

# Diosgenin and Its Analogs: Potential Protective Agents Against Atherosclerosis

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**Abstract:** Atherosclerosis is a chronic inflammatory disease of the artery wall associated with lipid metabolism imbalance and maladaptive immune response, which mediates most cardiovascular events. First-line drugs such as statins and antiplatelet drug aspirin have shown good effects against atherosclerosis but may lead to certain side effects. Thus, the development of new, safer, and less toxic agents for atherosclerosis is urgently needed. Diosgenin and its analogs have gained importance for their efficacy against life-threatening diseases, including cardiovascular, endocrine, nervous system diseases, and cancer. Diosgenin and its analogs are widely found in the rhizomes of *Dioscore*, *Solanum*, and other species and share similar chemical structures and pharmacological effects. Recent data suggested diosgenin plays an anti-atherosclerosis role through its anti-inflammatory, antioxidant, plasma cholesterol-lowering, anti-proliferation, and anti-thrombotic effects. However, a review of the effects of diosgenin and its natural structure analogs on AS is still lacking. This review summarizes the effects of diosgenin and its analogs on vascular endothelial dysfunction, vascular smooth muscle cell (VSMC) proliferation, migration and calcification, lipid metabolism, and inflammation, and provides a new overview of its anti-atherosclerosis mechanism. Besides, the structures, sources, safety, pharmacokinetic characteristics, and biological availability are introduced to reveal the limitations and challenges of current studies, hoping to provide a theoretical basis for the clinical application of diosgenin and its analogs and provide a new idea for developing new agents for atherosclerosis.

**Keywords:** atherosclerosis, diosgenin, dioscin, analogs

## Introduction

Cardiovascular diseases are the leading cause of death worldwide. According to the World Health Organization, about 17.9 million people die from cardiovascular disease each year,<sup>1</sup> among which atherosclerosis is responsible for the majority of cardiovascular events.

Atherosclerosis is a chronic inflammatory disease of the artery wall. It usually appears in the subcutaneous space of medium to large arteries and is associated with lipid metabolism imbalance and maladaptive immune responses.<sup>2</sup> In the early stage of the disease, various pathogenic factors lead to damage of the intima, which further increases the infiltration of inflammatory cells and lipids, and accelerates the formation of foam cells. In the later stage, plaque calcification and rupture occur, leading to the formation of thrombosis and, in turn, ischemic injury syndromes of vital organs, such as myocardial infarction, unstable angina (ischemic heartache), stroke, and other complications.<sup>3</sup> Lowering blood lipids, controlling blood pressure, dilating blood vessels, preventing platelet aggregation from preventing thrombotic complications, etc., are some of the treatment methods for atherosclerosis. Although these therapies alleviate the occurrence and progression of atherosclerosis, they do not target the inflammatory mechanisms responsible for the progression of atherosclerosis. Also, first-line drugs such as statins and antiplatelet drug aspirin in the secondary prevention of ACS have been associated with some adverse reactions such as myitis and myalgia (for statins),<sup>4</sup> gastrointestinal bleeding, ulcers, and increased drug resistance (for antiplatelet drug aspirin).<sup>5,6</sup> Thus, the development of new, safer, and less toxic agents for atherosclerosis is urgently required.

At present, phytochemicals have been attracting increasing attention due to their low toxicity and high yield. Diosgenin and its analogs are important natural steroidal saponins used as active ingredients of dioscin tablets, Di'ao Xin Xue Kang capsules, Dunye Guan Xin Ning, and other medicines which have been used in China for more than 20 years to treat coronary heart disease and other cardiovascular diseases.<sup>7–9</sup> Many experimental studies and some clinical trials have demonstrated that diosgenin and its analogs have anti-inflammatory, antioxidant, plasma cholesterol-lowering, anti-proliferation, and anti-thrombotic effects, thus suggesting that these drugs may be promising candidates for atherosclerosis treatment.<sup>10,11</sup> However, there is still a lack of relevant summaries.

