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Review Article

Functional roles and mechanisms of ginsenosides from Panax ginseng in atherosclerosis

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ABSTRACT

Atherosclerosis (AS) is a leading cause of cardiovascular diseases (CVDs) and it results in a high rate of death worldwide, with an increased prevalence with age despite advances in lifestyle management and drug therapy. Atherosclerosis is a chronic progressive inflammatory process, and it mainly presents with lipid accumulation, foam cell proliferation, inflammatory response, atherosclerotic plaque formation and rupture, thrombosis, and vascular calcification. Therefore, there is a great need for reliable therapeutic drugs or remedies to cure or alleviate atherosclerosis and reduce the societal burden. Ginsenosides are natural steroid glycosides and triterpene saponins obtained mainly from the plant ginseng. Several recent studies have reported that ginsenosides have a variety of pharmacological activities against several diseases including inflammation, cancer and cardiovascular diseases. This review focuses on describing the different pharmacological functions and underlying mechanisms of various active ginsenosides (Rb1,-Rd, -F, -Rg1, -Rg2, and -Rg3, and compound K) for atherosclerosis, which could provide useful insights for developing novel and effective anti-cardiovascular drugs.

1. Introduction

Cardiovascular disease (CVD) is among the leading causes of death worldwide, and the incidence and mortality of this disease are increasing annually. Cardiovascular disease is mainly caused by smoking, alcoholism, staying up late, lack of exercise, bad lifestyle habits, and so forth [1,2]. Therefore, the study of CVD has become a major task for the contemporary medical industry. Atherosclerosis (AS) is a kind of chronic progressive arterial disease, which is the basis of cardiovascular diseases, such as coronary heart disease, cerebral infarction, and other diseases. It is a severe threat to human life and health. In-depth research has been performed on the pathogenesis of atherosclerosis, and the widely accepted mechanisms of atherosclerosis are mainly inflammatory response, endothelial injury, vascular smooth muscle cells (VSMCs) phenotypic transformation, autophagy and apoptosis [3]. Lipid metabolism disorder is the pathological basis of atherosclerosis, and it is characterized by the accumulation of lipids and complex sugars, hemorrhage, thrombosis, the proliferation of fibrous tissue and calcium deposits, and the gradual degeneration and calcification of the middle layer of the artery, which leads to the thickening and hardening of the arterial wall and stenosis of the vascular cavity [4]. Inflammation is a vital self-defense mechanism of the body [5,6]. Atherosclerosis is a typical chronic progressive inflammatory response disease [7]. Inflammatory factors, which include tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and others, are often highly expressed during the formation of atherosclerotic plaque [8]. The injury-induced inflammatory sites of endothelial

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cells release various inflammatory factors, cell adhesion factors, such as vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1) and endothelium-selectin (E-selectin) [9]. Collectively, the inflammatory response plays a crucial role throughout the whole process of the occurrence, development, and pathogenesis of AS. In AS, macrophages can be polarized into two phenotypes, M1 and M2, which can regulate inflammation. Moreover, mature dendritic cell (DC) expression in AS is upregulated, and it is mainly involved in the early stage of AS through macrophage activation and immune response, and it can also promote the release of inflammatory factors and chemokines to mediate inflammatory response [10]. In addition, the VSMCs of the medial membrane undergo phenotypic transformation under physiological and pathological conditions. In AS, VSMCs present abnormal proliferation and migration, and foam cells formed by macrophages will gradually accumulate in endothelial cells, thereby forming atherosclerotic plaques. Besides, autophagy and apoptosis of VSMCs are also involved in the development process of AS and play a regulatory role in AS [11–16].

Panax ginseng Meyer is a perennial herb that belongs to the Acanthopanax family. It is a valuable tonic medicine that has mainly been produced in Northeast China, Korea, Japan and eastern Russia. In recent years, research on ginseng has increased in home country as well as abroad [17,18]. Ginseng has always been regarded as a "cure all" and thus has high medicinal value and a variety of pharmacological effects [19–21]. At present, there are 150 kinds of ginsenosides, which can be divided into the pentacyclic triterpenoid oleanolane type and tetracyclic triterpenoid dammarane type [22]. Ginsenosides are further differentiated according to whether there are hydroxyl groups at 6 positions of the chemical structure, with the six positions without the hydroxyl group considered protopanaxadiol (PPD) and the six positions with hydroxyl group considered protopanaxatriol (PPT). PPD mainly includes the ginsenosides Rb1, Rb2, Rb3, Rc, Rd, Rg3, and Rh2 and compound K, whereas PPT mainly includes the ginsenosides Re, G1, Rg2 and Rh1. The oleanolane-type ginsenoside that was originally considered the oleanolic acid-c type and the most typical representative is ginsenoside Ro [23]. These types have a significant difference in biological activity and can play different clinical roles [24–26]. Recently, growing evidence has shown that ginsenoside can be applied for the treatment of cardiovascular disease with a specific mechanism. Ginsenosides show a good protective effect on cardiovascular disease, especially in the formation and stability of atherosclerotic plaque, vascular remodeling and proliferation. It can be used in treating AS because it regulates cell proliferation cycle, inflammation-related gene pathways, lipid metabolism, cytokines and other molecular activity to alleviate the development of atherosclerotic disease [20,27].

In this review, we summarized the regulatory roles of ginsenosides followed by introducing the relationship between the occurrence and development of atherosclerosis and ginsenosides.

2. Ginsenosides and its anti-atherosclerotic activity

2.1. Ginsenoside Rb1 and its anti-atherosclerosis effect

Ginsenoside Rb1 is one of the most abundant active components in ginseng. As previous research has shown that Rb1 has a good therapeutic effect on the occurrence and development of atherosclerosis, especially in improving the compliance of the vascular wall, reducing the inflammatory response of endothelial cells, stabilizing plaque and attenuating the formation of plaque [28,29].

