). The p38 MAP kinase, a recently identified member of the MAP kinase family, is involved in apoptosis. Stress-activated protein kinase cascade involves the activation of JNK, which consequently induces activator protein-1 (AP-1)-mediated transactivation of the AP-1 responsible genes (Chang and Karin, 2001; Hommes *et al.*, 2003).

Therefore, to further characterize the action of both ginsenosides in cell growth and cell detachment, we investigated the effects of ginsenosides Rg_3 and Rh_2 on mitogen-activated protein (MAP) kinases. As shown in Fig. 5, ERKs in both prostate cell lines were activated by 10% serum treatment, however, Rg_3 and Rh_2 strongly inhibited ERKs in LNCaP cells. The basal activity of the enzymes in control cells was also inhibited by both ginsenosides in LNCaP cells. Furthermore, in the Rh_2-treated LNCaP cells, p38 MAP kinase was activated by 10% serum or 10 μM LPA, whereas Rg_3 did not show any significant effect on p38 MAP kinase activity. Finally, both ginsenosides displayed no effect on JNK activity in LNCaP cells.

LPA, a bioactive lysolipid mediator, activated ERKs in androgen independent PC3 cells (Fig. 6), however, ginsenosides Rg_3 and Rh_2 did not significantly modulate ERKs in this cell line (Fig. 6). LPA strongly inhibited p38 MAP kinase, and this inhibition was blocked by treatment with Rh_2 or Rg_3 (Fig. 6). In Rh_2-treated PC3 cells, LPA more strongly activated p38 MAP kinase than serum did. In contrast to LNCaP cells, JNK activation was observed

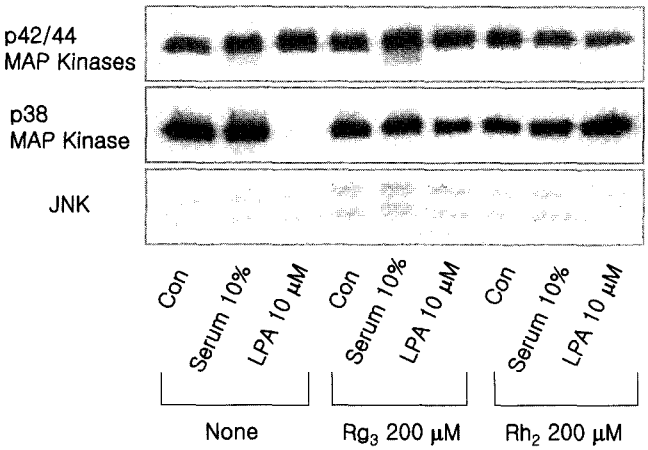

Fig. 5. Western blotting of three modules of MAP kinases in PC3 cells. PC3 cells suspended in HBM after trypsinization were incubated with vehicle or 200 μM ginsenoside Rg_3 or Rh_2. Then, the cells were further incubated with 10% serum or 10 μM LPA for 10 min. Activation of each MAP kinase was detected with the specific rabbit antibody, which recognized the active-phosphorylated forms of p44/42 MAP kinase (ERK), p38 MAP kinase, or JNK.

