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Research Article

Inhibitory effects of thromboxane A_2 generation by ginsenoside Ro due to attenuation of cytosolic phospholipase A_2 phosphorylation and arachidonic acid release

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ABSTRACT

Background: Thromboxane A_2 (TXA_2) induces platelet aggregation and promotes thrombus formation. Although ginsenoside Ro (G-Ro) from *Panax ginseng* is known to exhibit a Ca^{2+}-antagonistic antiplatelet effect, whether it inhibits Ca^{2+}-dependent cytosolic phospholipase A_2 ($cPLA_{2\alpha}$) activity to prevent the release of arachidonic acid (AA), a TXA_2 precursor, is unknown. In this study, we attempted to identify the mechanism underlying G-Ro-mediated TXA_2 inhibition.

Methods: We investigated whether G-Ro attenuates TXA_2 production and its associated molecules, such as cyclooxygenase-1 (COX-1), TXA_2 synthase (TXAS), $cPLA_{2\alpha}$, mitogen-activated protein kinases, and AA. To assay COX-1 and TXAS, we used microsomal fraction of platelets.

Results: G-Ro reduced TXA_2 production by inhibiting AA release. It acted by decreasing the phosphorylation of $cPLA_{2\alpha}$, p38-mitogen-activated protein kinase, and c-Jun N-terminal kinase1, rather than by inhibiting COX-1 and TXAS in thrombin-activated human platelets.

Conclusion: G-Ro inhibits AA release to attenuate TXA_2 production, which may counteract TXA_2-associated thrombosis.

1. Introduction

Platelets are activated via breakdown of phosphatidylinositol 4,5-bisphosphate in the plasma membrane (PM) to inositol-1,4,5-trisphosphate (IP_3) and diacylglycerol (DG) by phospholipase C (PLC) [1]. IP_3 mobilizes Ca^{2+} from the endoplasmic reticulum to the cytoplasm, and Ca^{2+} activates Ca^{2+}/calmodulin-dependent protein kinase [2]. Apart from phosphorylating pleckstrin by binding to protein kinase C, DG acts as a donor of arachidonic acid (AA) [3], a precursor of thromboxane A_2 (TXA_2) [4]. TXA_2 is an autacoid produced from AA by the actions of cyclooxygenase-1 (COX-1) and TXA_2 synthase (TXAS) and initiates thrombogenesis [5–7]. Antithrombotic drugs, such as aspirin, imidazole, and indomethacin, block TXA_2 production by inhibiting COX-1 or TXAS activity [8].

Mitogen-activated protein kinases (MAPKs), such as extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38-MAPK, are phosphorylated in thrombin-activated human platelets [9–11]. Phosphorylated p38-MAPK and ERK2 induce TXA_2 production [12–14]. Moreover, the phosphorylation of p38-MAPK is essential for the activation of cytosolic phospholipase $A_{2\alpha}$ ($cPLA_{2\alpha}$), leading to AA release [14].

Thrombin elevates the intracellular Ca^{2+} level, leading to the translocation of $cPLA_{2\alpha}$ from the cytosol to the PM. Subsequently, p38-MAPK activates $cPLA_{2\alpha}$ by phosphorylating it at Ser^{505} [15]. Therefore, it may be beneficial to evaluate the antiplatelet potential

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of a compound on TXA_2 production in relation to phosphorylation of MAPKs.

Roots of *Panax* (*P*) *ginseng* are used in traditional Oriental medicine. In a previous study, we reported that total saponin from Korean Red Ginseng inhibits both COX-1 and TXAS to reduce the production of TXA_2 [16]; however, its individual components have not yet been evaluated. Therefore, we evaluated the effects of ginsenoside Ro (G-Ro), an oleanane-type saponin (Fig. 1) in *P. ginseng*, on the production of TXA_2 along with its associated enzymes and signaling molecules.