Yang et al. [30] showed that Rb1 can activate G protein-coupled estrogen receptor (GPER) and alleviate AS. In the experiment, a high-fat-induced rabbit model was used to detect the therapeutic effect of Rb1 while exploring the regulatory mechanism of Rb1 for the mitigation of atherosclerosis. The measured rabbit total cholesterol (TC), low-density lipoprotein (LDL) and triglyceride (TG) levels were all elevated, while the high-density lipoprotein (HDL) levels were suppressed. The results of the treatment with Rb1 showed that blood lipids were significantly lower in rabbits, the levels of TC, TG and LDL were reversed. Observations of isolated ECs showed that the levels of IL-6, IL-1β and TNF-α were significantly increased in the high-fat induced rabbit model, which showed higher levels of apoptosis, but the levels of inflammatory factors were significantly reversed after Rb1 treatment. Moreover, changes were observed in the cells with Rb1 at 80 μM and GPER antagonists at different doses. In the next study, which mainly included cell experiments, Rb1 increased the expression level of proteins after phosphorylation by activating the GPER-mediated PI3K/Akt pathway, decreased the level of Bax-related apoptosis protein, and inhibit apoptosis. Collectively, Rb1 can reduce blood lipid levels, inflammatory factor expression and inflammatory response, which increase the anti-apoptotic effects and alleviate AS via the activation of GPER.

Moreover, research by Nanao Hamai Michiko et al. showed that Rb1 can prevent cardiovascular disease, inhibit apoptosis, and then decrease vascular calcification by transactivation of the growth inhibitor specific gene 6 (Gas6), which is mediated by androgen receptors in VSMCs [31]. Rb1 can inhibit the calcification of VSMCs induced by phosphate, restore the Gas6/Akt pathway, and can reduce the apoptosis induced by inorganic phosphate (Pi). Convincingly, androgen receptor antagonists can block the improvement of Rb1 at 1 μM, which provides evidence that the occurrence and development of atherosclerosis are inhibited via the transactivation of Gas6 mediated by androgen receptors. Therefore, Rb1 can be used as a selective androgen receptor modulator to inhibit calcification, however its effect on osteoblasts transformation (such as BMP2, Runx2) remains to be further studied. Moreover, Rb1 was also proved to inhibit the vascular neointimal hyperplasia induced by balloon-injury in rats via suppressing the VSMC proliferation and phenotypic switching, which may be mediated by the inhibition of ERK1/2-c-Jun/c-Fos-c-myc signaling pathway [32]. Besides, Rb1 treatment also dramatically suppressed AngII-induced diameter enlargement, extracellular matrix degradation, matrix metalloproteinase production, inflammatory cell infiltration, and VSMC dysfunction, and thus Rb1 can be a potential anti-abdominal aortic aneurysm agent [33].

In addition, Zhou et al. [34] demonstrated that Rb1 can attenuate AS in apolipoprotein E-deficient (Apo E−/−) mice treated with high-fat diet. Rb1 decreased body weight and food intake in ApoE−/− mice. And treatment of ApoE−/− mice with Rb1 substantially attenuated the atherosclerotic plaque area evidenced by oil Red O staining on the aortic root. Circulating levels of total cholesterol (TC), triglycerides (TG) and low-density lipoprotein cholesterol (LDL-C) were notably upregulated in ApoE−/− mice, and Rb1 administration significantly reduced these three parameters. In addition, they detected the inflammatory cytokines levels of IL-1β, IL-6, and TNF-α in the serum of ApoE−/− mice and found that these cytokine levels were lowered from this experiment. By regulating the expression of Bcl-2 family-related proteins, Rb1 inhibited the expression of caspase-3 and caspase-9. Surprisingly, Rb1 could improve the transformation of LC3-I to LC3-II [35], which suggests that Rb1 acts the treatment by reversing the imbalance between apoptosis and autophagy, inhibiting inflammation, reducing the oxidative stress response.

Recently, the role of non-coding RNA (ncRNA) in multiple diseases has been increasingly acknowledged [36,37]. Many ncRNAs have been reported to be closely related to the treatment or regulation of atherosclerosis. ncRNAs have important regulatory

functions in vascular inflammation and vascular smooth muscle cells; thus, ncRNA is expected to be a new strategy and target for the treatment of atherosclerosis [38–43]. As previously reported, pigment epithelium-derived factor (PEDF) has an effect on anti-angiogenesis and miR-33a can regulate the 3′-UTR of PEDF mRNA [44]. Lu et al. carried out experiments in human umbilical vein endothelial cells (HUVECs) to explore the role of Rb1 in PEDF to clarify the anti-angiogenic effect of PEDF in AS. *In vitro* assays revealed that PEDF is the target gene of miR-33a. The expression level between PEDF and miR-33a is negatively correlated *in vivo*. Furthermore, the effect of miR-33a in HUVECs *in vitro* was further clarified. Surprisingly, this report indicated that Rb1 can inhibit the effect of miR-33a *in vitro*. To clarify the role of nuclear receptors in Rb1-mediated miR-33a and PEDF, estrogen receptor antagonists, glucocorticoid receptor antagonists and peroxygenase proliferator activator antagonists were used to explore the related nuclear receptors. The protein expression level was investigated in the presence of Rb1, and the results confirmed that ginsenoside Rb1 regulates the expression of PEDF and miR-33a by activating peroxisome proliferator activator γ (PPARγ). PPARγ reduces the inhibition of miR-33a on PEDF, improves the expression of PEDF, promotes anti-angiogenesis, and further decreases the proliferation of abnormal blood vessels. MiR-33a provides a new therapeutic target and research direction for antiangiogenic therapy.