In this review, we summarized the effects of diosgenin and its analogs on vascular endothelial dysfunction, vascular smooth muscle cell proliferation, migration and calcification, lipid metabolism, and inflammation, thus providing a new overview of its anti-atherosclerosis mechanism. Besides, the structures, sources, safety, pharmacokinetic characteristics, and biological availability were introduced, hoping to provide a new idea for the development of new agents for atherosclerosis.

## Overview of the Structures, Sources, and Safety of Diosgenin and Its Analogs

Diosgenin is a natural steroidal saponin, whose structure is very similar to endogenous steroids (such as cholesterol, progesterone, and estrogen). Due to its estrogenic activity, it is often used as a precursor for producing norethindrone and progesterone and as a dietary supplement in hormone replacement therapy to improve menopausal symptoms.<sup>12</sup> Diosgenin has many structural analogs. Dioscin is the glycoside form of diosgenin obtained by connecting trisaccharide  $\alpha$ -L-Rha-(1 $\rightarrow$ 4)-[ $\alpha$ -L-Rha-(1 $\rightarrow$ 2)]- $\beta$ -D-Glc to diosgenin at position 3 through a glycosidic bond;<sup>13</sup> it can be converted to diosgenin by hydrolysis.<sup>14</sup> Considering that chemical structures and effects on lipid bilayer membranes of dioscin and diosgenin are similar to those of cholesterol, they have an essential role in cholesterol metabolism, ie, inhibition of the intestinal absorption of dietary cholesterol and the acceleration of the transformation of cholesterol into bile acids.<sup>15–17</sup>

Methylprotodioscin (MPD), pseudoprotodioscin (PPD), protodioscin (PD), yamogenin and tomatidine are other analogs of diosgenin that have similar pharmacological effects and are mainly extracted from the roots and stems of *Trigonella*, *Smilax*, *Dioscorea*, *Solanum*, and *Costus* species, among which *Dioscorea* has the highest content.<sup>18–20</sup> The molecular formula, molecular weight, source, and chemical structure of diosgenin and its main analogs are shown in Table 1 and Figure 1.

Safety is often the first step in drug development. Natural steroid saponins usually have high safety. Preclinical studies showed mild subchronic toxicity in male rats but not in female rats treated with diosgenin. Daily administration of diosgenin above 300 mg/kg may cause mild gastrointestinal distension, hemolytic anemia, and weight loss in rats, while long-term use of steroidal saponins in large doses has been reported to damage the liver, leading to liver damage such as acute icteric hepatitis.<sup>21</sup> However, at a moderate dose, diosgenin showed a significant protective effect on liver injury induced by ethanol and paracetamol.<sup>22</sup> Tohda et al suggested that the oral toxicity dosage (LD50) of diosgenin to mice and rats is > 8000 mg/kg (> 480g/ human).<sup>23</sup> Moreover, diosgenin derivative compound 5 did not show any toxicity in mice at an oral dose of 575.5 mg/ kg.<sup>24</sup> Therefore, diosgenin and its analogs are considered safe and non-toxic at the conventional dosage,<sup>25</sup> however, the safety of other analogs needs to be further explored.

## Material and Methods Regulation of Endothelial Dysfunction

Endothelial cells are an important barrier between the vascular wall and blood. When exposed to risk factors such as excess lipid (LDL), hypertension (shear stress), oxygen-free radicals, cigarette smoke constituents, high blood sugar, and stress, endothelial cell structure and function may change, which may result in endothelial dysfunction, which, in turn, can induce atherosclerosis. In the early stage of atherosclerosis, endothelial-dependent vasodilation is impaired, oxidative stress is enhanced, and leukocytes increase with the help of adhesion molecules. In the late stage, plaque rupture and thrombosis are formed.<sup>26–30</sup>