2. Materials and methods

2.1. Materials

G-Ro was obtained from Ambo Institute (Daejon, Korea). Thrombin was obtained from Chrono-Log Corporation (Havertown, PA, USA). Fura 2-AM was obtained from Invitrogen Molecular Probes (Eugene, OR, USA). Aspirin was obtained from Sigma Chemical Corporation (St. Louis, MO, USA). Thromboxane B_2 (TXB_2) enzyme immunoassay (EIA) kit, COX-1 fluorescence activity assay kit, ozagrel, and prostaglandin H_2 were purchased from Cayman Chemical Company (Ann Arbor, MI, USA). Anti-phosphor-cPLA$_2$ (Ser^{505}), anti-phosphor-p38-MAPK, anti-phosphor-JNK (1/2), anti-p38-MAPK, anti-JNK (1/2), anti-COX-1, anti-TXAS, anti-rabbit IgG-horseradish peroxidase conjugate, and lysis buffer were obtained from Cell Signaling Technology (Beverly, MA, USA). PD98059, SB203580, SP600125, and anti-β-actin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Polyvinylidene difluoride membrane and enhanced chemiluminescence solution were purchased from GE Healthcare (Piscataway, NJ, USA). Human AA EIA kit was obtained from Cusabio (Wuhan, Hubei, China).

2.2. Preparation of washed human platelets

Human platelet-rich plasma with acid–citrate–dextrose solution (0.8% citric acid, 2.2% sodium citrate, 2.45% glucose) was procured from Korean Red Cross Blood Center (Changwon, Korea). It was centrifuged for 10 min at 1,300×*g* to obtain the platelet pellets. The platelets were washed twice using 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 Na_2EDTA, pH 6.5) and resuspended in a suspension 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, and 0.25% gelatin, pH 6.9) to a final concentration of 5×10^8 cells/mL. All the aforementioned procedures were performed at 25°C to preserve platelet activity. These experiments were approved (PIRB12-072) by the National Institute for Bioethics Policy Public Institutional Review Board (Seoul, Korea).

2.3. Determination of platelet aggregation

Platelets (10^8 cells/mL) were preincubated, with or without G-Ro, in a $CaCl_2$ (2 mM) solution for 3 min at 37°C. They were stimulated with thrombin (0.05 U/mL) and allowed to aggregate for 5 min in an aggregometer (Chrono-Log Corporation). Platelet aggregation rate was determined as an increase in light transmission. G-Ro was dissolved in the platelet suspension buffer (pH 6.9), and MAPK inhibitors were dissolved in 0.1% dimethyl sulfoxide.

2.4. Western blot analysis of COX-1 and TXAS, and phosphorylation of p38-MAPK, JNK1/2, and cPLA$_{2\alpha}$

Platelet aggregation was terminated by adding an equal volume (250 μL) of lysis buffer (20 mM Tris-HCl, 150 mM NaCl, 1 mM Na_2EDTA, 1 mM ethylene glycol tetraacetic acid (EGTA), 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM ATPase, 1 mM Na_3VO_4, 1 μg/mL leupeptin, and 1 mM phenylmethanesulfonyl fluoride, pH 7.5). Protein content in the platelet lysate was measured using a bicinchoninic acid protein assay kit (Pierce Biotechnology, IL, USA). COX-1 and TXAS were analyzed by Western blotting after separating equal amounts of total protein (30 μg) in the lysate, microsomal, and cytosol fractions of platelets via sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (8%, 1.5 mm). Phosphorylation of p38-MAPK, JNK1/2, and cPLA$_{2\alpha}$ was evaluated by Western blotting after separating 15 μg of total protein by SDS-PAGE (6%, 1.5 mm). A Polyvinylidene difluoride membrane was used for protein transfer. The primary and secondary antibodies were diluted 1:1,000 and 1:10,000, respectively. The membranes were visualized using an enhanced chemiluminescence solution solution. The degrees of phosphorylation were analyzed using the Quantity One 1-D analysis software, Version. 4.5 (Bio-Rad, Hercules, CA, USA).

2.5. Measurement of TXB_2

Because TXA_2 is unstable and gets converted spontaneously to TXB_2, it was quantified by determining the TXB_2 content [4]. After platelet aggregation, the reaction was terminated by adding ice-cold EDTA (5 mM) and indomethacin (0.2 mM) to prevent the metabolism of AA to TXA_2. The amount of TXB_2, a stable metabolite of TXA_2, was determined using a TXB_2 EIA kit according to the procedure described by the manufacturer.