When monocytes enter a damaged tissue through the vascular endothelial cell layer, they undergo a series of transformations to become macrophages. Macrophages can be polarized into M1 and M2 phenotypes according to the stimulation of different types of cytokines, which play an important role in regulating plaque stability [45]. Zhang et al. [46] used ginsenoside Rb1 in C57BL/6 and Apo $E^{-/-}$ mice and they find Rb1 can increase the polarization of macrophages to M2. The research also showed Rb1 could increase IL-4 and IL-13 secretion and then continuously activate the phosphorylation level of signal activator 6 (STAT6), which promotes the polarization of macrophages to M2. The vulnerability index of plaque in Rb1-treated mice was significantly reduced compared to the control group. Besides, MMP-9, a pro-atherosclerosis cytokine secreted by macrophages, was also decreased significantly in atherosclerotic lesions of mice. Since the stability of atherosclerotic plaque is easy to rupture, Rb1 could be used as a promising drug to stabilize plaque and ameliorate the progression of atherosclerosis by regulating the polarization of macrophages.

TNF-α is a key cytokine in the inflammatory cascade and can promote the interaction between monocytes and ECs, thereby inducing the deposition of the extracellular matrix and then producing intracellular events, promoting the release of various cytokines, and increasing cell apoptosis [47,48]. TNF-α induces the production of ROS, leads to oxidative stress, and causes the activation of nuclear factor-κB (NF-κB). The JNK and p38 signaling pathways play a pivotal role in regulating gene expression. As previously reported by Zhou et al. [49], Rb1 can inhibit the expression of p38 and JNK, and then reduce the release of NF-κB-mediated inflammatory molecules and adhesion molecules, produce an anti-apoptosis effect and alleviate AS. These results for the first time indicated that Rb1 can significantly ameliorate TNF-α-induced HUVEC injury. In addition, Rb1 also inhibits fetal bovine serum-induced proliferation and TNF-α induced inflammatory responses through binding specifically to ERβ in VSMCs [50]; and it was the first study to prove that Rb1 possesses estrogenic properties. Changes of endothelial molecular function induced by homocysteine, the decrease in endothelial carbon monoxide synthase (eNOS), the inhibition of nitric oxide (NO) production, and the production of superoxide anion will further aggravate damage to the coronary artery, change the vasodilation and permeability of blood vessels, and increase the aggregation of platelets. Rb1 can improve the level of mRNA of eNOS, resist the reduction of eNOS induced by homocysteine, and reduce the production of superoxide anion to a certain extent, thereby providing a new treatment strategy for AS [51].

Nuclear factor erythroid-2-related factor2 (Nrf2) is an important nuclear transcription factor in the EC protection system and a potential pharmacological target for atherosclerosis [52]. When activated, Nrf2 can reduce inflammatory factors and Ox-LDL retention and inhibit adhesion events by inhibiting the regulation of the p38-VCAM-1 signaling pathway. Heme oxygenase-1 (HO-1) is the target gene of Nrf2 and plays an anti-inflammatory and anti-oxidant role in activated vascular endothelial cells (VECs), and it inhibits the migration of monocytes [53]. Fan et al. [54] studied the effect of Panax notoginseng saponins (PNS) and Rb1 on rats induced by high fat diet. In atherosclerotic rats, the relationship between Rb1 and Nrf2/HO-1 was investigated. First, after the formation of atherosclerosis induced by a cholesterol diet, PNS and Rb1 were applied to the treatment groups, respectively. Aortic histological changes in the animals were then observed *in vitro*, and the serum expression of related indexes, including SOD, NO and TNF-α, were assessed. The results showed that there were almost no plaques and fatty streaks on the aorta of rats after the PNS and Rb1 treatment, the levels of NO and SOD increased significantly after the Rb1 treatment, while the levels of TNF-α decreased, thus protecting ECs from oxidative damage. The antibodies of VCAM-1, p38 and HO-1 were used to determine the expression of the protein and the expression of HO-1 were increased while the VCAM-1 and p38 were decreased after the treatment of PNS and Rb1. They used anti-oxidant sulforaphane to demonstrate the same therapeutic effect as PNS and Rb1. Sulforaphane has been shown to interfere with the interaction with Keap1 to activate Nrf2 [55]. It was further confirmed that PNS and Rb1 treatment promoted the nuclear translocation of Nrf2 protein, which led to the increase of HO-1 level. Therefore, this report tells us that activating Nrf2 nuclear transcription can promote the activation of HO-1, which inhibit the ROS-TNF-α-p38-VCAM-1 pathway and reduce monocyte adhesion events.

Currently, Rb1 is one of the most studied ginsenosides in treating AS; Rb1 exhibits promising activities, and its regulation is mainly through mediating biological functions of macrophage, ECs, and VSMCs through multiple targets and signaling pathways. Indeed, Rb1 shows favorable therapeutic effects, such as anti-apoptosis, anti-proliferation, anti-inflammatory and anti-oxidant function; however, there are still several limitations needing further extensive study.

2.2. Ginsenoside Rd and its anti-atherosclerosis activity

The study of calcium channels is also very important for the regulation of atherosclerosis [56]. The abnormal function of transmembrane Ca^{2+} transport results in the increase of intracellular Ca^{2+}[Ca^{2+}]I, which is involved in the development of atherosclerosis. Transmembrane Ca^{2+} transport is mainly mediated through Ca^{2+} channels that are divided into voltage-dependent Ca^{2+} channels (VDCCs) and voltage-independent Ca^{2+} channels. In recent years, new roles of voltage-independent Ca^{2+} channels, storage manipulation Ca^{2+} channels (SOCCs) and receptor operated Ca^{2+} channels (ROCCs) in atherosclerosis have been studied [57]. In particular, hypercholesterolemia changes the content of calcium in the membrane. In atherosclerosis, the influx of calcium ions and the uptake of Ox-LDL by a scavenger receptor (SR-A) on the surface of macrophages are particularly important

Several reports have shown ginsenoside Rd as one of the main active components of ginseng. It can act as a voltage-independent calcium channel blocker and has good therapeutic effects on the improvement of disease, such as cardiovascular and

cerebrovascular diseases [58]. Ginsenoside Rd can improve injuries to mouse neurons, further demonstrating the potential of voltage-independent calcium channel blockers as novel neuroprotective drugs to prevent neuronal apoptosis and death induced by cerebral ischemia [59].

