

## Increase of Membrane Potential by Ginsenosides in Prostate Cancer and Glioma cells

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**Abstract :** Ginseng has an anti-cancer effect in several cancer models. As a mechanism study of ginsenoside-induced growth inhibition in cancer cells, we measured change of membrane potential in prostate cancer and glioma cells by ginsenosides, active constituents of ginseng. Membrane potential was estimated by measuring fluorescence change of DiBAC-loaded cells. Among 11 ginsenosides tested, ginsenosides Rb<sub>2</sub>, Rg<sub>3</sub>, and Rh<sub>2</sub> increased significantly and robustly the membrane potential in a concentration-dependent manner in prostate cancer and glioma cells. Ginsenosides Rc, Ro, and Rb<sub>1</sub> slightly increased membrane potential. The ginsenoside-induced membrane potential increase was not affected by treatment with pertussis toxin or U73122. The ginsenoside-induced membrane potential increase was not diminished in Na<sup>+</sup>-free or HCO<sub>3</sub><sup>-</sup>-free media. Furthermore, the ginsenoside-induced increase of membrane potential was not changed by EIPA (5-(*N*-ethyl-*N*-isopropyl)-amiloride), SITS (4-acetoamido-4'-isothiocyanostilbene-2,2'-disulfonic acid), and omeprazole. In summary, ginsenosides Rb<sub>2</sub>, Rg<sub>3</sub>, and Rh<sub>2</sub> increased membrane potential in prostate cancer and glioma cells in a GPCR-independent and Na<sup>+</sup> independent manner.

**Key words :** Prostate, glioma, ginseng, ginsenoside, membrane potential

### 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.<sup>1)</sup> 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.<sup>2,3)</sup> 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.<sup>4,5)</sup>

Gliomas represent about half of all brain tumors, and among them, glioblastoma multiformes is thought to be

the most malignant and common intracranial tumor.<sup>6)</sup> Although generally not metastatic, glioblastoma cells exhibit highly migratory and invasive behavior.<sup>7)</sup>

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.<sup>8-10)</sup> Ginsenosides, ginseng saponins, have been suggested to be the major effective ingredients in ginseng.<sup>8-10)</sup> Among them, ginsenoside Rh<sub>2</sub> with a dammarane skeleton is shown to have some biological effects on cell differentiation, such as stimulation of melanogenesis in melanoma cells,<sup>11)</sup> glucocorticoid-like action in growth suppressive effect on various cancer cells,<sup>12)</sup> and modulation of protein kinase C activity in HL-60 cells.<sup>13)</sup> Ginsenoside Rg<sub>3</sub> has inhibitory effects on Ca<sup>2+</sup> channel in sensory neurons and ventricular myocytes, cancer cells growth and platelet aggregation.<sup>14-16)</sup>

Modulation of membrane potential plays important roles in neuronal cells and smooth muscle cells. To clarify anticancer effects of ginseng, we investigated the effect of ginsenosides on membrane potential of prostate cancer

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and glioma cells. Among 11 ginsenosides (Rb<sub>1</sub>, Rb<sub>2</sub>, Rc, Rd, Re, Rf, Rg<sub>1</sub>, Rg<sub>2</sub>, Rg<sub>3</sub>, Rh<sub>2</sub>, and Ro) tested, ginsenoside Rb<sub>2</sub>, Rc, Rg<sub>3</sub>, and Rh<sub>2</sub> increased the membrane potential in a concentration-dependent manner.

## MATERIALS AND METHODS

### Agents

Ginsenosides (Rb<sub>1</sub>, Rb<sub>2</sub>, Rc, Rd, Re, Rf, Rg<sub>1</sub>, Rg<sub>2</sub>, Rg<sub>3</sub>, Rh<sub>2</sub>, and Ro) were purified in Ko'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. DiBAC<sub>4</sub>(3) was acquired from Biotium (Hayway, CA, USA). All other materials were purchased from Sigma-Aldrich (St. Louis, MO, USA).

### Prostate cancer and glioma cell 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<sub>2</sub>/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. Rat C6 glioma cells were maintained in high glucose DMEM containing 10% (v/v) fetal bovine serum, 100 units/ml penicillin, 50 µg/ml streptomycin, 2 mM of glutamine, and 1 mM of sodium pyruvate at 37°C in a humidified 5% CO<sub>2</sub> incubator.

### Measurement of membrane potential

The cells were sedimented, resuspended with a HEPES-buffered medium consisting of 20 mM of HEPES (pH 7.4), 103 mM NaCl, 4.8 mM KCl, 1.2 mM KH<sub>2</sub>PO<sub>4</sub>, 1.2 mM MgSO<sub>4</sub>, 0.5 mM CaCl<sub>2</sub>, 25 mM NaHCO<sub>3</sub>, 15 mM glucose and 0.1% bovine serum albumin (fatty acid free), and then incubated for 30 min with 5 µM of DiBAC<sub>4</sub>(3). Fluorescence emission at 530 nm wavelength from excitation wavelength (488 nm) were measured every 0.1 sec by F4500 fluorescence spectrophotometer (Hitachi, Japan). Membrane potential was estimated by measuring fluorescence change of DiBAC-loaded cells.<sup>17)</sup>

### Data presentation

Representative traces for membrane potential were chosen out of 3 separate experiments and shown in Fig 1-6.

