

Inhibitory Effects of Ginsenoside Ro on Clot Retraction through Suppressing PI3K/Akt Signaling Pathway in Human Platelets

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ABSTRACT: Glycoprotein IIb/IIIa (α IIb/ β ₃) is the most abundant integrin on platelet surfaces, which is involved in interaction between platelets, and triggers an intracellular signaling cascade, platelet shape changes, granule secretion, and clot retraction. In this study, we evaluated the effect of ginsenoside Ro (G-Ro) on the binding of fibronectin and fibrinogen to α IIb/ β ₃ and clot retraction. We found that G-Ro inhibited thrombin-induced platelet aggregation dose-dependently and attenuated the fibronectin-, and fibrinogen-binding to α IIb/ β ₃ through the dephosphorylation of phosphoinositide 3-kinase p85 and Akt, which influence clot retraction, reflecting the intensification of thrombus. We observed that G-Ro is involved in α IIb/ β ₃ in human platelets. These results suggest that G-Ro is beneficial, inhibiting fibronectin adhesion, fibrinogen binding, and clot retraction. Therefore, G-Ro in *Panax ginseng* may prevent platelet aggregation-mediated thrombotic disease.

Keywords: ginsenoside Ro, PI3K p85 (Tyr⁴⁵⁸), Akt (Ser⁴⁷³), fibrinogen-binding, fibronectin adhesion

INTRODUCTION

Platelets are activated at sites of vascular injury via several molecules, such as collagen, thrombin, and adenosine diphosphate. Full platelet aggregation is absolutely essential for normal hemostasis. Moreover, this physiological event can trigger circulatory disorders, such as thrombosis, atherosclerosis, and cardiovascular disease (1). Therefore, platelet function inhibition is a promising approach in preventing platelet-mediated circulatory disease. These signaling cascades are triggered by an inside-out signaling pathway and facilitate the activation of glycoprotein IIb/IIIa (α IIb/ β ₃). The α IIb/ β ₃ is abundant integrin at the platelet surface and acts as a receptor for adhesive proteins (i.e. fibrinogen, fibronectin, vitronectin, and thrombospondin). The activated α IIb/ β ₃ induces Ca²⁺ mobilization and granule secretion, and continuously intensifies the platelet aggregation and the formation of thrombus via outside-in signaling pathway (2). These two signaling-mediated molecules potentiate the formation of thrombus, the final reaction of platelets.

Human platelets contain class I phosphoinositide 3-kinase (PI3K) isoforms, which are subdivided into class IA and class IB. The class IA PI3Ks (PI3K α , β , and δ) is composed of a regulatory subunit (p50, p55, and p85) with a

catalytic subunit (p110 α , β , and δ), and class IB PI3K (PI3K γ) consists of a regulatory subunit (p101 and p84) with a catalytic subunit (p110 γ) (3). The p85 protein is known as the regulatory subunit of class IA PI3Ks, which is activated by binding tyrosine phosphorylation sites (4), GTP-binding proteins (5) and its phosphorylation (6,7). The activated p85 directly binds to p110 that enables the activation of PI3K. Upon platelet stimulation by thrombin, phosphatidylinositol 3,4,5 triphosphate (PIP₃) is accumulated by class I PI3Ks at the inner layer of the plasma membrane (8); then, a downstream effector Akt binds to PIP₃ and is phosphorylated for full enzyme activity on Thr³⁰⁸ and Ser⁴⁷³ by phosphatidylinositol dependent kinases (9). Several studies have revealed that the PI3K and Akt are the most important mediators in human platelets leading to adhesive function, filopodia formation, platelet spreading, and α IIb/ β ₃ activation (10,11). In addition, dephosphorylation of PI3K p85 (Tyr⁴⁵⁸) and Akt (Ser⁴⁷³) can suppress the binding of adhesive proteins to α IIb/ β ₃. Thus, to determine if inhibition of PI3K/Akt signaling pathway contributes to the α IIb/ β ₃ inhibitory action of ginsenoside Ro (G-Ro), PI3K/Akt inhibitors, wortmannin and miltetofosine were used for fibronectin adhesion, fibrinogen binding to α IIb/ β ₃, and clot retraction. The α IIb/ β ₃ activation contributes to estimate the antithrom-

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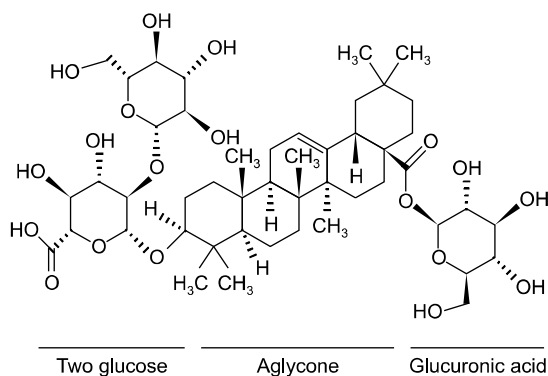


Fig. 1. Chemical structure of ginsenoside Ro (G-Ro). G-Ro is an oleanane-type saponin and contained in *Panax ginseng* Meyer, and is composed of oleanolic acid as aglycone, two glucose, and one glucuronic acid as sugar component.

botic effect of certain compounds (12,13). For instance, abciximab, etifibatide, tirofiban, and lamifiban have antithrombotic effects *via* suppression of the α IIB/ β ₃ (14).