An animal experiment was carried out by Li et al. [60], and changes of blood lipid protein were observed in the treatment group and the control group. The plaque area of mice treated with a high-fat diet increased significantly, whereas significant changes were not observed in the propylene glycol treatment group, and the plaque area of the mice treated with ginsenoside decreased significantly. Ginsenoside Rd was found to significantly inhibit the uptake of Ox-LDL, and Ca^{2+} induced by OAG and thapsigargin entered the macrophages of Apo $E^{-/-}$ mice. The degree of Ca^{2+} entering the cells was measured by Mn^{2+} quenching rate technology, and the content entering the cells was significantly reduced after the Rd treatment.

The expression of SR-A in the macrophages of RAW264.7 mice was also measured in an experiment to determine Ca^{2+} entry. With the increase of Ox-LDL treatment time, Ox-LDL increased Ca^{2+} entry and intracellular cholesterol levels. A positive correlation was observed between the calcium entry increase and cholesterol level. Treatment with ginsenoside Rd led to the simultaneous inhibition of Ox-LDL-induced Ca^{2+} entry and cholesterol accumulation. Ginsenoside Rd can reduce the entry of Ca^{2+} through a voltage-independent Ca^{2+} channel, thereby inhibiting the activity and expression of SR-A and the uptake of ox-LDL by SR-A, which reduces the formation of foam cells and the area of plaque formation. This review synthesizes the results of *in vivo* experiments and showed that ginsenoside Rd may reduce plaque formation by inhibiting voltage-independent calcium ion channels. *In vitro* experiments indicate that Rd reduces cholesterol uptake by macrophages and effectively alleviates atherosclerosis.

These results provide a direction for us to further study the relationship between calcium channels and AS. This report provides us with a therapeutic target for voltage-independent calcium channels, and ginsenoside Rd is expected to be effective in the treatment of AS.

2.3. Ginsenoside F1 and its anti-atherosclerosis effect

Zinc finger protein A20 (A20) is tumor necrosis factor-alpha inducible protein 3. Zinc finger protein is a transcription factor with a finger-like domain, and it plays an important role in cell differentiation, embryo development, and other life processes. As previously reported in the literature, A20 acts as an inhibitor of NF-κB to regulate anti-inflammatory effects in multiple cells [61].

Ginsenoside F1 (GF1) is derived from the dried roots of Panax quinquefolium, and can be obtained from the hydrolysis and metabolism of Rg1 and Re. Similar to other active components, GF1 also has anti-aging, anti-inflammatory and other pharmacological activities [62–65]. Thus, whether it has a unique regulatory role in the treatment of AS should be explored.

Qin Meng et al. used Apo $E^{-/-}$ mice to explore the A20-mediated NF-κB signal pathway of GF1 to verify the connection with AS [66]. They found that GF1 improves the expression of A20 and A20 inhibits nuclear translocation of NF-κB, which in turn prevents a series of inflammatory responses mediated by NF-κB and inhibits the pathogenesis of atherosclerosis. In this study, they found that GF1 reduced the plaque area inflammatory and adhesion factors expressed in mice, as well as the apoptosis of ECs. Further research showed that Ox-LDL significantly suppressed the expression of A20, which could be recovered after GF1 treatment. Meanwhile, A20 knockdown can markedly abolished the attenuation of NF-κB nuclear translocation and inflammatory factors induced by GF1. Hence, these evidence provided that GF1 can reduce the inflammation of ECs and prevent AS by inhibiting the A20-mediated NF-κB pathway. Collectively, GF1 is expected to be developed as a new promising drug, which will lead to huge benefits to society.

2.4. Ginsenosides Rg1, Rg2 and Rg3 and their anti-atherosclerotic effects

Ginsenoside Rg, including Rg1, Rg2 and Rg3, has been widely used in cardiovascular and cerebrovascular diseases due to its unique therapeutic characteristics [67–69].

Ginsenoside Rg1 can enhance the autophagy-related protein expression of macrophages and reduce the expression of apoptotic protein through the AMPK/mTOR pathway, thereby reducing macrophages apoptosis and enhancing autophagy [70], which helps maintain macrophages function in advanced atherosclerosis and protect lesions [71]. Besides, Rg1 can also inhibit VSMCs senescence as well as vascular intimal hyperplasia via suppressing VSMC proliferation [72,73].

Lipopolysaccharides (LPS) can induce the expression of adhesion molecules in human umbilical vein endothelial cells. VCAM-1, ICAM-1 and E-selectin play an important role in the development of AS [74]. Cho et al. [75] studied the effect of ginsenoside Rg2 on ECs. The expression of adhesion molecules in human umbilical vein blood cells was stimulated by 1 μg/ml LPS and the treatment of Rg2 was conentration-dependen in this report, finally, the cell experiment was carried out with 50 μM Rg2. Surprisingly, it provided direct protection of vascular inflammation by inhibiting the adhesion between leukocytes and the vascular wall. Rg2 can reduce the adhesion between monocytes and ECs by inhibiting the NF-κB pathway mediated the expression of VCAM-1, ICAM-1 and slow down the pathogenesis of atherosclerosis. Ginsenoside Rg2 is expected to be developed for the treatment of AS.