**Table I** Molecular Formula, Relative Molecular Weight and Main Sources of Diosgenin and Its Analogues

Compound	Molecular Formula	Relative Molecular Weight (g/mol)	Main Source	References
Diosgenin	C <sub>27</sub> H <sub>42</sub> O <sub>3</sub>	414.6	The roots of <i>Dioscorea villosa</i> , the seeds of fenugreek ( <i>T. foenum graecum</i> Linn), the rhizomes of <i>D. zingiberensis</i>	[163,164]
Dioscin	C <sub>45</sub> H <sub>72</sub> O <sub>16</sub>	869.05	The roots of <i>Dioscorea villosa</i> , the rhizomes of <i>D. zingiberensis</i> and <i>Dioscorea nipponica</i>	[165–168]
Pseudoprotodioscin.	C <sub>51</sub> H <sub>82</sub> O <sub>21</sub>	1031.2	The seeds of fenugreek ( <i>T. foenum graecum</i> Linn), the rhizomes of <i>Dioscorea panthaica</i>	[169,170]
Protodioscin	C <sub>51</sub> H <sub>84</sub> O <sub>22</sub>	1049.2	The Rhizome of <i>Dioscorea tokoro</i> , the rhizomes of <i>Dioscorea nipponica</i> , the seeds of fenugreek ( <i>T. foenum graecum</i> Linn), the seeds of <i>Tribulus Terrestris</i>	[171–174]
Methylprotodioscin	C <sub>52</sub> H <sub>86</sub> O <sub>22</sub>	1063.2	The rhizomes of <i>Dioscorea collettii</i> var. <i>hypoglauca</i> ( <i>Dioscoreaceae</i> )	[175]
Yamogenin	C <sub>27</sub> H <sub>42</sub> O <sub>3</sub>	414.6	The dried stems of <i>Asparagus officinalis</i> L, the seeds of fenugreek ( <i>T. foenum graecum</i> Linn)	[101,176,177]
Tomatidine	C <sub>27</sub> H <sub>45</sub> NO <sub>2</sub>	415.7	The unripe fruits, leaves, stems and roots of tomato plant	[178]