## RESULTS

### Effects of ginsenosides on membrane potential in prostate cancer and glioma cells

Among the ginsenosides tested (Rb<sub>1</sub>, Rb<sub>2</sub>, Rc, Rd, Re, Rf, Rg<sub>1</sub>, Rg<sub>2</sub>, Rg<sub>3</sub>, Rh<sub>2</sub>, and Ro), ginsenosides Rb<sub>2</sub>, Rh<sub>2</sub>, Rc, and Rg<sub>3</sub> induced significant increase of membrane potential in androgen dependent LNCaP prostate cancer cells (Fig. 1). Ginsenosides Ro and Rb<sub>1</sub> slightly increased membrane potential in the prostate cancer cells (Fig. 1). However, ginsenosides Rd, Re, Rf, Rg<sub>1</sub>, and Rg<sub>2</sub> did not increase membrane potential (Fig. 1), suggesting ginsenoside type-specific action of the increase of membrane

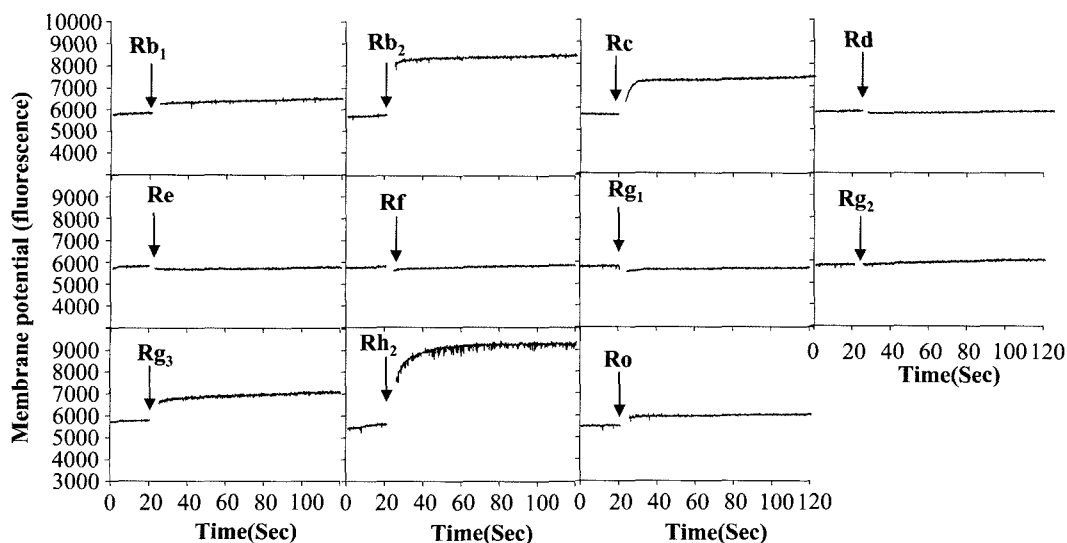
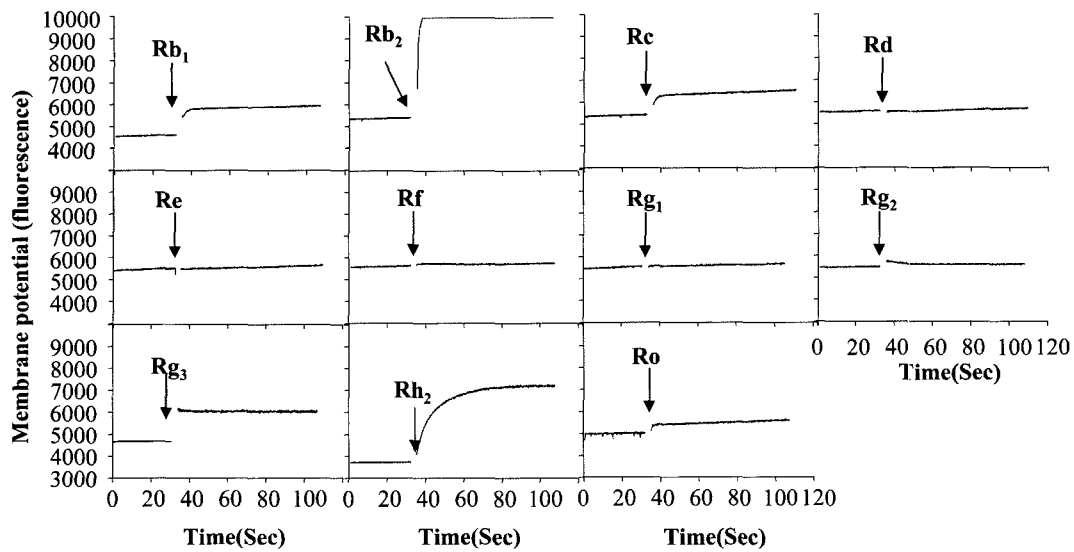


Fig. 1. Effects of ginsenosides on membrane potential in LNCaP prostate cells. Representative traces of membrane potential with 200 µM of each ginsenoside in DiBAC-loaded LNCaP cells were shown. Each ginsenoside was added at the arrow (30 sec).

