



Review Article

Anti-platelet role of Korean ginseng and ginsenosides in cardiovascular diseases

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ABSTRACT

Cardiovascular diseases prevail among modern societies and underdeveloped countries, and a high mortality rate has also been reported by the World Health Organization affecting millions of people worldwide. Hyperactive platelets are the major culprits in thrombotic disorders. A group of drugs is available to deal with such platelet-related disorders; however, sometimes, side effects and complications caused by these drugs outweigh their benefits. Ginseng and its nutraceuticals have been reported to reduce the impact of thrombotic conditions and improve cardiovascular health by antiplatelet mechanisms. This review provides (1) a comprehensive insight into the available pharmacological options from ginseng and ginsenosides (saponin and nonsaponin fractions) for platelet-originated cardiovascular disorders; (2) a discussion on the impact of specific functional groups on the modulation of platelet functions and how structural modifications among ginsenosides affect platelet activation, which may further provide a basis for drug design, optimization, and the development of ginsenoside scaffolds as pharmacological antiplatelet agents; (3) an insight into the synergistic effects of ginsenosides on platelet functions; and (4) a perspective on future research and the development of ginseng and ginsenosides as super nutraceuticals.

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1. Introduction

Cardiovascular diseases (CVDs) are considered among the leading causes of death in developed Western countries; however, they are prevailing in underdeveloped countries as well. Coronary heart disease alone caused almost one in every seven deaths, and heart failure caused one in nine deaths in the United States in 2013 [1–3]. The World Health Organization has also stated that CVD accounted for 30% of all the deaths that occurred because of CVD in 2005. In Europe, CVD remains the primary cause of death accounting for 42% of mortalities in men and 52% of deaths in women [4,5]. Although platelets play a key role in maintaining hemostasis

and preventing blood loss by promoting hemostatic plug formation following vascular injury, the underlying cause of CVD ailments is the hyperactivation of platelets due to various pathophysiological factors that may cause thrombotic complications, subsequently contributing to the development of atherosclerosis, thrombosis, coronary heart disease, stroke, or heart attack [6,7]. Platelets are also known to contribute to cerebral amyloid angiopathy as they express amyloid precursor protein (APP), and they are the primary source of amyloid beta ($A\beta$) deposition in Alzheimer disease, which affects almost 26 million people worldwide [8,9].

Generally, platelet activation is triggered by several intracellular signaling cascades stimulated by different adhesive proteins and

Abbreviations: AA, arachidonic acid; ADP, adenosine diphosphate; AC, adenyl cyclase; cAMP, cyclic adenosine monophosphate; ASA, acetylsalicylic acid; ATP, adenosine triphosphate; Akt, protein kinase B; $[Ca^{2+}]_i$, intracellular calcium ion; COX, cyclooxygenase; CRP, collagen-related peptide; CSF, crude saponin fraction; ERK, extracellular signal-regulated kinase; GPVI, glycoprotein VI; IC_{50} , half maximal (50%) inhibitory concentration; IP₃, inositol-1,4,5-triphosphate; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; MKK4, mitogen-activated protein kinase kinase 4; MLC, myosin light chain; PAF, platelet-activating factor; PAR, proteinase-activated receptor; PI3K, phosphatidylinositol 3-kinase; PKA, protein kinase A; PKC, protein kinase C; PKG, protein kinase G; cPLA_{2 α} , cytosolic phospholipase A_{2 α} ; PLA₂, phospholipase A₂; PLC γ 2, phospholipase C gamma-2; aPTT, activated partial thromboplastin time; PT, prothrombin time; PPD, protopanaxadiol; PPT, protopanaxatriol; ROCK, Rho-associated protein kinase; SFK, Src family kinase; Syk, spleen tyrosine kinase; TS, total saponin; TxA₂, thromboxane A₂; TxB₂, thromboxane B₂; TxAS, thromboxane-A synthase; TxR, thromboxane receptor; VASP, vasodilator-stimulated phosphoprotein; vWF, von Willebrand factor.

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soluble agonists. These signaling events can be subdivided into the following: (1) early receptor signaling induced by either adhesive proteins or agonists; (2) merging the signal transduction with other common pathways and amplification; (3) integrin $\alpha_{IIb}\beta_3$ -mediated inside-out signaling, which increases its affinity for fibrinogen binding and platelet adhesion; and (4) integrin $\alpha_{IIb}\beta_3$ -mediated outside-in signaling, which further enhances the later phases of platelet adhesion and activation and subsequently triggers clot retraction [7,10–12]. Collagen and thrombin are considered strong agonists that induce platelet aggregation by triggering granule secretion through Glycoprotein VI (GPVI) and proteinase-activated receptor signaling, respectively, whereas ADP is considered as a weak agonist but strongly induces platelet activation that mediates the P_2Y_{12} or P_2Y_1 receptor–signaling pathway. Collagen-related peptide (CRP) and convulxin are agonists that specifically stimulate GPVI and mediate downstream signaling events to induce platelet aggregation. The platelets secrete dense (δ) granules, which include Ca^{2+} , ADP, ATP, and serotonin, and α -granules loaded with adhesive proteins such as von Willebrand factor, fibrinogen, fibronectin, and p-selectin. There are different signaling cascades that contribute to granule secretion after being triggered by different agonists: (i) Src family kinase (SFK) signaling, which is generally considered as early signaling in platelet activation; (ii) calcium mobilization; (iii) cyclic nucleotide signaling [cAMP/cGMP, which stems protein kinase A (PKA)/protein kinase G (PKG)]; integrin $\alpha_{IIb}\beta_3$ -mediated inside-out and outside-in signaling; (iv) protein kinase C signaling; (v) mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) signaling; and (vi) RhoA signaling. All of these pathways promote granule secretion, which further enhances platelet shape change and aggregation, but the

mechanism remains unclear [10,13]. Platelets are constantly exposed to these activating factors while a strong symmetry between these factors is crucial for hemostasis; otherwise, any imbalance will produce fatal results. Fig. 1 summarizes the mechanistic aspects and signaling pathways of platelet activation.

One of the effective and proven ways to reduce thrombotic events is the pharmacological suppression of platelets, and a number of clinically approved drugs are available to treat and prevent CVD. However, the available options are often impeded by side effects and complications. In particular, the side effects of aspirin are gastric ulcers and bleeding, and clopidogrel sometimes results in aplastic anemia and thrombocytopenic purpura [14,15], thus necessitating the development of safer and efficacious agents to treat and prevent CVD with no or minimum drug-associated complications. Ethnomedicinal applications have gained interest to hamper CVD and its complications [16], and studies on medicinal plants and a traditional Mediterranean diet have also shown their positive effects on the treatment and prevention of CVD [17–19].

Panax ginseng Meyer (also called as Korean ginseng) is one of the most widely used medicinal herbs with several pharmacological compounds and a range of therapeutic applications. It contains nonsaponin and saponin (ginsenosides) fractions, among which ginsenosides are studied extensively for their role in platelet functions and cardiovascular health [20–24].