2.6. Isolation of microsomal fraction

Washed platelets (10^8 cells/mL), suspended in a buffer (pH 7.4) with 1% protease inhibitor, were sonicated 10 times at 100% sensitivity for 20 s on ice (Bandelin, HD2070, Germany) to obtain the platelet lysate. The microsomal fraction, containing endoplasmic reticulum membrane, was obtained by ultracentrifugation at 105,000×*g* for 1 h at 4°C [16].

2.7. AA release

The reaction was terminated after platelet aggregation, and the aggregates were centrifuged at 200×*g* at 4°C for 10 min. AA in the supernatant was quantified using an AA EIA kit (Cusabio), and the absorbance was measured at 450 nm using a Synergy HT multi-mode microplate reader (BioTek Instruments, Inc., Winooski, VT, USA).

2.8. COX-1 activity assay

The microsomal fraction of platelets was preincubated with aspirin (500 μM), a positive control, or with various concentrations of G-Ro and other reagents at 37°C for 30 min. COX-1 activity was assayed with a COX-1 fluorescence assay kit (Cayman Chemical Co).

2.9. TXAS activity assay

The microsomal fraction of platelets was preincubated with ozagrel (11 nM, IC_{50}), a positive control, or with various concentrations of G-Ro and other reagents at 37°C for 5 min. The reaction was initiated by adding prostaglandin H_2, and the samples were incubated at 37°C for 1 min; the reaction was terminated by adding citric acid (1 M). After neutralization with 1 N NaOH, the amount of TXB_2 was determined using a TXB_2 EIA kit according to the procedure described by manufacturer.

2.10. Statistical analyses

All experimental results are indicated as the mean ± standard deviation accompanied by the number of trials. Significant differences were determined by analysis of variance followed by the Newman–Keuls multiple comparisons method. All statistical analyses were conducted using the SPSS 21.0.0.0 software (SPSS, Chicago, IL, USA). A p value < 0.05 was considered to be statistically significant.

3. Results

3.1. Effects of G-Ro on platelet aggregation

We used thrombin at a dose of 0.05 U/mL, which induces maximum human platelet aggregation [17] to stimulate the platelets in this study. Thrombin increased platelet aggregation up to 92.5 ± 1.2%. However, G-Ro reduced the thrombin-induced platelet aggregation in a dose-dependent manner (Fig. 1B).

3.2. Effects of G-Ro on TXA_2 production

We determined whether G-Ro reduced platelet aggregation by inhibiting TXA_2 production (by measuring the TXB_2 level). As shown in Fig. 1C, thrombin increased TXB_2 level (49.2 ± 1.6 ng/10^8 platelets), whereas G-Ro dose-dependently (50–300 μM) reduced the TXB_2 level that was induced by thrombin; G-Ro (300 μM) inhibited the thrombin-mediated elevation in TXB_2 level by 94.9%.

3.3. Effects of G-Ro on activities of COX-1 and TXAS

We evaluated the activities of COX-1 (70 kDa) and TXAS (58 kDa) in the microsomal fraction to investigate whether they contributed to the reduction in TXB_2 by G-Ro (Fig. 2A, lane 2). COX-1 activity in the absence of G-Ro (negative control) was 2.3 ± 0.1 nmol/mg protein. However, G-Ro dose-dependently (50–300 μM) reduced its activity (Fig. 2B); at 300 μM, COX-1 activity was reduced by 26.4% of that of the negative control. TXAS activity in the absence of G-Ro (negative control) was 220.8 ± 1.8 ng/mg protein/min. However, G-Ro dose-dependently (50–300 μM) reduced its activity (Fig. 2C); at 300 μM, TXAS activity was reduced by 22.9% of that of the negative control. We observed that G-Ro (300 μM) reduced COX-1 (26.4%) and TXAS (22.9%) activities to similar extents.

3.4. Effects of G-Ro on $cPLA_{2\alpha}$ phosphorylation and AA release

The inhibitory effect of G-Ro (300 μM) on TXB_2 production (94.9%, Fig. 1C) was significantly higher than those on COX-1 (26.4%, Fig. 2B) and TXAS (22.9%, Fig. 2C) activities. This suggested that G-Ro might also inhibit AA release, a precursor of TXA_2, from PM phospholipids to reduce TXA_2 production in thrombin-activated platelets.