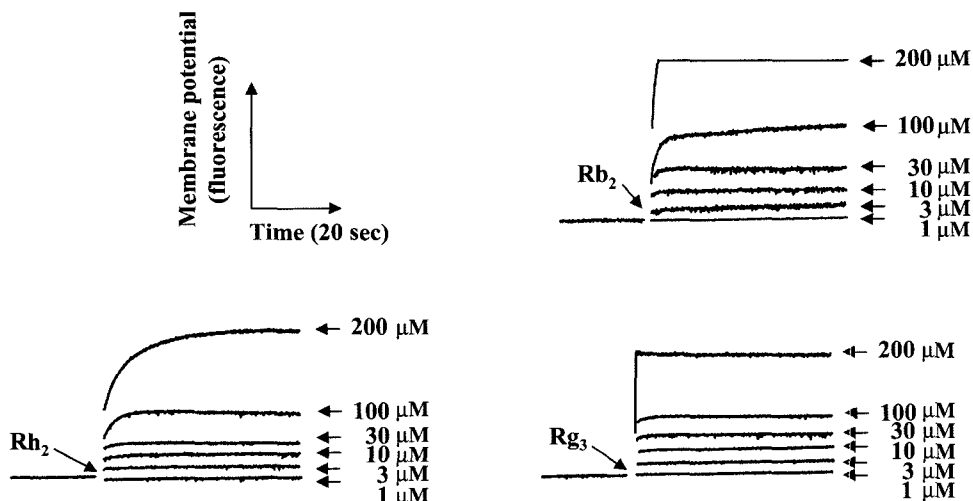
potential. In androgen independent PC3 prostate cancer cells, we observed similar specificity of ginsenosides on membrane potential increase (data not shown). In C6 glioma cells, membrane potential was increased by ginsenoside Rb<sub>2</sub> and Rh<sub>2</sub> most prominently. Ginsenoside Rg<sub>3</sub>, Rc, Rb<sub>1</sub>, and Ro also induced slight to modest increase of membrane potential (Fig. 2). However, ginsenosides Rd, Re, Rf, Rg<sub>1</sub>, and Rg<sub>2</sub> did not increase membrane potential (Fig. 2). In C6 glioma cells, increases of membrane potential by ginsenosides were stronger than those observed in

prostate cancer cells. Especially, ginsenoside Rb<sub>2</sub>-induced increase of membrane potential was above the limit of the instrument and recorded as the highest number on scale (Fig. 2). Therefore, we choose C6 cells and continued further studies.

Ginsenosides Rb<sub>2</sub>, Rh<sub>2</sub>, and Rg<sub>3</sub> increased membrane potential and the increase sustained in C6 cells (Fig. 2). As shown in Fig 3, the ginsenosides-induced increases of membrane potential were concentration-dependent. Above 3 μM, increase of membrane potential was observed by



**Fig. 2.** Effects of ginsenosides on membrane potential in C6 glioma cells. Representative traces of membrane potential with 200 μM of each ginsenoside in DiBAC-loaded C6 glioma cells were shown. Each ginsenoside was added at the arrow (30 sec).



**Fig. 3.** Concentration-dependence of ginsenosides Rb<sub>2</sub>, Rh<sub>2</sub>, and Rg<sub>3</sub> on increase of membrane potential in C6 glioma cells. Representative trace of membrane potential with indicated concentration of each ginsenoside in DiBAC-loaded C6 glioma cells was shown. Each ginsenoside was added at the arrow (30 sec).

three ginsenosides and above 100  $\mu\text{M}$ , robust increases were observed (Fig. 3).