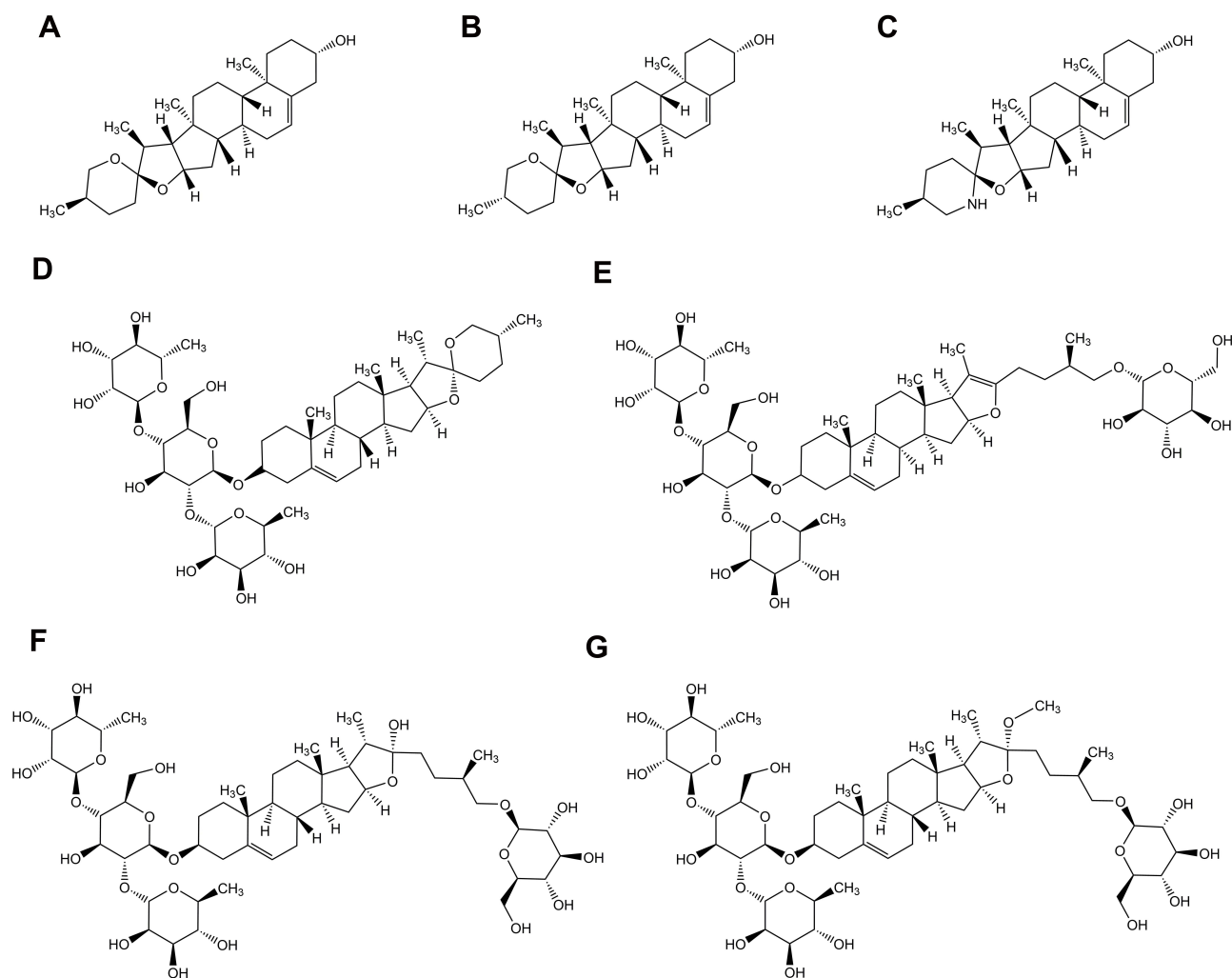
**Abbreviations:** MPD, Methylprotodioscin; PPD, pseudoprotodioscin; NO, nitric oxide; cGMP, cyclic guanosine monophosphate; eNOS, endothelial NO synthase; iNOS, inducible NO synthase; ET-1, endothelin-1; Arg-1, arginase-1; MDA, malondialdehyde; HUVECs, human umbilical vein endothelial cells; PAT, perivascular adipose tissue; ROS, reactive oxygen species; SOD, superoxide dismutase; CAT, catalase; GSH, glutathione; GPX, glutathione peroxidase; Sirt3, sirtuin 3; Nrf2, nuclear factor erythroid 2-related factor 2; GR, glutathione reductase; GST, glutathione S-transferase; oxLDL, low-density lipoprotein; MCP-1, monocyte chemoattractant protein-1; M-CSF, monocyte colony-stimulating factor; NF-κB, nuclear factor-κB; TNFR1, tumor necrosis factor receptor 1; VSMCs, vascular smooth muscle cells; PA, plasminogen activator; vWF, von Willebrand factor; Par, protease-activated receptor; TXA, tranexamic acid; TF, tissue factor; PAI-1, plasminogen activator inhibitor-1; PT, prolong prothrombin time; TT, thrombin time; APTT, activate partial thromboplastin time; FOXM1, Forkhead box protein M1; ADAM15, adamalysin metalloproteinase 15; Runx2, runt-related transcription factor 2; FC, free cholesterol; CE, cholesterol ester; TG, triglycerides; LDL-C, low-density lipoprotein cholesterol; HDL, high-density lipoprotein; VLDL-C, very-low-density lipoprotein cholesterol; PPARγ, peroxisome proliferation-activated receptor; LCAT, cholesterol acyltransferase; PL, pancreatic lipase; HL, hepatic proteinase; PGC1α, Peroxisome proliferator-activated receptor gamma coactivator 1-alpha; LPL, lipoprotein lipase; Erα, estrogen receptor α; SREBPs, sterol response element-binding proteins; LXRs, liver X receptors; PCSK9, proprotein convertase subtilisin-like/kexin type 9; LDLR, low-density lipoprotein receptor; ACAT, Acyl-Coenzyme A: Cholesterol Acyltransferase; SRs, Scavenger receptors; RCT, reverse cholesterol transport; NPC1L1, Niemann-Pick C1-Like 1; ABCG5/8, ATP-binding cassette G5/8; SRB1, scavenger receptor class B type 1; CES-1, carboxylesterase-1; CYP7A1, cholesterol 7α-hydroxylase; FXR, farnesoid X receptor; NICD, Notch intracellular domain; MDC, macrophage-derived chemokine, BLC, B lymphocyte chemokine; MIP-1α, macrophage inflammatory protein-1α; LPS, lipopolysaccharide; Pam3CSK4, palmitoyl-3-cysteine-serine-lysine-4; TF, tissue factor; CYP2E1, Cytochrome P450 2E1; COX-2, cyclooxygenase-2; HMGB1, high mobility group box-1; β-CD, β-cyclodextrin; SD rats, Sprague–Dawley rats; FeCl<sub>3</sub>, the ferric chloride; ADP, adenosine diphosphate; acLDL, Acetylated-low density lipoprotein; HAECs, human aortic endothelial cells; PBMCs, peripheral blood mononuclear cells; OxLDL, Oxidatively modified LDL; MPMs, Mouse peritoneal macrophages; LPS, Lipopolysaccharide; HUVECs, Human umbilical vein endothelial cells; HMDMs, Human monocyte-derived macrophages; CE, Cholesterol ester; EL, endothelial lipase; FC, fatty acid; GSH, Glutathione; GSSG, oxidized glutathione; E17G, estradiol-17β-(beta-D-glucuronide); Nrf2, nuclear factor E2-related factor-2; HO-1, heme oxygenase-1; SOD, superoxide dismutase; AMPK, AMP-activated protein kinase; GSS, glutathione synthetase; JNK, junNH2-terminal kinase; sirt1, sirtuin 1.