Because Ca^{2+}-dependent $cPLA_{2\alpha}$ is activated by phosphorylation [18] and releases AA from PM phospholipids in thrombin-activated human platelets [10], we investigated the effect of G-Ro on the phosphorylation of $cPLA_{2\alpha}$. As shown in Fig. 3A, G-Ro inhibited the thrombin-mediated phosphorylation of $cPLA_{2\alpha}$ (Ser^{505}) in a dose-dependent manner as it is reported that $cPLA_{2\alpha}$ is activated by phosphorylation of $cPLA_{2\alpha}$ at Ser^{505} [18,19]. At 300 μM, G-Ro inhibited the thrombin-induced $cPLA_{2\alpha}$ (Ser^{505}) phosphorylation by 96.5% (Fig. 3A). Moreover, it reduced the thrombin-induced AA release in a dose-dependent manner (Fig. 3B); at 300 μM, it inhibited AA release by 61.1% of that induced by thrombin (2159.2 ± 29.0 ng/10^8 platelets).

3.5. Effects of G-Ro on the phosphorylation of MAPKs

Platelets contain MAPKs, such as ERK, JNK, and p38-MAPK [20], that phosphorylate Ser^{505} of $cPLA_{2\alpha}$ [10,14,18,19,21–23].

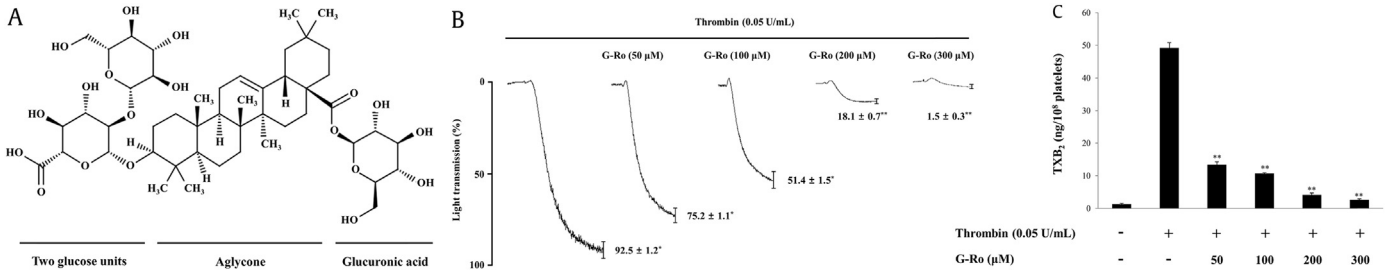

Fig. 1. Effects of G-Ro on thrombin-induced human platelet aggregation and thromboxane B_2 production. (A) Structure of G-Ro. (B) Effect of G-Ro on thrombin-induced human platelet aggregation. (C) Effect of G-Ro on thromboxane B_2 production. Platelet aggregation and thromboxane B_2 production were carried out as described in "Materials and methods" section. The data are expressed as the mean ± standard deviation ($n = 4$). $^{*}p < 0.05$ versus the thrombin-stimulated human platelets, $^{**}p < 0.01$ versus the thrombin-stimulated human platelets.
TXB_2, thromboxane B_2.