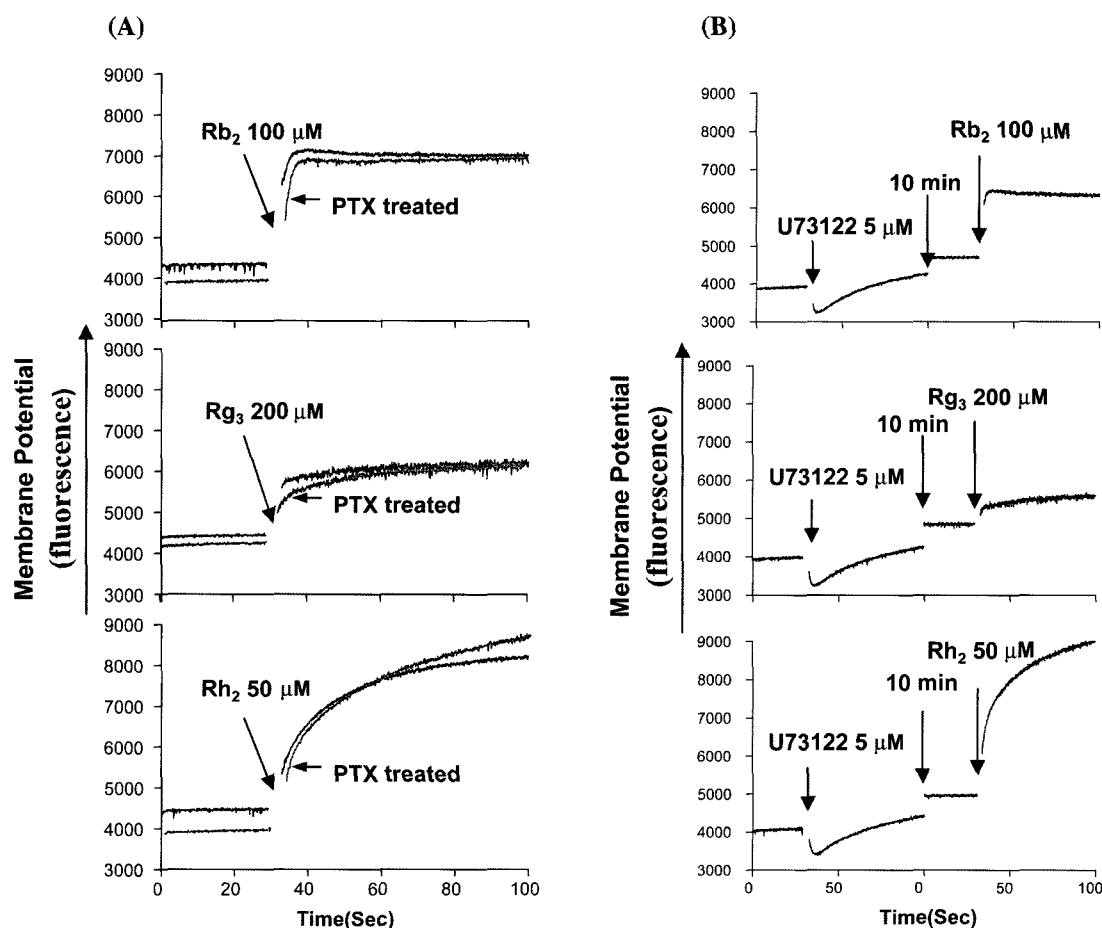
#### Involvement of G proteins and phospholipase C in ginsenoside-induced membrane potential increase

Pertussis toxin has been used to elucidate involvement of  $G_{i/o}$ -type G proteins.<sup>18)</sup> Since G-protein-coupled receptor (GPCR) for ginsenoside action has been suggested, we treated C6 glioma cells with pertussis toxin (100 ng/ml, 24 hr).<sup>19)</sup> However, ginsenoside-induced increase of membrane potential was not ameliorated, suggesting no involvement of GPCR coupling to  $G_{i/o}$ -type G proteins (Fig 4-A). We used U73122, a specific inhibitor of phospholipase C. Treatment of U73122 did not inhibit ginsenosides  $Rh_2$ -induced membrane potential increase,

however, ginsenoside  $Rg_3$ -induced increase of membrane potential was significantly blunted and ginsenoside  $Rb_2$ -induced one was slightly (Fig 4-B). However, because increase of intracellular  $Ca^{2+}$  concentration was not observed by three ginsenosides, it is hard to conclude that ginsenosides activate U73122-sensitive phospholipase C and consequently increase the membrane potential. It is possible that U73122 may have another action site and action on the site influences the ginsenoside  $Rg_3$ -induced increase of membrane potential.

