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Inhibitory Effects of Panaxatriol from *Panax ginseng* C. A. Meyer on Phosphoinositide Breakdown Induced by Thrombin in Platelets

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Abstracts : In this study, we have investigated the effect of panaxatriol (PT) on phosphoinositides (PIS) breakdown and Ca^{2+}-elevation in thrombin-induced platelet aggregation. Thrombin (5U/ml), a potent platelet agonist which activates phospholipase C_β via protease activated receptor (PAR), hydrolyzed PIS in platelet membrane. The phosphatidylinositol 4, 5-bisphosphate (PIP_2) was hydrolyzed after 10 sec of the thrombin-stimulation, and both the phosphatidylinositol 4-monophosphate (PIP) and phosphatidylinositol (PI) were brokendown after 30 sec of the thrombin-stimulation. However, PT inhibited the thrombin-stimulated hydrolysis of PIP_2, PIP, and PI. On the other hand, thrombin increased the level of phosphatidic acid (PA) which is phosphorylated from diacylglycerol (DG) generated by PIS-hydrolysis. However, PT inhibited the thrombin-increased PA level non-significantly. Thrombin increased cytosolic free Ca^{2+}($[Ca^{2+}]_i$) up to 72% as compared with control (30.8±0.9 nM) in intact platelet. However, PT (100 μg/ml) inhibited the thrombin-elevated $[Ca^{2+}]_i$ to 100%. These results suggest that PT may have a beneficial effect on platelet aggregation-mediated thrombotic disease by inhibiting thrombin-induced platelet aggregation *via* suppression of the $[Ca^{2+}]_i$ level and PIS breakdown.

Key words : Panaxatriol, Cytosolic free Ca^{2+}, Phosphoinositides, Phosphatidic acid

INTRODUCTION

Platelet aggregation is essential for haemostatic processes when blood vessels are injured. However, the interaction between platelets and agonists (i.e. collagen, thrombin, ADP, etc) can also cause circulatory disorders, such as thrombosis, atherosclerosis, and myocardial infarction[1].

Inhibition of the agonist-induced platelet aggregation might be a promising approach for the prevention of thrombosis. Especially, $[Ca^{2+}]_i$ in pltelets plays a central role in the activation of platelet aggregation and involves in the phosphodiesteratic cleavage of PIP_2, PIP, and PI *via* the activation of Ca^{2+}-dependent phospholipase C. This enzyme produces inositol 1, 4, 5-trisphosphate (IP_3) from PIP_2, inositol 1, 4-bisphosphate from PIP, and inositol phosphate from PI[2, 3]. DG is commonly produced by the breakdown of PIP_2, PIP, and PI, and is hydrolyzed by DG- and monoacylglycerol-lipase to produce arachidonic acid which is a precursor of thromboxane A_2 (TXA_2) [2-4]. TXA_2 is a potent platelet-aggregating agent[5]. In addition, DG binds to its intracellular receptor Ca^{2+}/DG-dependent protein kinase C (C-kinase), and thus phosphorylates 47 kDa pleckstrin to aggregate platelets[6]. IP_3 is very well

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known to mobilizing Ca^{2+} from Ca^{2+}-store (i.e. dense tubular system) in platelets or other cells[2-4] and the subsequently increased $[Ca^{2+}]_i$ is used for activation of phospholipase C, phospholipase A_2, C-kinase, and Ca^{2+}/calmodulin-dependent protein kinase (CaM-PK)[2-4]. CaM-PK phosphorylates 20 kDa myosin light chain to contract platelets, which is associated with the release of granule component such as serotonin[6]. Accordingly, it is interesting to determine whether breakdown of PIS such as PIP_2, PIP, and PI is an initial event to supply platelet-aggregating agents, such as TXA_2, phosphoproteins of pleckstrin and myosin light chain, and $[Ca^{2+}]_i$. It has been reported that non-saponin fraction, saponins, and polyacetylene compound from *Panax ginseng* C. A. Meyer have anti-platelet effects on thrombin- and collagen-induced platelet aggregation[7-9]. We previously reported that a lipophilic fraction from *Panax ginseng* C. A. Meyer had anti-platelet effect by inhibiting collagen-induced PI breakdown[10]. On the other hand, it is reported that total saponin from *Panax ginseng* C. A. Meyer might regulate PIS turnover in the liver or brain[11]. There are few reports that a component from *Panax ginseng* C. A. Meyer regulates PIS metabolism, an initial event in cellular signaling. In this study, we have observed that PT has an inhibitory effect on PIS breakdown, which is stimulated by thrombin.

MATERIALS AND METHODS

Materials

PT was manufactured by the Korean Tobacco and Ginseng Research Institute (present: Central Research Institute, KT&G Corporation, Daejon, Korea). Thrombin, Quin II/AM, HPTLC (silica gel G), and other reagents were obtained from Sigma Chemical Co. (St. Louis). Carrier-free phosphorus-32 was obtained from Amersham Life Science Co. (Buckinghamshire, UK). Platelet-rich plasma (PRP), obtained from the antecubital vein of normal healthy human volunteers, was purchased from Taejon Red Cross Blood Center, Korea.

