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REVIEW

Anti-atherosclerotic effects and molecular targets of ginkgolide B from *Ginkgo biloba*

Weile Ye[a,b,c,†], Jiaojiao Wang[a,b,c,†], Peter J. Little[d,e], Jiami Zou[a,b,c], Zhihua Zheng[a,b,c], Jing Lu[f], Yanjun Yin[g], Hao Liu[g], Dongmei Zhang[b,c], Peiqing Liu[f], Suowen Xu[g,h,*], Wencai Ye[b,c,*], Zhiping Liu[a,b,c,*]

[a]*International Cooperative Laboratory of Traditional Chinese Medicine Modernization and Innovative Drug Development of Ministry of Education (MOE) of China, Jinan University, Guangzhou 510632, China*
[b]*Guangdong Province Key Laboratory of Pharmacodynamic Constituents of Traditional Chinese Medicine and New Drugs Research, College of Pharmacy, Jinan University, Guangzhou 510632, China*
[c]*State Key Laboratory of Bioactive Molecules and Druggability Assessment, Jinan University, Guangzhou 510632, China*
[d]*Pharmacy Australia Centre of Excellence, School of Pharmacy, University of Queensland, Woolloongabba QLD 4102, Australia*
[e]*Sunshine Coast Health Institute and School of Health and Behavioural Sciences, University of the Sunshine Coast, Birtinya QLD 4575, Australia*
[f]*National-Local Joint Engineering Lab of Druggability and New Drugs Evaluation, Guangdong Provincial Key Laboratory of New Drug Design and Evaluation, Sun Yat-sen University, Guangzhou 510006, China*
[g]*School of Pharmacy, Bengbu Medical College, Bengbu 233030, China*
[h]*Institute of Endocrine and Metabolic Diseases, the First Affiliated Hospital of USTC, Division of Life Sciences and Medicine, University of Science and Technology of China, Hefei 230026, China*

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Abstract Bioactive compounds derived from herbal medicinal plants modulate various therapeutic targets and signaling pathways associated with cardiovascular diseases (CVDs), the world's primary cause of death. *Ginkgo biloba*, a well-known traditional Chinese medicine with notable cardiovascular actions, has been used as a cardio- and cerebrovascular therapeutic drug and nutraceutical in Asian countries for centuries. Preclinical studies have shown that ginkgolide B, a bioactive component in *Ginkgo biloba*, can ameliorate atherosclerosis in cultured vascular cells and disease models. Of clinical relevance, several

*Corresponding authors.
E-mail addresses: zhiping0414@163.com (Zhiping Liu), sxu1984@ustc.edu.cn (Suowen Xu), chywc@aliyun.com (Wencai Ye).
[†]These authors made equal contributions to this work.
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Endothelial dysfunction;
LOX-1;
PCSK9;
PAF-R antagonist

clinical trials are ongoing or being completed to examine the efficacy and safety of ginkgolide B-related drug preparations in the prevention of cerebrovascular diseases, such as ischemia stroke. Here, we present a comprehensive review of the pharmacological activities, pharmacokinetic characteristics, and mechanisms of action of ginkgolide B in atherosclerosis prevention and therapy. We highlight new molecular targets of ginkgolide B, including nicotinamide adenine dinucleotide phosphate oxidases (NADPH oxidase), lectin-like oxidized LDL receptor-1 (LOX-1), sirtuin 1 (SIRT1), platelet-activating factor (PAF), proprotein convertase subtilisin/kexin type 9 (PCSK9) and others. Finally, we provide an overview and discussion of the therapeutic potential of ginkgolide B and highlight the future perspective of developing ginkgolide B as an effective therapeutic agent for treating atherosclerosis.

1. Introduction

Atherosclerotic cardiovascular diseases (CVDs) are the major cause of human morbidity and mortality globally[1−3]. Atherosclerosis is a chronic inflammatory disease induced by interactions between endothelial cells, smooth muscle cells, various kinds of circulating blood cells (*e.g.*, platelets, neutrophils, and monocytes), the immune system, and the peripheral nervous system[4−13]; these interactions lead to altered vascular cell function and the development of life-threatening atherosclerotic plaques[14]. Initially, endothelial dysfunction allows low-density lipoprotein cholesterol particles (LDL-C) to infiltrate into the vascular neointima, where they are trapped and retained by atherogenic proteoglycans with modified (hyper-elongated) glycosaminoglycan chains[15,16]. The trapped LDL-C undergoes oxidative modifications to become oxidized low-density lipoprotein (ox-LDL)[17]. Monocyte-derived macrophages take up those lipids to form foam cells and transition into the intima to form "fatty streaks" lesions[18,19]. Finally, with the formation of a necrotic core and a fibrous cap, the lesion develops into an atherosclerotic plaque; plaques can be labile or stable depending upon their composition. Once the high-risk, vulnerable plaque ruptures, it can form a fatal arterial thrombus, eventually provoking tissue ischemia diseases due to insufficient blood supply to vital organs[20]. Because of the aging of the population worldwide and unhealthy lifestyles, the prevalence of atherosclerosis is increasing[21,22]. Therefore, there is a great medical need to identify potential drugs to prevent and treat atherosclerosis.

Ginkgo biloba L. (Fig. 1), a valuable herbal preparation from the dried leaves of ginkgo trees, has a medicinal history of over 2000 years and has been considered a "living fossil". Its extract, which has been commonly used in preventing and treating multiple cardiovascular diseases clinically, has the traditional effects of promoting blood circulation, activating meridians to relieve pain, melting turbidity, and lowering lipids[23−26]. *Ginkgo biloba* standardized extract (EGb 761) mainly contains ginkgo flavonoids, biflavonoids, and ginkgolides, with the latter being the most characteristic component of *Ginkgo biloba*. At present, ten ginkgolides have been discovered from *Ginkgo biloba*[27−32], among which ginkgolide A (GA, Fig. 1C), ginkgolide B (GB, Fig. 1D), and ginkgolide C (GC, Fig. 1E) are the primary substances regulating cardiovascular effects in *Ginkgo biloba*. As one of the most pharmacologically active and representative compounds in ginkgolides, GB can prevent or slow atherosclerosis progression in preclinical *in vitro* and *in vivo* models of atherosclerosis, suggesting its therapeutic potential in cardiovascular and cerebrovascular diseases that involve atherosclerosis as the pathological basis[33−42].

Considering the expanding appreciation of the pharmacological profile of GB in cardio- and cerebrovascular medicine, especially in atherosclerosis, a comprehensive review of the antiatherosclerotic impacts and mechanisms of action of GB is needed. The purpose of this review is to highlight the therapeutic potential of GB in reducing atherosclerosis and its clinical sequelae (ischemia and strokes) by providing an overview of the protective benefits and molecular mechanisms of action of GB in preventing or alleviating atherosclerosis (Fig. 2).

2. Emerging mechanisms of action and cellular targets for the protection against atherosclerotic vascular disorders by GB

Accumulating evidence has indicated that GB, the natural substance from *Ginkgo biloba,* possesses atheroprotective actions[33,34]. In particular, GB exerts antiatherosclerotic effects in various relevant models, including umbilical vein endothelial cells (HUVECs)[37,39], human platelets[40] and ApoE$^{-/-}$ mice[33,34]. GB reduces atherosclerosis by altering the functions of multiple vascular cells (Fig. 2) and modulating molecular targets (Fig. 3) associated with atherogenesis. It is noteworthy that GB also has pharmacological effects on myocardial ischemia/reperfusion injury-provoked inflammatory responses in rats by modulating the A20−NF-κB signaling pathway[43], suggesting that GB possibly has extra therapeutic advantages in treating coronary atherosclerotic disorders.

