The Journal of Applied Pharmacology, 14, 110-113(2006)

Effect of Sarcotride A on Membrane Potential in C6 Glioma Cells

Yun-Kyung LEE[1], Yonghong LIU[2], Jee H. JUNG[2], and Dong-Soon IM[1]*

[1]*Laboratories of Pharmacology and*

[2]*Marine Natural Product, College of Pharmacy and Research Institute for Drug Development, Pusan National University, Busan 609-735, Republic of Korea*

(Received May 10, 2006; Accepted June 12, 2006)

Abstract – We tested effect of sarcotride A, a bioactive cyclitol derivative from a marine sponge, on membrane potential in C6 glioma cells. Membrane potential was estimated by measuring fluorescence change of DiBAC-loaded glioma cells. Sarcotride A increased membrane potential in a concentration-dependent manner. We tested effects of pertussis toxin, U73122, EIPA, and Na^+-free media on sarcotride A-induced increase of membrane potential to investigate involvement of G proteins, phospholipase C, Na^+/H^+ exchanger, and Na^+ channels. However, we were not able to observe any significant effect of those pharmacological inhibitors, excluding the involvement of the molecules as candidate targets or signaling molecules of sarcotride A-induced increase of membrane potential. Further investigation is necessary to elucidate action mechanism of sarcotride A.

Keywords □ sarcotride A, membrane potential, glioma

INTRODUCTION

Marine sponges of the order Dictyoceratida are known to contain various furanosesterterpene derivatives that possess interesting biological properties(Faulkner, D. J. 2001). Dozens of new furano- and pyrroloterpenoids have been isolated from *Sarcotragus* sp. (Dictyoceratida) collected from Korean waters (Liu, Y. *et al.* 2001; Liu, Y. *et al.* 2002a). In our continuing study on the cytotoxic compounds of *Sarcotragus* sp., the cyclitol derivative sarcotride A has been isolated (Fig. 1)(Kim, D. K. *et al.* 1999; Liu, Y. *et al.* 2002b). Sarcotride A showed moderate to significant cytotoxicity against a small panel of five human tumor cell lines including XF498 human CNS tumor (Liu, Y. *et al.* 2002b). Sarcotride A exhibited inhibition on simian virus 40 (SV40) origin-dependent DNA replication *in vitro* at the level of initiation (Kim, D. K. *et al.* 1999). However, it did not show any inhibitory effect on topoisomerase I activity (Kim, D. K. *et al.* 1999).

Gliomas represent about half of all brain tumors, and among them, glioblastoma multiformes is thought to be the most malignant and common intracranial tumor (VandenBerg, S. R. 1992). Although generally not metastatic, glioblastoma cells exhibit highly migratory and invasive behavior(Ishiuchi, S. *et al.* 2002). Sarcotride A has been reported to be cytotoxic on several tumor cell lines including XF498 human CNS tumor.

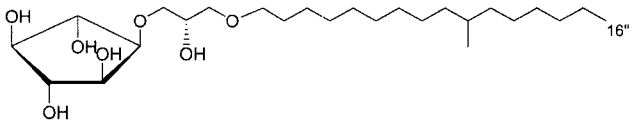

Fig. 1. Chemical structure of sarcotride A

Modulation of membrane potential plays important roles in neuronal cells and smooth muscle cells. However, it has been poorly investigated in glioma cells. Thus, in this study, we tested effect of sarcotride A on membrane potential in C6 glioma cells and further characterized the response with specific pharmacological inhibitors.

MATERIALS AND METHODS

Materials

Sarcotride A was isolated in Jung's laboratory(Kim, D. K. *et al.* 1999; Liu, Y. *et al.* 2002b). $DiBAC_4(3)$ was acquired from Biotium (Hayway, CA, USA). U73122 was from Biomol (Plymouth Meeting, PA, USA). All other materials were purchased from Sigma-Aldrich (St. Louis, MO, USA).

*Corresponding author
Tel: +82-51-510-2817, Fax: +82-51-513-6754
E-mail: imds@pusan.ac.kr

Cell culture

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_2 incubator (Lee, Y. K. and Im, D. S. 2006).

Measurement of membrane potential

The cells were trypsin-digested, sedimented, and 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_2PO_4, 1.2 mM $MgSO_4$, 0.5 mM $CaCl_2$, 25 mM $NaHCO_3$, 15 mM glucose and 0.1% bovine serum albumin (fatty acid free), and then incubated for 30 min with 5 μM of $DiBAC_4(3)$. 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 (Lee, Y. K. and Im, D. S. 2006).

Data presentation

Representative traces for membrane potential were chosen out of 3 separate experiments and shown in Fig. 2-4.

RESULTS

Sarcotride A induces increase of membrane potential in C6 glioma cells.

Sarcotride A largely increased membrane potential (Fig. 2). Increase of membrane potential by sarcotride A was observed in a concentration-dependent manner (Fig. 2). Significant increase was observed in higher concentrations than 10 μM (Fig. 2).