Methods

Preparation of ^{32}P-labeled washed human platelets

PRP was incubated with ^{32}P (0.2 mCi/ml PRP) at 37°C for 60min. The platelets were washed twice with a washing buffer (138 mM NaCl, 2.7 mM KCl, 12 mM $NaHCO_3$, 0.36 mM NaH_2PO_4, 5.5 mM glucose, and 1 mM EDTA, pH 6.5). The washed platelets were then suspended in suspending buffer (138 mM NaCl, 2.7 mM KCl, 12 mM $NaHCO_3$, 0.36 mM NaH_2PO_4, 0.49 mM $MgCl_2$, 5.5 mM glucose, 0.25% gelatin, pH6.9). The platelet number was adjusted to 5×10^8/ml in the suspending buffer. All of the above-mentioned procedures were carried out at 25°C in order to avoid platelet aggregation from any effect of low temperature.

Metabolism of ^{32}P-labeled phospholipids

^{32}P-labeled washed platelets (10^8/ml) were preincubated at 37°C for 3 min, and then were stimulated with 5 U/ml of thrombin for 10 sec or 30 sec while being gently stirred. The incubation was terminated by the addition of 2 ml of cold methanol. Then lipids were extracted according to the method of Agranoff *et al.* and Kito *et al.*[12, 13]. The extracted lipids were separated by the method of Agranoff *et al.*[12]. and Takamura *et al.*[14] The individual lipid classes were visualized by iodine vapor and autoradiography X-ray film, the phospholipids (PLS) were scraped off from thin layer chromatography (TLC) plate. Radioactivity of the separated PLS was determined by liquid scintillation counter in Aquasol/water/methanol (83:12:5, v/v).

Measurement of cytosolic free Ca^{2+} concentration

PRP was incubated with 25 μM Quin II/AM at 37°C for 60 min. Because Quin II/AM is light-sensitive, the tube containing PRP was covered with aluminum foil during loading Quin II/AM. The Quin II-loaded platelets were also prepared using the procedure described above. EDTA, a Ca^{2+} chelator, was removed by washing platelets twice with platelet suspending buffer (pH 6.9). Ca^{2+} was measured with gentle stirring at 37°C using the method of Tsien *et al.*,[15] and fluorescence spectrometer (Perkin Elmer, LS 50B). Dimethyl sulfoxide of PT vesicle was not affected by Ca^{2+} concentration.

Statistical Analysis

All data are shown as mean ± S.D. A Student's *t*-test was used for data analysis and paired or unpaired comparisons were used when necessary.

RESULTS AND DISCUSSION

Structural characteristics of PT

PT ($C_{30}H_{52}O_4$) is one of the tetracyclic triterpenoids in dammarane family of *Panax ginseng* C. A. Mayer (Fig. 1A), which is known to form by acid hydrolysis of ginsenoside (ex. ginsenoside-Rg_1) and is a different aglycone from 20(s) protopanaxatriol (PPT) (Fig. 1B) [16, 17]. PT ($C_{30}H_{52}O_4$) and PPT ($C_{30}H_{52}O_4$) have commonly three hydroxyl groups (-OH) at C-3, C-6, and C-12 of steroid parts (dammarane

