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

Muhammad Irfan, Minki Kim, Man Hee Rhee*

Laboratory of Veterinary Physiology and Cell Signaling, Department of Veterinary Medicine, College of Veterinary Medicine, Kyungpook National University, Daegu, Republic of Korea

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Cardiovascular diseases prevail among modern societies and underdeveloped countries, and a high mortality rate has also been reported by the World Health Organization affecting tens of 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 (Aβ) 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, adenylyl cyclase; cAMP, cyclic adenosine monophosphate; ASA, acetylsalicylic acid; ATP, adenosine triphosphate; Akt, protein kinase B; [Ca2+]i, intracellular calcium ion; COX, cyclooxygenase; CRP, collagen-related peptide; CSF, crude saponin fraction; CRP, collagen-related peptide; CSF, crude saponin fraction; ERK, extracellular regulated kinase; GPVI, glycoprotein VI; IC50, 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; cPLA2a, cPLA2, cytosolic phospholipase A2a; PLA2, phospholipase A2; PLCγ2, phospholipase C gamma-2; aPTT, activated partial thromboplastin time; PT, prothrombin time; PPD, protopanaxadiol; PPT, protopanaxatriol; ROCK, Rho-associated protein kinase; SFR, Src family kinase; Syk, spleen tyrosine kinase; TSH, total saponin; TxA2, thromboxane A2; TXB2, thromboxane B2; TXAS, thromboxane-A synthase; TXR, thromboxane receptor; VASP, vasodilator-stimulated phosphoprotein; VWF, von Willebrand factor.

* Corresponding author. Laboratory of Veterinary Physiology and Cell Signaling, Department of Veterinary Medicine, College of Veterinary Medicine, Kyungpook National University, 80 Daehakro, Buk-gu, Daegu, 41566, Republic of Korea.

E-mail address: rheemh@knu.ac.kr (M.H. Rhee).
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 αIIbβ3-mediated inside-out signaling, which increases its affinity for fibrinogen binding and platelet adhesion; and (4) integrin αIIbβ3-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 P2Y12 or P2Y1 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 (D) granules, which include Ca2+, 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 αIIbβ3-mediated inside-out and outside-in signaling; (iv) protein kinase C signaling; and (v) platelet-activating protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) 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...
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 µg/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 (PLCγ2) 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 responses to collagen-induced granule secretion and aggregation, and calcium mobilization, granule secretion (both calcium- and ATP-dependent), and cAMP elevation, and vasodilator-stimulated phosphoprotein (VASP)ser phosphorylation [45]. 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 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 µg/mL) potently inhibited collagen-induced platelet aggregation via the inhibition of calcium mobilization, ATP secretion, and fibrinogen binding to integrin αIIbβ3. It also reduced the phosphorylation of MAPK (ERK1/2, JNK, and p38MAPK), 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 1 and adhesion proteins to GPIIb/IIIa via VASPser [45] and the PI3K/Akt pathway in human platelets. It was found that TS (from 25 to 150 µg/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-cGMP), 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 p38MAPK phosphorylation in a dose-dependent manner against potent 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 Rh1, Rc, Rd, 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 µg/mL) was examined in collagen (1.25 µg/mL)-induced rat platelets [49]. It was found to inhibit platelet aggregation in a significant and dose-dependent manner. It exerted a broad-spectrum antiplatelet effects by inhibiting fibrinogen binding to integrin αIIbβ3, [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 µg/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 aspirin 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 salvinionic 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 use of Rp-8-Br-cAMPS (A-kinase inhibitor) and the enhanced phosphorylation of inositol 1,4,5-

3.3. G-Rg5 and G-Rg6

The antiplatelet activity of G-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. Oleane-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-

M. Irfan et al / Antiplatelet effects of ginsenosides
tripolyphosphate receptor I (Ser1756) that inhibits $[\text{Ca}^{2+}]$, mobilization, which consequently inhibited platelet aggregation. Broad-spectrum antiplatelet effects of Ro were observed; it elevated cAMP levels and inhibited $[\text{Ca}^{2+}]$, 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 ($\alpha_{\text{IIb}}\beta_3$) and the upregulation of cAMP-dependent VASP$^{\text{Ser157}}$, 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 $\alpha_{\text{IIb}}\beta_3$ 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 $\mu$M) significantly inhibited platelet aggregation. TxA$_2$ generation lead to vasoconstriction and thromboxenogenesis, and Ro strongly inhibited TxA$_2$ generation via inhibiting AA release in a dose-dependent manner. TxA$_2$ is produced by AA, which is produced by COX-1 and thromboxane-A synthase (TxA$_S$), and initiates thrombus formation [62,63]. It is known that phosphorylated MAPK (ERK2 and p38$^{\text{MAPK}}$) induces TxA$_2$ production, and the activation of cytosolic phospholipase A$_2$A ($\text{cPLA}_2A$) ultimately leads to AA release [64–67]. Ro treatment has reduced all of the aforementioned parameters involved in TxA$_2$ production in a dose-dependent manner (Fig. 1). It was proved that AA and TxB$_2$ inhibition was attributed to Ca$^{2+}$-dependent cPLA$_2A$ phosphorylation by p38$^{\text{MAPK}}$ evidenced by the use of SB203580 (p38$^{\text{MAPK}}$ 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 IC$_{50}$ of about 18.8 ± 0.4 $\mu$M against thrombin and 20.0 ± 0.9 $\mu$M against collagen. 2H-Rg3 exerted antiplatelet effects through the elevation of cAMP and the inhibition of the MAPK (ERK2 and p38$^{\text{MAPK}}$) pathway (Figs. 1 and 2) [42].
5.2. G-Rp1