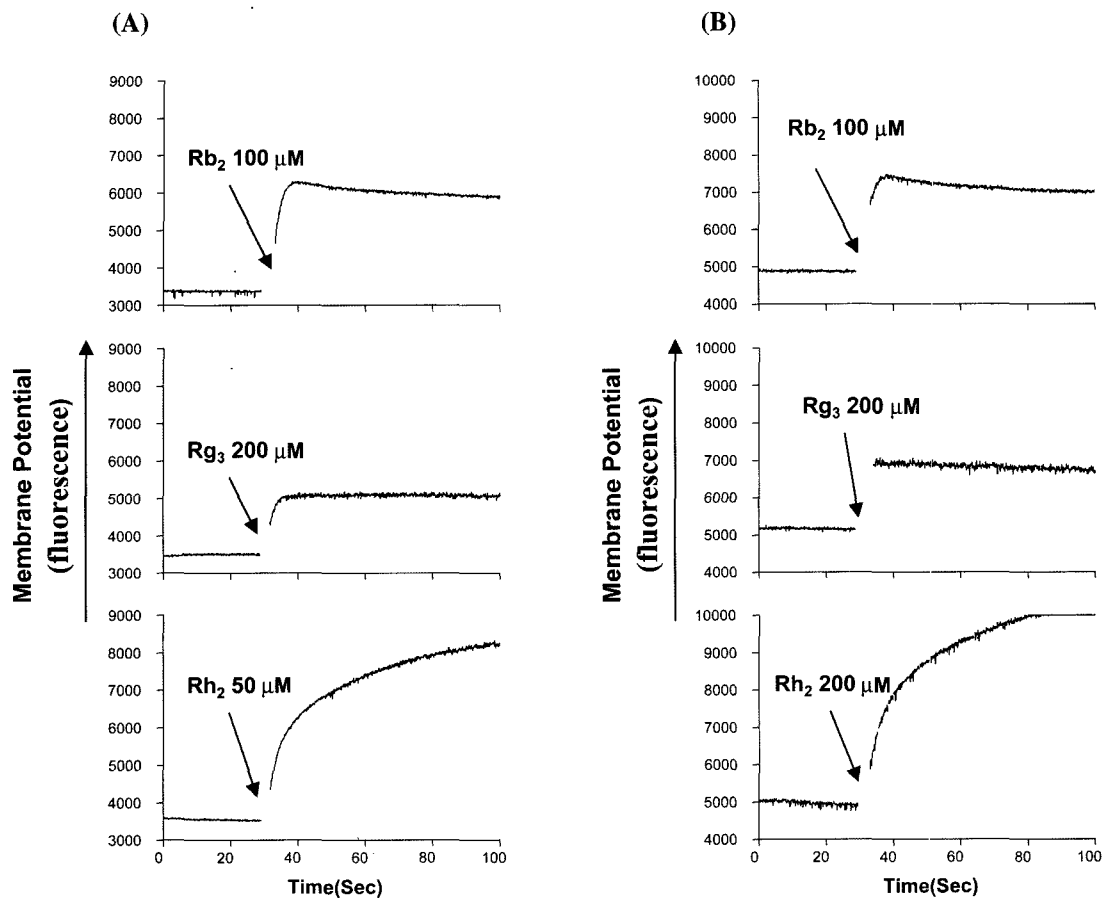
#### Effect of $Na^+$ -free and $HCO_3^-$ -free media on ginsenoside-induced membrane potential increase

Intracellular pH is tightly regulated by pH regulator proteins such as  $Na^+/H^+$  exchanger (NHE), bicarbonate



**Fig. 4. Effects of pertussis toxin and U73122 on ginsenoside-induced increase of membrane potential.**

A. Representative traces of membrane potential with 100  $\mu\text{M}$  of ginsenoside  $Rb_2$ , 200  $\mu\text{M}$  of ginsenoside  $Rg_3$ , or 50  $\mu\text{M}$  of ginsenoside  $Rh_2$  in DiBAC-loaded C6 glioma cells treated with pertussis toxin (100 ng/ml, 24 hr, dotted lines) or without pertussis toxin treatment (lines) were shown. B. Representative traces of membrane potential with 100  $\mu\text{M}$  of ginsenoside  $Rb_2$ , 200  $\mu\text{M}$  of ginsenoside  $Rg_3$ , or 50  $\mu\text{M}$  of ginsenoside  $Rh_2$  in DiBAC-loaded C6 glioma cells. The cells were treated with U73122 (5  $\mu\text{M}$ , first arrow), after 10 min (second arrow) cells were treated with indicated ginsenosides (third arrow).



**Fig. 5. Effects of  $\text{Na}^+$ -free media and  $\text{HCO}_3^-$ -free media on ginsenoside-induced increases of membrane potential.**