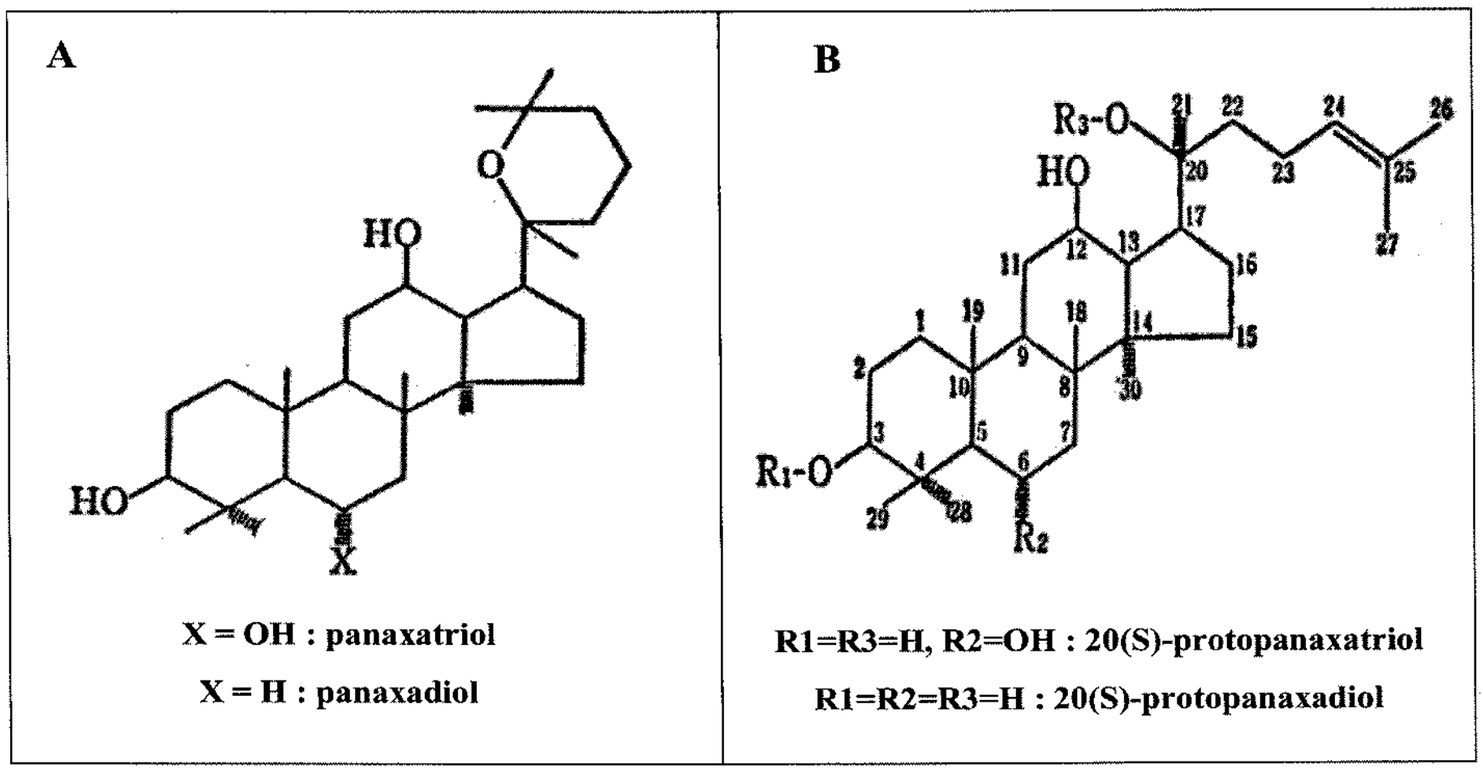

Fig. 1. Structures of panaxatriol and 20(s)-protopanaxatriol from Panax ginseng C. A. Mayer.

skeleton) in their structures, respectively (Fig. 1A, B).

Effect of PT on $[Ca^{2+}]_i$

Since the 100 μg of panaxadiol (PD)/ml, an analogue of PT, did not inhibited the thrombin-elevated $[Ca^{2+}]_i$ in platelet cytosol[18], we have used 100 μg of PT/ml in this study, equivalent to PD concentration, and this was compared with the effect of PD (100 μg/ml) [18] on the following successive experiments. As shown in Fig. 2, basal $[Ca^{2+}]_i$ in intact platelets (10^8/ml) were 30.8 ± 0.9 nM (Fig. 2). This level was in agreement with the previous reports [18, 19]. However, thrombin (5 U/ml) increased the $[Ca^{2+}]_i$ from 30.8 ± 0.9 nM to 53.0 ± 3.7 nM (Fig. 2) indicating that average 22 nM of $[Ca^{2+}]_i$) is increased into the cytosol by thrombin. This $[Ca^{2+}]_i$ (22 nM) is resulted from Ca^{2+}-influx or Ca^{2+}-mobilization. Ca^{2+}-influx is increased through plasma membrane from extracellular domain, and Ca^{2+}-mobilization is increased through dense tubular system membrane or endoplasmic reticulum. PT (100 μg/ml) completely inhibited thrombin-induced $[Ca^{2+}]_i$ level by 100 % (Fig. 2). Because the $[Ca^{2+}]_i$ requiring for platelet activation was increased by IP_3-mediated Ca^{2+} release from internal stores (dense tubular system) via the IP_3 receptor[20], the inhibitory effect of PT on $[Ca^{2+}]_i$ might be associated with the inhibition of $[^{32}P]$-PIP_2 breakdown increased by thrombin (Fig. 4A). In addition, thrombin activates Ca^{2+}-dependent-phospholipase C or -phospholipase A_2 to produce a precursor arachidonic acid of TXA_2, another platelet-aggregating molecule. Since thrombin-elevated $[Ca^{2+}]_i$ was inhibited by PT (Fig. 2), PT seems to inhibit the activity of phospholipase C or phospholipase A_2, and subsequently might inhibit the production of TXA_2 via the suppression of arachidonic acid release from PIS or phosphatidylcholine in platelet membrane. This hypothesis is supported from our previous report that PT (100 μg/ml) potently inhibits adrenaline-produced TXA_2 level[19]. However, it is noteworthy that ginsenoside Rg_1 (Rg_1), a PPT type saponin, did not inhibit collagen-

	$[Ca^{2+}]_i$ (nM)	Increase (%)	Inhibition (%)
Basal	30.8 ± 0.9[a]	0	-
Thrombin (5 U/ml)	53.0 ± 3.7[b]	72	0
PT (1 μg/ml)+ Thrombin (5 U/ml)	51.1 ± 2.2[c]		8.6①
PT (100 μg/ml)+ Thrombin (5 U/ml)	30.2 ± 1.1[d]		100②