Based on the classification, saponin fractions are divided into four categories: (1) protopanaxadiol (PPD) type, which includes ginsenoside (G)-Ra1-3, G-Rb1-3, G-Rc, G-Rd, G-Rg3, G-Rg5, G-Rh2-3, and G-Rs1-3; (2) protopanaxatriol (PPT) type, which includes G-Re, G-Rf, and G-Rg1-2, G-Rg6, G-Rh1, and G-Rh4; (3) oleanane type, which includes G-Ro; and (4) ocotillol type, which includes

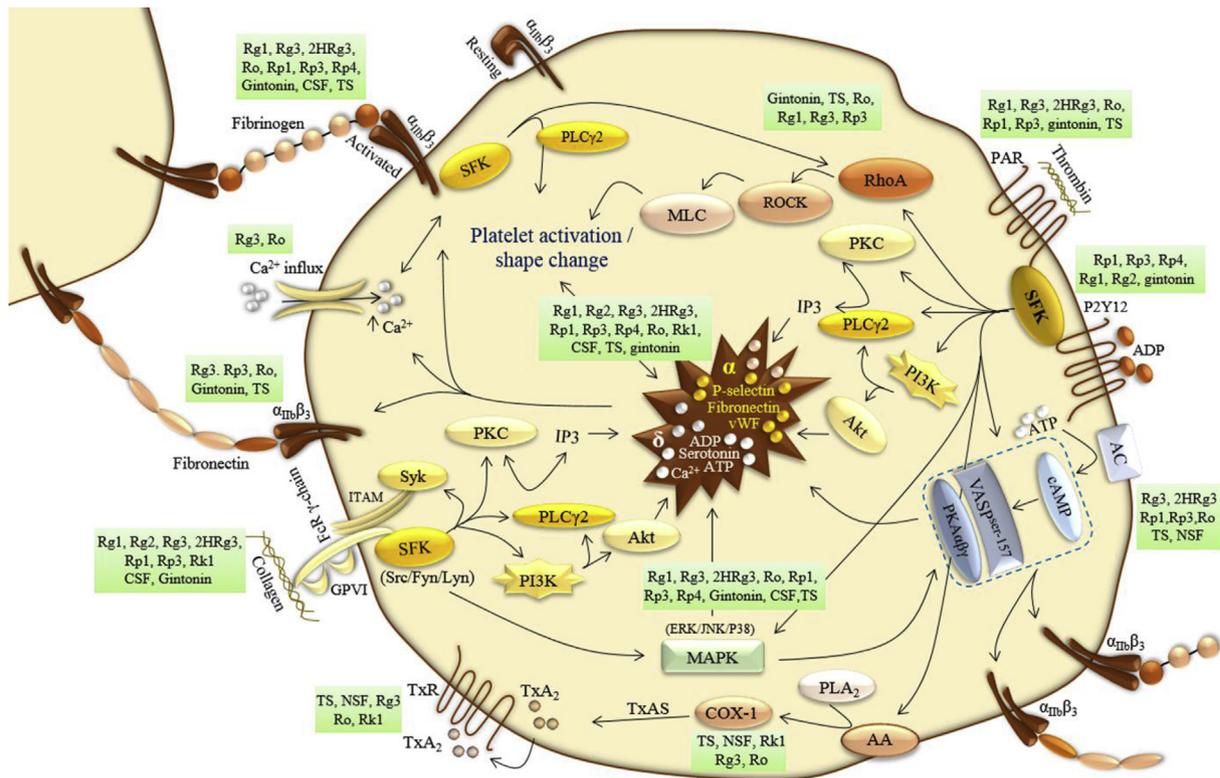


Fig. 1. Mechanism of platelet activation showing intracellular signaling pathways and effects of different ginsenosides on the modulation of platelet functions that affect various receptors or downstream signaling events. AA, arachidonic acid; AC, adenylyl cyclase; Akt, protein kinase B; COX, cyclooxygenase; CSF, crude saponin fraction; IP3, inositol-1,4,5-triphosphate; MAPK, mitogen-activated protein kinase; MLC, myosin light chain; PAR, proteinase-activated receptor; PKA, protein kinase A; PKC, protein kinase C; PLA₂, phospholipase A₂; PLCγ₂, phospholipase C gamma-2; ROCK, Rho-associated protein kinase; SFK, Src family kinase; TS, total saponin; TxAS, thromboxane synthase; TxA₂, thromboxane A₂; VASP, vasodilator-stimulated phosphoprotein; vWF, von Willebrand factor.

Makonoside-Rs [25,26]. Many of them have been extensively studied for their pharmacological and therapeutic properties [21–23,25] while nonsaponin fractions (NSFs), which include gintonin and some other lipophilic fractions, have also been studied [27–30].

Previously, we have shown that NSFs and several ginsenosides from ginseng modulate platelet functions and inhibit thrombus formation *via* broad-spectrum antiplatelet properties. This review provides a comprehensive insight into the available ginseng-related options to treat and prevent CVD. It also explains how the structural modification of ginsenosides leads to the development of new drug compounds (ginsenoside derivatives) and affects antiplatelet activity with improved efficacy. Finally, there is a discussion about how structural modification may further advance the drug design, optimization, research, and development of ginsenoside scaffolds as strong antiplatelet agents with fewer drug-associated complications.

2. Nonsaponin and crude/total saponin fractions from ginseng modulate platelet functions

2.1. Nonsaponin fractions

Our group prepared gintonin (a NSF) from a subset of glycolipoprotein components from ginseng, which has been known for its pharmacological properties [28,29,31,32]. We investigated the antiplatelet effects of gintonin in mice, rats, and human platelets and found its potential inhibitory effects on platelet functions and thrombus formation [27]. Gintonin (from 12.5 to 50 $\mu\text{g}/\text{mL}$) was able to significantly inhibit platelet aggregation against several potential agonists, that is, collagen, convulxin, ADP, and thrombin. We explored the mechanistic aspects of its inhibitory pathway and found that it mainly targeted the GPVI downstream signaling pathway affecting SFK (Fyn, Lyn) and Syk activation, MAPK [extracellular signal-regulated kinase (ERK)1/2 and c-Jun N-terminal kinase (JNK)], mitogen-activated protein kinase kinase 4 (MKK4), PI3K/Akt, and PI3K-dependent phospholipase C gamma-2 (PLC γ 2) expression. All of these factors are critical for functional responses to collagen-induced granule secretion and aggregation, that is, calcium mobilization and ATP release, which transduce signals to integrin $\alpha_{\text{IIb}}\beta_3$ and GPIIb/IIIa mediating inside-out and outside-in signaling and clot retraction, thereby increasing platelet activation [10,11,33–35]. A mixture of collagen and epinephrine has been recognized to evaluate antithrombotic drugs [36]. Gintonin inhibited platelet aggregation and thrombus formation in mice by attenuating these collagen-induced GPVI signaling events. Another NSF (lipophilic fraction) has been reported to inhibit platelet aggregation by regulating cGMP, calcium mobilization, and thromboxane A₂ (TxA₂) in thrombin-induced human platelets [30] (Fig. 1).

2.2. Crude/total saponin fractions

We have previously shown the inhibitory effects of different ginseng fractions (NSF, aqueous fraction, acidic polysaccharides, PPD, PPT, and 2H-Rg3 on platelet functions) [30,37–42], so our group explored the antiplatelet properties of total saponins (TSs). The TS fraction contained G-Rg1, G-Re, G-Rb1, G-Rc, G-Rg2, G-Rb2, G-Rd, and G-Rg3 as evidenced by HPLC. Among these ginsenosides, Rg1, Rg2, and Rg3 were known to inhibit platelet aggregation [43]. TS proved effective in inhibiting thrombin-induced platelet aggregation *via* the inhibition of cyclooxygenase (COX)-1, thromboxane synthase, and TxA₂ [44], calcium mobilization, granule secretion, cAMP elevation, and vasodilator-stimulated phosphoprotein (VASP)^{ser-157} phosphorylation [43]. The antiplatelet effects of the

crude saponin fraction were examined on rat platelets, and it was found that the crude saponin fraction (from 50 to 400 $\mu\text{g}/\text{mL}$) potently inhibited collagen-induced platelet aggregation *via* the inhibition of calcium mobilization, ATP secretion, and fibrinogen binding to integrin $\alpha_{\text{IIb}}\beta_3$. It also reduced the phosphorylation of MAPK (ERK1/2, JNK, and p38^{MAPK}), MEK1/2, (an upstream molecule of MAPK), and the PI3K/Akt pathway [45] (Fig. 1).

Our group then examined further mechanistic aspects of the antiplatelet properties of TS and proved its effects on calcium mobilization through the cAMP-dependent protein kinase catalytic subunit and inositol 1,4,5-triphosphate receptor type I and adhesive proteins to GPIIb/IIIa *via* VASP^{ser-157} and the PI3K/Akt pathway in human platelets. It was found that TS (from 25 to 150 $\mu\text{g}/\text{mL}$) has a strong ability to antagonize calcium mobilization through the cAMP-dependent pathway evidenced by using a PKA inhibitor (Rp-8-Br-cAMPS) and PKG inhibitor (Rp-8-Br-cGMPS), while it also reduced granule secretion (ATP and serotonin release), PI3K/Akt phosphorylation, fibrinogen binding and fibronectin adhesion, clot retraction, and enhanced PKAc phosphorylation [46,47] (Fig. 1).