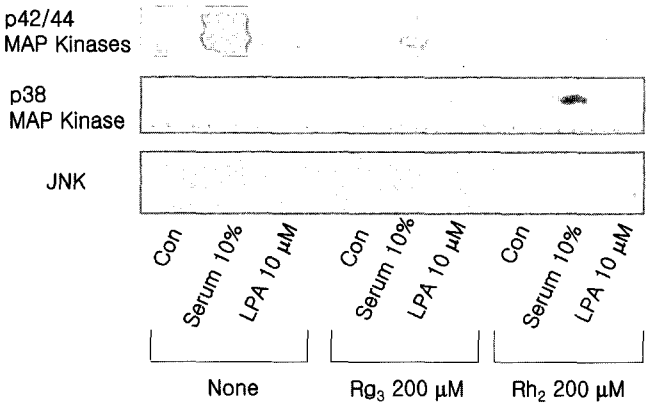

Fig. 6. Western blotting of three modules of MAP kinases in LNCaP cells. LNCaP cells suspended in HBM after trypsinization were incubated with vehicle or 200 μM ginsenoside Rg_3 or Rh_2. Then, the cells were further incubated with 10% serum or 10 μM LPA for 10 min. Activation of each MAP kinase was detected with the specific rabbit antibody, which recognized the active-phosphorylated forms of p44/42 MAP kinase (ERK), p38 MAP kinase, or JNK.

in PC3 cells. Furthermore, Rg_3 activated JNK, whereas Rh_2 did not (Fig. 6).

Effects of ginsenosides Rg_3 and Rh_2 on intracellular Ca^{2+} concentration in prostate cancer cells

Intracellular Ca^{2+} concentration plays important roles in cell proliferation, differentiation, and apoptosis. Previous reports suggest that some ginsenosides induce changes of intracellular Ca^{2+} concentration through modulation of Ca^{2+} channels (Rhim *et al.*, 2002; Nah *et al.*, 1995; Bai *et al.*, 2003; Choi *et al.*, 2002). Therefore, effects of ginsenosides Rg_3 and Rh_2 on intracellular Ca^{2+} concentration were investigated in both prostate cell lines, by using with fura-2, a Ca^{2+}-sensing fluorescent dye. However, there was no change in intracellular Ca^{2+} concentration by Rg_3 and Rh_2 in PC3 cells, although LPA increased Ca^{2+} concentration (data not shown). In the case of LNCaP cells, it was not able to measure the intracellular Ca^{2+} increase even with LPA, in agreement with the previous report (Wasilenko *et al.*, 1997): There might be a defect on Ca^{2+} signaling in LNCaP cells. Since voltage-dependent Ca^{2+} channels have been implicated as the target of ginsenosides in neuronal cells (Rhim *et al.*, 2002), we measured Ca^{2+} increase under the condition of membrane depolarization by changing the extracellular medium to a high-potassium one. Under such a condition, no significant change in intracellular Ca^{2+} concentration was observed by both ginsenosides (data not shown).

DISCUSSION

Major findings of this communication are four folds. First, two ginsenosides, ginsenosides Rg_3 and Rh_2, were found to inhibit proliferation of androgen dependent and independent prostate cancer cells. Second, the growth inhibition was related with inhibition of cell adhesion. Third, the both ginsenosides modulated three modules of MAP kinases activities differently in androgen dependent and independent prostate cells. Last, intracellular Ca^{2+} concentration was not changed by the ginsenosides even in the membrane-depolarized condition.

Recently, Liu *et al.* reported that gingenoside Rg_3 inhibited proliferation of androgen dependent LNCaP cells (Liu *et al.*, 2000), however, the effect of ginsenoside Rh_2 on the prostate cancer cells was not observed (Liu *et al.*, 2000). In the present study, effects of both ginsenosides Rg_3 and Rh_2 on androgen independent PC3 prostate cells are for the first time characterized in our report, and this would be useful fundamental data for the future development of ginsenosides as chemotherapeutic agents for the prostate cancer, which is resistant to hormone therapy.

Inhibitory effects of ginsenosides Rg_3 and Rh_2 on the cell growth have previously been observed in other types of cancers. In particular, ginsenoside Rh_2 has been shown to be an apoptotic agent in rat glioma (Kim *et al.*, 2000), human melanoma (Fei *et al.*, 2002), ovarian cancer (Nakata *et al.*, 1998), breast cancer (Oh *et al.*, 1999), hepatoma (Park *et al.*, 1997), and neuroblastoma (Kim *et al.*, 2000). Also, ginsenoside Rh_2 has been reported to induce differentiation of melanoma (Odashima *et al.*, 1985), teratocarcinoma (Lee *et al.*, 1996), and leukemia (Kim *et al.*, 1998). Nevertheless, the relationship of cell detachment with growth inhibition by ginsenosides has not been described in early studies. There were differential cell