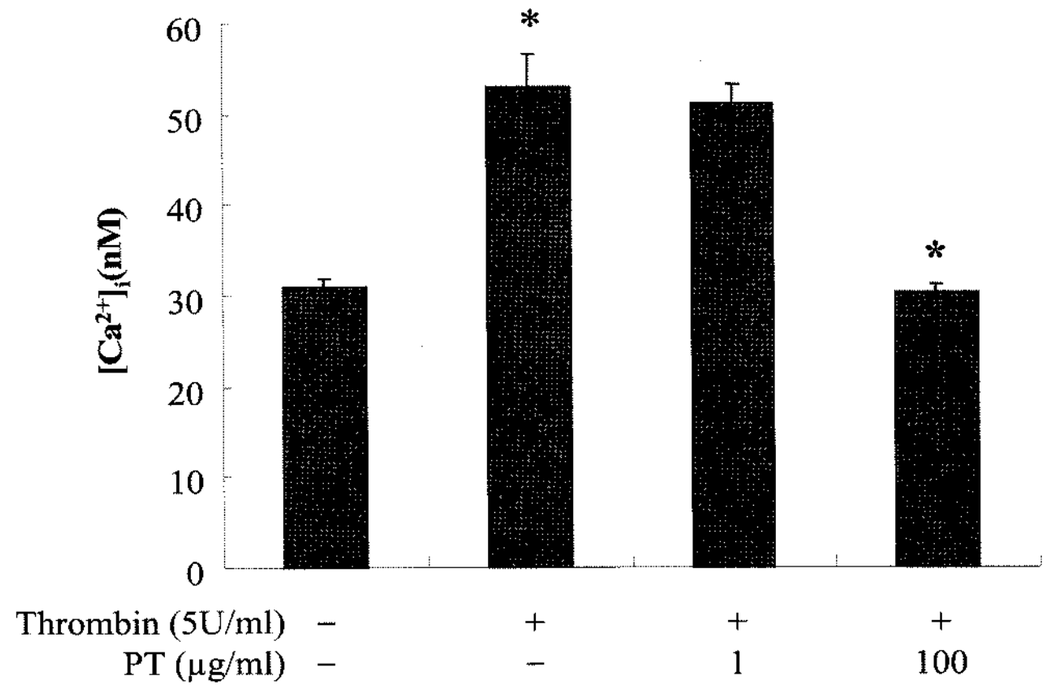

Fig. 2. Effect of PT on thrombin-elevated $[Ca^{2+}]_i$. $[Ca^{2+}]_i$ was determined as described in the "MATERIALS AND METHODS". ① Inhibition (%)=b-c / b-a × 100. ② Inhibition (%) = b-d / b-a × 100. * $P < 0.05$ as compared to the control or PT+ thrombin