Roots of *Panax ginseng* are used in traditional oriental medicine. G-Ro (Fig. 1) in *Panax ginseng* Meyer, is known to inhibit the fibrin formation (15), but the anti-platelet mechanism of G-Ro is not fully understand. Therefore, we investigated G-Ro inhibit-signaling pathways in human platelets using specific inhibitors.

MATERIALS AND METHODS

Materials

G-Ro (molecular weight=957.1) was obtained from Ambo Institute (Daejeon, Korea). Thrombin was obtained from Chrono-Log Corporation (Havertown, PA, USA). Anti-PI3K, anti-phosphor-PI3K p85 (Tyr⁴⁵⁸), anti-Akt, anti-phosphor-Akt (Ser⁴⁷³), anti-rabbit IgG-horseradish peroxidase, and lysis buffer were purchased from Cell Signaling (Beverly, MA, USA). Wortmannin (PI3K inhibitor) and miltefosine (Akt inhibitor) were purchased from Cayman Chemical (Ann Arbor, MI, USA). CytoSelect 48-well cell adhesion assay kits (fibronectin-coated, colorimetric format) were purchased from Cell Biolabs (San Diego, CA, USA). Eptifibatide (α IIB/ β ₃ inhibitor), GR 144053 (α IIB/ β ₃ inhibitor), and anti- β -actin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Polyvinylidene difluoride (PVDF) membrane and enhanced chemiluminescence solution (ECL) were purchased from General Electric Healthcare (Amersham, Buckinghamshire, UK). Fibrinogen Alexa Fluor 488 conjugate was obtained from Invitrogen Molecular Probes (Eugene, OR, USA).

Preparation of washed human platelets

Human platelet-rich plasma (PRP) was obtained from

the Korean Red Cross Blood Center (Changwon, Korea) and centrifuged for 10 min at 1,300 g. The platelets were then washed twice with washing buffer (138 mM NaCl, 2.7 mM KCl, 12 mM NaHCO₃, 0.36 mM NaH₂PO₄, 5.5 mM glucose, and 1 mM ethylenediaminetetraacetic acid disodium salt dihydrate, pH 6.5), and resuspended in suspension buffer (138 mM NaCl, 2.7 mM KCl, 12 mM NaHCO₃, 0.36 mM NaH₂PO₄, 0.49 mM MgCl₂, 5.5 mM glucose, and 0.25% gelatin, pH 6.9). The platelet concentrations were adjusted to a final concentration of 5×10^8 /mL. All aforementioned procedures for platelet activity were performed at 25°C. Experimental approval (PIRB12-072) was obtained from the Public Institutional Review Board at the National Institute for Bioethics Policy (Seoul, Korea).

Determination of platelet aggregation

Platelets (10^8 /mL) were preincubated with or without substances in the 2 mM CaCl₂ for 3 min at 37°C followed by thrombin stimulation (0.05 U/mL). The aggregation was performed for 5 min using an aggregometer (Chrono-Log Corporation). Platelet aggregation rate was determined as an increase in light transmission. PI3K/Akt inhibitors were dissolved in dimethyl sulfoxide to a final concentration of 0.1%.

Western blots for analyzing of PI3K- and Akt-phosphorylation

Platelet aggregation was stopped by adding 1×lysis buffer. The platelet lysates were then measured using a bicinchoninic acid protein assay kit (Pierce Biotechnology, Rockford, IL, USA). Proteins (15 μ g) were analyzed *via* sodium dodecyl sulfate-polyacrylamide gel electrophoresis (6%, 1.5 mm), and PVDF membranes were used for protein transfer. The dilutions for the primary and secondary antibodies were 1:1,000 and 1:10,000, respectively. The membranes were visualized using the ECL solution.

Determination of fibronectin adhesion

The plates were coated with fibronectin or bovine serum albumin (BSA) as a negative control. Washed human platelets (10^8 /mL) were incubated for 60 min at 37°C in the presence of thrombin (0.05 U/mL) with or without various concentrations of G-Ro. After five times washing with phosphate buffered saline (PBS), cell stain solution was added and the plates were incubated at room temperature for 10 min. After washing five times, extraction solution was added and the plates were incubated for 10 min. Each sample was transferred to a 96-well microtiter plate and absorbance measured with a Synergy HT Multi-Model Microplate Reader (BioTek Instruments, Winooski, VT, USA) at 560 nm.