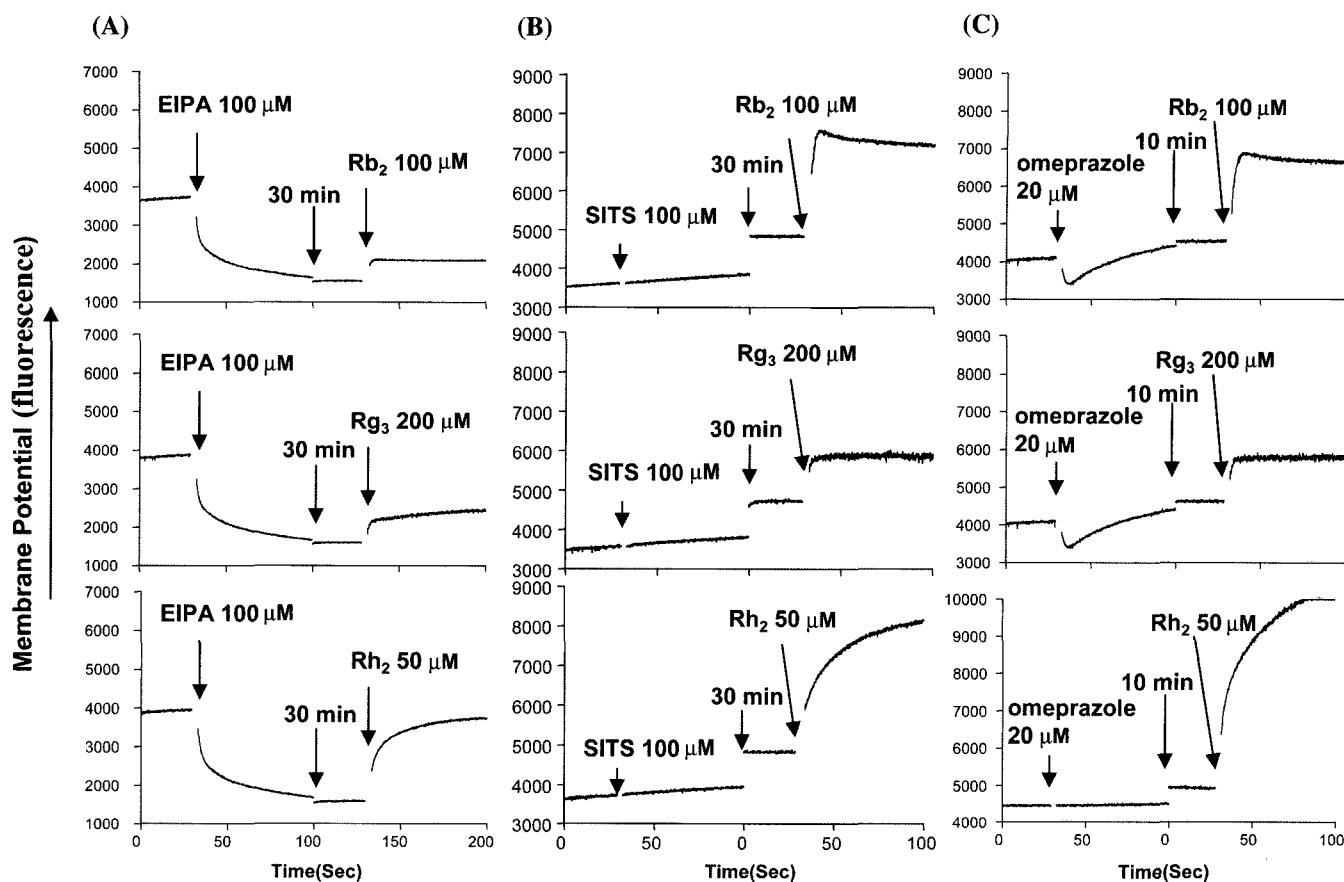
Representative traces of membrane potential with 100  $\mu\text{M}$  of ginsenoside  $\text{Rb}_2$ , 200  $\mu\text{M}$  of ginsenoside  $\text{Rg}_3$ , or 50  $\mu\text{M}$  of ginsenoside  $\text{Rh}_2$  in DiBAC-loaded C6 glioma cells in  $\text{Na}^+$ -free media (A) or in  $\text{HCO}_3^-$ -free media (B) were shown.

transporter (BCT), and proton pump.<sup>20)</sup> Because such a regulation of ions could affect membrane potential, involvement of such regulators was studied. The effect of ginsenosides on membrane potential was measured in  $\text{Na}^+$ -free or  $\text{HCO}_3^-$ -free medium. Depletion of  $\text{Na}^+$  in the extracellular medium did not abrogate ginsenoside-induced increase of membrane potential, and also the membrane potential response in  $\text{HCO}_3^-$ -free medium was intact (Fig 5). To further confirm that NHE was not involved in ginsenoside-induced pH increase, EIPA (5-(*N*-ethyl-*N*-isopropyl)-amiloride), a specific inhibitor of NHE, was used. As shown in Fig 6-A, EIPA treatment decrease the membrane potential dramatically by itself and such decrease of membrane potential lasted for 30 min. After 30 min of the EIPA treatment, ginsenosides were tested. Ginsenoside-induced increases of membrane potential were observed, however, the increments were lesser than non-treated groups. It is not possible to say that NHE is a component of ginsenoside-induced increase of membrane

potential, because the resting membrane potential was decreased by the treatment of EIPA. It is sure that EIPA-sensitive NHE is a regulator of membrane potential in C6 cells. Application of SITS (4-acetoamido-4'-isothiocyano-stilbene-2,2'-disulfonic acid), a specific inhibitor of BCT, or omeprazol, a specific inhibitor of proton pump, did not influence the ginsenoside activity significantly (Fig 6-B and C), excluding the possibility of involvement of BCT and proton pump in the response and supporting the result obtained in  $\text{HCO}_3^-$ -free medium. Therefore, the above results excluded the involvement of NHE, BCT, or proton pump in ginsenoside-induced increase of membrane potential.

## DISCUSSION

Recently, Liu *et al.* reported that ginsenoside  $\text{Rg}_3$  inhibited proliferation of androgen dependent LNCaP cells.<sup>21)</sup> Previously, we characterized effects of both ginsenosides



**Fig. 6.** Effects of EIPA, SITS, and omeprazole on ginsenoside-induced increase of membrane potential.

Representative traces of membrane potential with 100  $\mu\text{M}$  of ginsenoside  $\text{Rb}_2$ , 200  $\mu\text{M}$  of ginsenoside  $\text{Rg}_3$ , or 50  $\mu\text{M}$  of ginsenoside  $\text{Rh}_2$  in DiBAC-loaded C6 glioma cells. The cells were treated with EIPA (A, 100  $\mu\text{M}$ , first arrow), SITS (B, 100  $\mu\text{M}$ ), or omeprazole (C, 20  $\mu\text{M}$ ), after 30 min (second arrow) cells were treated with indicated ginsenosides (third arrow).