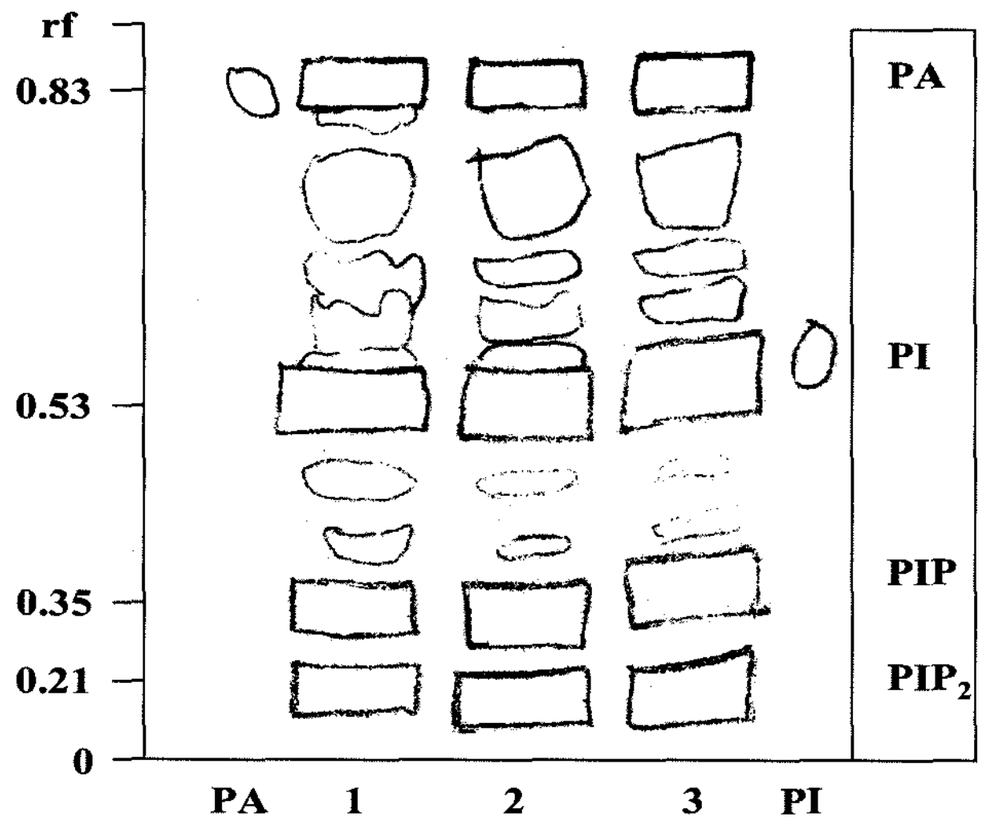

Fig. 3. Separation of PIS on thrombin-induced platelet activation. TLC (silica gel G) plate was impregnated with CH_3OH/water (2:3, v/v) containing potassium oxalate (1 %) at room temperature, and was dried. This plate was baked at 150°C for 8 min, and subsequently was developed by $CHCl_3$/acetone/CH_3OH/acetic acid/water (100:50:100:4:10, v/v). [^{32}P]-PIS were observed with X-ray film. TLC chromatogram is [^{32}P]-disposed X-ray film. lane 1: Control. lane 2: Thrombin (5 U/ml). lane 3: PT (100 μg/ml) + thrombin (5 U/ml). PIP_2: Phosphatidylinositol 4,5-bisphosphate. PIP: Pho-sphatidylinositol 4-monophosphate. PI: Phosphatidy-linositol (standard). PA: Phosphatidic acid (standard).

elevated TXA_2 or thrombin-released arachidonic acid in platelets[21, 22].

Separation of phosphoinositides and phosphatidic acid

As shown in Fig. 3, [^{32}P]-labeled-PIP_2, -PIP, and -PI were observed at Rf 0.21, 0.35, and 0.53 in the TLC, respectively. [^{32}P]-PA was observed at Rf 0.83, which is phosphorylated from DG produced by hydrolysis of PIP_2, PIP, and PI with phospholipase C in agonist (i.e: thrombin)-activated platelets. The phospholipids (PLS) were separated by scraping each PLS band in TLC, and were used to investigate the metabolism of PIS.

Effect of PT on PIS breakdown in thrombin-induced platelet aggregation

Three kinds of PIS, such as PIP_2, PIP, and PI in platelets and other cells, have very important roles in terms of cellular signal transduction, and thus supply second messengers such as IP_3 and DG in response to all cellular reactions[2-4]. In fact, IP_3 increases Ca^{2+} and DG then stimulates the activity of C-kinase to phosphorylate pleckstrin in platelets. Accordingly, we have investigated the effect of PT on breakdown of PIP_2, PIP, and PI in thrombin-induced platelet aggregation. When platelets (10^8/ml) were stimulated by thrombin (5 U/ml), after 10 sec, [^{32}P]-PIP_2 was decreased significantly by 9.0 ± 1.0% (Fig. 4A) as compared with control (13 ± 3.0%) in intact platelets (Fig. 4A). This is in accord with the previous report that [^{32}P]-PIP_2 breakdown by phospholipase C was observed within 10 sec after the stimulation[23]. In contrary to [^{32}P]-PIP_2, [^{32}P]-PIP and [^{32}P]-PI were significantly decreased at 30 sec after thrombin (5 U/ml)-stimulation (Fig. 4A and 4B). However, the levels of [^{32}P]-PIP_2, [^{32}P]-PIP, and [^{32}P]-PI decreased by thrombin (5 U/ml) were reversed to the control level in the presence of PT (100 μg/ml) (Fig. 4A, 4B, and 4C). These results suggest that PT could inhibit phospholipase C activity to suppress the breakdown of PIP_2, PIP, and PI by thrombin. It is known that thrombin receptor is PAR, which is G-protein coupled receptor and mediates a Gq-linked response leading to the activation of phospholipase C_β. Phospholipase C_β hydrolyzed the PIP_2 [24]. It could not excluded that PT could inhibit phospholipase C_β activity to suppress the breakdown of PIP_2 by thrombin. DG is commonly generated by the cleavage of PIP_2, PIP, and PI, and then DG is phosphorylated subsequently to PA by DG-kinase. Accordingly, we investigated the effect of PT on PA-production. As shown in Fig. 5, after 10 sec or 30 sec of thrombin-stimulation, [^{32}P]-PA level was increased significantly by 12 ± 2.0% or 18 ± 3.0%, respectively (Fig. 5), compared to that (5.5 ± 1.0%) of the control. This result reflected that increased level of [^{32}P]-PA was due to the increased hydrolysis of [^{32}P]-PIP_2, [^{32}P]-PIP, and [^{32}P]-PI by thrombin-activated phospholipase C at 10 sec or 30 sec after thrombin-stimulation (Fig. 4A, 4B, 4C). The increase of [^{32}P]-PA level by thrombin was inhibited non significantly by PT (Fig. 5). The results, however, suggest that PT might inhibit phospholipase C, and in turn inhibits the production of DG. Usually, PIP_2 breakdown accompanies the generation of IP_3 besides DG. Subsequently, IP_3 mobilizes Ca^{2+} from Ca^{2+} store (dense tubular system), and thus involves directly in platelet aggregation. It is reported that ginsenoside Rg_1 did not inhibit the production of DG from PIP_2 but inhibited thrombin-elevated $[Ca^{2+}]_i$ in human platelets[22]. We have reported that ginsenoside Rg_1 inhibited C-kinase activity, which was induced by phorbol-12-myristate-13-acetate, a tumor promoter[25]. Considering the previous- or our-results, it is thought that PT might inhibit Gq-linked response inducing to the activa-