Determination of fibrinogen binding to α IIb/ β ₃

The platelet aggregation was conducted in the presence of Alexa Flour 488-human fibrinogen (30 μ g/mL) for 5 min at 37°C. The reaction was stopped by the addition of 0.5% paraformaldehyde in cold PBS, and the aforementioned procedures were implemented under dark conditions. The assay of fibrinogen binding was carried out using flow cytometry (BD Biosciences, San Jose, CA, USA), and its degree was determined with cellQuest software (BD Biosciences).

Assay of platelet-mediated fibrin clot retraction

Human PRP 250 μ L were preincubated with or without G-Ro (300 μ M) for 10 min at 37°C, and incubated with thrombin (0.05 U/mL) for 20 min at 37°C. Photographs of fibrin clots were taken by a digital camera, and its area (at 20 min) was measured by NIH Image J Software (v1.46, National Institutes of Health, Bethesda, MD, USA). Percentage of clot retraction was calculated as follows:

$$\text{Retraction (\%)} \text{ by thrombin} = \frac{\text{Control area} - \text{Thrombin area}}{\text{Control area}} \times 100$$

Measurement of PT and APTT

To investigate whether G-Ro shows anticoagulant characteristics, we measured prothrombin time (PT) and activated partial thromboplastin time (APTT), markers of blood coagulation. The platelet poor plasma (PPP) (100 μ L) was preincubated in a two-channel coagulator (Behnk Elektronik GmbH & Co., KG, Norderstedt, Germany) with gentle stirring for 1 min at 37°C. PT was determined as the time interval between the addition of PT reagent (100 μ L) to the PPP and the formation of a fibrin clot. After preincubation of PPP for APTT measurement, 100

μ L of APTT reagent was added to the PPP (100 μ L) and incubated for 3 min at 37°C. Following incubation, 100 μ L of 25 mM CaCl₂ was immediately added to the PPP containing APTT reagent. APTT was determined as the time required to form a fibrin clot.

Statistical analyses

The experimental results are reported as the mean \pm standard deviation accompanied by the number of observations. The data were compared via analysis of variance (ANOVA). Significant differences among the group means were compared using the Tukey-Kramer method. Statistical analysis was performed using SPSS 21.0.0.0 (SPSS, Chicago, IL, USA). $P < 0.05$ was considered to be statistically significant.

RESULTS

Effects of G-Ro on thrombin-induced human platelet aggregation

Based on a previous report, 0.05 U/mL of thrombin was used as it maximally aggregates human platelets (16). The thrombin-induced aggregation rate was $95.8 \pm 1.7\%$, however, G-Ro dose-dependently reduced (50, 100, 200, and 300 μ M) the light transmission. PI3K/Akt inhibitors, wortmannin (10 μ M) and miltefosine (10 μ M) also suppressed thrombin-induced light transmission, the aggregation rates were $45.8 \pm 3.6\%$ and $35.5 \pm 1.3\%$, respectively. Moreover, PI3K/Akt inhibitors with G-Ro (300 μ M) showed synergistic inhibitory effects (Fig. 2).

Effects of G-Ro on thrombin-induced PI3K- and Akt-phosphorylation

We could not directly confirm the activity of the regulatory subunit (p50, p55, and p85) and a catalytic subunit