Another study revealed that the panaxatriol saponin (PTS) fraction (including G-Rg1, G-R1, and G-Re) from *Panax notoginseng* is effective in the inhibition of rat and human platelet aggregation [48]. PTS inhibited platelet aggregation, intracellular calcium mobilization, and ERK2 and p38^{MAPK} phosphorylation in a dose-dependent manner against potential agonists collagen, thrombin, and ADP. The antiplatelet effects of PTS were comparable in concentration with those of the ginsenoside contents against collagen, ADP, and thrombin; however, a higher dosage of PTS (from 1 to 3 mg/mL) than that of the ginsenosides (from 50 to 100 μM) was necessary to elicit the same results, indicating that PTS has weaker antiplatelet effects compared with the single ginsenosides.

Rg3-enriched red ginseng extract (Rg3-RGE) was prepared, which contained Rb1, Rc, Rd, Rf, Rg2, 20(S)-Rg3, 20(R)-Rg3, and Rh1 (67.41 mg/g total); 20(S)-Rg3 constituted 44.91 mg/g of the total. Rg3-RGE (from 50 to 200 $\mu\text{g}/\text{mL}$) was examined in collagen (1.25 $\mu\text{g}/\text{mL}$)-induced rat platelets [49]. It was found to inhibit platelet aggregation in a significant and dose-dependent manner. It exerted broad-spectrum antiplatelet effects by inhibiting fibrinogen binding to integrin $\alpha_{\text{IIb}}\beta_3$, [Ca²⁺]_i mobilization, granule secretion (both dense and alpha granules) such as ATP, and serotonin and P-selectin in a significant dose-dependent manner. Rg3-RGE effectively reduced phosphorylation of MAPK (ERK1/2, JNK, and p38MAPK), MKK4, and the PI3K/Akt pathway (Fig. 1). The antithrombotic activity of Rg3-RGE (at 50 mg/kg) was further confirmed in mice using a collagen- and epinephrine-induced acute pulmonary thromboembolism model in which the mice significantly survived thrombosis compared with the control.

3. Antiplatelet effects of the Rg, Rh, Rs, and Rk ginsenoside series

Ginsenosides are major active pharmacological components of ginseng that have been reported to have numerous preventive and therapeutic properties against CVDs. More than 40 ginsenosides have been identified, which primarily include Rb1-2, Rc, Rd, Re, Rf, Rg1-3, Rh1-2, Rp1-4, and Ro. These ginsenosides are divided into the PPD, PPT, and oleanane types according to their structural orientations [22,23].

3.1. G-Rg1 and G-Rg2

A previous study has reported weak antiplatelet effects of Rg1 and Rg2 at a concentration of 1 mg/mL against collagen (10 $\mu\text{g}/\text{mL}$), ADP (20 μM), arachidonic acid (AA; 100 μM), and platelet-activating factor (2 ng/mL), induced rabbit and human platelets, observed by

the inhibition of ATP release. Rg2 also reduced calcium mobilization [50]. Later, Zhou et al [51] examined the antiplatelet effects of Rg1 (at 4 mg/mL) on human platelets against collagen (1 and 2 µg/mL), ADP (10 and 20 µM), U46619 (0.3 and 0.6 µM), and thrombin (0.05 and 0.1 U/mL) and found a significant inhibition of platelet aggregation at both concentrations of agonists, although inhibition was more evident at lower dosages of the agonists. Rg1 was also tested in combination with apyrase and indomethacin to exclude the possibility of observed effects due to ADP or TxA₂ secretion. It was found that the combination greatly inhibited thrombin (0.1 U/mL)-induced platelets, which showed ADP- and TxA₂-independent effects of Rg1. In combination with salvianolic acid A (a known antiplatelet compound), Rg1 showed synergistic inhibition of platelet aggregation. Inhibitory effects of Rg1 on fibrinogen binding, collagen and fibrinogen adhesion, clot retraction, and ERK, Akt, and protein kinase C phosphorylation (Fig. 1) were also reported. An *in vivo* arterial thrombosis model showed potent inhibitory effects of Rg1 (10 mg/kg, PO) on arterial occlusion time, which was almost 46% higher than that of the control.

3.2. G-Rg3

G-Rg3 stands among the frequently studied ginsenosides and has been known to have an abundance of therapeutic effects against several diseases, especially CVDs [23]. Studies have proved several cardioprotective and antiplatelet effects of G-Rg3 through various pharmacological pathways. Previously, we have shown that Rg3 dose dependently inhibited thrombin- and collagen-induced (0.1 U/mL and 2.5 µg/mL, respectively) rat platelet aggregation with a half maximal (50%) inhibitory concentration (IC₅₀) of 40.2 ± 0.9 µM and 35.2 ± 1.2 µM, respectively (Fig. 1) [42].

Recently, Kwon [52,53] explored other mechanistic aspects of Rg3 antiplatelet effects on human platelets. Rg3 (from 50 to 300 µM) effectively inhibited thrombin-induced platelet aggregation, [Ca²⁺]_i, and thapsigargin-induced Ca²⁺ influx in a significant dose-dependent manner. The study specifically proved that Rg3 inhibited [Ca²⁺]_i and Ca²⁺ influx via the phosphorylation of inositol 1,4,5-triphosphate receptor I and ERK, in a cAMP-dependent manner evidenced by the fact that the A-kinase inhibitor (Rp-8-Br-cAMPS) dose dependently increased [Ca²⁺]_i while no significant difference was observed when the platelets were incubated with the G-kinase inhibitor (Rp-8-Br-cGMPS). The downregulation of [Ca²⁺]_i levels also suppressed granule secretion, especially that of P-selectin (a marker for α-granule secretion) surface expression. Rg3 also elevated cAMP levels and VASP^{ser-157} phosphorylation, and correspondingly, Rg3 reserved its binding affinity of fibrinogen and fibronectin adhesion to integrin α_{IIb}β₃ in a significant dose-dependent manner along with the inhibition of clot retraction (Fig. 1).

3.3. G-Rg5 and G-Rg6

The antiplatelet activity of Rg5 was tested against collagen (4 µg/mL), ADP (4 µM), U46619 (4 µM) with a threshold of collagen (1 µg/mL), and AA (50 µM) with a threshold of collagen (1 µg/mL), and it was found effective against collagen-, AA-, and U46619-induced rat platelet aggregation with an IC₅₀ of 409 µM, 8 µM, and 102 µM, respectively [54]. Similarly, Rg6 was found to be effective against AA- and U46619-induced rat platelet aggregation with an IC₅₀ of 76 µM and 286 µM, respectively [55]. However, their mechanism of exerting antiplatelet effects needs to be explored.

3.4. G-Rh1, G-Rh2, and G-Rh4

G-Rh1 and G-Rh2 were studied for their antiplatelet effects in collagen- and ADP-stimulated rat platelets. Rh1 (at a concentration of 1 mM) mildly inhibited platelet aggregation and also increased the thrombin-induced clotting time of fibrinogen, indicating its weak potential as antiplatelet agents [56]. Later, Shin et al [46] also examined the effects of Rh1 and Rh2 (at 300 µM) on thrombin-induced human platelet aggregation and did not observe any inhibitory effect at the tested given concentration. Rh4 was found to be effective against AA- and U46619-induced rat platelet aggregation with an IC₅₀ of 200 µM and 119 µM, respectively, compared with acetylsalicylic acid (ASA) that showed an IC₅₀ of 63 µM and 468 µM, respectively [55].

3.5. G-Rs3, G-Rs4 and G-Rs5

Lee et al [55] stated that G-Rs3–5 showed mild antiplatelet effects against collagen (4 µg/mL), U46619 (4 µM) with a threshold of collagen (1 µg/mL), and AA (50 µM) with a threshold of collagen (1 µg/mL). The results indicated that these ginsenosides are weak antiplatelet agents.