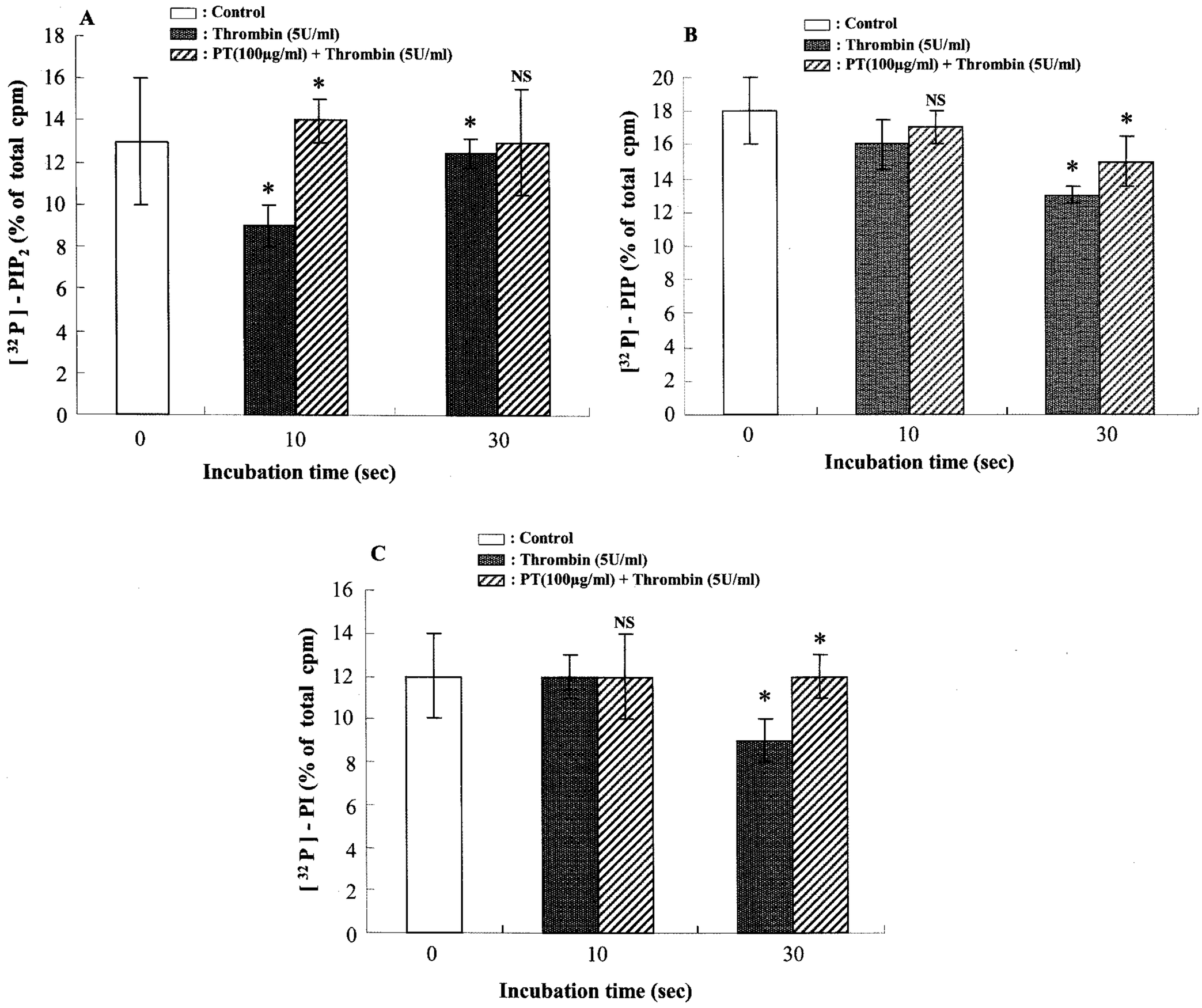

Fig. 4. Effect of PT on thrombin-induced PIS cleavage. 2A: Effect of PT on thrombin-hydrolyzed phosphatidylinositol 4,5-bisphosphate (PIP_2). 2B: Effect of PT on thrombin-hydrolyzed phosphatidylinositol 4-monophosphate (PIP). 2C: Effect of PT on thrombin-hydrolyzed phosphatidylinositol (PI). [^{32}P]-labeled PIP_2, PIP, and PI were analyzed as described in the "MATERIALS AND METHODS". *$P < 0.05$ as compared to the control or thrombin (5 U/ml). NS: Not significant