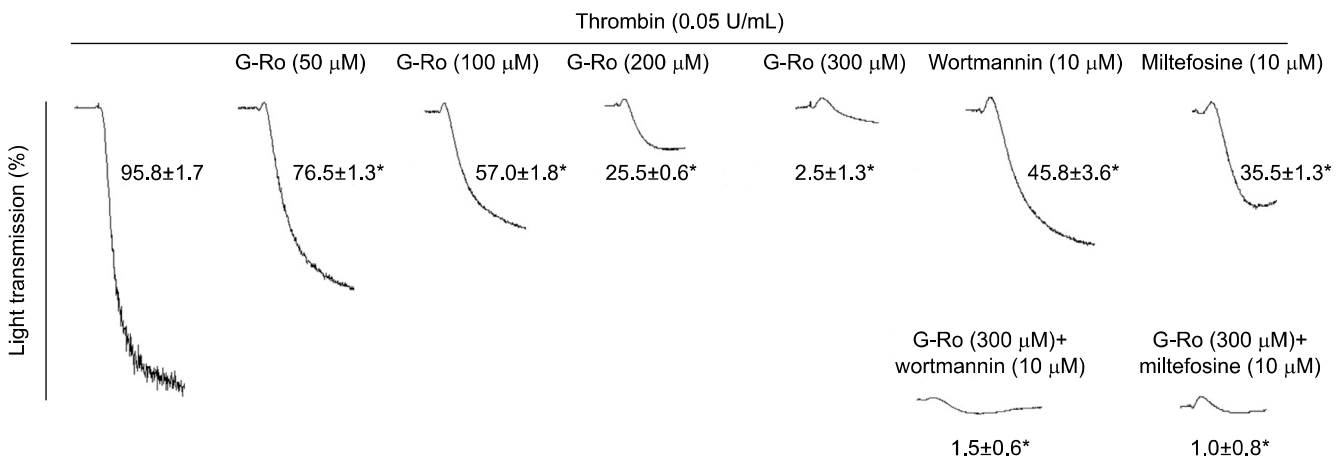


Fig. 2. Effects of ginsenoside Ro (G-Ro) and phosphoinositide 3-kinase (PI3K)/Akt inhibitors on thrombin-induced human platelet aggregation. Measurement of platelet aggregation was carried out as described in "MATERIALS AND METHODS" section. The rate of inhibition was expressed as the percentage of the thrombin-induced aggregation rate. The data are expressed as the mean \pm standard deviation (n=4). * $P < 0.05$ versus the thrombin-stimulated human platelets.

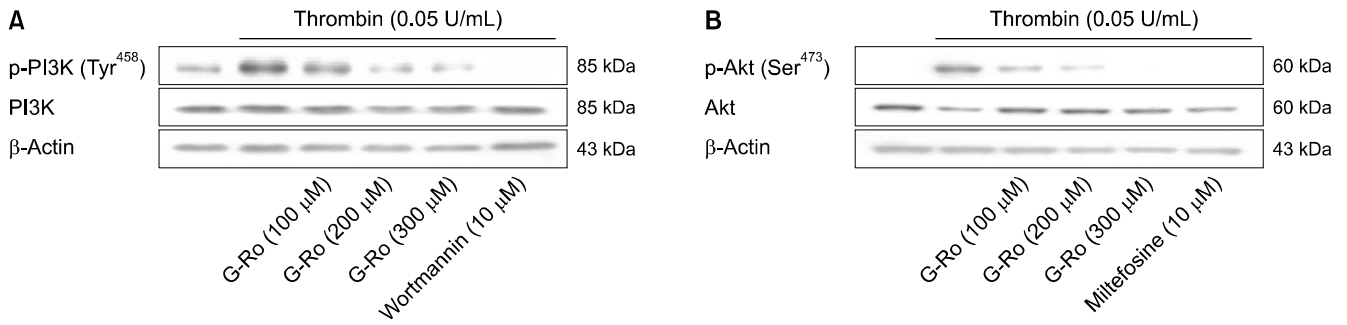


Fig. 3. Effects of ginsenoside Ro (G-Ro) on thrombin-induced phosphoinositide 3-kinase (PI3K)- and Akt-phosphorylation. (A) Effects of G-Ro on thrombin-induced PI3K (Tyr⁴⁵⁸) phosphorylation. (B) Effects of G-Ro on thrombin-induced Akt (Ser⁴⁷³) phosphorylation. Western blotting was performed as described in "MATERIALS AND METHODS" section.

(p110 α , β , and δ) of PI3K. Thus, we investigated that G-Ro affects the PI3K signaling through phosphorylation and dephosphorylation. Because the stimulation of thrombin in human platelets has been shown to increase the phosphorylation of p85 (7,17), the experiment focused on the inhibitory effect of G-Ro on p85 phosphorylation. Thrombin accelerated PI3K-phosphorylation (Fig. 3A) compared to unstimulated platelets. However, G-Ro dose-dependently dephosphorylated (100 to 300 μ M) thrombin-phosphorylated PI3K (Fig. 3A). In addition, thrombin-induced the phosphorylation of Akt (Fig. 3B), a downstream molecule, was dephosphorylated in a dose-dependent manner of G-Ro (100 to 300 μ M) (Fig. 3B). Each positive control, wortmannin (10 μ M) and miltefosine (10 μ M) decreased PI3K and Akt phosphorylation (Fig. 3).

Effects of G-Ro on fibronectin adhesion

Next, we investigated fibronectin adhesion to α IIb/ β ₃, which is an important reaction in outside-in signaling.

Thrombin strongly stimulated the fibronectin adhesion to α IIb/ β ₃ as compared with unstimulated platelets, but not BSA, a negative control against fibronectin (Fig. 4A). G-Ro decreased thrombin-increased the adherence of fibronectin in a dose (50 to 300 μ M)-dependent manner (Fig. 4A). The positive controls of α IIb/ β ₃, eptifibatid, GR 144053, suppressed the adhesion, and those inhibitory degrees were equal to that by G-Ro (300 μ M) (Fig. 4A). Thrombin-induced fibronectin adhesion was also inhibited by wortmannin (10 μ M) and miltefosine (10 μ M) (Fig. 4A), and showed synergistic inhibitory effects with G-Ro (300 μ M) on fibronectin adhesion (Fig. 4B).