## Regulation of Vascular Tone

At the early stage, atherosclerosis is often accompanied by the reduced secretion and lower activity of NO, which leads to abnormal vasoconstriction and spasm, changes in blood flow shear stress, thrombosis, and even vascular proliferation. Therefore, NO is extremely important in the development of atherosclerosis.

As an important endothelium-derived relaxation factor, NO can dilate blood vessels through the nitric oxide/cyclic guanosine monophosphate (NO/cGMP) signaling pathway.<sup>31</sup> Endothelial cells catalyze the production of NO through endothelial NO synthase (eNOS),<sup>32</sup> while inducible NO synthase (iNOS) mediates endothelium-dependent vasodilatation dysfunction.<sup>33</sup> When exposed to free fatty acids and hypercholesterolemia, superoxide anion is overexpressed in endothelial cells, and NO activity is reduced, thus attenuating NO-induced relaxation and causing endothelial dysfunction.<sup>34,35</sup> Endothelin-1 (ET-1) is an endothelial cell-derived peptide that inhibits eNOS activity and promotes vasoconstriction. ET-1 is increased in atherosclerotic lesions.<sup>36</sup>

Diosgenin can improve vascular resistance and regulate arterial tension, and this beneficial effect may be mediated by the increasing NO production and activation of eNOS.<sup>37,38</sup> Studies have discovered that diosgenin improves endothelial



**Figure 1** Structure of diosgenin and its analogs. (A) diosgenin; (B) yamogenin; (C) tomatidine; (D) dioscin (E) Pseudoprotodioscin; (F) Protodioscin (G) Methylprotodioscin.

dysfunction by reducing the increase of MCP-1 and iNOS induced by atherogenic diet, promoting the expression of arginase-1 (Arg-1), inhibiting eNOS phosphorylation, and increasing NO production.<sup>39,40</sup> Diosgenin also restores insulin-mediated NO production and inhibits ET-1 expression via activation of the Akt pathway.<sup>41</sup> Furthermore, in vitro experiments suggested that PPD can regulate the levels of eNOS, NO, and malondialdehyde (MDA) in human umbilical vein endothelial cells (HUVECs), and reduce the oxidative stress of endothelial cells, which is mediated by the ER $\alpha$  pathway.<sup>42</sup> Another study suggested that perivascular adipose tissue (PAT) can regulate vascular endothelial function through endocrine or paracrine functions.<sup>43</sup> Also, adiponectin secreted by PAT can promote NO production in endothelial cells and dilate blood vessels.<sup>44,45</sup> Moreover, diosgenin can alleviate the impairment of endothelial-dependent vasodilation and improve endothelial dysfunction via affection of endothelial cells by modulating the paracrine function of PAT.<sup>40</sup> Thus, it is believed that diosgenin and its analogs control vascular tension by regulating enzymes related to NO metabolism and have a positive role in preventing AS at an early stage.