Macrophages play an important role in the development of AS [76]. Arterial plaque in diabetic patients is unstable, and hyperglycemia can easily lead to the negative polarization of macrophages and inflammation. Ginsenoside Rg3 is a natural ligand of PPARγ, and diabetic $ApoE^{-/-}$ mice treated with Rg3 showed a decrease of M1 macrophages and an increase of M2 anti-inflammatory cells. The combination of PPARγ antagonist GW9662 can block the polarization of M2, and the inflammatory response is aggravated again. Therefore, it is clear that Rg3 can promote the polarization of M1 to M2 via targeting to PPARγ. Moreover, Rg3 can suppress the development of AS by reversing the effects of advanced glycation end products (AGEs) on the polarization of macrophages. Rg3 abated the pro-inflammatory effects (TNF-α and IL-6) of AGEs and promoted the secretion of anti-inflammatory cytokines (IL-10 and TGF-β). Encouragingly, Rg3 could enhance plaque stability by regulating macrophages (MOMA2), the phenotypic transformation of VSMCs, and collagen expression in ApoE−/− mice. Hence, these data supported that Rg3 might be developed as a potential therapeutic drug for treatment of AS.

In brief, Rg1 and Rg3 have therapeutic effects on AS via mainly targeting macrophages and VSMCs, while Rg2 was proved to inhibit inflammation of AS by mediating monocytes-ECs adhesion. Specifically, Rg3 also has promising therapeutic effects on AS combined with diabetes. However, the underlying mechanisms are still not fully clear and are should be further analyzed.

2.5. Compound K and its anti-atherosclerosis effect

Compound K (CK) is a 20(S)-protopanaxadiol ginsenoside and the metabolite of Rb1 in the gastrointestinal tract. It has a variety of pharmacological activities. Therefore, the effects of CK on AS have been studied in depth. CK plays an important role in the

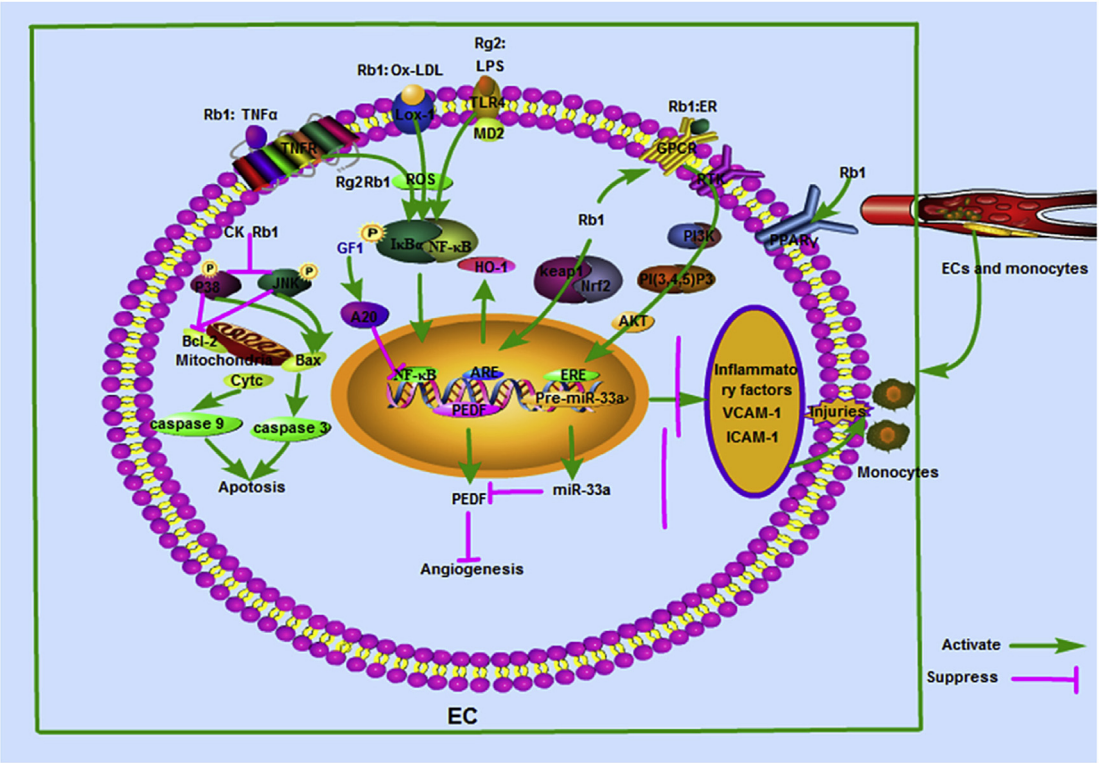

Fig. 1. The regulatory mechanisms of ginsenosides in HUVEC. (1) Compound K(CK) and ginsenoside Rb1 (Rb1) similarly regulate the expression of Bcl-2 by inhibiting the phosphorylation of p38 and JNK to regulate the role of mitochondria in anti-apoptosis induced by TNFα or by Ox-LDL. (2) Ginsenoside F1 improves the expression of A20 by targeting A20 when induced by Ox-LDL, which inhibits the inflammatory factors secreted by the NF-κB pathway and cell adhesion molecules, suppresses the adhesion of monocytes to ECs, and inhibits the inflammatory response. (3) Ginsenoside Rg2 could inhibit the inflammatory factors secreted by the NF-κB pathway and cell adhesion molecules when induced by LPS. (4) Rb1 can active the Nrf2-HO-1 pathway and then suppress the ROS-VCAM-1 pathway to protect from aggrevating AS. (5) Rb1 could activate the PI3K/Akt pathway by targeting GPER and then suppress the apoptosis and inflammation. (6) Rb1 can suppress the expression of miR-33a and then improve the expression of PEDF to alleviate the angiogenesis.

development of atherosclerosis [77]. Lee et al. [78] showed that HUVECs were injuried by NF-κB-mediated signaling pathway under the induction of TNF-α, the level of IκB after phosphorylation was increased, and the expression of adhesion molecules, integrin and matrix metalloproteinases were significantly increased. After treatment with 25 μM of CK, the adhesion and transmigration of monocytes mediated by NF-κB were inhibited. The treatment of HUVECs with 25 μM CK dampened the TNF-α-enhanced induction of VCAM-1, ICAM-1, and E-selectin. CK also significantly repressed the induction of α4/β1 integrin VLA-4 and αL/β2 integrin LFA-1 in TNF-α-treated THP-1 cells. Meanwhile, metalloproteinase-9 (MMP9), IL-8 and its receptor, and CXCR1 expression were blunted by CK, which encumbering the solid adhesion of monocytes to ECs. Thus, CK can reduce the release of integrin and adhesion factors, effectively block the transport and exosmosis of circulating monocytes, alleviate the adhesion between ECs and monocytes, and mitigate the development of atherosclerosis by inhibiting the NF-κB pathway induced by inflammatory factors.