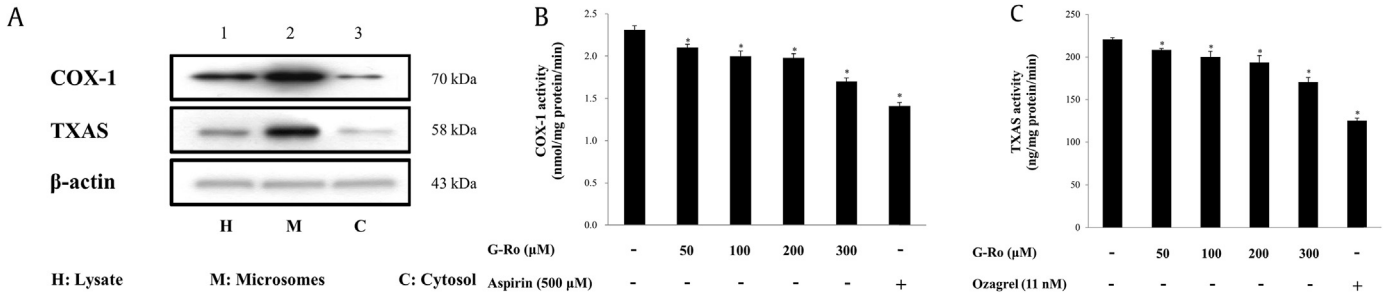

Fig. 2. Effects of G-Ro on COX-1 and TXAS activities. (A) Determination of the effects of the enzyme sources on COX-1 and TXAS activities. (B) Determination of the effects of G-Ro on COX-1. (C) Determination of the effects of G-Ro on TXAS activities. Western blot analysis and COX-1 and TXAS activities were determined as described in "Materials and methods" section. The data are expressed as the mean ± standard deviation ($n = 4$). $^{*}p < 0.05$ versus the thrombin-stimulated human platelets.
COX-1, cyclooxygenase-1; TXAS, thromboxane A_2 synthase.

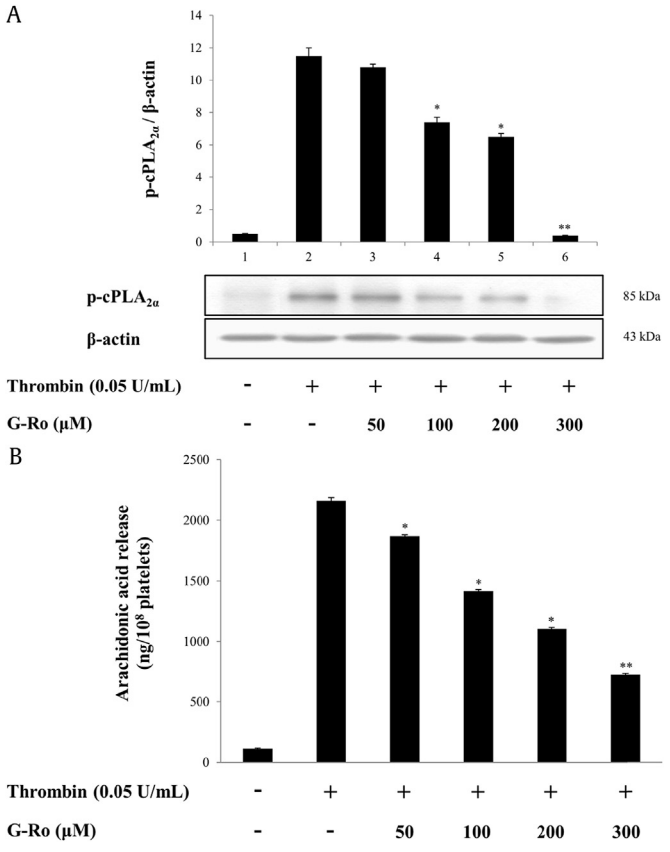

Fig. 3. Effects of G-Ro on $cPLA_{2\alpha}$-phosphorylation and AA release. (A) Effects of G-Ro on $cPLA_2$-phosphorylation. (B) Effects of G-Ro on AA release. Western blot and AA release assay were determined as described in "Materials and methods" section. The data are expressed as the mean ± standard deviation ($n = 3$). $^{*}p < 0.05$ versus the thrombin-stimulated human platelets, $^{**}p < 0.01$ versus the thrombin-stimulated human platelets.
AA, arachidonic acid; $cPLA_2$, cytosolic phospholipase A_2.