Effects of G-Ro on fibrinogen binding to α IIb/ β ₃

Because G-Ro decreased fibronectin adhesion, G-Ro may influence fibrinogen binding to α IIb/ β ₃. Thus, we investigated inhibitory effects of fibrinogen binding to α IIb/ β ₃ by G-Ro (300 μ M), and PI3K/Akt inhibitors. Thrombin elevated fibrinogen binding to α IIb/ β ₃ (Fig. 5A-b and 5B), and its degree was 95.7 ± 0.5 . However, G-Ro (300 μ M)

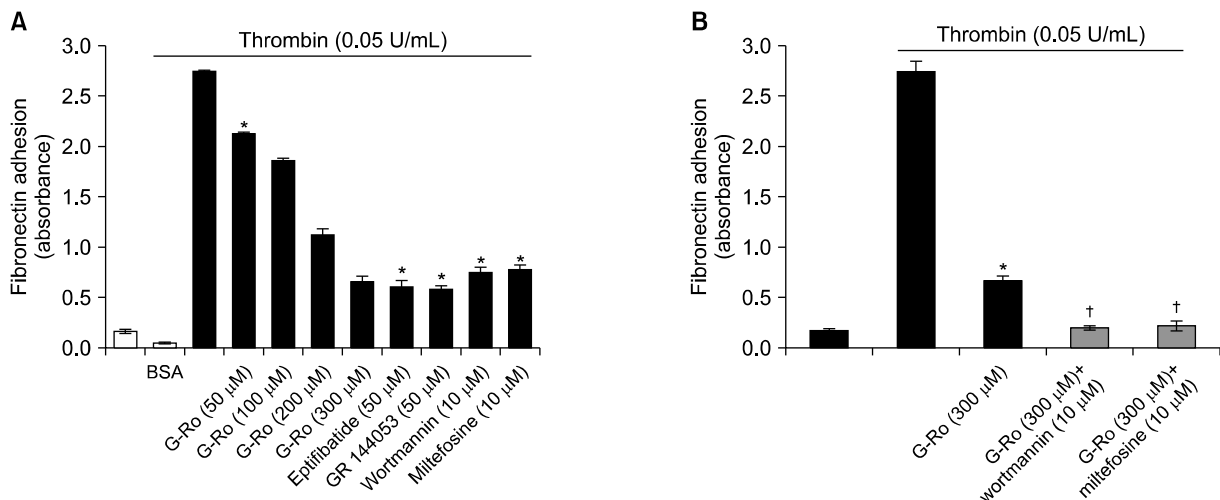


Fig. 4. Effects of ginsenoside Ro (G-Ro) and phosphoinositide 3-kinase (PI3K)/Akt inhibitors on thrombin-induced fibronectin adhesion. (A) Inhibitory effects of G-Ro and PI3K/Akt inhibitors on fibronectin adhesion. (B) Synergistic effects of G-Ro with PI3K/Akt inhibitors on fibronectin adhesion. Measurement of fibronectin adhesion was carried out as described in "MATERIALS AND METHODS" section. The data are expressed as the mean \pm standard deviation (n=4). * $P < 0.05$ versus the thrombin-stimulated human platelets and † $P < 0.05$ versus the thrombin-stimulated human platelets in the presence of G-Ro (300 μ M).