In AS, the abnormal proliferation and migration of VSMCs caused by platelet derived growth factor-BB (PDGF-BB) accelerate pathological changes after arterial injury. Park Eun Seok et al [79] studied the relationship between CK and vascular smooth muscle injury. When VSMCs were treated with PDGF-BB, the proliferation and differentiation level of VSMCs were significantly improved, and the number of cells in the G0/G1, S, and G2/M phases was observed and compared. The results showed that the cell cycle progression was promoted and the content of cell cycle-related proteins was significantly increased. To verify the effect of CK on the improvement of VSMCs, CK was used to treat VSMCs, and the results indicated that it inhibited the synthesis and differentiation of G0/G1 phase RNA and related proteins, suppressed cyclin dependent kinase 2 (CDK 2), cyclin E, CDK4, cyclin D1 and proliferating cell nuclear antigen (PCNA), and inhibited synchronous cell growth. Park Eun Seok et al used the rat carotid balloon injury model to further verify the relationship between CK and neointima after VSMC injury. Treatment with CK significantly reduced the formation of neointima and inhibited the proliferation of cells in the injured area. *In vitro* experiments showed that CK reduced the destruction of the extracellular matrix by inhibiting the release of matrix metalloproteinase-2 (MMP-2) and MMP-9, weakened the migration of VSMCs to the intima to form neointima induced by PDGF-BB. Therefore, in vivo and *in vitro* experiments verified that CK can reduce the migration of VSMCs, inhibit the formation of neointima and alleviate the formation of atherosclerotic plaque by regulating the cell cycle. In addition, Ginsenoside Re also inhibited PDGF-BB induced VSMC proliferation via the eNOS/NO/cGMP pathway [80]. Collectively, these evidence provide promising pharmacological activities of Re to support the possible prevention and treatment of cardiovascular diseases targeting abnormal VSMC.

Ox-LDL acts on the human hemagglutinoid oxidized LDL receptor-1 (LOX-1) of ECs. LOX-1 can inhibit the protein kinase of NF-κB, which will improve the nuclear transcription of NF-κB to promote the production of apoptotic protein and reduce the expression of Bcl-2. In the experiment of Lu et al. [77], HUVECs were pretreated with 80 mg/ml Ox-LDL. The activity of cells decreased based on the MTT method and were then treated with 0.625 μM, 1.25μM, 2.5μM CK, successively. The activity of cells were increased significantly. CK has been shown to reduce the

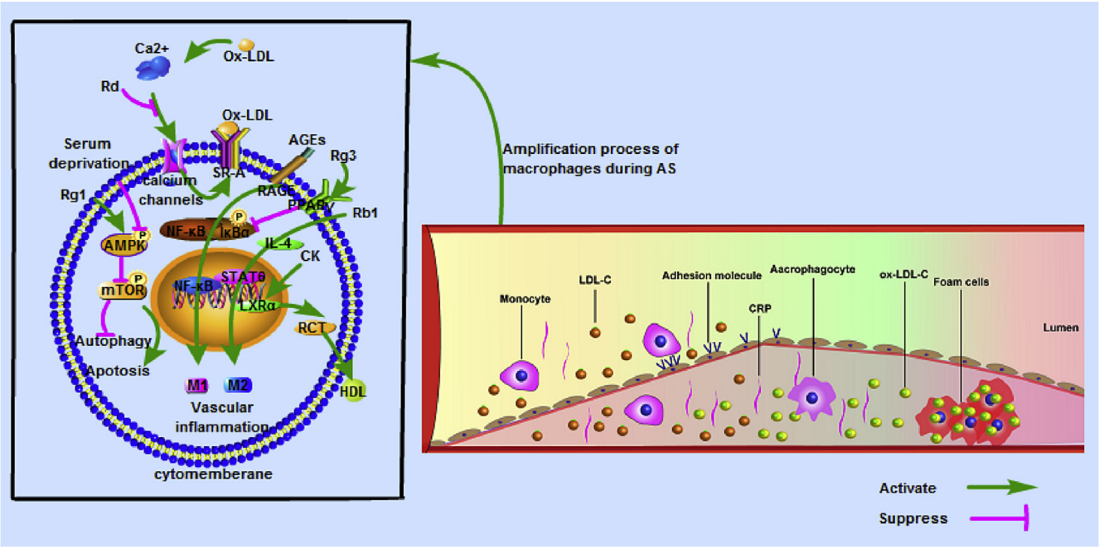

Fig. 2. The regulatory network of ginsenosides in macrophage. (1) Ginsenoside Rg1 (Rg1) activates autophagy in macrophages in serum-deficient environment through the AMPK/mTOR pathway, which suppresses apoptosis and improves the stability of late atherosclerotic plaques. (2) Ginsenoside Rd (Rd) suppresses the formation of foam cells by inhibiting calcium influx and reducing the uptake of Ox-LDLmacrophages. (3) Ginsenoside Rg3 (Rg3) promotes macrophages polarization to M2 by targeting PPARγ and inhibits nuclear transcription of NF-κB, which suppresses macrophage polarization to pro-inflammatory M1 and the release of inflammatory factors, and then alleviates inflammatory responses in diabetic atherosclerosis. (4) Ginsenoside Rb1 (Rb1) promotes the activation of stat6 by increasing the secretion of IL-4, prompting macrophages to polarize to M2 and play an anti-inflammatory role. (5) CK promotes the effect of RCT by activating the LXRα and suppresses the amount of cholesterol in cells.

expression of p38 and JNK-MAPK, thus inhibiting the nuclear transcription of NF-κB, reversing the response of the LOX-1-Iκκ-IκB−NF−κB signal pathway, reducing the production of apoptotic cells in endothelial cells, and effectively slowing down the progression of atherosclerosis.