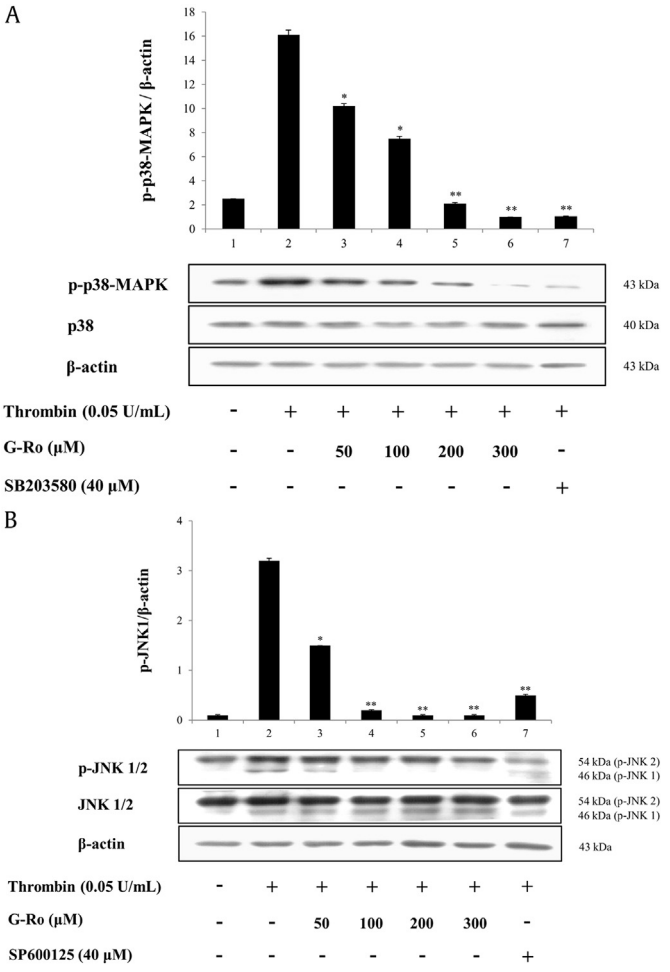

Fig. 4. Effects of G-Ro on the phosphorylation of MAPKs. (A) Effects of G-Ro on the phosphorylation of p38-MAPK. (B) Effects of G-Ro on JNK1/2 phosphorylation. Western blot was determined as described in "Materials and methods" section. The data are expressed as the mean ± standard deviation ($n = 3$). $^{*}p < 0.05$ versus the thrombin-stimulated human platelets, $^{**}p < 0.01$ versus the thrombin-stimulated human platelets.
JNK, Jun N-terminal kinase; MAPK, mitogen-activated protein kinases.

Therefore, we investigated whether G-Ro inhibited the phosphorylation of $cPLA_{2\alpha}$ (Ser^{505}) in thrombin-activated human platelets. Thrombin-mediated p38-MAPK phosphorylation (Fig. 4A, lane 2) was dose-dependently (50–300 μM) inhibited by G-Ro (Fig. 4A, lanes 3–6). Furthermore, the p38-MAPK inhibitor, SB203580, attenuated the thrombin-induced phosphorylation of p38-MAPK (Fig. 4A, lane 7).

Thrombin phosphorylated JNK1 (46 kDa), but not JNK2 (54 kDa), as shown (Fig. 4B, lane 2). G-Ro attenuated the thrombin-induced phosphorylation of JNK1 in a dose-dependent manner (Fig. 4B, lanes 3–6). The inhibitor of JNK, SP600125, inhibited the phosphorylation of both JNK1 and JNK2 in thrombin-activated human platelets (Fig. 4B, lane 7).

3.6. *Effects of MAPK inhibitors on $cPLA_{2\alpha}$ phosphorylation, AA release, and TXA_2 production*

Furthermore, we investigated whether MAPK inhibitors inhibited the phosphorylation of $cPLA_{2\alpha}$. Thrombin extensively phosphorylated $cPLA_{2\alpha}$; however, it was inhibited by SB203580 (40 μM). Nevertheless, PD98059 (40 μM) and SP600125 (40 μM) did not influence the thrombin-induced $cPLA_{2\alpha}$ phosphorylation (Fig. 5A). Among the MAPK inhibitors, only SB203580 (40 μM), a p38-MAPK inhibitor, strongly inhibited the thrombin-mediated $cPLA_{2\alpha}$ phosphorylation. This suggested that p38-MAPK induces $cPLA_{2\alpha}$ phosphorylation and may stimulate TXA_2 production by promoting AA release. Therefore, we tested this hypothesis using SB203580.

We observed that it inhibited the thrombin-induced AA release and TXA_2 production by 75.2% and 91.6%, respectively (Figs. 5B, 5C).