detachment between the two drugs: Ginsenoside Rg_3-induced detachment was greater than ginsenoside Rh_2-induced one after 1 h of treatment, however, the detachment 24 h later was greater in ginsenoside Rh_2-treated group than ginsenoside Rg_3-treated group. The differential changes of cell detachment observed by both ginsenosides imply different action modes between ginsenosides Rg_3 and Rh_2.

Implication of the different action modes was further supported by differential modulations of MAP kinases by the two ginsenosides: Treatment of LNCaP cells with ginsenoside Rh_2 modulated p38 MAP kinase activation, but not with ginsenosides Rg_3. On the other hand, ginsenoside Rg_3 induced JNK activation in PC3 cells, but not ginsenosides Rh_2. The differential modulations were further observed between two cell lines: ERKs were strongly inhibited by both ginsenosides in LNCaP cells, but not in PC3 cells. p38 MAP kinase in PC3 cells was modulated by both ginsenosides, but only by ginsenoside Rh_2 in LNCaP cells. Furthermore, JNK was activated by ginsenoside Rg_3 in PC3 cells, but not in LNCaP cells. Therefore, in androgen dependent LNCaP cells, inhibition of ERKs by ginsenosides Rg_3 and Rh_2 was an important trigger for cell detachment and growth inhibition, and modulation of p38 MAP kinase was additive in the case of the ginsenoside Rh_2. In androgen independent PC3 cells, modulation of p38 MAP kinase was important for the effects of both ginsenosides, and activation of JNK was additive in the case of ginsenoside Rg_3. These differential modulations in two prostate cancer cells may explain why the cell detachment was different between the two ginsenosides. Activities of three MAP kinases modules were observed after 10 min treatment of ginsenosides, suggesting that the modulation of the activities could be early events triggering the cell detachment and growth inhibition.

Serum or LPA activated p38 MAP kinase in ginsenoside Rh_2-treated LNCaP cells. This observation may imply that serum have two components: one signal to inhibit p38 MAP kinase and the other activates p38 MAP kinase. The former signal might be strong enough to mask the effect of the latter signal without treatment of ginsenoside Rh_2. In the cells treated with ginsenoside Rh_2, however, inhibitory signal of the former might have been blocked, and the stimulatory signal was observable. Similar enhanced activation of p38 MAP kinase by LPA was also observed in ginsenoside Rh_2-treated PC3 cells.

Ginsenoside Rg_3 has been reported to inhibit platelet aggregation, relaxation of rat aorta (Lee *et al.*, 1997; Kim *et al.*, 1999; Kim *et al.*, 1999), and Ca^{2+} channels in rat sensory neurons (Rhim *et al.*, 2002). However, in this study, intracellular Ca^{2+} concentration in PC3 prostate cells was not affected by ginsenoside Rg_3 or Rh_2. Since PC3 prostate cells are epithelial, but not excitable cells, they may not express the voltage-sensitive Ca^{2+} channels, which are modulated by ginsenosides (Putney and Bird, 1993).

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REFERENCES

Bai, C. X., Sunami, A., Namiki, T., Sawanobori, T., and Furukawa, T., Electrophysiological effects of ginseng and ginsenoside Re in guinea pig ventricular myocytes. *Eur. J. Pharmacol.*, 476, 35-44 (2003).

Chang, L. and Karin, M., Mammalian MAP kinase signalling cascades. *Nature*, 410, 37-40 (2001).

Choi, S., Jung, S. Y., Ko, Y. S., Koh, S. R., Rhim, H., and Nah, S. Y., Functional expression of a novel ginsenoside Rf binding protein from rat brain mRNA in Xenopus laevis oocytes. *Mol. Pharmacol.*, 61, 928-935 (2002).