In addition, Yan et al [81]synthesized CK to improve the solubility of CK to improve the bioavailability of CK after administration. CK has been reported to activate the Liver X receptor α (LXRα), ATP binding cassette (ABC) transporters, including ATP binding cassette transporter A1 (ABCA1), and promote reverse cholesterol transport by activating LXRα. Reverse cholesterol transport (RCT) regulates the reverse transport of cholesterol. ABCA1 plays an important role in RCT and is the speed limiting step, thereby reducing the content of total cholesterol in blood, reducing blood viscosity, and relieving AS [82]. Zhou et al. [83] also demonstrated that CK can active LXRα and attenuate the development of AS in ApoE−/− mice. CK over activation of LXRα receptor and increased expression of RCT and related proteins reduced macrophages forming foam cells, and reduced the expression of NLRP3, caspase-1 and nuclear NF-κB p65 protein in mouse aorta; thereby reducing the level of IL-1β, IL-6 and TNF-α in the serum and the plaque formation in the western diet of ApoE−/− mice. But unlike LXRα agonist, CK does not cause the increase of triglyceride and the degeneration of fatty liver in mice. Collectively, CK reduced the systemic inflammatory response of mice to alleviate AS.

In terms of the current reports, CK exhibits an excellent therapeutic effect on several stages of AS, including the adhesion of monocyte-ECs, proliferation and migration of VSMCs, cholesterol efflux in macrophages, the formation of foam cells, and inflammatory response. All of them were suppressed significantly by CK, indicated CK could be developed as a potential clinical agent with promising properties in the treatment of AS in future.

2.6. Other ginsenosides and their anti-atherosclerosis effect

Total Ginsenosides (TG) can suppress the rat carotid artery neointimal hyperplasia induced by balloon injury through enhancing the anti-oxidative action and the inhibition effects of NO/cGMP in VSMC [84]. Besides, Ginsenoside Rb3 significantly inhibited Ang II-induced VSMC proliferation by inhibiting the transition of G0⁄G1 to S phase and the expression of c-fos, c-jun, and c-myc [85].

3. Discussion

Atherosclerosis is the basis of a variety of fatal cardiovascular diseases. As a result of long-term poor eating habits and work and relaxation rules as well as the aging of the population, artery wall vessels gradually calcify, and the reduced pliability causes AS [86,87]. Patients with hypertension, hyperglycemia and hyperlipidemia have a high risk of occurrence and development of atherosclerosis and will gradually exhibit a variety of complications. All the risk factors form the foundation of coronary heart disease, myocardial infarction, and other cardiovascular diseases [7].

Ginseng has rich and diverse pharmacological effects and can improve a variety of diseases [88]. The total ginsenosides, which are hydrolytic extracts of ginseng, are the main active ingredients. The monomer ginsenosides separated from total ginsenosides present activities against cardiovascular disease. Studies on the mechanisms of atherosclerotic protection by ginseng have mostly focused on the action of ginsenosides (Figs. 1 and 2). Surprisingly, the therapeutic effect of these monomer ginsenosides is quite good. The effect of ginsenosides on some diseases can be well explained at the molecular and animal levels. Single ginsenoside can be used in the treatment of diabetes, and many scientists have elucidated the molecular mechanisms of ginsenosides in the treatment of diabetes [89]. Ginsenosides can be also used in the treatment of obesity, and it can better target the adipose cell as compared to other available medications, thus playing a great anti-obesity role [39]. Also, ginsenosides can facilitate vasoactive substances to promote NO production and alleviate symptoms in spontaneous hypertensive mice [90]. In addition, ginsenosides can also be used to alleviate ischemia-reperfusion injury [91]. The formation of

Table 1
The regulatory function and mechanisms of ginsenosides in the progression of AS

Compound	Effects	Experimental models	Ref
Rb1	downregulate eNOS and prevent AS	Porcine coronary arteries	[51]
	upregulate the expression of GPER and p-PI3K、p-Akt and Bax; improve the expreesion of Bcl-2	High cholesterol diet plus balloon catheter-injured rabbit model	[30]
	restore growth arrest specific gene 6 expression, suppress apoptosis and inhibits vascular calcification	Human vascular (aortic) smooth muscle cells	[31]
	attenuates apoptosis and enhance autophagy	ApoeE −/− mice	[34]
	suppress the expression of mir-33a, upregulate the expression of PDEF	HUVECs	[44]
	sensitize the STAT6 and promote the secretion of IL-4 and IL-13	macrophages from C57BL/6 mice	[46]
	suppress the expression of p38 and the secretion of VCAM-1	HUVECs	[49]
	activate Nrf2 increase of NO and SOD in serum, suppress the level of TNFα	Male Wistar rats	[54]
Rd	suppress the influx of Ca^{2+}	$ApoE^{-/-}$ and RAW264.7 cells	[60]
GF1	upregulate A20,decrease the transcription of NF-κB	male C57BL/6 mice and $ApoE^{-/-}$ mice	[66]
Rg1	activate the AMPK/mTOR signaling pathway.	The murine Raw264.7 macrophages	[70]
Rg2	downregulate the expression of VCAM-1, ICAM-1 and E-selectin; inhibit the pathway of NF-κB	HUVECs	[75]
Rg3	swith macrophages to the M2 Phenotype	Apo E−/− mice	[76]
CK	inhibit the expression of MMP-2 and MMP-9; attenuates neointimal hyperplasia	VSMCs and Rat carotid balloon injury	[78]
	inhibit the expression of VCAM-1 and ICAM-1, E-selectin; inhibit the NF-κB nuclear translocation	HUVEC and THP-1	[79]
	suppress NF-κB, P38 and JNK-MAPK pathways	HUVECs	[77]
	activate LXRα and improve the expression of ABCA1	HUVECs	[81]
	suppress the secretion of IL-1β、IL-6 and TNFα; activate LXRα	ApoE −/− mice	[83]

atherosclerotic plaque is the basis of many cardiovascular diseases with high mortality, and ginsenosides play a unique therapeutic role in alleviating the occurrence and development of atherosclerosis.