tion of phospholipase C_β, upstream pathway involving in PIS-breakdown, and ginsenoside Rg_1 might inhibit C-kinase activity by DG/Ca^{2+}, downstream pathway involving in pleckstrin phosphorylation. Effective concentration of antiplatelet drugs such as aspirin, indomethacin, and imidazole on human platelet is 1~200 μM *in vitro*. The concentration of PT (100 μg/ml, $C_{30}H_{52}O_4$, Mw 476)[16] used in our study is almost equivalent to 210 μM. Because PT, which is produced by acid hydrolysis of Rg_1[16, 17], has antiplatelet effect at low concentration (100 μg/ml) than Rg_1 (500 μg/ml)[22], it is thought that PT might become a beneficial saponin aglycone to inhibit platelet aggregation. Considering the fact that PT inhibits PIS breakdown and $[Ca^{2+}]_i$ increase by thrombin (Fig. 2, 4), PT might be involved in inhibition of Ca^{2+}-mediated platelet aggregation, and thus might have a beneficial effect on platelet aggregation-mediated cardiovascular disease.

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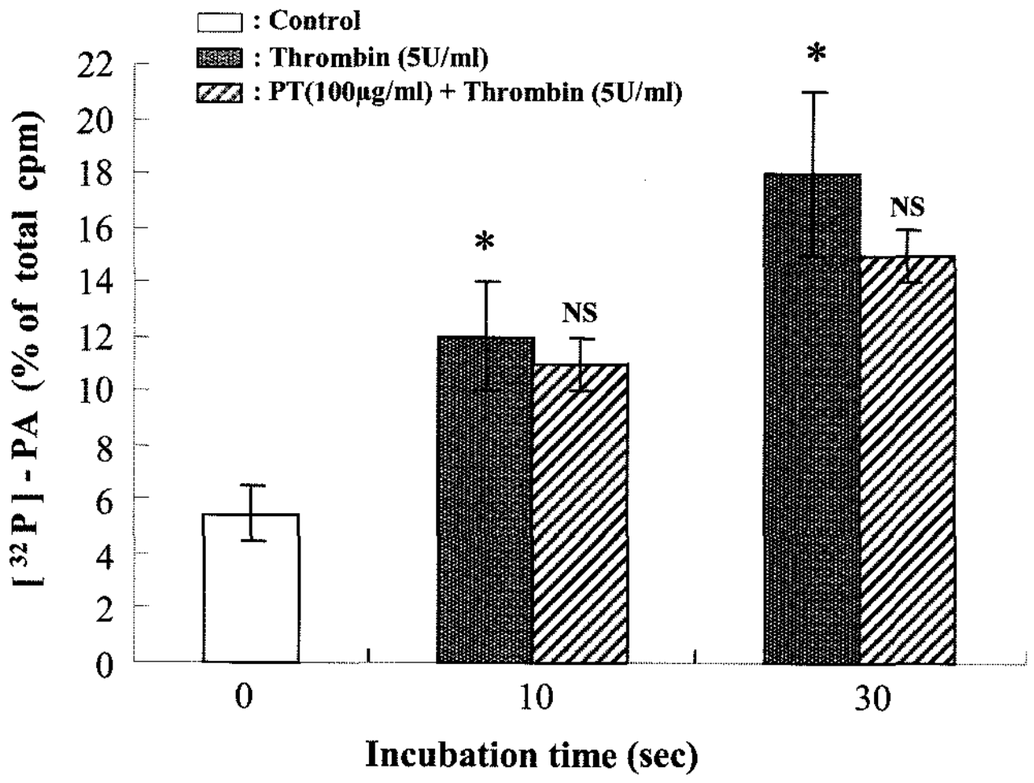

Fig. 5. Effect of PT on thrombin-produced phospatidic acid (PA). PA was analyzed as described in the "MATERIALS AND METHODS". * $P < 0.05$ as compared to the control or thrombin (5 U/ml). NS: Not significant.