Involvement of G proteins and phospholipase C in lysophospholipids-induced membrane potential

Pertussis toxin has been used to elucidate involvement of $G_{i/o}$-type G proteins(Im, D. S. *et al.* 1997; Lee, Y. K. and Im, D. S. 2006). We treated C6 glioma cells with pertusis toxin (100 ng/ml, 24 hr). However, sarcotride A-induced change of membrane potential was not blunted, suggesting no involvement of G-protein-coupled receptors coupling to $G_{i/o}$-type G proteins (Fig. 3-A). U73122 is a pharmacological tool to test involvement of phospholipase C in the plasma membrane such as $G_{q/11}$-protein-coupled receptors(Im, D. S. *et al.* 1997). We treated C6 glioma cells with U73122 (5 μM, 10 min) and found no change (Fig. 3-B).

Effect of EIPA and Na^+ free media on sarcotride A-induced membrane potential

Next, we tested effect of EIPA and Na^+ free media on sarcotride A-induced membrane potential change. EIPA is an inhibitor of Na^+/H^+ exchanger. However, EIPA did not inhibit sarcotride A-induced increase of membrane potential. Furthermore, in Na^+-free media, sarcotride A-induced action was not

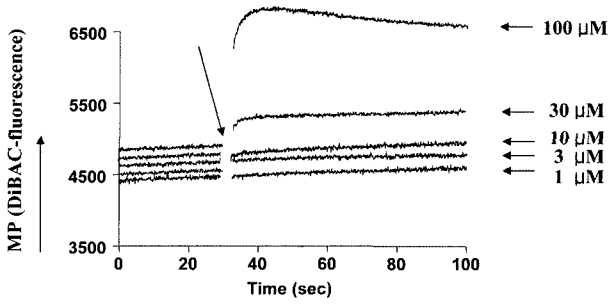

Fig. 2. Effect of sarcotride A on membrane potential in C6 glioma cells. Representative traces of membrane potential with different concentrations of sarcotride A in DiBAC-loaded C6 glioma cells were shown. sarcotride A was added at the arrow (30 sec).

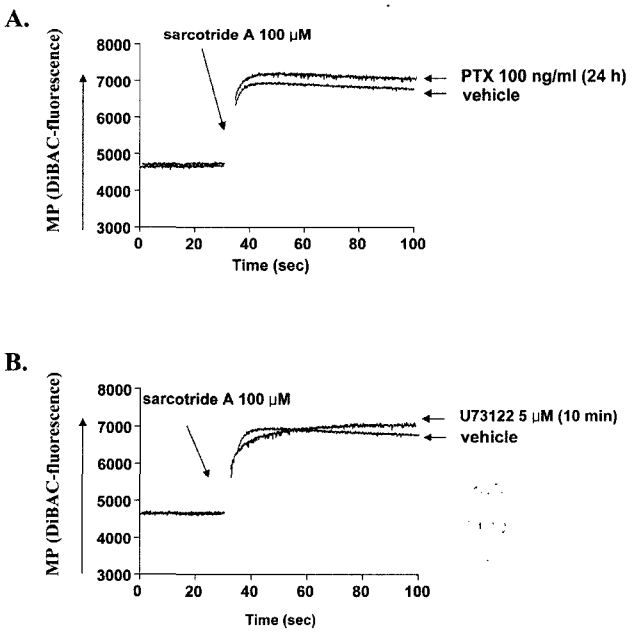

Fig. 3. Effects of pertussis toxin and U73122 on sarcotride A-induced increase of membrane potential. Representative traces of membrane potential with 100 μM of sarcotride A in DiBAC-loaded C6 glioma cells treated with pertusiss toxin (A, 100 ng/ml, 24 hr) or U73122 (B, 5 μM, 10 min) were shown.

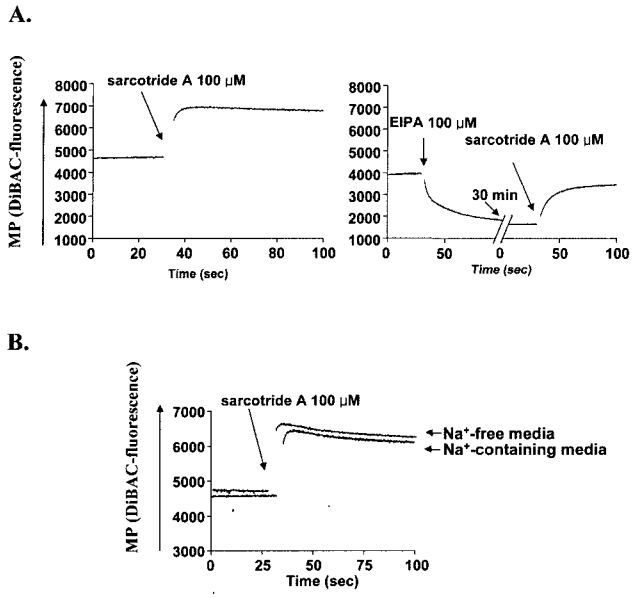

Fig. 4. Effects of EIPA or Na^+-free media on sarcotride A-induced increase of membrane potential. Representative trace of membrane potential with 100 μM of sarcotride A (A), in EIPA-treated cells (B, 100 μM, 30 min), or in Na^+-free media (C) in DiBAC-loaded C6 glioma cells were shown.

diminished (Fig. 4).