$\text{Rg}_3$  and  $\text{Rh}_2$  on androgen independent PC3 prostate cells.<sup>22)</sup> Inhibitory effects of ginsenosides  $\text{Rg}_3$  and  $\text{Rh}_2$  on the cell growth have previously been observed in other types of cancers. In particular, ginsenoside  $\text{Rh}_2$  has been shown to be an apoptotic agent in rat glioma,<sup>23)</sup> human melanoma,<sup>24)</sup> ovarian cancer,<sup>25)</sup> breast cancer,<sup>26)</sup> hepatoma,<sup>27)</sup> and neuroblastoma.<sup>23)</sup> Also, ginsenoside  $\text{Rh}_2$  has been reported to induce differentiation of melanoma,<sup>11)</sup> teratocarcinoma,<sup>12)</sup> and leukemia.<sup>13)</sup>

Previously, we also reported cell detachment by ginsenoside  $\text{Rg}_3$  and  $\text{Rh}_2$ .<sup>22)</sup> There was differential cell detachment between ginsenoside  $\text{Rg}_3$  and  $\text{Rh}_2$ : Ginsenoside  $\text{Rg}_3$ -induced detachment was greater than ginsenoside  $\text{Rh}_2$ -induced one after 1 h of treatment, however, the detachment 24 h later was greater in ginsenoside  $\text{Rh}_2$ -treated group than ginsenoside  $\text{Rg}_3$ -treated group.<sup>22)</sup> Implication of the different action modes was further supported by differential modulations of MAP kinases by the

two ginsenosides.<sup>22)</sup> In androgen dependent LNCaP cells, inhibition of ERKs by ginsenosides  $\text{Rg}_3$  and  $\text{Rh}_2$  was an important trigger for cell detachment and growth inhibition, and modulation of p38 MAP kinase was additive in the case of the ginsenoside  $\text{Rh}_2$ . 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 ginsenosides  $\text{Rg}_3$ .<sup>22)</sup>

As a mechanism study of ginsenoside-induced growth inhibition in cancer cells, we measured membrane potential in prostate cancer and glioma cells in the present study. Major findings of this communication are four folds. First, three ginsenosides, ginsenosides  $\text{Rb}_2$ ,  $\text{Rh}_2$  and  $\text{Rg}_3$ , were found to increase significantly and robustly membrane potential of androgen dependent and independent prostate cancer cells and C6 glioma cells. Second, the increase of membrane potential may not be related with  $\text{G}_{i/o}$  type G proteins. Third,  $\text{Na}^+$  ion or  $\text{HCO}_3^-$  ion is

not involved with the ginsenoside-induced increase of membrane potential. Last, NHE, BCT, and proton pump might not be related with the ginsenoside-induced increase of membrane potential. We could not observe any effect of ginsenosides on intracellular  $Ca^{2+}$  concentration in prostate cancer cells, excluding a possibility of that ginsenoside-induced  $Ca^{2+}$  increase causes increase of membrane potential.<sup>22)</sup>

In the previous study, we showed increases of membrane potential by LPA, LPC, and SPC in C6 glioma cells. Although the precise mechanism for the increases was not elucidated, we found involvement of  $Na^+$  influx in the process and independence of suramin-sensitive GPCRs and pertussis toxin-sensitive G proteins.<sup>17)</sup> However, in our study, ginsenoside-induced increase of membrane potential was not much changed by treatment of PTX, U73122, EIPA, SITS, or omeprazole. Further study is necessary to elucidate action mechanism of ginsenosides on membrane potential and to find relationship between anti-cancer effect of ginsenosides and increase of membrane potential by ginsenosides.

### FOOTNOTE

Abbreviations used are: HBM hepes-buffered medium, EIPA 5-(*N*-ethyl-*N*-isopropyl)-amiloride, DiBAC<sub>4</sub>(3) bis-(1,3-dibarbituric acid)-trimethine oxanol, SITS 4-acetamidido-4'-isothiocyanostilbene-2,2'-disulfonic acid, PTX pertussis toxin, GPCR G-protein-coupled receptor, NHE  $Na^+/H^+$  exchanger, BCT bicarbonate transporter, LPA 1-oleoyl-*sn*-2-lysophosphatidic acid, LPC 1-palmitoyl-*sn*-2-lysophosphatidylcholine, SPC sphingosylphosphorylcholine.