Fei, X. F., Wang, B. X., Tashiro, S., Li, T. J., Ma, J. S., and Ikejima, T., Apoptotic effects of ginsenoside Rh_2 on human malignant melanoma A375-S2 cells. *Acta Pharmacol. Sin.*, 23, 315-322 (2002).

Hommes, D. W., Peppelenbosch, M. P., and van Deventer, S. J., Mitogen activated protein (MAP) kinase signal transduction pathways and novel anti-inflammatory targets. *Gut*, 52, 144-151 (2003).

Hooks, S. B., Santos, W. L., Im, D. S., Heise, C. E., Macdonald, T. L., and Lynch, K. R., Lysophosphatidic acid-induced mitogenesis is regulated by lipid phosphate phosphatases and is Edg-receptor independent. *J. Biol. Chem.*, 276, 4611-4621 (2001).

Im, D. S., Heise, C. E., Harding, M. A., George, S. R., O'Dowd, B. F., Theodorescu, D., and Lynch, K. R., Molecular cloning and characterization of a lysophosphatidic acid receptor, Edg-7, expressed in prostate. *Mol. Pharmacol.*, 57, 753-759 (2000).

Kim, N. D., Kang, S. Y., Kim, M. J., Park, J. H., and Schini-Kerth, V. B., The ginsenoside Rg_3 evokes endothelium-independent relaxation in rat aortic rings: role of K^+ channels. *Eur. J. Pharmacol.*, 367, 51-57 (1999).

Kim, N. D., Kang, S. Y., Park, J. H., and Schini-Kerth, V. B., Ginsenoside Rg3 mediates endothelium-dependent relaxation in response to ginsenosides in rat aorta: role of K^+ channels. *Eur. J. Pharmacol.*, 367, 41-49 (1999).

Kim, Y. S., Kim, D. S., and Kim, S. I., Ginsenoside Rh_2 and Rh_3 induce differentiation of HL-60 cells into granulocytes: modulation of protein kinase C isoforms during differentiation by ginsenoside Rh2. *Int. J. Biochem. Cell Biol.*, 30, 327-338 (1998).

Kim, Y. S., Jin, S. H., Lee, Y. H., Park, J. D., and Kim, S. I.,

Differential expression of protein kinase C subtypes during ginsenoside Rh_2-Induced apoptosis in SK-N-BE(2) and C6Bu-1 cells. *Arch. Pharm. Res.*, 23, 518-524 (2000).

Lee, S. R., Park, J. H., Choi, K. J., and Kim, N. D., Inhibitory effects of ginsenoside Rg_3 on platelet aggregation and its mechanism of action. *Kor. J. Ginseng Sci.*, 21, 132-140 (1997).

Lee, Y. N., Lee, H. Y., Chung, H. Y., Kim, S. I., Lee, S. K., Park, B. C., and Kim, K. W., *In vitro* induction of differentiation by ginsenoides in F9 teratocarcinoma cells. *Eur. J. Cancer*, 32A, 1420-1428 (1996).

Liao, B., Newmark, H., and Zhou, R., Neuroprotective effects of ginseng total saponin and ginsenosides Rb_1 and Rg_1 on spinal cord neurons *in vitro*. *Exp. Neurol.*, 173, 224-234 (2002).

Liu, W. K., Xu, S. X., and Che, C. T., Anti-proliferative effect of ginseng saponins on human prostate cancer cell line. *Life Sci.*, 67, 1297-1306 (2000).

Nah, S. Y., Park, H. J., and McCleskey, E. W., A trace component of ginseng that inhibits Ca^{2+} channels through a pertussis toxin-sensitive G protein. *Proc. Natl. Acad. Sci. U. S. A.*, 92, 8739-8743 (1995).