3.6. G-Rk1 and G-Rk3

Lee et al [54,55] reported that Rk1 exhibited 8- to 22-fold stronger antiplatelet effects than ASA against collagen (4 µg/mL), ADP (4 µM), U46619 (4 µM) with a threshold of collagen (1 µg/mL), and AA (50 µM) with a threshold of collagen (1 µg/mL) with an IC₅₀ of 197, 555, 78, and 3.0 µM, respectively. Rk3 also exhibited stronger antiplatelet effects than ASA against collagen (4 µg/mL), U46619 (4 µM) with a threshold of collagen (1 µg/mL), and AA (50 µM) with a threshold of collagen (1 µg/mL) with an IC₅₀ of 192, 187, and 128 µM, respectively.

Another study subsequently revealed the antiplatelet effects of Rk1 and explored its mechanistic aspects in detail [57]. Rk1 (from 10 to 50 µM) was able to inhibit collagen (1 µg/mL) + AA (3 µM)-induced rat platelet aggregation in a dose-dependent manner. The study revealed that Rk1 reduced thromboxane B₂ (TxB₂) generation *via* the inhibition of COX (Fig. 1), and it also decreased 12-hydroxy-5,8,10,14-eicosatetraenoic acid, which is one of the metabolites of AA. Intracellular calcium concentrations decreased with Rk1 treatment. It was reported that a decreased 12-hydroxy-5,8,10,14-eicosatetraenoic acid level induced by ginsenoside Rk1 to study antiplatelet aggregation was related to the translocation of 12-LOX from decreased Ca²⁺ levels because the translocation of 12-LOX from the cytosol to the membrane is related to intracellular calcium levels. The study showed that metabolomics could be a potential tool to study antiplatelet drugs, and Rk1 proved to be a potent candidate for platelet-related cardiovascular disorders.

4. Oleanane-type G-Ro modulates platelet functions in human platelets

It was reported earlier that G-Ro (1 mg/mL) possesses antiplatelet effects *via* the inhibition of ATP release against collagen (10 µg/mL)-, ADP (20 µM)-, AA (100 µM)-, and platelet-activating factor (2 ng/mL)-induced rabbit platelets [50]. Kwon et al [58] analyzed the inhibitory effects of Ro (from 50 to 300 µM) on thrombin (0.05 U/mL)-stimulated human platelets and found a dose-dependent inhibition of platelet aggregation with an IC₅₀ of 155 µM. Ro remarkably inhibited Ca²⁺ concentrations in a cAMP-dependent manner, evidenced by the use of Rp-8-Br-cAMPS (A-kinase inhibitor) and the enhanced phosphorylation of inositol 1,4,5-

triphosphate receptor I (Ser1756) that inhibits $[Ca^{2+}]_i$ mobilization, which consequently inhibited platelet aggregation. Broad-spectrum antiplatelet effects of Ro were observed; it elevated cAMP levels and inhibited $[Ca^{2+}]_i$, ATP, and serotonin release and P-selectin expression (Fig. 1). Interestingly, most of the pathways revolved around cAMP as evidenced by the use of Rp-8-Br-cAMPS (A-kinase inhibitor).

Shin et al [59] reported antiplatelet and antiadhesive properties of Ro and showed that it dose dependently inhibited thrombin-induced human platelet aggregation *via* the downregulation of glycoprotein IIb/IIIa ($\alpha_{IIb}\beta_3$) and the upregulation of cAMP-dependent VASP^{ser-157}, and it strongly abolished clot retraction (Fig. 1). Kwon [60] subsequently reported that Ro inhibited clot retraction and the binding of adhesive ligands fibronectin and fibrinogen to integrin $\alpha_{IIb}\beta_3$ *via* the downregulation of the PI3K/Akt pathway.

Recently, Shin et al [61] examined the antiplatelet effects of Ro on thrombin-stimulated human platelets and found that Ro (from 50 to 300 μ M) significantly inhibited platelet aggregation. TxA₂ generation lead to vasoconstriction and thrombogenesis, and Ro strongly inhibited TxA₂ generation *via* inhibiting AA release in a dose-dependent manner. TxA₂ is produced by AA, which is produced by COX-1 and thromboxane-A synthase (TxAS), and initiates thrombus formation [62,63]. It is known that phosphorylated MAPK (ERK2 and p38^{MAPK}) induces TxA₂ production, and the activation of cytosolic phospholipase A2 α (cPLA_{2 α}) ultimately leads to AA release [64–67]. Ro treatment has reduced all of the aforementioned parameters involved in TxA₂ production in a dose-

dependent manner (Fig. 1). It was proved that AA and TXB₂ inhibition was attributed to Ca^{2+} -dependent cPLA_{2 α} phosphorylation by p38^{MAPK} evidenced by the use of SB203580 (p38^{MAPK} inhibitor). Therefore, G-Ro could be considered as a potent preventive therapeutic candidate against thrombotic CVD.

5. Antiplatelet effects of structurally modified/derived ginsenosides

Ginsenosides are relatively unstable and show low bioavailability [68]. Therefore, to overcome these limitations, we aimed to synthesize novel ginsenoside compounds with improved chemical stability, bioavailability, and mass production rate.

5.1. Dihydroxy-G-Rg3

Rg3 is unstable under acidic and high-temperature conditions. To overcome this instability, 2H-Rg3 was chemically derived from Rg3 by means of reduction with hydrogenation and verified to be a relatively stable, potent, and two times stronger antiplatelet agent than its parent compound with an IC₅₀ of about 18.8 ± 0.4 μ M against thrombin and 20.0 ± 0.9 μ M against collagen. 2H-Rg3 exerted antiplatelet effects through the elevation of cAMP and the inhibition of the MAPK (ERK2 and p38^{MAPK}) pathway (Figs. 1 and 2) [42].