## Regulation of Oxidative Stress

Vascular endothelial cells are highly sensitive to oxidative stress. Endothelial function is impaired when the production of antioxidants such as reactive oxygen species (ROS) exceeds the endogenous antioxidant capacity. Superoxide dismutase (SOD) is an antioxidant protein that helps remove superoxide;<sup>46</sup> catalase (CAT) can directly decompose H<sub>2</sub>O<sub>2</sub>;<sup>47</sup> glutathione (GSH) and glutathione peroxidase (GPX) c hyperlipidemia and pig models of coronary heart disease,

which leads to maintain the reductive state of the cell environment, remove hydrogen peroxide and lipid peroxides, and mitigate the toxicity of toxic metal ions and the damage of toxic oxygen products to cells.<sup>48</sup> Preclinical studies have shown that diosgenin can inhibit the generation of oxidation product MDA and oxygen free radicals by activating antioxidant enzymes (such as SOD, CAT, GPX, and eNOS) in plasma and liver of rats with to the improvement of lipid peroxidation and combats oxidative stress in the aorta.<sup>49–55</sup>

Sirtuin 3 (Sirt3) is closely associated with oxidative stress, lipid metabolism, and inflammation and is conducive to superoxide clearance.<sup>56</sup> The Nuclear factor erythroid 2-related factor 2 (Nrf2) is used as the main transcription regulator of GPX, GST, SOD, CAT, and GR.<sup>57</sup> Dioscin can enhance antioxidant capacity and reduce oxidative stress by up-regulating Sirt3, thereby promoting the expression of antioxidant enzymes Nrf2, SOD2, and GST, and inhibiting the expression of Keap1.<sup>58</sup> Also, diosgenin preconditioning can reduce the production of ROS and GSH in H<sub>2</sub>O<sub>2</sub>-induced HUVECs cells, which have an important role in protecting endothelial integrity.<sup>49</sup>

To sum up, dioscin and diosgenin are good antioxidants that can inhibit oxidative stress, protect endothelium and enhance vascular function by enhancing the antioxidant system composed of SOD, GSH, CAT, GST, GR and GPX.

## Inhibition of Leukocyte Adhesion

When the vascular endothelium is activated by pro-inflammatory cell signaling pathways and stimulated by oxidized lipids and low-density lipoprotein (oxLDL), endothelial cells can secrete a variety of adhesion molecules and chemokines, promoting leukocytes (monocytes and T lymphocytes) that adhere to the atherosclerotic sites. These cells then migrate and differentiate into macrophages,<sup>59</sup> which have an important role in initiating atherosclerosis and promoting plaque instability. In addition, endothelial cells can also secrete monocyte chemoattractant protein-1 (MCP-1) and monocyte colony-stimulating factor (M-CSF) under the stimulation of oxLDL, which further induces the recruitment of monocytes. In vitro studies have shown that dioscin and diosgenin can inhibit the expression of ICAM-1 and VCAM-1 in macrophages and HUVECs induced by TNF- $\alpha$  by blocking the activation of nuclear factor- $\kappa$ B (NF- $\kappa$ B), thus reducing the adhesion of monocytes to HUVEC stimulated by TNF- $\alpha$ .<sup>60</sup> Moreover, in vivo studies found that diosgenin can reduce the expression of MCP-1 in the aorta of atherosclerotic rats, reduce inflammation,<sup>61</sup> and reduce TNF- $\alpha$  reactivity by inducing the shedding of the extracellular domain of tumor necrosis factor receptor 1 (TNFR1). This, in turn, reduces the expression of ICAM-1 induced by TNF- $\alpha$  and the inflammatory response of vascular endothelial cells.<sup>62</sup> At the same time, PPD can reduce the inflammatory response of the arterial wall of HUVECs and ovariectomized apoE<sup>-/-</sup> mice by regulating the estrogen receptor ER $\alpha$ , promoting the production of NO, inhibiting the NF- $\kappa$ B signaling pathway, down-regulating the expression of MCP-1 and adhesion molecules; thus, having a potential therapeutic effect on atherosclerosis associated with estrogen deficiency.<sup>42</sup>