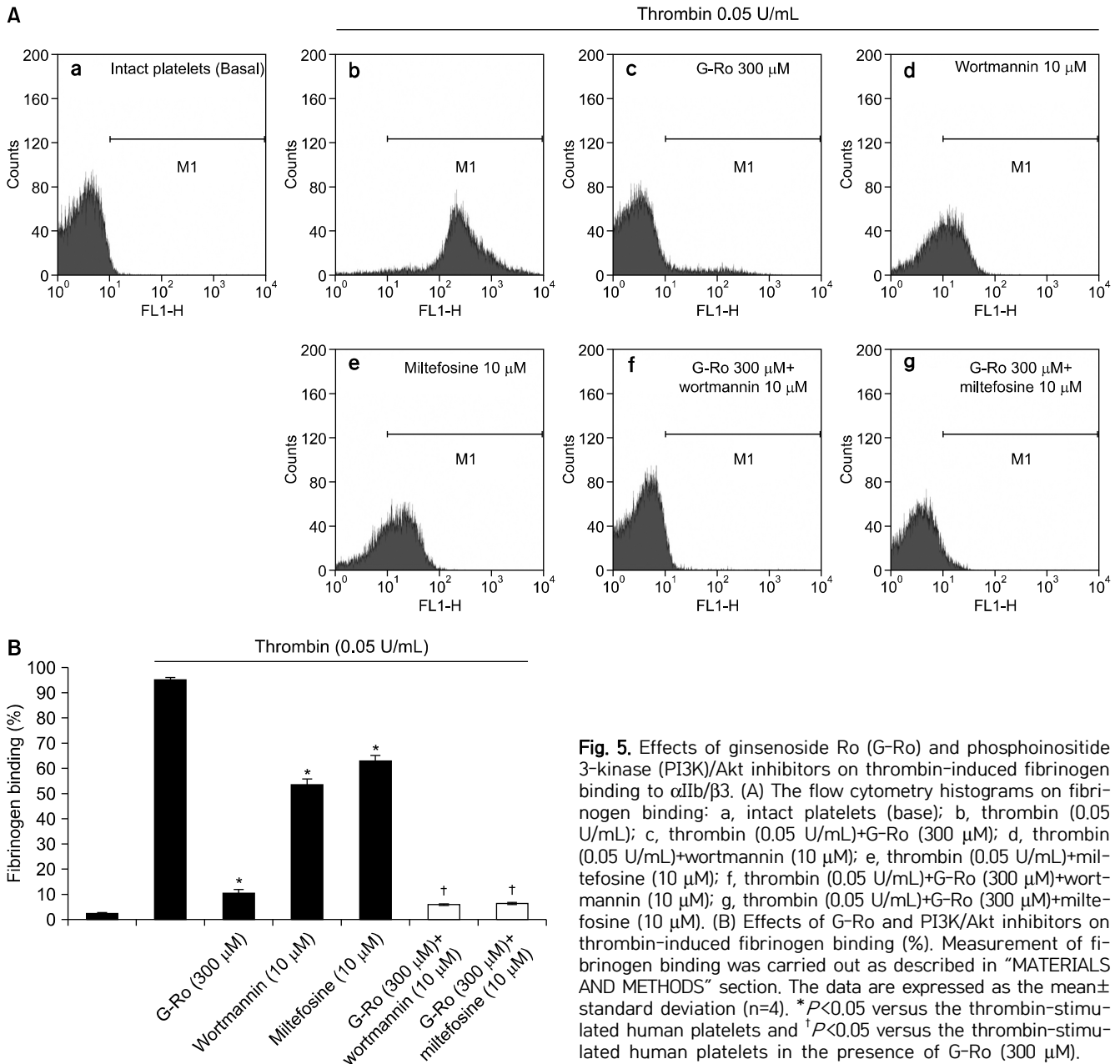


Fig. 5. Effects of ginsenoside Ro (G-Ro) and phosphoinositide 3-kinase (PI3K)/Akt inhibitors on thrombin-induced fibrinogen binding to α IIb/ β 3. (A) The flow cytometry histograms on fibrinogen binding: a, intact platelets (base); b, thrombin (0.05 U/mL); c, thrombin (0.05 U/mL)+G-Ro (300 μ M); d, thrombin (0.05 U/mL)+wortmannin (10 μ M); e, thrombin (0.05 U/mL)+miltefosine (10 μ M); f, thrombin (0.05 U/mL)+G-Ro (300 μ M)+wortmannin (10 μ M); g, thrombin (0.05 U/mL)+G-Ro (300 μ M)+miltefosine (10 μ M). (B) Effects of G-Ro and PI3K/Akt inhibitors on thrombin-induced fibrinogen binding (%). Measurement of fibrinogen binding was carried out as described in "MATERIALS AND METHODS" section. The data are expressed as the mean \pm standard deviation (n=4). * P <0.05 versus the thrombin-stimulated human platelets and $^{\dagger}P$ <0.05 versus the thrombin-stimulated human platelets in the presence of G-Ro (300 μ M).

strongly attenuated the fibrinogen binding to α IIb/ β 3 (Fig. 5A-c), and its inhibitory degree was 88.1%. The fibrinogen binding to α IIb/ β 3 was also inhibited by both inhibitors, wortmannin (10 μ M) and miltefosine (10 μ M) (Fig. 5A-d and -e) and G-Ro (300 μ M) plus wortmannin (10 μ M) or miltefosine (10 μ M) showed strong synergistic effects on fibrinogen binding to α IIb/ β 3 (Fig. 5A-f, -g, and 5B).

Effects of G-Ro on retraction of fibrin clot

The binding of fibronectin and fibrinogen is known to stimulate clot retraction for thrombus formation (18,19). Thus, we investigated whether G-Ro inhibits clot retraction. Thrombin accelerated the clot retraction (Fig. 6A), and its degree (at 20 min) was decreased 95% compared to (55.4 \pm 1.3 mm²) no thrombin, control (Fig. 6B). How-

ever, G-Ro very potently inhibited clot retraction that occurred within thrombin (Fig. 6A and 6B), which was attenuated up to 225% against that (2.7 \pm 0.2 mm²) occurred by thrombin (Fig. 6B). PI3K/Akt inhibitors, wortmannin (10 μ M) and miltefosine (10 μ M) also strongly inhibited clot retraction (Fig. 6A and 6B), which were attenuated up to 270% and 248% compared with thrombin (2.7 \pm 0.2 mm²) (Fig. 6B). In addition, G-Ro (300 μ M) plus wortmannin (10 μ M) or miltefosine (10 μ M) had a synergistic effects on clot retraction, which were attenuated up to 507% and 510%, respectively compared with thrombin plus G-Ro (300 μ M) (8.8 \pm 0.2 mm²) (Fig. 6C).