The cited research (Table 1) has provided information can be used to alleviate the occurrence and development of atherosclerosis based on the total ginsenosides and single ginsenosides, and the mechanisms underlying the anti-atherosclerosis activity of the ginsenosides Rb1, Rd, ginsenoside Rg, GF1 and the metabolite CK have been increasingly clarified. Although several ginsenosides exhibit potential therapeutic effect on AS, the involved cells and mechanisms are different which were discussed above. Besides, Rb1 has a strong anti-angiogenic effect on endothelial cells even at very low concentration (nanomolar level); while others can inhibit cellular inflammation and oxidative stress at micromolar level [30,44]. Rg3 can play a protective role in diabetic atherosclerosis [76], while other ginsenosides were not reported to be involved yet. In addition, Rd specially regulates the development of AS via calcium channel. Interestingly, differential binding modes of Rg3 stereoisomers in the PPARc-LBD contribute to differential PPARc activation at the cellular level [92]. Rg3, which contains 2 neighbouring hydroxyl groups near and on the chiral centre C-20, can act as a natural ligand of PPARc, whereas the PPARc agonist activity of 20(S)-Rg3 is 10 times stronger than that of 20(R)-Rg3 in angiogenesis assay.

Most of the associated research focuses either on the effect of total ginsenosides on the occurrence and development of a certain disease or on the investigation of a single active component of ginsenosides to determine its ability to regulate the disease by a certain pathway and to know whether these factors play a certain role in treatment and alleviation. Therefore, we need to further explore ginsenosides, and study the control of ginsenosides related to two or more pathways on the development of AS as well as to determine whether the total ginsenosides have a stronger therapeutic effect than a single active ingredient. Besides, many active components, such as ginsenoside Ro and ginsenoside Rh, remain and whether they have an active role in the treatment of AS has a clear therapeutic mechanism is still unclear. The complex interaction mechanisms of the active components of ginsenosides limits our understanding of the relationship between ginsenoside and AS, therefore, further studies are required on the therapeutic effects of different components of ginsenosides to develop more therapeutic targets and contribute to the development of new drugs for cardiovascular disease treatment. Moreover, the effect of ginsenosides on certain regulatory pathways is dose-dependent and time-dependent [93,94]. We can further explore the dose-effect relationship to obtain new drugs with high bioavailability [95]. Our researchers also can modify the structure-activity relationship of ginsenoside to develop new therapeutic drugs, which will provide more possibilities for the development of the medical industry.

In conclusion, ginsenosides have a promising effect on the treatment of cardiovascular diseases based on atherosclerosis, and they are especially important in ameliorating the occurrence and development of atherosclerosis, inhibiting the formation of atherosclerotic plaques, and effectively slowing down the pathogenesis of cardiovascular diseases. Definitely, ginsenosides provide new targets and ideas for the treatment of disease. However, our scientists still need to study ginsenosides rigorously due to existing scientific research problems to develop safe, efficient and low-toxicity drugs using natural active ingredients.

Conflicts of interest

The authors declare that they have no competing interests.

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Abbreviations

AS	Atherosclerosis
NF−κB	nuclear transcription factor-κB
Iκκ	IκB kinase
CK	Compound K
Ox-LDL	Oxidized low-density lipoprotein
DC	Dendritic cells

IL-13	Interleukin-13
TNF-α	tumor necrosis factor-α
IL-1β	interleukin-1β
VCAM-1	vascular cell adhesion molecule-1
ICAM-1	intercellular adhesion molecule-1
E-selectin	Endothelium-selectin
PPD	protopanaxadiol
PPT	protopanaxatriol
LOXα	liver x-nuclear receptor
GPER	G protein-coupled estrogen receptor
Gas6	growth arrest-specific gene 6
Apo E−/−	apolipoprotein E-deficient
PEDF	Pigment epithelium derived factor
HUVECs	Human umbilical vein endothelial cells
PPARγ	Peroxisome Proliferator Activator Receptor γ
STAT6	Signal transducer and activator of transcription-6
eNOS	endothelial carbon monoxide synthase
Nrf2	nuclearfactorerythroid-2-relatedfactor2
HO-1	Hemeoxygenase-1
VECs	vascular endothelial cells
SR-A	scavenger receptor A
A20	Zinc finger protein A20 (A20)
AGEs	advanced glycation end products
SOCCs	storage manipulation Ca2+ channels
ROCCs	receptor operated Ca2+ channels
GF1	Ginsenoside F1
Rg1	Ginsenosides Rg1
Rg2	Ginsenosides Rg2
Rg3	Ginsenosides Rg3
PDGF-BB	platelet derived growth factor BB
CDK 2	cyclin dependent kinase 2
PCNA	proliferating cell nuclear antigen
MMP-2	matrix metalloproteinase-2
MMP-9	matrix metalloproteinase-9
LOX-1	human hemagglutinoid oxidized LDL receptor-1
ABCA1	ATP binding cassette transporter A1
RCT	Reverse cholesterol transport
PCNA	proliferating cell nuclear antigen
TC	total cholesterol
LDL	low-density lipoprotein
TG	triglyceride
HDL	high-density lipoprotein
LXRα	Liver X receptor α
TG	Total Ginsenosides
NO	nitric oxide

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