The disease process of atherosclerosis is complex and involves several interconnected processes, such as endothelial cell dysfunction, platelet adhesion and activation, and leukocyte function and activation[14,44−47]. Upon biochemical insults to endothelial cells, the expression of inflammatory cytokines is enhanced, which attracts monocytes to congregate at the injury site[48−50]. By interacting with the endothelium, monocytes attach to the endothelium surface and promote further inflammation[51]. After moving into the neointima from the endothelium, monocytes develop into macrophages. By ingesting ox-LDL, these macrophages transform into foam cells, a key component in the development of atherosclerotic plaques[52]. Additionally, von Willebrand factor (vWF) and collagen, which trigger platelet adhesion, are exposed in injured endothelial cells[53,54]. For platelets to adhere to

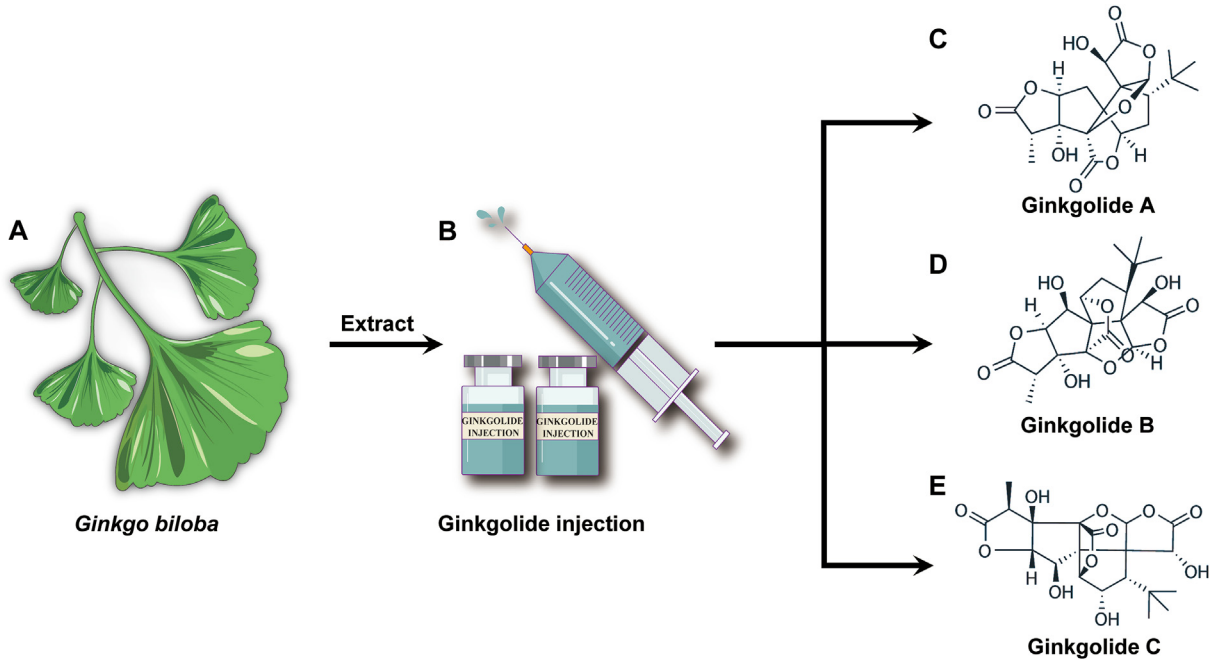

Figure 1 *Ginkgo biloba* L. and its major active ginkgolide compounds' chemical structures mediate cardiovascular protection actions. (A) *Ginkgo biloba* is a valuable herbal medicine from the dried leaves of ginkgo trees. (B−E) Ginkgolide injection is a traditional remedy for CVDs, and its main bioactive components are ginkgolide A (PubChem CID# 9909368), ginkgolide B (PubChem CID# 6324617) and ginkgolide C (PubChem CID# 24721502).

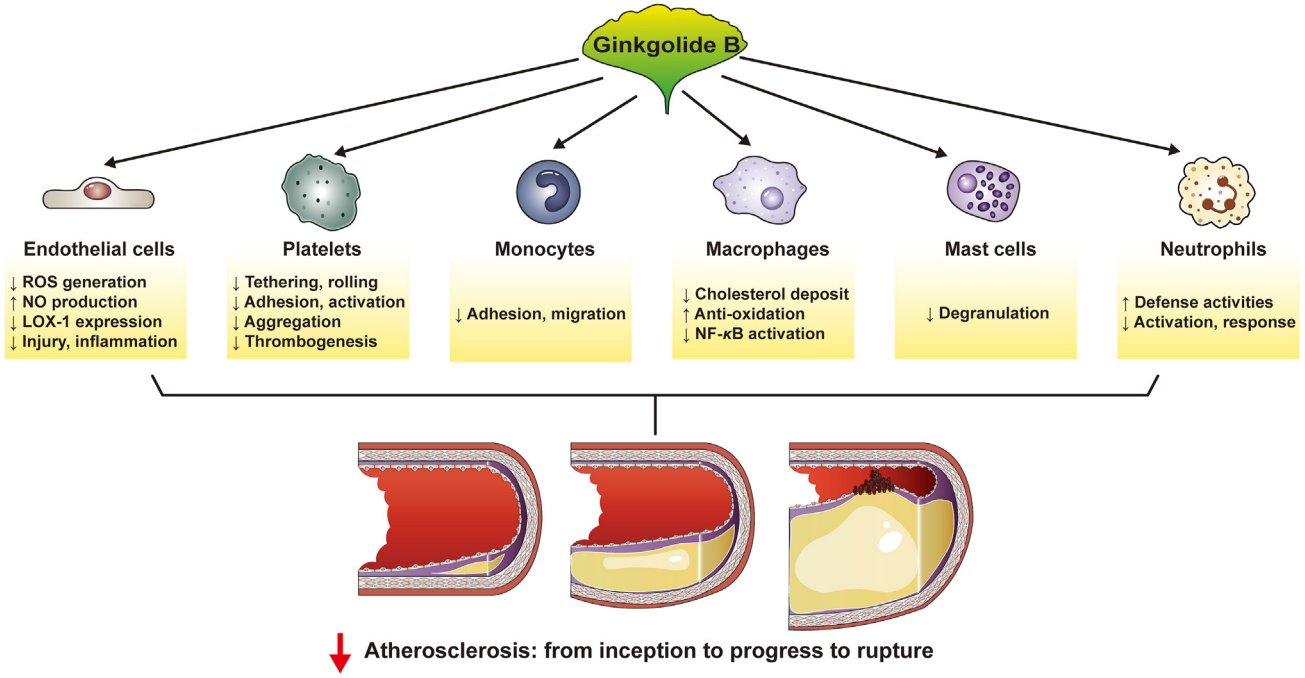

Figure 2 Ginkgolide B regulates important cellular processes in atherosclerosis. Ginkgolide B inhibits the progression of atherosclerosis *via* a variety of mechanisms. Ginkgolide B, in particular, corrects endothelial dysfunction, inhibits thrombin and platelet function, inhibits inflammation, reduces macrophage-derived foam cell formation, and so on.

the endothelium, vWF and collagen bind to GPIIb/IIIa receptors on the surface of platelets[45]. Once attached, platelets are activated and then release growth factors and proinflammatory substances while aggregating to form blood clots[55]. In addition to monocytes, other leukocytes in the circulation are also recruited into the vascular wall at sites of lipid accumulation, which further exacerbates plaque formation and worsens the development of atherosclerosis[56,57]. Therefore, multiple biochemical and cellular