DISCUSSION

Previously, moderate to significant cytotoxicity of sarcotride A, a bioactive cyclitol derivative from marine sponge, was shown against a small panel of five human tumor cell lines including XF498 human CNS tumor(Liu, Y. *et al.* 2002b), and inhibition of simian virus 40 (SV40) origin-dependent DNA replication *in vitro* was observed at the level of initiation(Kim, D. K. *et al.* 1999). However, no inhibitory effect on topoisomerase I activity was reported (Kim, D. K. *et al.* 1999). And also we previously observed distinct changes of membrane potential by bioactive lysophospholipids such as LPA, LPC, and SPC, suggesting different action mechanisms between bioactive lysophospholipids (Lee, Y. K. and Im, D. S. 2006). Because sarcotride A has a long hydrophobic chain and an interesting cyclitol structure instead of a phosphate in lysophospholipids, as a mechanism study, we tested influence of sarcotride A on membrane potential. We, for the first time, observed increase of membrane potential by sarcotride A by using $DiBAC_4(3)$ fluorescence dye in C6 rat glioma cells.

Previously, membrane potential changes by lysophospholipids were partially blunted in Na^+-free media (Lee, Y. K. and Im, D. S. 2006). However, sarcotride A-induced increase of membrane potential was not abrogated by EIPA, an inhibitor of Na^+/H^+ exchanger, and in Na^+-free media, suggesting different mechanisms involved in membrane potential increases by sarcotride A and lysophospholipids. Furthermore, we found independence of pertussis toxin-sensitive G proteins and U73122-sensitive phospholipase C in the sarcotride A-induced increase of membrane potential in C6 glioma cells. To increase membrane potential, movement of positive or negative ions such as Ca^{2+} influx, K^+ efflux or blockage of Cl^- influx should be modulated by sarcotride A. Further studies are necessary to find exact mechanism of membrane potential increase by sarcotride A.

In summary, we showed increase of membrane potential by sarcotride A in C6 glioma cells. Although the precise mechanism for the increase was not elucidated, the present study would be useful for elucidation of action mechanism of sarcotride A and development of sarcotride A or related compounds as an anti-cancer drug in the future.

ACKNOWLEDGEMENT

This work was supported for two years by Pusan National University Research Grant.

Footnote

Abbreviations: U73122 1-[6-((17-3-methoxyestra-1,3,5(10)-trien-17-yl)amino-hexyl)-1*H*-pyrrole-2,5-dione, HBM hepes-buffered medium, EIPA 5-(*N*-ethyl-*N*-isopropyl)-amiloride, $DiBAC_4(3)$ bis-(1,3-dibarbituric acid)-trimethine oxanol, LPA lysophosphatidic acid, LPC lysophosphatidylcholine, SPC sphingosylphosphorylcholine,

REFERENCES

Faulkner, D. J. (2001). Marine natural products. *Nat Prod Rep* **18**, 1-49.

Im, D. S., Fujioka, T., Katada, T., Kondo, Y., Ui, M. and Okajima, F. (1997). Characterization of sphingosine 1-phosphate-induced actions and its signaling pathways in rat hepatocytes. *Am J Physiol* **272**, G1091-9.

Ishiuchi, S., Tsuzuki, K., Yoshida, Y., Yamada, N., Hagimura, N., Okado, H., Miwa, A., Kurihara, H., Nakazato, Y., Tamura, M., Sasaki, T. and Ozawa, S. (2002). Blockage of Ca(2+)-permeable AMPA receptors suppresses migration and induces apoptosis in human glioblastoma cells. *Nat Med* **8**, 971-8.

Kim, D. K., Lim, Y. J., Kim, J. S., Park, J. H., Kim, N. D., Im, K. S., Hong, J. and Jung, J. H. (1999). A cyclitol derivative as a

replication inhibitor from the marine sponge Petrosia sp. *J Nat Prod* **62**, 773-6.

Lee, Y. K. and Im, D. S. (2006). Distinct effects of lysophospholipids on membrane potential in C6 glioma cells. *J Appl Pharmacol* **14**, 25-29.

Liu, Y., Bae, B. H., Alam, N., Hong, J., Sim, C. J., Lee, C. O., Im, K. S. and Jung, J. H. (2001). New cytotoxic sesterterpenes from the sponge Sarcotragus species. *J Nat Prod* **64**, 1301-4.

Liu, Y., Hong, J., Lee, C. O., Im, K. S., Kim, N. D., Choi, J. S. and Jung, J. H. (2002a). Cytotoxic pyrrolo- and furanoterpenoids from the sponge sarcotragus species. *J Nat Prod* **65**, 1307-14.

Liu, Y., Lee, C. O., Hong, J. and Jung, J. H. (2002b). Cyclitol derivatives from sponge Sarcotragus species. *Bull Kor Chem Soc* **23**, 1467-1469.

VandenBerg, S. R. (1992). Current diagnostic concepts of astrocytic tumors. *J Neuropathol Exp Neurol* **51**, 644-57.