### ACKNOWLEDGEMENT

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### REFERENCE

- Rhim, J. S. and Kung, H. F.: Human prostate carcinogenesis, *Crit Rev Oncog* **8**(4), 305-328 (1997).
- See, W. A.: Adjuvant hormone therapy after radiation or surgery for localized or locally advanced prostate cancer, *Curr Treat Options Oncol* **4**(5), 351-362 (2003).
- Sandler, H. M., Narayan, S. and Smith, D. C.: Combined modality treatment for prostate cancer: role of chemotherapy, *Semin Oncol* **30**(4 Suppl 9), 95-100 (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**(6), 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**(5 Pt 1), 1544-1547 (1993).
- VandenBerg, S. R.: Current diagnostic concepts of astrocytic tumors, *J Neuropathol Exp Neurol* **51**(6), 644-657 (1992).
- Ishiyuchi, S., Tsuzuki, K., Yoshida, Y., Yamada, N., Hagimura, N., Okado, H., Miwa, A., Kurihara, H., Nakazato, Y., Tamura, M., Sasaki, T. and Ozawa, S.: Blockage of  $Ca^{2+}$ -permeable AMPA receptors suppresses migration and induces apoptosis in human glioblastoma cells, *Nat Med* **8**(9), 971-978 (2002).
- 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 Korean Med Sci* **16** Suppl, S6-18 (2001).
- 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**(19), 8739-8743 (1995).
- Liao, B., Newmark, H. and Zhou, R.: Neuroprotective effects of ginseng total saponin and ginsenosides Rb1 and Rg1 on spinal cord neurons in vitro, *Exp Neurol* **173**(2), 224-234 (2002).
- 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**(6), 2781-2784 (1985).
- 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 ginsenosides in F9 teratocarcinoma cells, *Eur J Cancer* **32A**(8), 1420-1428 (1996).
- Kim, Y. S., Kim, D. S. and Kim, S. I.: Ginsenoside Rh2 and Rh3 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**(3), 327-338 (1998).
- Lee, S. R., Park, J. H., Choi, K. J. and Kim, N. D.: Inhibitory effects of ginsenoside Rg3 on platelet aggregation and its mechanism of action, *Kor J Ginseng Sci* **21**(2), 132-140 (1997).
- Rhim, H., Kim, H., Lee, D. Y., Oh, T. H. and Nah, S. Y.: Ginseng and ginsenoside Rg3, a newly identified active ingredient of ginseng, modulate  $Ca^{2+}$  channel currents in rat sensory neurons, *Eur J Pharmacol* **436**(3), 151-158 (2002).
- 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**(1-2), 35-44 (2003).
17. Lee, Y. K. and Im, D. S.: Distinct effects of lysophospholipids on membrane potential in C6 glioma cells, *J Appl Pharmacol* **14**(1), 25-29 (2006).
  18. Im, D. S., Fujioka, T., Katada, T., Kondo, Y., Ui, M. and Okajima, F.: Characterization of sphingosine 1-phosphate-induced actions and its signaling pathways in rat hepatocytes, *Am J Physiol* **272**(5 Pt 1), G1091-9 (1997).
  19. Sugo, T., Tachimoto, H., Chikatsu, T., Murakami, Y., Kikukawa, Y., Sato, S., Kikuchi, K., Nagi, T., Harada, M., Ogi, K., Ebisawa, M. and Mori, M.: Identification of a lysophosphatidylserine receptor on mast cells, *Biochem Biophys Res Commun* **341**(4), 1078-1087 (2006).
  20. Izumi, H., Torigoe, T., Ishiguchi, H., Uramoto, H., Yoshida, Y., Tanabe, M., Ise, T., Murakami, T., Yoshida, T., Nomoto, M. and Kohno, K.: Cellular pH regulators: potentially promising molecular targets for cancer chemotherapy, *Cancer Treat Rev* **29**(6), 541-549 (2003).
  21. 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**(11), 1297-306 (2000).
  22. Kim, H. S., Lee, E. H., Ko, S. R., Choi, K. J., Park, J. H. and Im, D. S.: Effects of ginsenosides Rg3 and Rh2 on the proliferation of prostate cancer cells, *Arch Pharm Res* **27**(4), 429-435 (2004).
  23. 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 Rh2-induced apoptosis in SK-N-BE(2) and C6Bu-1 cells, *Arch Pharm Res* **23**(5), 518-524 (2000).
  24. Fei, X. F., Wang, B. X., Tashiro, S., Li, T. J., Ma, J. S. and Ikejima, T.: Apoptotic effects of ginsenoside Rh2 on human malignant melanoma A375-S2 cells, *Acta Pharmacol Sin* **23**(4), 315-322 (2002).
  25. Nakata, H., Kikuchi, Y., Tode, T., Hirata, J., Kita, T., Ishii, K., Kudoh, K., Nagata, I. and Shinomiya, N.: Inhibitory effects of ginsenoside Rh2 on tumor growth in nude mice bearing human ovarian cancer cells, *Jpn J Cancer Res* **89**(7), 733-740 (1998).
  26. 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 Rh2 on MCF-7 human breast cancer cells, *Int J Oncol* **14**(5), 869-875 (1999).
  27. 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 Rh2-induced apoptosis, *Cancer Lett* **121**(1), 73-81 (1997).