Nakata, H., Kikuchi, Y., Tode, T., Hirata, J., Kita, T., Ishii, K., Kudoh, K., Nagata, I., and Shinomiya, N., Inhibitory effects of ginsenoside Rh_2 on tumor growth in nude mice bearing human ovarian cancer cells. *Jpn. J. Cancer Res.*, 89, 733-740 (1998).

Odashima, S., Ohta, T., Kohno, H., Matsuda, T., Kitagawa, I., Abe, H., and Arichi, S., Control of phenotypic expression of cultured B16 melanoma cells by plant glycosides. *Cancer Res.*, 45, 2781-2784 (1985).

Oh, M., Choi, Y. H., Choi, S., Chung, H., Kim, K., Kim, S. I., Kim, D. K., and Kim, N. D., Anti-proliferating effects of ginsenoside Rh_2 on MCF-7 human breast cancer cells. *Int. J. Oncol.*, 14, 869-875 (1999).

Ohta, H., Sato, K., Murata, N., Damirin, A., Malchinkhuu, E., Kon, J., Kimura, T., Tobo, M., Yamazaki, Y., Watanabe, T., Yagi, M., Sato, M., Suzuki, R., Murooka, H., Sakai, T., Nishitoba, T., Im, D. S., Nochi, H., Tamoto, K., Tomura, H., and Okajima, F., Ki16425, a subtype-selective antagonist for EDG-family lysophosphatidic acid receptors. *Mol. Pharmacol.*, 64, 994-1005 (2003).

Park, J. A., Lee, K. Y., Oh, Y. J., Kim, K. W., and Lee, S. K., Activation of caspase-3 protease *via* a Bcl-2-insensitive pathway during the process of ginsenoside Rh_2-induced apoptosis. *Cancer Lett.*, 121, 73-81 (1997).

Putney, J., W., Jr. and Bird, G. S., The inositol phosphate-calcium signaling system in nonexcitable cells. *Endocr. Rev.*, 14, 610-631 (1993).

Rhim, H., Kim, H., Lee, D. Y., Oh, T. H., and Nah, S. Y., Ginseng and ginsenoside Rg_3, a newly identified active ingredient of ginseng, modulate Ca^{2+} channel currents in rat sensory neurons. *Eur. J. Pharmacol.*, 436, 151-158 (2002).

Rhim, J. S. and Kung, H. F., Human prostate carcinogenesis. *Crit. Rev. Oncog.*, 8, 305-328 (1997).

Sandler, H. M., Narayan, S., and Smith, D. C., Combined modality treatment for prostate cancer: role of chemotherapy. *Semin. Oncol.*, 30, 95-100 (2003).

See, W. A., Adjuvant hormone therapy after radiation or surgery for localized or locally advanced prostate cancer. *Curr. Treat. Options Oncol.*, 4, 351-362 (2003).

Teicher, B. A., Kakeji, Y., Ara, G., Herbst, R. S., and Northey, D., Prostate carcinoma response to cytotoxic therapy: *in vivo* resistance. *In Vivo*, 11, 453-461 (1997).

Theyer, G., Schirmbock, M., Thalhammer, T., Sherwood, E. R., Baumgartner, G., and Hamilton, G., Role of the MDR-1-encoded multiple drug resistance phenotype in prostate cancer cell lines. *J. Urol.*, 150, 1544-1547 (1993).

Wasilenko, W. J., Cooper, J., Palad, A. J., Somers, K. D., Blackmore, P. F., Rhim, J. S., Wright, G. L., Jr., and Schellhammer, P. F., Calcium signaling in prostate cancer cells: evidence for multiple receptors and enhanced sensitivity to bombesin/GRP. *Prostate*, 30, 167-173 (1997).

Yun, T. K., Lee, Y. S., Lee, Y. H., Kim, S. I., and Yun, H. Y., Anticarcinogenic effect of Panax ginseng C.A. Meyer and identification of active compounds. *J. Kor. Med. Sci.*, 16 Suppl, S6-18 (2001).