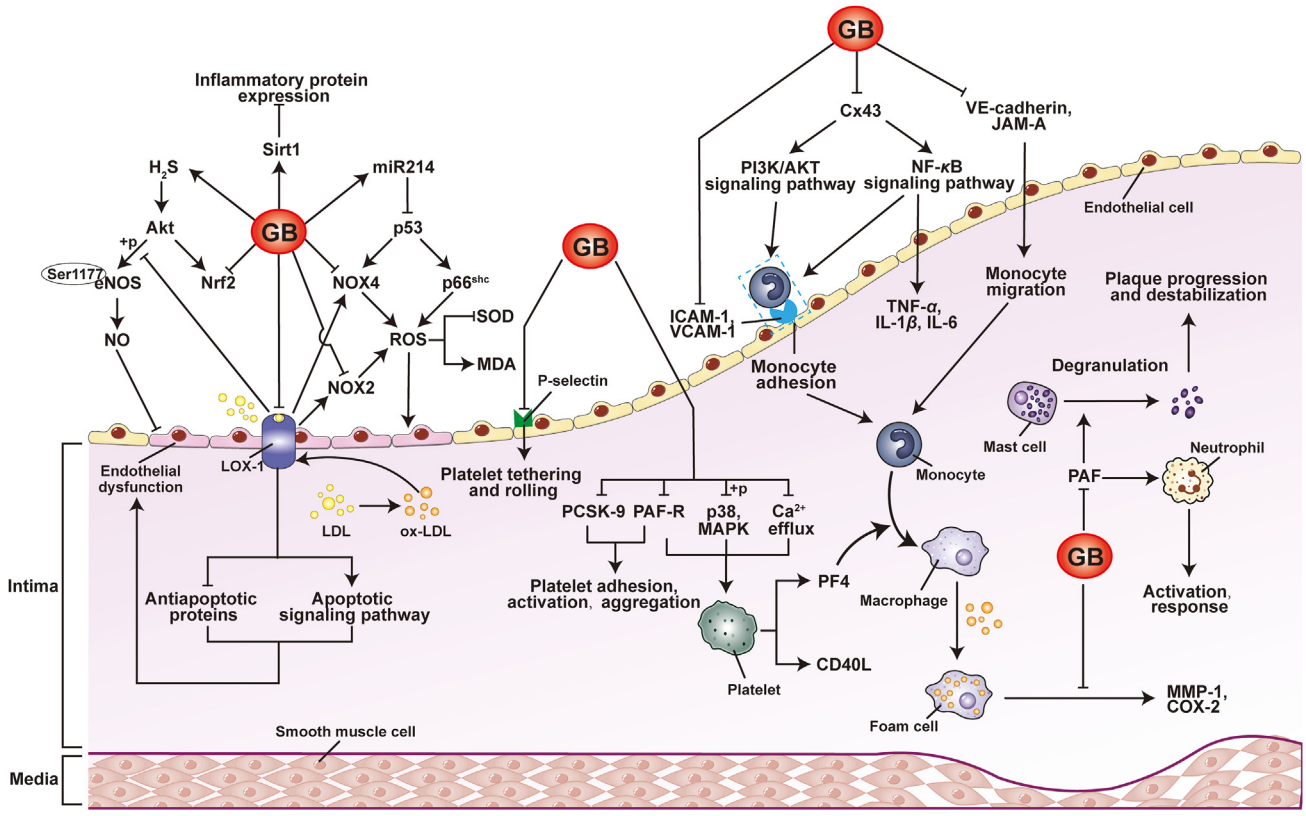

Figure 3 Novel vascular targets of ginkgolide B. Ginkgolide B regulates a range of therapeutic targets during atherosclerosis. Endothelial cells, platelets, monocytes, macrophages, neutrophils, and other cells targeted by ginkgolide B are summarized. The regulatory actions of ginkgolide B on various cellular targets indicate its therapeutic promise in atherosclerotic vascular disorders.

events can be addressed in the treatment and prevention of atherosclerosis to intervene in these steps and mitigate or even prevent the potentially devastating consequences of atherosclerosis and its clinical sequelae. It is worth noting that GB not only plays a protective role in endothelial injury, an early event in atherosclerosis, but also inhibits other links that promote the further development of atherosclerosis, including the adhesion and activation of platelets and the activation of leukocyte function. A key point herein is that GB could orchestrate the complex crosstalk between the injured endothelium, platelets, and leukocytes against atherosclerotic vascular disorders. To demonstrate the orchestration ability of GB more clearly, the parts that follow break these interconnected processes down and provide further details.

2.1. GB corrects endothelial dysfunction

The vascular endothelium, the innermost layer of blood vessels, not only plays a crucial role in regulating endothelium-dependent vasodilation, but also hinders platelet and leukocyte adherence to the artery wall and regulates thrombosis and inflammation[58]. Endothelial dysfunction, caused by free radicals such as reactive oxygen species (ROS), transforming growth factor-β[48,59], high ox-LDL levels, and other risk factors, is an early stage in the development of atherosclerosis.

One central mechanism of alleviating endothelial dysfunction is decreasing the expression and/or activity of nicotinamide adenine dinucleotide phosphate (NADPH) oxidases (NOXs)[60,61]. Abnormal NOX activity results in the overproduction of ROS, specifically superoxide ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2), which impair vascular function by damaging endothelial cells and subsequently promote further endothelial dysfunction[62]. Since therapeutic strategies based on scavenging ROS using antioxidants have been ineffective in delaying the development of arteriosclerosis in humans, blocking their enzymatic production by inhibiting NOXs may represent an effective strategy for restoring endothelial function and ultimately preventing or treating atherosclerosis[61]. These findings highlight the critical role of NOXs in the development of endothelial dysfunction and offer a promising avenue for new therapeutic approaches to combat atherosclerosis. Notably, due to its highly conserved histidine residue, NOX4 produces H_2O_2 rather than $O_2^{\bullet-}$ [63,64], and it is expressed at a higher level than the other four members of the NOX family[65]. In addition, a recent study[66] showed that endothelial NOX4 dysfunction aggravates atherosclerosis *via* endoplasmic reticulum stress, and more importantly, NOX4 was thought to be involved in the incidence and progression of human coronary atherosclerotic disease by increasing intracellular oxidative stress[67]. Furthermore, NOX4 overexpression was linked to smooth muscle cell growth inhibition, apoptosis, and senescence, factors that contribute to plaque destabilization[68]. Given these findings, the targeting of NOXs, especially NOX4, with innovative therapeutic agents may hold significant promise for the prevention and treatment of coronary artery diseases. Two independent studies[35,42] have demonstrated that GB has a pharmacological effect on intracellular oxidative stress in combination with a reduction in ROS by reducing NOX4 expression. Additionally, GB also significantly elevated SOD activity and decreased the expression of GPX1 protein and NOX2[69], indicating that GB has potential cardiovascular benefits conferred

by its antioxidant effect. Further mechanistic studies indicated that GB suppressed apoptosis and lowered superoxide production by reducing the expression of p53-regulated NOX4 and $p66^{Shc}$ [70]. Notably, NOX4-derived H_2O_2 could activate NOX2, boosting mitochondrial ROS (mtROS) generation *via* $pSer36\text{-}p66^{Shc}$ [71]. These findings suggest that the antioxidative mechanism of GB may operate through a complex interplay of the NOX4/NOX2/$pSer36\text{-}p66^{Shc}$/mtROS axis, which highlights the intricate and multifaceted nature of its therapeutic potential for cardiovascular disease. Although the precise antioxidant mechanism of GB is unclear, current research indicates that GB may help mitigate the pathological effects of oxidative stress by targeting this pathway, ultimately offering new avenues for the prevention and treatment of atherosclerotic vascular disorders.

NO, as a dissolved gas, is a key vascular protective factor of the endothelium and regulates many domains of atherosclerotic processes[72]. Thus, endothelial NO synthase (eNOS)-dependent NO production is another essential mechanism that counteracts endothelial dysfunction and atherosclerosis[73–75]. The expression of eNOS can be regulated by different mechanisms, principally through kinase-mediated multisite phosphorylation[76,77]. Among various phosphorylation sites, Ser1177 is one of the most studied sites associated with endothelial dysfunction[44,78,79], and inhibition of its phosphorylation attenuated the H_2S-stimulated increase in endothelial NO production[80]. Wang et al.[69] found that GB alleviated endothelial dysfunction in diabetic rats by increasing NO bioavailability and H_2S generation, the mechanism of which might be linked to eNOS Ser1177 phosphorylation. Additionally, GB had protective effects on the revascularization of blood vessels by promoting the proliferation and activity of endothelial progenitor cells from bone marrow *via* activation of the Akt/eNOS and mitogen-activated protein kinase (MAPK)/p38 signaling pathways[81], further confirming the regulatory effects of GB on eNOS-dependent endothelial function. These findings provide additional valuable insight into the molecular mechanisms underlying the counteracting actions of GB on endothelial dysfunction and atherosclerosis.

In addition to regulating the generation of ROS and NO, GB also protects the endothelium by downregulating lectin-like oxidized LDL receptor-1 (LOX-1) expression[41]. LOX-1 is an ox-LDL scavenger receptor with a critical role in endothelial dysfunction and the progression of atherosclerosis[82,83]. *In vitro*, ox-LDL-treated increased LOX-1 expression triggers endothelial cell apoptosis by reducing anti-apoptotic proteins (including c-IAP-1 and Bcl-2) and activating pro-apoptotic pathways (containing caspase-3 and caspase-9)[84], thereby causing a direct injurious biochemical effect on the endothelium. In addition to this direct effect on endothelial cells, LOX-1 knockdown dramatically lowered ROS levels, which was linked to decreased NOX2 and NOX4 expression[85]. Moreover, endothelial cell-specific overexpression of LOX-1 blunted eNOS-activating phosphorylation in $ApoE^{-/-}$ mice, suggesting that LOX-1 might induce endothelial dysfunction by reducing NO production[82]. Thus, targeting LOX-1 may be explored as a novel therapeutic method to correct endothelial dysfunction by influencing multiple targets. Interestingly, GB is capable of decreasing LOX-1 expression. A recent study[41] indicated that GB supplementation inhibited ROS production in ox-LDL-activated HUVECs by suppressing LOX-1 and NOX4 expression. In line with this result, Feng et al.[35] reported that GB improved endothelial dysfunction by targeting LOX-1, NOX4, monocyte chemotactic protein-1, intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and inflammatory cascade-associated markers. In ox-LDL-treated endothelial cells, GB not only suppressed LOX-1 expression but also increased the expression of Sirtuin-1 (Sirt1), a deacetylase with antiaging properties whose mechanism is tied to the inhibition of Akt activation[38]. Notably, the upstream kinase cyclin-dependent kinase 5 (CDK5) phosphorylates Sirt1, which promotes endothelial cell senescence and atherosclerosis[86]. Therefore, it will be intriguing to further study whether GB suppresses Sirt1 function in senescent endothelial cells and inhibits atherogenesis by affecting CDK5-mediated phosphorylation of Sirt1.