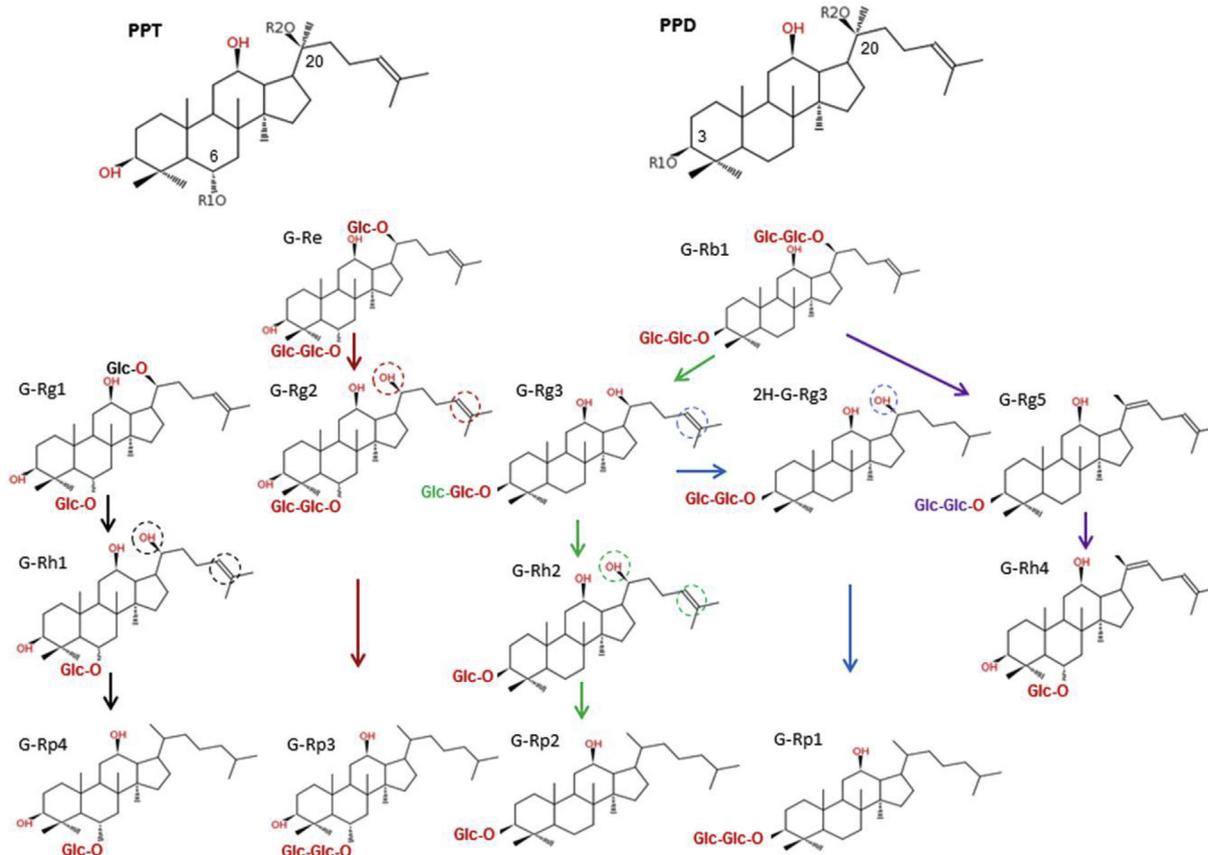


Fig. 2. Chemical structures and schematic conversion of the ginsenosides with respect to the structural modification among specific functional groups. Rb1 → Rg5 → Rh4, Rg1 → Rh1 → Rp4, Re → Rg2 → Rp3, Rb1 → Rg3 → Rh2 → Rp2, and Rg3 → 2H-Rg3 → Rp1. PPD, protopanaxadiol; PPT, protopanaxatriol.

5.2. G-Rp1

Chemically stable Rp1 was prepared from other ginsenosides (Rg3, 2h-Rg3, Rg5, and Rk1) by means of reduction with hydrogenation (Fig. 2). It has been shown to have 10 times more potent anticancer effects than its parent compounds Rg3 and Rg5 [69,70]. We examined the antiplatelet effects of Rp1 in rat and mouse models, and many experiments were conducted to explore its underlying mechanisms [71]. Rp1 (from 2.5 to 20 μ M; *in vitro* and 50 and 100 mg/kg; *ex vivo*) was able to inhibit platelet aggregation against collagen (2.5 μ g/mL), ADP (10 μ M), and thrombin (0.1 U/mL) in a significant and dose-dependent manner with an IC_{50} of 10.1 ± 0.1 μ M, 6.1 ± 0.3 μ M, and 6.8 ± 0.9 μ M, respectively. Intracellular calcium concentrations play a crucial role in platelet activation and thrombus formation [72], granule (α - and δ -granules) secretion is considered as a marker of platelet activation and aggregation [15,73], and TxA₂ amplifies signal transduction and ensures thrombus formation [74]. After stimulation with an agonist, the binding affinity of integrin $\alpha_{IIb}\beta_3$ to fibrinogen increases, which causes the adhesion of platelets [11]. Rp1 significantly inhibited $[Ca^{2+}]_i$, ATP secretion, P-selectin surface expression, TxA₂ generation, and fibrinogen binding to integrin $\alpha_{IIb}\beta_3$ in a dose-dependent manner. Further mechanistic aspects were explored, and it was found that Rp1 specifically elevated cAMP and enhanced phosphorylation of PKA $\alpha\beta\gamma$ and VASP^{ser157}, whereas it reduced phosphorylation of MAPK (ERK2 and p38^{MAPK}), PI3K, and PLC γ 2, thereby inhibiting platelet activation. SFKs (Fyn and Lyn) and Syk are involved in early platelet signaling, and they become activated by several agonists such as collagen, ADP, and thrombin, which further activate linker for the activation of T cells (LAT) and downstream PLC γ 2 [34]. Rp1 inhibited the activation of all of these proteins in a dose-dependent manner, indicating its broad-spectrum, antiplatelet effects (Fig. 1). Antithrombotic effects were evaluated *in vivo* using a rat arteriovenous shunt model and a mouse bleeding time assay. Rp1 at 15, 30, and 50 mg/kg significantly inhibited thrombus weight by 20, 33, and 47%, respectively. Rp1 did not affect bleeding time, prothrombin time (PT), and activated partial thromboplastin time (aPTT), indicating that it does not affect hemostasis. Observed antiplatelet effects showed the potential of Rp1 as a therapeutic antiplatelet agent for platelet-related cardiovascular disorders.

5.3. G-Rp3

Rp3 was prepared from the structural modification of Re with the same method that was used for Rp1 production (Fig. 2). We explored the antiplatelet effects of Rp3 in rat, mouse, and human models and reported the detailed underlying mechanism. Rp3 (from 6.25 to 50 μ M) was tested against potential agonists such as collagen (2.5 μ g/mL), ADP (10 μ M), and thrombin (0.1 U/mL) in rats and humans, and a significant and dose-dependent inhibition of platelet aggregation was found, which was confirmed by scanning electron microscopy [75]. It also inhibited $[Ca^{2+}]_i$, ATP secretion, P-selectin surface expression, fibrinogen binding, and fibronectin adhesion to integrin $\alpha_{IIb}\beta_3$ in a dose-dependent manner. When fibrinogen binds to integrin $\alpha_{IIb}\beta_3$, it transduces signals into the cell and triggers platelet spreading. Then, in the latter phase of thrombus formation, binding triggers clot retraction, which is termed as outside-in signaling. Rp3 significantly inhibited clot retraction *via* the inhibition of the Rho kinase pathway evidenced by the use of Y-27637 (Rho-associated protein kinase or Rho kinase inhibitor) and Src-dependent PLC γ 2, which is also involved in clot retraction. Furthermore, Rp3 inhibited the activation of early signaling events, that is, SFK (Src, Fyn, and Lyn), PLC γ 2, and Src-dependent PLC γ 2 activation, and reduced the phosphorylation of

late signaling molecules including MAPK (ERK2 and JNK), MKK4, and PI3K/Akt. It also specifically elevated cAMP levels and enhanced the phosphorylation of PKA $\alpha\beta\gamma$ and VASP^{ser157} (Fig. 1). Antithrombotic effects were evaluated using an acute pulmonary thromboembolism mouse model, which confirmed the survival of mice against collagen and epinephrine thrombus induction, consequently reflecting its therapeutic potential as an antithrombotic agent to prevent and treat platelet-related cardiovascular disorders.

5.4. G-Rp4

Rp4 was derived from Rg1 by a similar method as stated previously (Fig. 2). The antiplatelet effects of Rp4 (from 6.25 to 50 μ M) were analyzed in ADP (10 μ M)-stimulated rat platelets, and a significant dose-dependent inhibition of platelet aggregation was found [76]. It remarkably inhibited $[Ca^{2+}]_i$ and P-selectin surface expression, even at 6.25 to 25 μ M. Rp4 also inhibited fibrinogen binding to integrin $\alpha_{IIb}\beta_3$ and reduced the phosphorylation of MAPK (ERK1/2, JNK, and p38^{MAPK}), PI3K/Akt, and PLC γ 2, showing the potential inhibitory mechanism involved in the modulation of platelet function (Fig. 1).

6. Synergistic effects of ginsenosides on platelet functions

We evaluated the synergistic effects of different ginsenosides to assess whether structural modification affects their antiplatelet activities and to what extent one ginsenoside provides additional synergistic antiplatelet properties to those of another. To analyze synergistic effects, 10–20% inhibitory concentrations of the ginsenosides G-Rg3 (20 μ M), 2H-Rg3 (12.5 μ M), G-Rp1 (2.5 μ M), G-Rp3 (12.5 μ M), and G-Rp4 (12.5 μ M) were selected, and their additional or synergistic effects on collagen (2.5 μ g/mL)- and ADP (10 μ M)-induced platelet aggregation were assessed [75]. Table 1 summarizes the synergistic effects of the ginsenosides. G-Rp3 proved to be effective at inhibiting platelet aggregation by exerting synergistic effects with G-Rp1 and 2H-Rg3, whereas additional effects were observed when Rp3 was cotreated with Rp4.

Similarly, Zhou et al [51] had reported that Rg1 synergistically inhibited thrombin-induced human platelet aggregation when cotreated with salvianolic acid A compared with no cotreatment or cotreatment with Rg1.

7. Structural modification affects the efficacy of ginsenosides

Change in the specific functional group of a compound remarkably alters its properties, and structural–activity

Table 1
Synergistic effects of ginsenosides.