4. Discussion

The autacoid TXA_2, produced in platelets, constricts blood vessels and initiates thrombogenesis [7,24,25]. *P. ginseng* compounds, such as ginsenoside Rp1 [26], panaxadiol, and panaxatriol saponins [27–29], inhibit TXA_2 production and attenuate platelet aggregation. In this study, we evaluated whether G-Ro inhibits thrombin-induced platelet aggregation by decreasing TXA_2 production and investigated the mechanisms underlying the attenuation of AA release. We sought to identify the TXA_2 antagonistic potential of G-Ro for development into an antiplatelet agent.

G-Ro inhibited TXA_2 production to abolish thrombin-induced platelet aggregation. We determined the activities of COX-1 (70 kDa) and TXAS (58 kDa) in the microsomal fraction, which has the highest activity of cytochrome c reductase (an endoplasmic reticulum marker enzyme) to justify this inhibitory effect [16]. G-Ro reduced the production of TXA_2 more than it reduced the activities of COX-1 and TXAS, suggesting that it may also inhibit AA release by $cPLA_{2\alpha}$ and AA utilization by COX-1 and TXAS in thrombin-activated platelets. As expected, G-Ro strongly inhibited both thrombin-

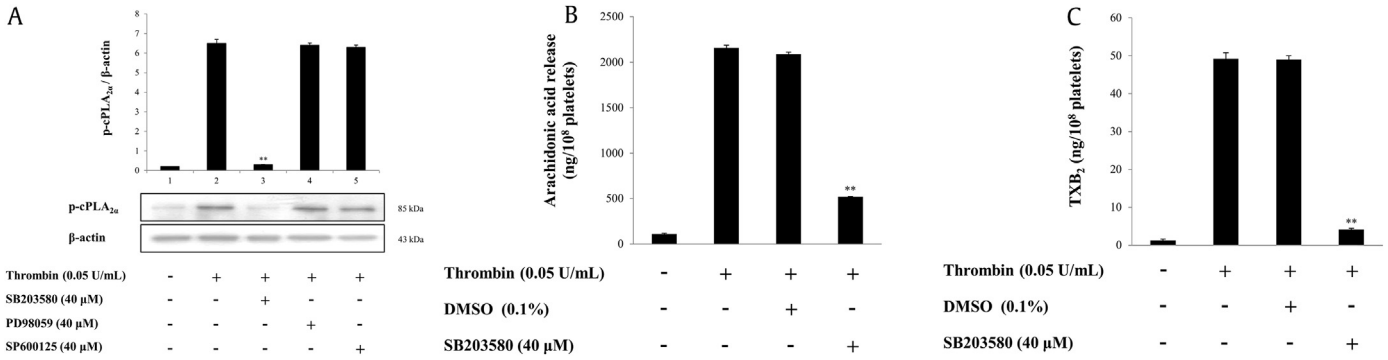

Fig. 5. Effects of MAPK inhibitors on $cPLA_{2\alpha}$-phosphorylation, AA release, and TXA_2 production. (A) Effects of MAPK inhibitors on $cPLA_2$-phosphorylation. (B) Effects of SB203580 on AA release. (C) Effects of SB203580 on TXA_2 production. Western blot, AA release assay, and TXA_2 production were determined as described in "Materials and methods" section. The data are expressed as the mean ± standard deviation ($n = 3$). $^{*}p < 0.05$ versus the thrombin-stimulated human platelets in the presence of 0.1% DMSO, $^{**}p < 0.01$ versus the thrombin-stimulated human platelets in the presence of 0.1% DMSO.
AA, arachidonic acid; $cPLA_{2\alpha}$, cytosolic phospholipase $A_{2\alpha}$; DMSO, dimethyl sulfoxide; MAPK, mitogen-activated protein kinases; TXB_2, thromboxane B_2.

induced Ca^{2+}-dependent $cPLA_{2\alpha}$ (Ser^{505}) phosphorylation and AA release. These results verify that the reduction in intracellular Ca^{2+} level by G-Ro [30] prevents the binding of $cPLA_{2\alpha}$ to its PM substrates, such as phosphatidylcholine (PC), phosphatidylserine (PS), and phosphatidylethanolamine (PE). Accordingly, the Ca^{2+}-antagonistic effects of G-Ro [30] reduce AA release from $cPLA_{2\alpha}$ substrates (PC, PS, and PE) to decrease TXA_2 production. Moreover, thrombin-elevated intracellular Ca^{2+} hydrolyzes the AA bond at position 2 of PS, PC, and PE in the PM of human platelets [31], indicating that the AA, bound at position 2 of glycerophospholipids, is attacked by Ca^{2+}-dependent $cPLA_{2\alpha}$. Thrombin also activates Ca^{2+}-dependent PLC_β to produce DG and IP_3 from phosphatidylinositol 4,5-bisphosphate in the PM. DG is hydrolyzed to AA and glycerol via the DG– and monoacylglycerol–lipase pathway [1]. Accordingly, we cannot rule out G-Ro-mediated inhibition of the PLC_β/DG–lipase/monoacylglycerol–lipase pathway to reduce AA release in thrombin-activated platelets.