Chemically stable Rp1 was prepared from other ginsenosides (Rg3, 2h-Rg3, Rg5, and Rk1) by means of reduction with hydrogenezation (Fig. 2). It has been shown to have 10 times more potent antiplatelet 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 μM in vitro and 50 and 100 mg/kg; ex vivo was able to inhibit platelet aggregation against collagen (2.5 μg/mL), ADP (10 μM), and thrombin (0.1 U/mL) in a significant and dose-dependent manner with an IC50 of 10.1 ± 0.1 μM, 6.1 ± 0.3 μM, and 6.8 ± 0.9 μM, respectively. Intra-cellular calcium concentrations play a crucial role in platelet activation and thrombus formation \[72\], and TXA2 amplifies signal transduction and ensures thrombus formation \[74\]. After stimulation with an agonist, the binding affinity of integrin αIIbβ3 to fibrinogen increases, which causes the adhesion of platelets \[11\]. Rp1 significantly inhibited \([\text{Ca}^{2+}]_{i}\), ATP secretion, P-selectin surface expression, TXA2 generation, and fibrinogen binding to integrin αIIbβ3 in a dose-dependent manner. Further mechanistic aspects were explored, and it was found that Rp1 specifically elevated cAMP and enhanced phosphorylation of PKAβγ and VASP \[15,73\], whereas it reduced phosphorylation of MAPK (ERK2 and p38MAPK), P3K, and PLCγ2, thereby inhibiting platelet activation. SFKs (Fyn and Lyn) and Src are involved in early platelet signaling, and they become activated by inhibiting platelet aggregation. SFKs (Fyn and Lyn) and Src are involved in early platelet signaling, and they become activated by inhibiting platelet aggregation. SFKs (Fyn and Lyn) and Src are involved in early platelet signaling, and they become activated by inhibiting platelet aggregation. SFKs (Fyn and Lyn) and Src are involved in early platelet signaling, and they become activated by inhibiting platelet aggregation. SFKs (Fyn and Lyn) and Src are involved in early platelet signaling, and they become activated by inhibiting platelet aggregation. SFKs (Fyn and Lyn) and Src are involved in early platelet signaling, and they become activated by inhibiting platelet aggregation. SFKs (Fyn and Lyn) and Src are involved in early platelet signaling, and they become activated by inhibiting platelet aggregation.

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 μM was tested against potential agonists such as collagen (2.5 μg/mL), ADP (10 μM), 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 \([\text{Ca}^{2+}]_{i}\), ATP secretion, P-selectin surface expression, fibrinogen binding, and fibrinectin adhesion to integrin αIIbβ3 in a dose-dependent manner. When fibrinogen binds to integrin αIIbβ3, 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 PLCγ2, which is also involved in clot retraction. Furthermore, Rp3 inhibited the activation of early signaling events, that is, SFK (Src, Fyn, and Lyn), PLCγ2, and Src-dependent PLCγ2 activation, and reduced the phosphorylation of late signaling molecules including MAPK (ERK2 and JNK), MKK4, and P3K/Akt. It also specifically elevated cAMP levels and enhanced the phosphorylation of PKAβγ and VASP \[15,73\] (Fig. 1). Anti-thrombotic 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 anti-thrombotic 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 μM) were analyzed in ADP (10 μM)-stimulated rat platelets, and a significant dose-dependent inhibition of platelet aggregation was found \[76\]. It remarkably inhibited \([\text{Ca}^{2+}]_{i}\) and P-selectin surface expression, even at 6.25 to 25 μM. Rp4 also inhibited fibrinogen binding to integrin αIIbβ3 and reduced the phosphorylation of MAPK (ERK1/2, JNK, and p38MAPK), P3K/Akt, and PLCγ2, 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 μM), 2H-Rg3 (12.5 μM), G-Rp1 (2.5 μM), G-Rp3 (12.5 μM), and G-Rp4 (12.5 μM) were selected, and their additional or synergistic effects on collagen (2.5 μg/mL)- and ADP (10 μM)-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
 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 PPP 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 IC50 of 40.2 ± 0.9 μM and 35.2 ± 1.2 μM 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 IC50 of about 18.8 ± 0.4 μM against thrombin and 20.0 ± 0.9 μM 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 hydroxylation (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 IC50 of 10.1 ± 0.1 μM, 6.1 ± 0.3 μM, and 6.8 ± 0.9 μM, 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 therapeu- tic 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 [Ca2+] mobilization, granule secretion, integrin αIIbb3 activation, and elevation of CAMP followed by the phosphorylation of VASP[92,93]. 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 therapeu- tic agents to treat CVD. Ginsenosides are also reported to have therapeutic effects in neurodegenerative diseases such as Alz- heimer 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.

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