Although the protective effects of GB on the endothelium have not been extensively investigated, it is possible to speculate that it could act as a multitargeting agent to regulate endothelial function through a variety of mechanisms, including diminished ROS production derived from NOXs, enhanced eNOS-derived NO production, decreased expression of LOX-1, and upregulation of Sirt1 expression. The net result of these actions would be the maintenance of endothelial cell homeostasis. These initial findings concerning the regulation of endothelial function by GB shed new light on the molecular mechanisms that underpin the antiatherosclerotic potential of GB.

2.2. *GB alleviates thrombosis by inhibiting platelet activation*

Early atherosclerosis is caused by endothelial cell malfunction, and platelets adhere to these cells in vulnerable places[45,87]. After platelet adherence and aggregation, platelets are activated and secrete chemokines and cytokines, which leads to further aggravation of endothelial cell dysfunction and inflammation[88,89]. Of utmost significance, platelets are involved in atherosclerotic thrombosis, increasing the likelihood of vascular occlusion that may provoke life-threatening atherosclerotic complications[90–93], such as myocardial infarction and angina pectoris. In summary, the role of platelets in atherosclerosis is to promote endothelial dysfunction, inflammation, and thrombosis. The process by which platelets adhere to the vascular endothelium and promote thrombus formation can be divided into four stages: platelet tethering and rolling, platelet firm adhesion and activation, platelet aggregation, and ultimately thrombus stabilization. GB is an interesting molecule because it can inhibit all of these steps. Below, we explore these actions in detail.

2.2.1. *The effect of GB on platelet tethering and rolling*

The first step in thrombus formation is mostly regulated by activated platelets- and endotheliocyte-expressed P-selectin (CD62P), whose ligands are P-selectin glycoprotein ligand-1 (PSGL-1) and glycoprotein Ib (GPIb)[94–97]. P-selectin is involved in platelet aggregation as well as platelet and leukocyte rolling on activated endothelium[98–100]. Besides regulating platelet-endothelial cell interactions, P-selectin also has a major impact on platelet-leukocyte interactions[101]. Platelet-P-selectin binds to leukocyte-PSGL-1, forming platelet-leukocyte complexes that encourage the production of chemokines (including CCL2 and CCL5) and cytokines [such as interleukin-1β (IL-1β)], ultimately further activating leukocytes and promoting atherosclerosis[102].

Despite the complex nature of thrombus formation, there is promise in treating thrombosis and atherosclerosis by blocking P-selectin/PSGL-1 interactions. For example, injection of monoclonal antibodies against P-selectin or PSGL-1 holds potential for treating thrombosis and atherosclerosis[103]. In aortic plaques in $ApoE^{-/-}$ mice, GB attenuated atherosclerotic lesions and

P-selectin by possibly regulating the PI3K/Akt pathway, and interestingly, its effect was similar to that of aspirin, the most promising antiatherosclerosis agent for preventing thrombosis[33]. A study[104] has shown that GB is an inhibitor of P-selectin translocation, which suggests that GB inhibits the transfer of P-selectin from Weibel-Palade bodies of ECs to the cell surface during inflammation. The studies reveal a crucial new insight into the effects of GB on thrombosis, specifically that GB possesses an inhibitory effect on the initial step of thrombosis—the tethering and rolling of platelets—by attenuating P-selectin translocation. These results shed light on one of the mechanisms of action of GB in alleviating thrombosis. Furthermore, there exists a gap in knowledge in this area regarding the potential inhibitory effect of GB on platelet-leukocyte interactions through its regulation of P-selectin translocation. Further research in this area of vascular biology would provide a deeper understanding of the pharmacological and potential therapeutic actions of GB.

2.2.2. *The effect of GB on platelet adhesion and activation*

Platelet adhesion to endothelial cells requires activation of integrins (crucially platelet $\alpha_{IIb}\beta_3$ integrin) on the surface of both cell types[105–107], and adhesion leads to platelet activation. As the major integrin in platelets, $\alpha_{IIb}\beta_3$ is referred to as the glycoprotein GPIIb/IIIa (CD41/CD61) complex, which can bind to vWF, fibrinogen, and other ligands[108], showing that platelet activation is a multifactorial process. Notwithstanding the strong inhibitory effects of GB on platelet adhesion[33,39,109], its mechanism of action is unclear. Since GBE preparations can reduce the level of vWF in plasma[110,111], it can be speculated that GB might inhibit platelet adhesion by reducing the binding of vWF to $\alpha_{IIb}\beta_3$. Nevertheless, it remains to be explored whether GB can modulate vWF *in vivo* and whether such regulation can inhibit platelet adhesion in animal models of relevant vascular diseases.

Known as a natural product antagonist of platelet-activating factor receptor (PAF-R)[112], GB exerts a strong inhibitory effect on inflammation and platelet activation by inhibiting the elevation of PAF levels and reducing the interaction/binding of PAF with PAF-R[113], for which the IC_{50} value of GB is 0.273 μmol/L[114]. PAF is a powerful inflammatory mediator that is involved in a series of diseases caused by inflammatory pathways in combination with PAF-R[115,116]. Other biomolecules, such as ox-LDL and lipopolysaccharide, can exhibit PAF-like activity by serving as ligands/activators of PAF-R[117,118], indicating that ox-LDL formed in the endothelium could bind PAF-R and induce inflammation. Consistent with this notion, GB inhibits the PAF-induced cascade in inflammatory reactions[119] and substantially suppresses inflammation caused by lipopolysaccharide by acting on the PAF-R downstream signaling pathway[120]. Additionally, PAF promotes platelet activation[121], while GB pretreatment antagonizes this effect[122,123]. Multiple pathways contribute to platelet activation[124], and a recent study[55] demonstrated that proprotein convertase subtilisin/kexin 9 (PCSK9) promotes platelet activation and thrombosis in combination with CD36, an effect that is abolished by PCSK9 inhibitors or aspirin. PCSK9 expression was increased in ox-LDL-exposed HUVECs, whereas GB downregulated this elevation in mRNA and protein levels, which was verified both *in vitro* and *in silico*[41]. This evidence implies that GB might also suppress platelet activation and thrombosis by inhibiting PCSK9 levels in addition to PAF blockade. Overall, GB demonstrates potential as a multitarget therapeutic agent with the ability to inhibit PAF-induced inflammatory injuries, platelet activation, and thrombosis through its multifunctional mechanisms.

2.2.3. *The effect of GB on platelet aggregation and thrombogenesis*

GB-mediated inhibition of platelet aggregation is another key point in thrombus prevention. PAF exerts a stimulatory effect on platelet aggregation in rabbits and dogs, leading to changes in circulatory flow at the site of arterial stenosis and endothelial injury, and PAF-stimulated platelet aggregation is inhibited by GB treatment[125]. A previous study[40] showed that GB inhibited platelets from releasing platelet factor 4 (PF4) and CD40 ligand (CD40L), the mechanism of which is related to the suppression of Syk and p38 MAPK phosphorylation, as well as calcium efflux, and the blocking of PAF-R[40]. Platelet-released PF4 plays a role in thrombogenesis, and PF4 neutralization is a major part of the anticoagulant action of heparin[126]. Studies[127,128] have shown that PF4 acts as a monocyte chemokine to induce monocytes to differentiate into macrophages. Furthermore, platelet CD40L mediates thrombogenesis and inflammation in atherosclerosis by enhancing platelet-leukocyte and leukocyte–endothelial interactions, apart from platelet–platelet interactions[129]. Given the abovementioned evidence, it is feasible that GB, as well as a direct inhibitory effect on platelet aggregation and thrombosis, could have an indirect suppressive effect on atherogenesis by inhibiting leukocyte–endothelial interactions, monocyte differentiation into macrophages, and subsequent foam cell formation.

2.3. *GB inhibits leukocyte action in atherosclerosis*

Increasing evidence[130,131] has shown that leukocyte subsets aggregate in different stages of atherosclerotic lesions and take part in pathological reactions during the formation of atherosclerosis, supporting the concept that atherosclerosis is a chronic inflammatory disorder. Endothelial damage and dysfunction lead directly to leukocyte infiltration. Once bound to endothelial cells, leukocytes migrate from the endothelium to atherosclerotic plaques under the action of various chemokines[132]. During the process of leukocyte recruitment, the expression of ICAM-1 and VCAM-1 are two major determinants[133], which participate jointly in multiple steps that mediate leukocyte–endothelial cell interactions, including slow rolling, crawling and arrest[134]. Many studies[135–137] have indicated that blocking the adhesion molecules ICAM-1 and VCAM-1 reduces atherosclerotic lesions, and these two molecules provide potentially valuable targets for therapeutic interventions in atherosclerosis. Interestingly, evidence shows that GB is effective in inhibiting both ICAM-1[35,38] and VCAM-1[41,138]. The effect of GB on ICAM-1 action has been confirmed in other studies[139–141], which showed that GB reduced ICAM-1, TNF-α, IL-1β, and IL-6, probably related to blocking NF-κB activation[36]. However, although GB has been shown to downregulate VCAM-1 levels in TNF-α-treated HUVECs, the underlying mechanism of action has not yet been further explored. Overall, the study results above revealed a new mechanism of action of GB in protecting against leukocyte invasion and inflammation by suppressing both ICAM-1 and VCAM-1 under diseased conditions. Not only does GB regulate both of these targets, but it has also been shown to impede the entry of leukocytes into the intima or directly regulate a variety of leukocytes, such as monocytes, macrophages, mast cells, and neutrophils, *via* action on different molecular targets (see Table 1). Given the critical role of leukocyte subsets in the beginning and developing stages of atherosclerotic lesions, it is possible that GB has an impact on atherosclerosis by favorably modulating leukocyte functions.