Compound	Collagen (2.5 μ g/ml)	ADP (10 μ M)
Vehicle	100 \pm 0.0	100 \pm 0.0
G-Rp3	80 \pm 4.08*	85 \pm 4.0
G-Rg3	80 \pm 2.0*	90 \pm 5.0
G-Rp3+G-Rg3	65 \pm 5.0*	77.5 \pm 2.5*
2H-Rg3	85 \pm 4.08	86.66 \pm 4.71
G-Rp3+2H-Rg3	9 \pm 4.32**	12.33 \pm 5.24**
G-Rp1	91.66 \pm 4.7	81.6 \pm 6.23
G-Rp3+G-Rp1	3.33 \pm 1.24**	38.33 \pm 6.2**
G-Rp4	86.6 \pm 4.7	88.33 \pm 2.35
G-Rp3+G-Rp4	40.66 \pm 7.36**	53.33 \pm 4.7**

G, ginsenoside.

* $p < 0.05$.

** $p < 0.001$.

relationships are critical in the development and modification of existing compounds to make more effective and potent antiplatelet agents [77]. Ginsenosides are primarily divided into PPT types (e.g., Re, Rg1, Rg2, Rh1, Rh4, Rp3, and Rp4) and PPD types (e.g., Rb1, Rg3, 2H-Rg3, Rg5, Rh2, Rp1, and Rp2) because of the orientation of specific functional groups at C3, C6, and C20 (Fig. 2). Among these ginsenosides, Re and Rb1 do not possess antiplatelet effects, whereas Rg2, Rh1, Rh2, Rh, and Rg5 have shown mild antiplatelet activity [46,55].

7.1. Conversion of Rb1 to Rg3, following 2H-Rg3, and Rp1

Rb1 and Re do not possess antiplatelet effects, but the deletion of two glucose (Glc–Glc) molecules from Rb1 at C20 produces Rg3, which is a strong antiplatelet agent with an IC_{50} of $40.2 \pm 0.9 \mu\text{M}$ and $35.2 \pm 1.2 \mu\text{M}$ against thrombin and collagen, respectively. Rg3 became stronger when converted to 2H-Rg3 via the reduction of the double bond between C24–25. 2H-Rg3 has strong antiplatelet activity with an IC_{50} of about $18.8 \pm 0.4 \mu\text{M}$ against thrombin and $20.0 \pm 0.9 \mu\text{M}$ against collagen, indicating that 2H-Rg3 is 2 times more potent than Rg3. 2H-Rg3 was converted to Rp1 after deletion of the hydroxyl group (-OH) at C20 by means of reduction and hydrogenation; (Fig. 2; i.e., Rb1 → Rg3 → 2H-Rg3 → Rp1), which was shown to have antiplatelet effects 2–4 times stronger than those of 2H-Rg3 and Rg3 against collagen, ADP, and thrombin with an IC_{50} of $10.1 \pm 0.1 \mu\text{M}$, $6.1 \pm 0.3 \mu\text{M}$, and $6.8 \pm 0.9 \mu\text{M}$, respectively [71].

7.2. Conversion of Re to Rg2 and Rp3

Re was converted into Rg2 by the deletion of the glucose moiety from Re at C20. It is a weak antiplatelet agent, as discussed earlier. But the deletion of the -OH group at C20 and the reduction of bonds at C24–25 produces Rp3, which is a strong antiplatelet agent [75], (Fig. 2; i.e., Re → Rg2 → Rp3).

7.3. Conversion of Rg1 to Rh1 and Rp4

Rg1 is a weak antiplatelet agent [50,51], but when it is converted to Rh1 by the deletion of one glucose moiety from C20, it becomes weaker. Rh1 transforms into Rp4 after the deletion of -OH at C20 and reduction at C24–25. Rp4 is a much stronger antiplatelet agent than Rg1 [76], (Fig. 2; i.e., Rg1 → Rh1 → Rp4).

7.4. Structural analogs of Rp ginsenoside series

Ginsenosides Rp1 and Rp2 are categorized as the PPD type with a difference of one and two glucose moieties at C3 in Rp2 and Rp1, respectively. This difference affects their antiplatelet efficacies and renders Rp1 the strongest among those in the Rp series. Rp3 and Rp4 have glucose moieties at C6, but their antiplatelet efficacy is almost the same [71,75,76] (Fig. 2).

8. Conclusion and future perspectives

Ginseng has been used as a traditional preventive and therapeutic herbal medicine against several diseases, especially CVD, while numerous ginsenosides have been characterized as emerging nutraceuticals. Broad-spectrum antiplatelet effects of ginsenosides could be attributed to their ability to attenuate $[Ca^{2+}]_i$ mobilization, granule secretion, integrin $\alpha_{IIb}\beta_3$ activation, and elevation of cAMP followed by the phosphorylation of VASP^{ser-157}, as evidenced by the antiplatelet mechanism of several potent ginsenosides, for example, Rg3, 2H-Rg3, Rp1, Rp3, and Ro. Structural modification proved to be effective in improving the efficacy of ginsenosides. This may be helpful in the drug design, optimization, and

development of new antithrombotic strategies that use the activity relationships among the ginsenoside scaffolds as potential therapeutic agents to treat CVD. Ginsenosides are also reported to have therapeutic effects in neurodegenerative diseases such as Alzheimer disease, but further studies are required to unveil antiplatelet-oriented inhibitory mechanisms using potential ginsenosides.

Conflicts of interest

The authors declare no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jgr.2019.05.005>.

References

- Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, Das SR, de Ferranti S, Despres JP, Fullerton HJ, et al. Heart disease and stroke statistics-2016 update: a report from the American heart association. *Circulation* 2016;133(4):e38–360.
- Pagidipati NJ, Gaziano TA. Estimating deaths from cardiovascular disease: a review of global methodologies of mortality measurement. *Circulation* 2013;127(6):749–56.
- Shafiq G, Tatinati S, Ang WT, Veluvolu KC. Automatic identification of systolic time intervals in seismocardiogram. *Sci Rep* 2016;6:37524.
- Gielen S, Landmesser U. The Year in Cardiology 2013: cardiovascular disease prevention. *Eur Heart J* 2014;35(5):307–12.
- Nichols M, Townsend N, Scarborough P, Rayner M. Cardiovascular disease in Europe: epidemiological update. *Eur Heart J* 2013;34(39):3028–34.
- Smith JN, Negrelli JM, Manek MB, Hawes EM, Viera AJ. Diagnosis and management of acute coronary syndrome: an evidence-based update. *J Am Board Fam Med* 2015;28(2):283–93.
- Andrews RK, Berndt MC. Platelet physiology and thrombosis. *Thromb Res* 2004;114(5–6):447–53.
- Gowert NS, Donner L, Chatterjee M, Eisele YS, Towhid ST, Munzer P, Walker B, Ogorek I, Borst O, Grandoch M, et al. Blood platelets in the progression of Alzheimer's disease. *PLoS One* 2014;9(2):e90523.
- Donner L, Gremer L, Ziehm T, Gertzgen CGW, Gohlke H, Willbold D, Elvers M. Relevance of N-terminal residues for amyloid-beta binding to platelet integrin $\alpha\text{IIb}\beta_3$, integrin outside-in signaling and amyloid-beta fibril formation. *Cell Signal* 2018;50:121–30.
- Estevez B, Du X. New concepts and mechanisms of platelet activation signaling. *Physiology (Bethesda, Md)* 2017;32(2):162–77.
- Calderwood DA. Integrin activation. *J Cell Sci* 2004;117(Pt 5):657–66.
- Davi G, Patrono C. Platelet activation and atherothrombosis. *N Engl J Med* 2007;357(24):2482–94.
- Golebiewska EM, Poole AW. Platelet secretion: from haemostasis to wound healing and beyond. *Blood Rev* 2015;29(3):153–62.
- Barrett NE, Holbrook L, Jones S, Kaiser WJ, Moraes LA, Rana R, Sage T, Stanley RG, Tucker KL, Wright B, et al. Future innovations in anti-platelet therapies. *Br J Pharmacol* 2008;154(5):918–39.
- Mackman N. Triggers, targets and treatments for thrombosis. *Nature* 2008;451(7181):914–8.
- Badimon L, Vilahur G, Padro T. Nutraceuticals and atherosclerosis: human trials. *Cardiovasc Ther* 2010;28(4):202–15.
- Estruch R, Ros E, Salas-Salvado J, Covas MI, Corella D, Aros F, Gomez-Gracia E, Ruiz-Gutierrez V, Fiol M, Lapetra J, et al. Primary prevention of cardiovascular disease with a Mediterranean diet. *N Engl J Med* 2013;368(14):1279–90.
- Rastogi S, Pandey MM, Rawat AK. Traditional herbs: a remedy for cardiovascular disorders. *Phytomedicine* 2015;23(11):1082–9.
- Irfan M, Kwon T-H, Yun B-S, Park N-H, Rhee MH. Eisenia bicyclis (brown alga) modulates platelet function and inhibits thrombus formation via impaired P2Y12 receptor signaling pathway. *Phytomedicine* 2018;40:79–87.
- Kim SK, Park JH. Trends in ginseng research in 2010. *J Ginseng Res* 2011;35(4):389–98.
- Ernst E. Panax ginseng: an overview of the clinical evidence. *J Ginseng Res* 2010;34(4):259–63.
- Lee CH, Kim J-H. A review on the medicinal potentials of ginseng and ginsenosides on cardiovascular diseases. *J Ginseng Res* 2014;38(3):161–6.
- Kim J-H. Pharmacological and medical applications of Panax ginseng and ginsenosides: a review for use in cardiovascular diseases. *J Ginseng Res* 2018;42(3):264–9.
- Lu J-M, Yao Q, Chen C. Ginseng compounds: an update on their molecular mechanisms and medical applications. *Curr Vasc Pharmacol* 2009;7(3):293–302.