Effects of G-Ro on blood coagulation

Blood coagulation is connected to platelet aggregation and thrombosis (20,21). Accordingly, we investigated the

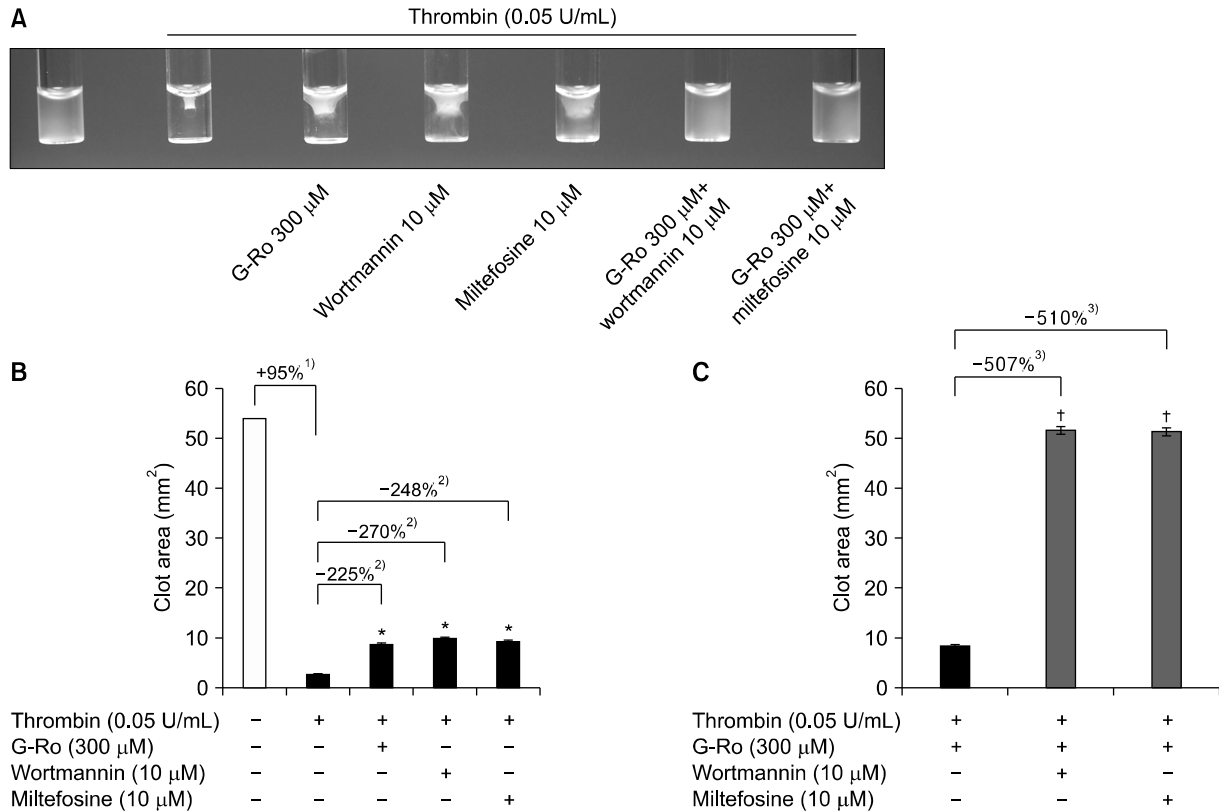


Fig. 6. Effects of ginsenoside Ro (G-Ro) and phosphoinositide 3-kinase (PI3K)/Akt inhibitors on fibrin clot retraction. (A) Photographs of fibrin clot and (B) effects of G-Ro on thrombin-retracted fibrin clot (%). (C) Synergistic effects of G-Ro with PI3K/Akt inhibitors on thrombin-retracted fibrin clot (%). Quantification of fibrin clot retraction was performed as describe in "MATERIALS AND METHODS" section. ¹⁾(base-thrombin)/base \times 100. ²⁾[thrombin-(thrombin+G-Ro or PI3K/Akt inhibitors)]/thrombin \times 100. ³⁾[(thrombin+G-Ro)-(thrombin+G-Ro+inhibitor)]/(thrombin+G-Ro) \times 100. The data are expressed as the mean \pm standard deviation (n=4). * P <0.05 versus the thrombin-stimulated human platelets and [†] P <0.05 versus the thrombin-stimulated human platelets in the presence of G-Ro (300 μ M).

Table 1. Effects of ginsenoside Ro (G-Ro) on blood coagulation (unit: s)

	PT	APTT
PPP	12.8 \pm 0.1	29.9 \pm 0.1
G-Ro 50 μ M	12.7 \pm 0.7	30.1 \pm 0.3
G-Ro 100 μ M	13.1 \pm 0.2	30.7 \pm 0.5
G-Ro 200 μ M	13.1 \pm 0.3	30.0 \pm 0.4
G-Ro 300 μ M	13.2 \pm 0.1 ^{NS}	31.2 \pm 0.5 ^{NS}

The results were expressed as the mean \pm standard deviation (n=4).