Table 1 The effects of GB on leukocyte function.

Leukocyte subset	Target modulated by GB	Influence of GB on leukocyte subsets in atherosclerosis
Monocytes	VCAM-1, VE-cadherin, Cx43	GB suppressed monocyte adhesion in TNF-α-stimulated HUVECs under varied degrees of laminar shear stress[39]
	JAM-A, Cx43, and VE-cadherin	GB inhibited junction protein expression and decreased monocyte transmigration in ox-LDL-treated HUVECs[37]
	VCAM-1, ICAM-1	GB suppressed THP-1 monocyte adhesion to TNF-α-induced HUVECs in a dose-dependent manner[142]
Macrophages	LOX-1, NOX4, MMP-1, COX-2	GB decreased cholesterol deposits and showed atherosclerotic protective property[35]
	iNOS, NO	GB antagonized homocysteine-stimulated iNOS-mediated NO production in macrophages through antioxidation and attenuating NF-κB activation[143]
Mast cells	PAF-R	GB pretreatment significantly reduced the increase of mast cell degranulation in the rat isolated omentum stimulated by adenosine analogue[144]
	PAF-R	Pretreatment with GB observably decreased PAF's degranulating actions in omental mast cells while significantly reducing PAF's histamine-releasing impact on peritoneal mast cells[145]
Neutrophils	PAF-R	GB completely inhibited PAF-induced neutrophil response and subsequent neutrophil-mediated endothelial injury[146]
	$[Ca^{2+}]_i$, PAF-R	GB stimulated intracellular signaling events in neutrophils through tyrosine phosphorylation of proteins, phosphorylation of P38 map kinase, calcium transience, and phospholipase D activity, thus enhancing neutrophils' defense activities[147]
	PAF-R, CD18, CDllb, superoxide anion	Neutrophils pretreated with GB reduced plasma-mediated neutrophil stimulation after myocardial infarction in the presence of high plasma PAF levels[148]
	PAF-R	GB specifically suppressed neutrophil activation by blocking the binding of PAF to its receptor on neutrophil[149]
	PAF-R	GB inhibited PAF-induced chemotaxis of neutrophils[150]

2.3.1. *The effects of GB on monocytes*

In the process of atherogenesis, after adhering to endothelial cells, monocytes migrate to the intima and differentiate into macrophages. Subsequently, monocyte-derived macrophages take up ox-LDL, leading to foam cell development. As shown in Table 2, GB had a suppressive impact on the TNF-α/ox-LDL-stimulated elevation of ICAM-1 and VCAM-1 in HUVECs. In addition to these two targets, GB can also affect monocytes by regulating other entities, such as connexin 43 (Cx43), junctional adhesion molecule (JAM-A), and vascular endothelial cadherin (VE-cadherin). As one of the most significant major initiators of atherosclerosis, monocyte-endothelial cell adhesion can not only be modulated by VCAM-1 and ICAM-1 expression, which is regulated by endotheliocyte-expressed Cx43[151] but also be rapidly decreased by monocytic Cx43 channel function through ATP release and conversion to adenosine[152]. In 2019, a study found that Cx43 expression in monocytes could attenuate cell adhesion by activating the PI3K/AKT/NF-κB signaling pathway[153]. In terms of JAM-A, a previous study[154] reported that inhibition of endothelial JAM-A expression inhibited the inflow of monocytes into the artery wall and limited the development of atherosclerotic lesions. Furthermore, Allingham et al.[155] discovered that ICAM-1-mediated VE-cadherin phosphorylation on tyrosines 658 and 731 contributes to leukocyte transendothelial migration. These findings highlight junction proteins as critical control targets of leukocyte adhesion and migration, underscoring their therapeutic promise as potential targets to treat various inflammation-related diseases. Interestingly, recent evidence[37] has shown that GB substantially reduced monocyte migration by inhibiting JAM-A, Cx43, and VE-cadherin expression, and the mechanism of GB-induced inhibition of these proteins was connected with inhibition of the PI3K/Akt pathway. These studies provide yet another basis for the potential of GB in the treatment of atherosclerotic CVDs and reveal the importance of targeting monocyte adhesion and migration in the treatment of inflammation-related diseases, including atherosclerosis.

2.3.2. *The effect of GB on macrophages*

As a major inflammatory cell type, macrophages derived from monocytes are critical in the progression of atherosclerosis. The progression of atherosclerosis is marked by two main events, namely, macrophage infiltration and macrophage-derived foam cell production[156,157]. Therefore, pharmacological intervention, which targets the inhibition of macrophage inflammation and its behavior and function, should be regarded as a potential therapy for atherosclerosis. An earlier study[143] showed that homocysteine, an independent risk factor for atherosclerotic disease[158], stimulated iNOS-mediated NO production in macrophages at pathophysiological concentrations, while GB blocked this action through antioxidant properties and reduced NF-κB activation. Subsequently, Feng et al.[35] reported that GB also exhibited atheroprotective properties by inhibiting the upregulation of MMP-1 and cyclooxygenase-2 (COX-2) expression in RAW264.7 macrophages. The increased expression of MMP-1, contributing to plaque expansion and fibrous cap destruction, has been associated with intraplaque hemorrhage in macrophages in the plaque area[159]. For COX-2, an earlier study[160] provided pharmacological and genetic proof that COX-2 stimulated the early formation of atherosclerotic lesions in LDL receptor-deficient mice. The aforementioned research illuminates the potential of GB as a promising therapeutic strategy for inhibiting the conversion of monocytes into macrophages, as well as for modulating macrophage inflammation and behavior, thus highlighting its potential as an effective intervention for preventing the progression of

Table 2 Pharmacokinetic properties of GB and its preparations.

Species	Dose	Pharmacokinetic parameter of GB	Ref.
Humans	3 EGb 761™ tablets (the amount of GB is 836.46 μg)	C_{max} = 10.90 ± 5.59 ng/mL T_{max} = 2.00 ± 1.63 h AUC_{last} = 37.54 ± 17.35 ng/mL·h AUC_{tot} = 71.73 ± 26.98 ng/mL·h $\%AUC_{extra}$ = 38.10 ± 12.20 $t_{1/2}$ = 3.94 ± 1.42 h	Karin et al.[187]
	90 drops (2.73 mL) Geriaforce™ tincture (the amount of GB is 147.45 μg)	C_{max} = 1.45 ± 0.98 ng/mL T_{max} = 0.88 ± 0.38 h AUC_{last} = 2.99 ± 3.22 ng/mL·h AUC_{tot} = 6.17 ± 3.77 ng/mL·h $\%AUC_{extra}$ = 20.53 ± 12.05 $t_{1/2}$ = 2.34 ± 0.81 h	
	4 Ginkgo fresh plant extract tablets (the amount of GB is 524.56 μg)	C_{max} = 4.61 ± 2.87 ng/mL T_{max} = 2.35 ± 2.27 h AUC_{last} = 15.45 ± 13.44 ng/mL·h AUC_{tot} = 29.86 ± 25.08 ng/mL·h $\%AUC_{extra}$ = 43.48 ± 27.64 $t_{1/2}$ = 4.01 ± 2.58 h	
Beagle dogs	20 mg/kg conventional GB tablet	AUC_{0-t} = 236.2 ± 81.14 μg/L·h $AUC_{0-\infty}$ = 246.8 ± 92.08 μg/L·h MRT_{0-t} = 5.65 ± 3.42 h $MRT_{0-\infty}$ = 6.58 ± 3.54 h $t_{1/2}$ = 4.45 ± 3.04 h T_{max} = 3.73 ± 1.21 h CL_z/F = 90.94 ± 33.97 L/h/kg V_z/F = 560.2 ± 405.4 L/kg C_{max} = 66.64 ± 29.30 μg/L	Zhao et al.[188]
	20 mg/kg GB capsule made from hot-melt extrusion technology	AUC_{0-t} = 606.7 ± 125.03 μg/L·h $AUC_{0-\infty}$ = 672.0 ± 59.89 μg/L·h MRT_{0-t} = 4.18 ± 1.55 h $MRT_{0-\infty}$ = 8.75 ± 7.63 h $T_{1/2}$ = 4.14 ± 1.81 h T_{max} = 0.80 ± 0.18 h CL_z/F = 29.96 ± 2.70 L/h/kg V_z/F = 405.7 ± 398.5 L/kg C_{max} = 309.2 ± 106.0 μg/L	
	20 mg/kg GB pellet created using liquid layer technology	AUC_{0-t} = 419.1 ± 55.58 μg/L·h $AUC_{0-\infty}$ = 438.9 ± 71.14 μg/L·h MRT_{0-t} = 3.53 ± 1.77 h $MRT_{0-\infty}$ = 4.30 ± 2.38 h $t_{1/2}$ = 3.14 ± 1.80 h T_{max} = 1.20 ± 1.04 h CLz/F = 46.68 ± 8.65 L/h/kg Vz/F = 216.1 ± 132.4 L/kg C_{max} = 192.4 ± 84.22 μg/L	
Beagle dogs	80 mg/kg EGb	C_{max} = 16.23 ± 8.39 ng/mL t_{max} = 0.25 ± 0.37 h AUC_{0-t} = 19.86 ± 6.33 ng/mL·h $AUC_{0-\infty}$ = 20.49 ± 6.26 ng/mL·h MRT = 2.03 ± 0.26 h F = 33.70%	Chai et al.[191]
Rats	40 mg/kg EGb	C_{max} = 13447.10 ± 4137.57 ng/mL T_{max} = 2.08 ± 1.02 h $t_{1/2}$ = 3.86 ± 3.53 h AUC_{0-t} = 82680.76 ± 22792.87 ng/mL·h	Guan et al.[192]
Rats	3 mg/kg total ginkgolides for 7 days	$C_{5\ min}$ = 4608.3 ± 755.6 ng/mL $T_{1/2}$ = 2.0 ± 0.7 h AUC_{0-6t} = 2016.9 ± 671.9 μg/L·h $AUC_{0-\infty}$ = 2117.7 ± 739.5 μg/L·h	Wang et al.[189]