- [25] Kim JH, Yi Y-S, Kim M-Y, Cho JY. Role of ginsenosides, the main active components of Panax ginseng, in inflammatory responses and diseases. *J Ginseng Res* 2017;41(4):435–43.
- [26] CHOI Kt. Botanical characteristics, pharmacological effects and medicinal components of Korean Panax ginseng CA Meyer. *Acta Pharmacol Sin* 2008;29(9):1109–18.
- [27] Irfan M, Jeong D, Saba E, Kwon H-W, Shin J-H, Jeon B-R, Kim S, Kim S-D, Lee D-H, Nah S-Y. Gintonin modulates platelet function and inhibits thrombus formation via impaired glycoprotein VI signaling. *Platelets* 2018;1–10.
- [28] Hwang SH, Shin TJ, Choi SH, Cho HJ, Lee BH, Pyo MK, Lee JH, Kang J, Kim HJ, Park CW, et al. Gintonin, newly identified compounds from ginseng, is novel lysophosphatidic acids-protein complexes and activates G protein-coupled lysophosphatidic acid receptors with high affinity. *Mol Cells* 2012;33(2):151–62.
- [29] Saba E, Jeon BR, Jeong DH, Lee K, Goo YK, Kwak D, Kim S, Roh SS, Kim SD, Nah SY, et al. A novel Korean red ginseng compound gintonin inhibited inflammation by MAPK and NF-kappaB pathways and recovered the levels of mir-34a and mir-93 in RAW 264.7 cells. *Evid Based Complement Alternat Med* 2015;2015:624132.
- [30] Park H-J, Rhee M-H, Park K-M, Nam K-Y, Park K-H. Effect of non-saponin fraction from Panax ginseng on cGMP and thromboxane A2 in human platelet aggregation. *J Ethnopharmacol* 1995;49(3):157–62.
- [31] Choi SH, Shin TJ, Lee BH, Hwang SH, Kang J, Kim HJ, Park CW, Nah SY. An edible gintonin preparation from ginseng. *J Ginseng Res* 2011;35(4):471–8.
- [32] Pyo MK, Choi S-H, Hwang SH, Shin T-J, Lee B-H, Lee S-M, Lim Y-H, Kim D-H, Nah S-Y. Novel glycolipoproteins from ginseng. *J Ginseng Res* 2011;35(1):92–103.
- [33] Adam F, Kauskot A, Rosa JP, Bryckaert M. Mitogen-activated protein kinases in hemostasis and thrombosis. *J Thromb Haemost* : JTH 2008;6(12):2007–16.
- [34] Senis YA, Mazharian A, Mori J. Src family kinases: at the forefront of platelet activation. *Blood* 2014;124(13):2013–24.
- [35] Pankov R, Yamada KM. Fibronectin at a glance. *J Cell Sci* 2002;115(20):3861–3.
- [36] Canobbio I, Cipolla L, Consonni A, Momi S, Guidetti G, Oliviero B, Falasca M, Okigaki M, Balduini C, Greslele P, et al. Impaired thrombin-induced platelet activation and thrombus formation in mice lacking the Ca(2+)-dependent tyrosine kinase Pyk2. *Blood* 2013;121(4):648–57.
- [37] Park H-J, Rhee M-H, Park K-M, Nam K-Y, Park K-H. Panaxadiol from Panax ginseng CA Meyer inhibits synthesis of thromboxane A₂ in platelet aggregation induced by thrombin. *J Ginseng Res* 1993;17(2):131–4.
- [38] Rhee M-H, Park K-M, Park H-J, Nam K-Y, Park K-H. Inhibition of peritonin release by lipophilic fraction from Korean red ginseng. *J Ginseng Res* 1993;17(2):127–30.
- [39] Park H-J, Rhee M, Park K, Park K, No Y. Effects of protein fractions and ginsenosides from Panax ginseng CA Meyer on substrate phosphorylation by a catalytic fragment of protein kinase. *Korean Biochem J (Korea Republic)* 1994;27(4):280–3.
- [40] Lee W-M, Kamruzzaman S, Song Y-B, Cho J-Y, Park H-J, Rhee M-H. Inhibitory activities of red ginseng acidic polysaccharide in platelet aggregation. *J Ginseng Res* 2008;32(1):73–8.
- [41] Park K-M, Rhee M-H, Shin H-J, Song Y-B, Hyun H-C, Park K-H, Cho H-J, Choi S-A, Kang H-C, Kim K-J. Inhibitory effects of Panaxatriol from Panax ginseng CA Meyer on phosphoinositide breakdown induced by thrombin in platelets. *J Ginseng Res* 2008;32(2):107–13.
- [42] Lee WM, Kim SD, Park MH, Cho JY, Park HJ, Seo GS, Rhee MH. Inhibitory mechanisms of dihydroginsenoside Rg3 in platelet aggregation: critical roles of ERK2 and cAMP. *J Pharm Pharmacol* 2008;60(11):1531–6.
- [43] Lee D-H, Cho H-J, Kim H-H, Rhee MH, Ryu J-H, Park H-J. Inhibitory effects of total saponin from Korean red ginseng via vasodilator-stimulated phospho-protein-Ser157 phosphorylation on thrombin-induced platelet aggregation. *J Ginseng Res* 2013;37(2):176–86.
- [44] Lee D-H, Cho H-J, Kang H-Y, Rhee MH, Park H-J. Total saponin from Korean red ginseng inhibits thromboxane A2 production associated microsomal enzyme activity in platelets. *J Ginseng Res* 2012;36(1):40–6.
- [45] Jeon BR, Kim SJ, Hong SB, Park H-J, Cho JY, Rhee MH. The inhibitory mechanism of crude saponin fraction from Korean Red Ginseng in collagen-induced platelet aggregation. *J Ginseng Res* 2015;39(3):279–85.
- [46] Shin J-H, Kwon H-W, Cho H-J, Rhee MH, Park H-J. Inhibitory effects of total saponin from Korean Red Ginseng on [Ca²⁺]_i mobilization through phosphorylation of cyclic adenosine monophosphate-dependent protein kinase catalytic subunit and inositol 1, 4, 5-trisphosphate receptor type I in human platelets. *J Ginseng Res* 2015;39(4):354–64.
- [47] Kwon H-W, Shin J-H, Cho H-J, Rhee MH, Park H-J. Total saponin from Korean Red Ginseng inhibits binding of adhesive proteins to glycoprotein IIb/IIIa via phosphorylation of VASP (Ser157) and dephosphorylation of PI3K and Akt. *J Ginseng Res* 2016;40(1):76–85.
- [48] Qi H, Huang Y, Yang Y, Dou G, Wan F, Zhang W, Yang H, Wang L, Wu C, Li L. Anti-platelet activity of panaxatriol saponins is mediated by suppression of intracellular calcium mobilization and ERK2/p38 activation. *BMC Complement Altern Med* 2016;16(1):174.
- [49] Jeong D, Irfan M, Kim S-D, Kim S, Oh J-H, Park C-K, Kim H-K, Rhee MH. Ginsenoside Rg3-enriched red ginseng extract inhibits platelet activation and in vivo thrombus formation. *J Ginseng Res* 2017;41(4):548–55.