In the present study, G-Ro inhibited the activities of both the AA release enzyme ($cPLA_{2\alpha}$) and AA utilization enzymes (COX-1 and TXAS) to decrease the thrombin-induced TXA_2 production. These enzymes are known to be activated by phosphorylated MAPKs [12–14,19–21,32–39]. Therefore, we used MAPK inhibitors to investigate whether G-Ro requires inhibition of thrombin-phosphorylated MAPKs for attenuating TXA_2 production. SB203580 (a p38-MAPK inhibitor) inhibited the thrombin-induced p38-MAPK phosphorylation, $cPLA_{2\alpha}$ phosphorylation, AA release, and TXA_2 production. These results confirm that thrombin-phosphorylated p38-MAPK increases AA release and TXA_2 production by promoting $cPLA_{2\alpha}$ phosphorylation.

Similar to SB203580, G-Ro attenuated thrombin-induced p38-MAPK phosphorylation, $cPLA_{2\alpha}$ phosphorylation, AA release, and TXA_2 production. Therefore, we can assume that G-Ro inhibits thrombin-induced AA release and TXA_2 production by preventing the phosphorylation of both p38-MAPK and $cPLA_{2\alpha}$. Moreover, G-Ro is reported to inhibit the thrombin-mediated phosphorylation of ERK2 [30] and JNK1. However, G-Ro failed to inhibit AA release through suppression of ERK2- and JNK1-induced $cPLA_{2\alpha}$ phosphorylation in thrombin-activated platelets. Furthermore, both PD98059 (ERK inhibitor) and SP600125 (JNK inhibitor) did not inhibit thrombin-induced $cPLA_{2\alpha}$ phosphorylation. Therefore, other platelet-activating mechanisms, such as Ca^{2+} influx [9,40,41] and COX-1 activation by ERK2 [39] and serotonin release by JNK1 [20], might have led to the suppression of ERK2 and JNK1 by G-Ro. Many compounds of ginseng, such as G-Ro, G-Rp4, Rg3-enriched red ginseng extract, and G-Rp1, inhibit the phosphorylation of MAPKs to attenuate Ca^{2+} influx and serotonin release in platelets [26,42,43].

We previously showed that G-Ro inhibits thrombin-induced Ca^{2+}-dependent platelet-activating reactions, including granule secretion, fibrinogen binding, and fibrin clot retraction, by upregulating the cyclic adenosine monophosphate (cAMP)-dependent phosphorylation of IP_3 (Ser^{1756}) and vasodilator-stimulated phosphoprotein (Ser^{157}) [44]. In this study, we observed that G-Ro attenuated thrombin-induced TXA_2 production by inhibiting AA release, and this effect was due to the inhibition of Ca^{2+}-dependent $cPLA_{2\alpha}$ phosphorylation by p38-MAPK. In addition, G-Ro abolishes Ca^{2+}-dependent p-selectin expression in thrombin-activated platelets [30]. Because its expression in activated platelets causes leukocytic inflammatory atherosclerosis, G-Ro may counteract inflammation and atherosclerosis [45–47]. The *in vitro* and *in vivo* antiinflammatory activities of G-Ro and Korean Red Ginseng are reported [48–50].

In conclusion, G-Ro attenuates TXA_2 production by inhibiting p38-MAPK-mediated $cPLA_{2\alpha}$ phosphorylation and AA release. It also reduced the activities of microsomal COX-1 and TXAS in thrombin-activated human platelets. Combined with previous reports [30,44,48,49], G-Ro holds significant antiplatelet potential.

Conflicts of interest

The authors declare no conflict of interest.

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