C_{max}: maximum plasma concentration; T_{max}: time of maximum plasma concentration; AUC: area under the plasma concentration−time curve; $\%AUC_{extra}$: percentage of the area extrapolated for calculation of AUC from time zero to infinity; AUC_{tot}: the total AUC; AUC_{last}: AUC from the time of dosing to the time of the last measurable concentration; $t_{1/2}$: elimination half-time; CL: clearance; MRT: mean residence time; V_z: apparent volume of distribution; F: bioavailability.

atherosclerosis. The relationship between atherosclerotic macrophages and exosomes has attracted significant attention in recent years. These small vesicles released by macrophages play a critical role in atherosclerosis by facilitating intercellular communication and regulating several cellular processes, including cell proliferation, apoptosis, the inflammatory response, and hematopoiesis[161,162]. This would provide evidence that GB might regulate intercellular communication to affect different vascular cells in atherosclerosis if GB modulates the production of macrophage-derived exosomes.

2.3.3. *The effect of GB on mast cells*

Activated mast cells release various biochemical mediators, such as proteases, histamines, and proinflammatory agents[163,164]. These mediators facilitate plaque progression and instability by stimulating cholesterol uptake, inhibiting cholesterol efflux, increasing leukocyte infiltration (including monocytes, neutrophils, and T cells), and inducing the apoptosis of plaque cells (such as endothelial cells, macrophages, and smooth muscle cells), which may eventually contribute to plaque rupture with an acute thrombotic event[164]. In omental mast cells, two independent studies[144,145] consistently showed that GB pretreatment markedly reduced PAF-mediated mast cell degranulation. Although the finding that GB is a prospective drug for decreasing mast cell degranulation is encouraging, it is still unknown whether a similar effect of GB on mast cells exists in the intima and how GB acts on mast cells and their released granules, areas that deserve further investigation.

2.3.4. *The effect of GB on neutrophils*

Neutrophils have diverse effects on different stages of atherosclerosis, such as causing early atherosclerosis, promoting the instability of plaques, and facilitating the shedding of endothelial cells in atherosclerotic plaques[165]. It has been reported in the earlier literature[166] that PAF activates neutrophils either directly or by enhancing TNF-α and neutrophil chemoattractant production, implying that PAF antagonists have an inhibitory effect on neutrophils. In line with this evidence, in plasma samples taken from patients with acute myocardial ischemia, GB, utilized as a specific PAF antagonist, showed a strong inhibitory effect on neutrophil activation[148,149]. Moreover, trace amounts of PAF substantially enhance the neutrophil response to subsequent activation by agonists, including respiratory bursts characterized by enhanced superoxide release and degranulation as measured by elastase release. These actions can be ameliorated by GB by inhibiting superoxide production, elastase release and cell aggregation[146]. In addition to inhibition of neutrophil activation, GB also suppressed PAF-stimulated chemotaxis of neutrophils and blocked the specific binding of [^{3}H]-PAF to eosinophils and neutrophils[150]. Given the importance of the neutrophil extracellular trap in atherosclerosis and atherothrombosis[56], the potential role of GB in the neutrophil extracellular traps should be further investigated.

Taken together, the evidence above points to the ability of GB to mitigate the deleterious vascular effects of inflammation caused by leukocytes. Considering the various protective roles of GB in a range of leukocyte types, including monocytes, macrophages, mast cells, and neutrophils, it is reasonable to suggest that GB has potential as a therapeutic agent for treating atherosclerotic CVDs.

3. The influence of GB on risk factors for atherosclerosis and cardiovascular disease

In the initial stages of vascular disease, many risk factors, such as the biochemical parameters of hyperlipidemia and hyperglycemia, can affect the critical role of the endothelium in maintaining vascular quiescence and homeostasis. Addressing hyperlipidemia with appropriate medical treatments can slow the development and progression of atherosclerosis and resultant cardiovascular disease[167,168]. In patients with diabetes and hence hyperglycemia, broad alterations in vascular homeostasis are the main feature of vasculopathy, eventually leading to atherothrombosis[169,170]. Since these are two important parameters of the multitude of risk factors for atherosclerosis and cardiovascular disease, GB could mitigate the progression of atherosclerosis by ameliorating these risk factors (Fig. 4).

3.1. *Hyperlipidemia*

According to statistics by the International Atherosclerosis Society, those with the highest levels of LDL-C are at higher risk for atherosclerotic CVD[171]. Additionally, Nordestgaard et al.[167] also observed that patients with familial hypercholesterolemia reached the LDL-C burden threshold earlier and developed premature atherosclerotic diseases. This evidence points to LDL playing a causative role in atherosclerosis. Thus, computed plasma LDL-C has become a promising therapeutic strategy for atherosclerosis and the emphasis is on appraising the risk and therapeutic effect of atherosclerotic CVDs[172,173]. As reported, GB could significantly decrease the levels of TC, TG, and LDL-C, as well as raise the level of high-density lipoprotein in hyperlipidemic mouse serum, an action that is very interestingly related to the manipulation of gut microbiota by GB[34]. A recent paper[174] reported that the gut microbiota is associated with atherosclerosis, and hence, this emerging factor needs to be included as a risk factor for cardiovascular disease. Of note, one of the pathways by which the gut microbiota has a beneficial impact on the progression of atherosclerosis is to modulate host metabolism (such as cholesterol and lipid metabolism)[175], which implies that GB-regulated gut microbiota might affect atherosclerotic plaque formation by reducing cholesterol and lipid levels in the body. Furthermore, Yang et al.[176] discovered that fat droplet deposition, occurring in hepatic steatosis, and the number of necrotic liver cells were reduced after consuming GB, showing that GB affects lipid accumulation, liver injury, and fatty acid metabolism. They also found that GB effectively upregulated HO-1 expression *via* the Nrf2 pathway, thereby increasing resistance to ferroptosis[176]. Ferroptosis is an iron-dependent necrosis that causes oxidative damage to phospholipids. It has been reported that disturbances in intracellular iron can injure macrophages, vascular smooth muscle cells, and vascular endothelial cells and alter atherosclerosis *via* lipid peroxidation, oxidative stress, inflammation, dyslipidemia, and other risk factors or pathological processes[177]. The evidence above indicates that GB, by regulating the gut microbiota and increasing resistance to ferroptosis, can have an impact on atherosclerosis by reducing hyperlipidemia.