REFERENCES

1. Schwartz, S. M., Heinmark, R. L. and Majesky, M.W.: Developmental mechanisms underlying pathology of arteries. *Physiol. Rev.* **70**, 1177-1209 (1990).
2. Berridge, M. J. and Irvine, R. F.: Inositol trisphosphate, a novel second messenger in cellular signal transduction. *Nature*. **312**, 315-321(1984).
3. Majerus, P. W., Neufeld, E. J. and Wilson, D. B.: Production of phosphoinositide-derived messenger. *Cell*. **37**, 701-703 (1984).
4. Nishizuka, Y.: The role of protein kinase C in cell surface signal transduction and tumor promotion. *Nature*. **308**, 693-697 (1984).
5. Nishikawa, M., Tanaka, T. and Hidaka, H.: Ca^{2+}-calmodulin-dependent phosphorylation and platelet secretion. *Nature*. **287**, 863-865 (1980).
6. Cattaneo, M., Tenconi, P. M., Lecchi, A. and Mannucci, P. M.: In vitro effects of picotamide on human platelet aggregation, the release reaction and thromboxane B_2 production. *Thromb. Res.* **62**, 717-724 (1991).
7. Park, H. J., Rhee, M. H., Park, K. M., Nam, K. Y. and Park, K. H.: Effect of non-saponin fraction from *Panax ginseng* on cGMP and thromboxane A_2 in human platelet aggregation. *J. Ethano. Pharmacol*. **49**, 157-162 (1995).
8. Park, H. J., Lee, J. H., Song, Y. B. and Park, K. H.: Effects of dietary supplementation of lipophilic fraction from *Panax ginseng* on cGMP and cAMP in rat platelets and on blood coagulation. *Biol. Pharm. Bull*. **19**, 1434-1439 (1996).
9. Kuo, S. C., Teng, C. M., Lee, J. C., Ko, F. N., Chen, S. C. and Wu, T. S.: Antiplatelet components in *Panax ginseng*. *Planta Medica*. **56**, 164-167 (1990).
10. Lee, J. H. and Park, H. J.: Effect of lipophilic fraction from *Panax ginseng* on phosphatidylinositol breakdown in rat platelets activated by collagen. *Kor. J. Gerontol*. **7**, 47-51 (1997).
11. Rim, K. T., Choi, J. S., Lee, S. M. and Cho, K. S.: Effects of ginsenosides from red ginseng on the enzymes of cellular signal transduction system. *Korean J. Ginseng. Sci*. **21**, 19-27 (1997).
12. Agranoff, B. W., Murthy, P. and Seguim, E. B.: Thrombin-induced phosphodiesteratic cleavage of phosphatidylinositol bisphosphate in human platelets. *J. Biol. Chem*. **258**, 2076-2078 (1983).
13. Kito, M., Narita, H., Ishinaga, M., Park, H. J. and Takamura, H.: Phosphoinositide breakdown as an indirect link between stimulation and aggregation of rat platelets by thrombin and collagen. *J. Biochem*. **97**, 765-772 (1985).
14. Takamura, H., Narita, H., Park, H. J., Tanaka, K. I., Matsuura, T. and Kito, M.: Differential hydrolysis of phospholipid molecular species during activation of human platelets with thrombin and collagen. *J. Biol. Chem*. **262**, 2262-2269 (1987).
15. Tsien, R. Y., Pozzan, T. and Rink, T. J.: Calcium homeostasis in intact lymphocytes: cytoplasmic free calcium monitored with a new intracellulary trapped fluorescent indicator. *J. Cell Biol*. **94**, 325-334 (1982).
16. Shibata, S., Tanaka, O., Soma, K., Ando, T., Iida, Y. and Nakamura, H.: Studies on saponins and sapogenins of ginseng. The structure of panaxatriol. *Tetrahedron Lett*. **42**, 207-213 (1965).
17. Tanaka, O., Nagai, M. and Shibata, S.: Chemical studies on the oriental plant drugs. XVI. The stereochemistry of protopanaxadiol, a genuine sapogenin of ginseng. *Chem. Pharm. Bull*. **14**, 1150-1156 (1966).
18. Park, H. J., Rhee, M. H., Park, K. M., Nam, K. Y. and Park, K. H.: Panaxadiol from *Panax ginseng* C. A, Meyer inhibits synthesis of thromboxane A_2 in platelet aggregation induced by thrombin. *Korean J. Ginseng Sci*. **17**, 131-124 (1993).
19. Park, K. M., Rhee, M. H. and Park, H. J.: Panaxadiol and panaxatriol from *Panax ginseng* C. A. Meyer inhibit the synthesis of thromboxane A_2 in adrenaline-stimulated human platelet aggregation. *Korean J. Ginseng Sci*. **18**, 44-48 (1994).
20. Furuichi, T. and Mikoshiba, K.: Inositol 1,4,5-trisphosphate receptor-mediated Ca^{2+}-signaling in the brain. *J. Neurochem*. **64**, 953-960 (1995).
21. 田村泰, 平井愛山, 山本恭平: 高麗人蔘の抗血小板作用の解析, p.109-121. in: 山本雄一, 熊谷朗(ed). 薬用人蔘'89. 共立出版株式會社, 日本 (1989).
22. Kimura, Y., Okuda, H. and Arichi, S.: Effects of various ginseng saponins on 5-hydroxytryptamine release and aggrega-

tion in human platelets. *J. Pharm. Pharmacol.* **40**, 838-843 (1988).

23. Narita, H., Park, H. J., Tanaka, K. I., Matsuura, T. and Kito, M.: Insufficient mobilization of calcium by early breakdown of phosphatidylinositol 4,5-bisphosphate for aggregation of human platelets by collagen. *J. Biochem.* **98**, 1063-1068 (1985).
24. Savage, B., Cattaneo, M. and Ruggeri Z. M.: Mechanisms of platelet aggregation. *Curr Opin Hematol.* **8**, 270-26 (2001).
25. Park, H. J., No, Y. H., Rhee, M. H., Park, K. M. and Park, K. H.: Effects of protein fraction and ginsenosides from *Panax ginseng* C. A. Meyer on substrate phosphorylation by a catalytic fragment of protein kinase C. *Korean Biochem. J.* **27**, 280-283 (1994).