- [50] Teng C-M, Kuo S-C, Ko F-N, Lee J-C, Lee L-G, Chen S-C, Huang T-F. Antiplatelet actions of panaxynol and ginsenosides isolated from ginseng. *Biochimica et Biophysica Acta (BBA) - General Subjects* 1989;990(3):315–20.
- [51] Zhou Q, Jiang L, Xu C, Luo D, Zeng C, Liu P, Yue M, Liu Y, Hu X, Hu H. Ginsenoside Rg1 inhibits platelet activation and arterial thrombosis. *Thromb Res* 2014;133(1):57–65.
- [52] Kwon H-W. 20(S)-ginsenoside Rg3 inhibits glycoprotein IIb/IIIa activation in human platelets. *J Appl Biol Chem* 2018;61(3):257–65.
- [53] Kwon H-W. Inhibitory effect of 20(S)-Ginsenoside Rg3 on human platelet aggregation and intracellular Ca(2+) levels via cyclic adenosine monophosphate dependent manner. *Prev Nutr and Food Sci* 2018;23(4):317–25.
- [54] Lee JG, Lee YY, Kim SY, Pyo JS, Yun-Choi HS, Park JH. Platelet antiaggregating activity of ginsenosides isolated from processed ginseng. *Pharmazie - An Int J Pharmaceut Sci* 2009;64(9):602–4.
- [55] Lee JG, Lee YY, Wu B, Kim SY, Lee YJ, Yun-Choi HS, Park JH. Inhibitory activity of ginsenosides isolated from processed ginseng on platelet aggregation. *Pharmazie - An Int J Pharmaceut Sci* 2010;65(7):520–2.
- [56] Matsuda H, Namba K, Fukuda S, Tani T, Kubo M. Pharmacological study on Panax ginseng C. A. Meyer. IV. Effects of red ginseng on experimental disseminated intravascular coagulation. (3). Effect of ginsenoside-Ro on the blood coagulative and fibrinolytic system. *Chem Pharm Bull* 1986;34(5):2100–4.
- [57] Ju HK, Lee JG, Park MK, Park S-J, Lee CH, Park JH, Kwon SW. Metabonomic investigation of the anti-platelet aggregation activity of ginsenoside Rk1 reveals attenuated 12-HETE production. *J Proteome Res* 2012;11(10):4939–46.
- [58] Kwon H-W, Shin J-H, Lee D-H, Park H-J. Inhibitory effects of cytosolic Ca²⁺ concentration by ginsenoside Ro are dependent on phosphorylation of IP3R1 and dephosphorylation of ERK in human platelets. Evidence-based complementary and alternative medicine. *eCAM* 2015;2015:764906.
- [59] Shin J-H, Kwon H-W, Cho H-J, Rhee MH, Park H-J. Vasodilator-stimulated phosphoprotein-phosphorylation by ginsenoside Ro inhibits fibrinogen binding to α IIb/ β 3 in thrombin-induced human platelets. *J Ginseng Res* 2016;40(4):359–65.
- [60] Kwon H-W. Inhibitory effects of ginsenoside Ro on clot retraction through suppressing PI3K/Akt signaling pathway in human platelets. *Prev Nutr Food Sci* 2019;24(1):1.
- [61] Shin J-H, Kwon H-W, Rhee MH, Park H-J. Inhibitory effects of thromboxane A2 generation by ginsenoside Ro due to attenuation of cytosolic phospholipase A2 phosphorylation and arachidonic acid release. *J Ginseng Res* 2019;43(2):236–41.
- [62] Ruggeri ZM. Mechanisms initiating platelet thrombus formation. *Thromb Haemost* 1997;78(1):611–6.
- [63] Jennings LK. Role of platelets in atherothrombosis. *Am J Cardiol* 2009;103(3 Suppl):4a–10a.
- [64] Yacoub D, Theoret JF, Villeneuve L, Abou-Saleh H, Mourad W, Allen BG, Merhi Y. Essential role of protein kinase C delta in platelet signaling, alpha IIb beta 3 activation, and thromboxane A2 release. *J Biol Chem* 2006;281(40):30024–35.
- [65] Garcia A, Shankar H, Murugappan S, Kim S, Kunapuli SP. Regulation and functional consequences of ADP receptor-mediated ERK2 activation in platelets. *Biochem J* 2007;404(2):299–308.
- [66] Kudo I, Murakami M. Phospholipase A2 enzymes. *Prostagl Other Lipid Mediat* 2002;68–69:3–58.
- [67] Kwon H-W. Inhibitory effects of phytochemicals on thromboxane A2 generation via MAPK signaling pathways in platelet aggregation. Republic of Korea: Department of Smart Foods and Drugs, Graduate School, Inje University Gimhae; 2017.
- [68] Tawab MA, Bahr U, Karas M, Wurglics M, Schubert-Zsilavecz M. Degradation of ginsenosides in humans after oral administration. *BMC Complement Altern Med* 2003;31(8):1065.
- [69] Park TY, Park MH, Shin WC, Rhee MH, Seo DW, Cho JY, Kim HM. Anti-metastatic potential of ginsenoside Rp1, a novel ginsenoside derivative. *Biol Pharm Bull* 2008;31(9):1802–5.
- [70] Kang J-H, Song K-H, Woo J-K, Park MH, Rhee MH, Choi C, Oh SH. Ginsenoside Rp1 from Panax ginseng exhibits anti-cancer activity by down-regulation of the IGF-1R/Akt pathway in breast cancer cells. *Plant Foods Hum Nutr* 2011;66(3):298.
- [71] Endale M, Lee WM, Kamruzzaman SM, Kim SD, Park JY, Park MH, Park TY, Park HJ, Cho JY, Rhee MH. Ginsenoside-Rp1 inhibits platelet activation and thrombus formation via impaired glycoprotein VI signalling pathway, tyrosine phosphorylation and MAPK activation. *Br J Pharmacol* 2012;167(1):109–27.
- [72] Varga-Szabo D, Braun A, Nieswandt B. Calcium signaling in platelets. *J Thromb Haemost* : JTH. 2009;7(7):1057–66.
- [73] Zarbock A, Polanowska-Grabowska RK, Ley K. Platelet-neutrophil-interactions: linking hemostasis and inflammation. *Blood Rev* 2007;21(2):99–111.
- [74] Offermanns S. Activation of platelet function through G protein-coupled receptors. *Circ Res* 2006;99(12):1293–304.

- [75] Irfan M, Jeong D, Kwon H-W, Shin J-H, Park S-J, Kwak D, Kim T-H, Lee D-H, Park H-J, Rhee MH. Ginsenoside-Rp3 inhibits platelet activation and thrombus formation by regulating MAPK and cyclic nucleotide signaling. *Vasc Pharmacol* 2018;109:45–55.
- [76] Son YM, Jeong DH, Park HJ, Rhee MH. The inhibitory activity of ginsenoside Rp4 in adenosine diphosphate-induced platelet aggregation. *J Ginseng Res* 2017;41(1):96–102.
- [77] Ravishankar D, Salamah M, Akimbaev A, Williams HF, Albadawi DAI, Vaiyapuri R, Greco F, Osborn HMI, Vaiyapuri S. Impact of specific functional groups in flavonoids on the modulation of platelet activation. *Sci Rep* 2018;8(1):9528.