3.2. *Hyperglycemia*

The hallmarks of the dysmetabolic state of diabetes (type 2 diabetes) are insulin resistance and hyperglycemia, which cause endothelial and smooth muscle cell dysfunction through inflammatory responses, oxidative stress, and platelet-activating mechanisms, which are drivers for CVDs in diabetes mellitus. Some research has indicated the therapeutic potential of GB in glucose metabolism disorders. For instance, the protective effect of GB on pancreatic β cells was dose-dependent, and GB significantly reduced the likelihood of severe insulitis in rats, suggesting that GB could be used as a PAF antagonist in the treatment of insulin-dependent diabetes mellitus[178]. In addition to preventing endothelial dysfunction in diabetic model rats[69], GB also has a protective effect on high glucose-treated endothelial cells by inhibiting the binding of PAF-R and TLR4[179]. The potential mechanism was linked to the suppression of JAK2/STAT3 and p38 MAPK phosphorylation[179]. Notably, regulating the JAK/STAT signaling pathway contributes to improving the progression of

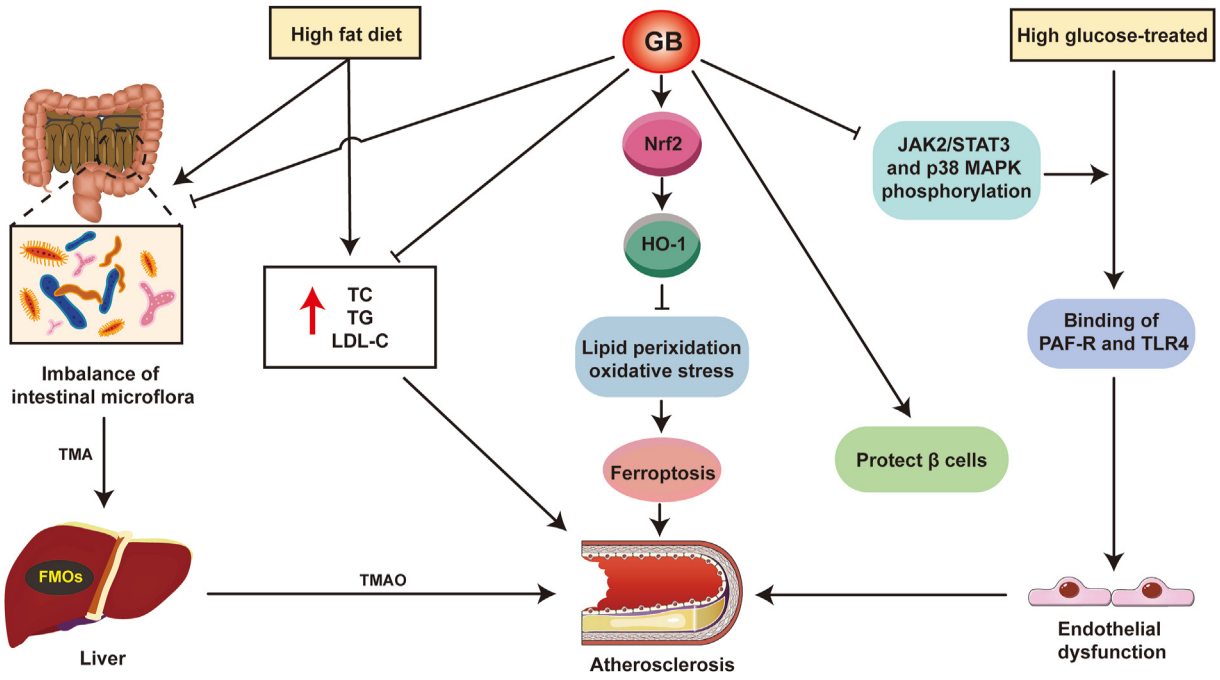

Figure 4 Diagram of the mechanism of GB on independent risk factors for atherosclerosis. Trimethylamine *N*-oxide (TMAO) levels in the blood can be utilized as a predictor of early atherosclerosis. Trimellitic anhydride (TMA) is derived from digested and absorbed foods containing choline or trimethylamine ingredients and then oxidized to TMAO by flavin monooxygenases (FMOs) secreted by the liver. This process could be inhibited by regulating the intestinal flora after GB treatment. In addition, a high-fat diet (HFD) resulted in higher levels of TG, TC, and LDL-C in serum, which were positively correlated with atherosclerosis. GB significantly attenuated the abnormal lipid and cholesterol metabolism induced by HFD. On the other hand, GB can improve resistance to ferroptosis through the Nrf2/HO-1 signaling pathway, thereby improving the pathological process of atherosclerosis. In addition to ameliorating HFD-induced atherosclerosis, GB also reduces endothelial dysfunction after high glucose treatment by inhibiting PAF-R and TLR4 binding, and it can treat diabetes mellitus by protecting β cells.

obesity and diabetes[180,181], which not only reveals the therapeutic significance of targeting the JAK/STAT signaling pathway by GB in the treatment of obesity and diabetes but also provides a new strategy to ameliorate hyperlipidemia and hyperglycemia to treat atherosclerosis. In addition, the activation of p38 MAPK is a driving factor of atherosclerosis in humans and is involved in multiple cell types that influence atherosclerosis (such as endotheliocytes[182], smooth muscle cells[183], and various immune cells[184,185]), and targeting p38 MAPK by GB or other medicinal plant-derived bioactive compounds is expected to be an option for future therapeutic intervention in atherosclerosis. The potential effects of GB on micro- and macrovascular complications associated with hyperglycemia require further evaluation, especially considering that 40% of deaths in patients with diabetes arise from CVDs in China[186].

4. Pharmacokinetic characteristics of GB and its preparations

The pharmacokinetic parameters of GB in both animals and humans have been investigated. As indicated in Table 2, several investigations have demonstrated that GB and its preparations have potential therapeutic value. Woelkart et al.[187] studied volunteers who consumed three different GB-containing formulations and discovered that GB was of low bioavailability in humans, but its bioavailability could be improved by preparing it in a variety of dosage forms. The reason for the limited oral bioavailability of GB, as for many drugs, is its poor solubility, which impacts its absorption, distribution, metabolism and elimination. The developments of pharmaceutical sciences and the development of new GB formulations can overcome this problem. A study on beagle dogs discovered that GB in plasma has good stability within 4 h and that GB preparations prepared by hot-melt extrusion or liquid layer technology can significantly improve the AUC and plasma concentrations of GB *in vivo*[188], implying that the development of novel oral GB formulations that possess high bioavailability is possible. Distribution studies show that GB is present in many organs and tissues, with the largest concentrations in the lungs, heart, spleen, kidney, liver, and gut and the lowest concentrations in the brain[189,190]. However, interestingly, borneol-modified ginkgolide liposomes can contribute to promoting the transport of ginkgolides across the blood−brain barrier and increasing the drug concentration of GB in the brain[190], implying that GB could become a therapeutic drug for the treatment of cerebrovascular disease with the assistance of borneol.

5. Clinical trials of ginkgolide B-related drug preparations in the prevention of acute ischemic cerebrovascular disease

A wide variety of pharmaceutical preparations that contain GB are commercially available. To date, mounting clinical evidence has demonstrated the therapeutic value of GB-related drug preparations for stroke or ischemia (Table 3). Notably, two clinical trials specifically focusing on safety have demonstrated the positive impact of GB injection on ischemic stroke disorders, providing promising evidence of the potential of GB as a medicinal agent.

Table 3 Clinical trials of a GB-related product for acute ischemic cerebrovascular disease.

Registration No.	Recruitment status	Study title	Target disease	Phase	Interventions
CTR20200390	Completed	A phase I clinical trial to evaluate the safety and tolerability of single and multiple intravenous ginkgolide B injections in healthy Chinese subjects	Acute ischemic stroke	I	Ginkgolide B injection
CTR20200487	Recruiting	A phase I clinical study to evaluate the safety, tolerability, and pharmacokinetics of ginkgolide B injection in healthy Chinese volunteers	Acute ischemic stroke	I	Ginkgolide B injection
ChiCTR-IPR-17012310	Recruiting	Ginkgolide in ischemic stroke patients with large artery atherosclerosis (GISAA)[193]	Stroke	IV	Ginkgolide injection + oral aspirin
NCT03772847	Completed	Ginkgolide with intravenous alteplase thrombolysis in acute ischemic stroke neurological improving trial[200]	Intravenous alteplase thrombolysis Neurological improving	IV	Ginkgolide plus alteplase
ChiCTR-ONC−13003247	Completed	A multicenter clinical trial assessment of Baiyu Ginkgolide injection, in the treatment of ischemic stroke with blood stasis syndrome	Ischemic stroke	IV	Baiyu Ginkgolide injection
NCT01958957	Completed	A safety study of ginkgolides meglumine injection in the treatment of ischemic stroke	Ischemic stroke	IV	Ginkgolides Meglumine Injection
CTR20160610	Completed	Single and multiple administration of dimethylaminoethyl ginkgolide B mesylate tablets in a randomized double-blind placebo-controlled PK/PD study and the effect of eating on PK/PD	Acute ischemic cerebrovascular disease	I	Dimethylaminoethyl ginkgolide B mesylate tablets
CTR20140805	Completed	Research protocol of multiple administration, dose-escalation, randomized, double-blind, placebo-controlled phase I clinical trial of dimethylaminoethyl ginkgolide B mesylate injection	Acute ischemic stroke	I	Dimethylaminoethyl ginkgolide B mesylate injection
CTR20210113	Recruiting	A phase I clinical study on the safety, tolerability and pharmacokinetics/pharmacodynamics (PK/PD) of a single dose of dimethylaminoethyl ginkgolide B mesylate injection in healthy adult subjects.	Acute ischemic cerebrovascular disease	I	Dimethylaminoethyl ginkgolide B mesylate injection
CTR20210115	Recruiting	Phase I clinical study of safety, tolerability, and pharmacokinetics/pharmacodynamics (PK/PD) of multidose dimethylaminoethyl ginkgolide B mesylate tablets in healthy adult subjects.	Acute ischemic cerebrovascular disease	I	Dimethylaminoethyl ginkgolide B mesylate tablets