PT, prothrombin time; APTT, activated partial thromboplastin time; PPP, platelet poor plasma.

NS, not significant.

effects of G-Ro on blood coagulation time (PT and APTT) as an index of bleeding. As shown in Table 1, both PT and APTT were not significantly prolonged by G-Ro (50 to 300 μ M) compared with those (PT, 12.8 \pm 0.1 s; APTT, 29.9 \pm 0.1 s) by PPP. These results suggest that G-Ro does not function as an anticoagulant.

DISCUSSION

It has been reported that Korean red ginseng has anticoagulation effects by PT and APTT *in vitro* (22). Thus, we focused on the anticoagulation effect by G-Ro, however, G-Ro did not prolong PT and APTT *in vitro* (Table 1), which shows that G-Ro has no anticoagulant characteristics and may not influence fibrin production. The clot retraction is the most crucial step in repair and healing on damaged portions of blood vessels. The clot formation that is produced through coagulation factors and platelet aggregation is composed of fibrin and platelets. The coagulation phase, extrinsic and intrinsic pathways, generates thrombin that converts circulating fibrinogen in plasma into the fibrin. At the same time, the activated platelets are accumulated in the damaged blood vessel and build up a fibrin-platelet meshwork. Additional circulatory platelets and blood cells are trapped in the meshwork, which accelerate the extension of clot formation. The clot formation that seals off the damaged vessel begins to undergo retraction over a period of 30 to 60 min and pulls the cut edges together. Thus, this is clear evidence that the inhibitory effect of G-Ro on clot retraction is due to the downregulation of α IIb/ β 3 activity.

The interaction between α IIb/ β 3 and fibrin is a key role for the clot formation and the α IIb/ β 3 inhibitors strongly suppress the clot retraction (23). The α IIb/ β 3 continuously intensifies the platelet aggregation (24) and the formation of thrombus via outside-in signaling pathway and influence the progression of atherosclerosis (25,26). Activation of α IIb/ β 3 is connected to inflammation, a cause of atherosclerosis, and its associated proteins (platelet-derived growth factor, vascular endothelial growth factor, p-selectin, and interleukin 1 β) are secreted from α -granules (18,27). Even though G-Ro inhibits fibronectin- and fibrinogen-binding to α IIb/ β 3 and fibrin clot retraction, if G-Ro could not influence inflammation, anti-platelet effects by G-Ro would be doubtful. In our previous study, we showed that G-Ro significantly inhibited the expression of p-selectin (28), which means that G-Ro may be involved in the inhibition of inflammation by suppressing thrombin-induced p-selectin expression. Moreover, some experiments showed that G-Ro had anti-inflammatory activity *in vivo* and *in vitro* (29,30). Therefore, our study suggests that G-Ro may influence thrombosis and atherosclerosis.

With regard to the synergistic effect of G-Ro with PI3K/Akt inhibitors on thrombin-induced human platelet function, as shown in Fig. 2, platelet aggregation was strongly inhibited by G-Ro (300 μ M) plus wortmannin (10 μ M) or miletefosine (10 μ M). As shown in Fig. 4B, the inhibitory effects of fibronectin adhesion by G-Ro (300 μ M) plus wortmannin (10 μ M) or miletefosine (10 μ M) was higher compared with G-Ro (300 μ M) alone. In addition, the fibrinogen binding inhibition by G-Ro (300 μ M) plus wortmannin (10 μ M) or miletefosine (10 μ M) was higher compared with G-Ro (300 μ M) alone (Fig. 5B).

Furthermore, thrombin-induced clot retraction is inhibited by G-Ro (300 μ M), wortmannin (10 μ M), and miletefosine (10 μ M) (Fig. 6B). Therefore, it is thought that G-Ro (300 μ M), wortmannin (10 μ M), and miletefosine (10 μ M) showed similar inhibitory effect in thrombin-induced human platelets and two similar substances interacted with each other and showed a strong synergistic effect (Fig. 6C). These results suggest that G-Ro inhibits platelet function by inhibiting PI3K/Akt signaling from the thrombin-mediated G protein-coupled receptor.

Our results demonstrate that G-Ro inhibits fibronectin- and fibrinogen-binding to α IIb/ β 3, via dephosphorylation of PI3K/Akt. The downregulation of α IIb/ β 3 activity by G-Ro suppressed the thrombin-induced clot retraction. Therefore, our study elucidated the α IIb/ β 3 inhibitory mechanism by G-Ro. Thus, G-Ro may be used as a therapeutic agent for prevention of thrombosis and other platelet-mediated cardiovascular diseases.

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AUTHOR DISCLOSURE STATEMENT

The author declares no conflict of interest.

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