Ginkgolide injection is one representative GB preparation. Assessed from the composition of raw materials that have been marketed in China, GA and GB are the main components of ginkgolide injection (the content of both is greater than 95%), of which GB has the strongest pharmacological activity[193,194]; it appears that the contribution of GA and GC provides a synergistic effect with GB. Based on the aforementioned evidence, it can be inferred that GB likely plays a crucial role as the primary therapeutic agent in ginkgolide injections. Currently, several ginkgolide injections have completed clinical phase IV trials that investigated the impact of ginkgolide injections on the treatment of ischemic stroke. Additionally, an ongoing clinical study is actively recruiting patients to evaluate the impact of ginkgolide injections on ischemic stroke patients with large artery atherosclerosis, which is expected to be concluded shortly. Furthermore, two small-sample studies[195,196] demonstrated that the combination of rosuvastatin and ginkgo biloba extract tablets has demonstrated clear clinical efficacy in treating cerebral infarction patients with dyslipidemia or diabetes. This combination therapy effectively reduces carotid atherosclerotic plaque, alleviates inflammation, improves blood lipid profiles, and exhibits a favorable safety profile. These clinical studies have confirmed the therapeutic effect of GB-related drug preparations in the treatment of stroke or ischemia and have also suggested their potential for reducing atherosclerotic plaques. However, it remains unclear whether the administration of GB-related drug preparations alone, especially the isolated GB compound, can effectively reduce atherosclerotic plaques or even treat atherosclerosis in clinical practice.

In addition, a larger percentage of the total were clinical trials involving dimethylaminoethyl ginkgolide B mesylate (XQ-1H), a derivative of GB, in ischemic cerebrovascular disorders. This might be because GB is a hexacyclic cage diterpenoid molecule with high structural stiffness, low water solubility, and low bioavailability, all of which restrict its potency and efficacy[187,197]. XQ-1H has superior water solubility and bioavailability and a higher safety threshold than GB, allowing it to be used in more clinical settings[198,199]. It is possible to propose that if the issue of the poor solubility of GB can be resolved by structural changes, it may considerably increase its efficacy and broaden its therapeutic value. As a result, structural changes in GB to improve water solubility are a key direction for advancing clinical research in this field.

Unfortunately, there have been no clinical studies specifically addressing the prevention and treatment of atherosclerosis using GB monomer or XQ-1H. However, with increased focus on GB monomers and structurally modified compounds, more in-depth preclinical investigations and clinical trials are anticipated to define their role in atherosclerosis.

6. Concluding remarks and future perspectives

GB is a ginkgolide with favorable pharmacological and therapeutic effects on preventing atherosclerosis. Multiple studies have demonstrated that GB has antiatherosclerotic actions that merit further exploration. The functions of endothelial cells, platelets, and other immunological cells, all of which are involved in atherosclerosis, can be regulated by GB, thus reducing the formation and development of atherosclerotic plaques. GB could also be beneficial in the management of hyperlipidemia and hyperglycemia, both of which are important risk factors in atherosclerosis. In short, GB possesses the therapeutic advantage of "multitarget and multipathway", which is closely linked to its modulation of lipid and glucose metabolism, inflammation, oxidative stress, endothelial function, leukocyte accumulation, platelet aggregation, thrombosis, and other processes. This scenario is therapeutically important in light of the complex pathological mechanisms of atherosclerosis and the intricate interaction among various vascular cells.

Atherosclerosis has the potential to precipitate additional types of CVDs, the most alarming of which are heart and brain damage. The regulation of the PI3K/AKT/NF-κB, p38 MAPK, JAK/STAT, and PAF-R signaling pathways by GB is not only beneficial for the attenuation of atherosclerosis but also protective against myocardial ischemia/reperfusion injury[43,201], ischemic stroke[202–204] and cerebral ischemia/reperfusion injury[205,206]. It is also of interest that GB acts as a PAF antagonist affecting multiple proatherosclerotic cellular processes, including inhibiting thrombosis, suppressing neutrophil activation and reducing mast cell degranulation. As an early contributor to atherosclerosis, PAF promotes atherosclerotic plaque formation through inflammation[207,208], endothelial dysfunction[146], oxidative stress[209], and platelet activity[210], which indicates that PAF antagonists might help prevent and cure atherosclerosis. There is evidence that GB exhibits potent PAF antagonism by hindering the binding of PAF to its receptor. For example, GB reduced the incidence of ventricular fibrillation by 40% during ischemia and reperfusion by inhibiting the binding of PAF to PAF-R receptors on platelets[123]. Furthermore, activated platelets stimulated smooth muscle cell proliferation by releasing PAF, but this proliferation declined by approximately 50% after GB treatment[211]. Since VSMCs are a key component in the genesis and establishment of atherosclerosis, their proliferation may be helpful as the disease progresses[212]. It has been reported that GB can also reduce VSMC growth in a concentration-dependent manner[213,214], which implies that GB might improve VSMC function to treat atherosclerosis. While GB affects a variety of cells involved in the atherosclerotic process, it is uncertain whether it can reverse atherosclerosis by affecting proliferation or phenotype on VSMCs.

In the last decade, our knowledge of the atheroprotective benefits of GB and biological targets has continued to expand. However, there are many limitations and challenges to the clinical application of GB in atherosclerosis. For example, although mounting clinical evidence has demonstrated the therapeutic value of GB-related drug preparations for acute ischemic cerebrovascular disease, mainly focusing on the safety and tolerability of GB, there is still no clinical trial that proves the effectiveness of GB for the treatment of atherosclerosis, which needs to be conducted in the future. Besides, the low oral bioavailability of GB has been addressed by nanoparticle encapsulation[215,216] or structural molecular modifications[217]. Nevertheless, those strategies are currently only used to study the prevention of Parkinson's disease[215,216] or ischemic stroke[218,219] and have not been studied in atherosclerosis models, which might be the future direction for the GB study area. In view of a study[220] showing that loading GB into liposomes boosted the therapeutic efficacy in the cerebral ischemia-reperfusion injury model, it is plausible that liposome formulations for GB will possess improved antiatherosclerosis efficacy. It is unknown if recombination with other vectors, particularly nanomaterials with distinct ROS-regulating capacities that may precisely cure atherosclerosis[221], can improve GB's therapeutic effectiveness. Despite the fact that we have some initial understanding of the actions of GB in the prevention of atherosclerosis, many fundamental issues remain to be addressed.

For instance, are structurally modified GB analogues more efficacious in preventing and treating atherosclerosis? Do GB and its derivatives affect the activation of NLRP3-mediated inflammasomes[222] and the cGAS−STING pathway[223] in the context of atherosclerosis? Is there a function for GB in controlling other apoptotic and nonapoptotic programs of death of vascular cells or immune cells, such as pyroptosis, necroptosis, or copper-dependent death[224], in response to diverse pathogenic stimuli within the atherosclerotic microenvironment, in addition to ferroptosis?

In summary, *in vivo* and *in vitro* research demonstrates that GB has the potential to prevent and treat atherosclerosis by acting *via* a variety of targets and pathways. It can also help to mitigate the impact of other risk factors that contribute to atherosclerosis. GB regulates a range of cell functions and risk factors, providing considerable vascular protection and serving as a candidate medication to prevent and treat atherosclerosis. Future considerations can center on improving GB bioavailability, intestinal absorption, and *in vivo* stability, which would provide a valuable drug template for advanced clinical applications.

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Author contributions

Weile Ye, Jiaojiao Wang and Zhiping Liu conceived and designed the manuscript. Weile Ye, Jiaojiao Wang, Jiami Zou and Zhihua Zheng prepared figures and tables and wrote the manuscript. Peter J Little, Jing Lu, Dongmei Zhang, Peiqing Liu, Yanjun Yin, Hao Liu, Suowen Xu, Wencai Ye and Zhiping Liu provided the idea and revised the manuscript. Wencai Ye and Zhiping Liu directed the study. All of the authors have read and approved the final manuscript.

Conflicts of interest

The authors declare no conflict of interests.

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