Overall, the above data suggest that diosgenin and its analogs can reduce endothelial inflammation and inhibit endothelial dysfunction by inhibiting leukocyte adhesion.

## Inhibition of Platelet Aggregation and Prevention of Thrombosis

Atherosclerosis is rarely fatal. However, if it remains untreated, VSMCs will be progressively lost and foam cells will undergo apoptotic disintegration and release matrix metalloproteinases (MMPs), especially MMP-2 and -9, which gradually thins and degrades the fibrous cap, induces angiogenesis, and increases susceptibility to plaque rupture.<sup>63</sup> Plaque rupture leads to leakage of prothrombotic substances, inducing thrombosis at the site of rupture, which, in turn, may lead to fatal cardiovascular events. Therefore, the prevention of thrombosis helps reduce the occurrence of life-threatening cardiovascular and cerebrovascular events.

Endothelial cells express various molecules with anticoagulant, antiplatelet, and fibrinolytic properties, and their integrity is of great importance for clotting inhibition and thrombosis.<sup>64</sup> The plasminogen activator (PA) synthesized by endothelial cells promotes plasmin production and inhibits thrombosis. When stimulated or injured, endothelial cells can synthesize clotting substances, such as von Willebrand factor (vWF), platelet-activating factor (such as Par), and thromboxane (such as TXA) to promote platelet adhesion and aggregation on the damaged vascular wall. This then promotes the expression of tissue factor (TF), coagulation factor V, IX, X, etc., which facilitates blood coagulation and

blood flow obstruction.<sup>65</sup> In addition, plasminogen activator inhibitor-1 (PAI-1) in endothelium and platelets can inhibit PA expression and promotes atherosclerotic thrombosis.<sup>66</sup>

Diosgenin can reduce platelet aggregation rate, prolong prothrombin time (PT), thrombin time (TT) and activate partial thromboplastin time (APTT), inhibit thrombus formation, and increase the dissolution of blood clots in a dose-dependent manner, which, in turn, improve anticoagulant function in rats.<sup>67</sup> Besides, after structural modification of diosgenin, the anti-thrombotic effect of diosgenin is further enhanced with no side effects; moreover, the anti-thrombotic effect of diosgenin seems to be superior to that of aspirin.

In vitro and in vivo studies have shown that prodrug micelles containing derivatives diosgenin and polyethylene glycol (PEG) can inhibit the adhesion, aggregation, apoptosis, and activation of platelets and regulate the APTT value by prolonging the activity of factor VIII (FVIII), which is involved in the intrinsic coagulation pathway, and increases the anti-thrombotic effect without causing excessive bleeding and obvious histological damage.<sup>68</sup>

Compound 5, a diosgenin derivative formed by substituting the carboxyl group in aspirin structure with diosgenin, shows a high inhibitory rate on platelet aggregation and could regulate the activation of FVIII and prolong APTT. Compared to aspirin, it has a lower risk of bleeding and fewer gastric mucosal lesions.<sup>24</sup> A similar effect was observed using compound 3 formed by combining the C3 position of diosgenin with a disaccharide consisting of glucose and galactose residues.<sup>69</sup> Therefore, these compounds may potentially be used as antiplatelet inhibitors.

Diosgenin can also inhibit the expression of PAI-1 and improve the endothelial dysfunction induced by palmitic acid. This mechanism may be related to the recovery of the anticoagulant effect of PA and the improvement of the endothelial pre-thrombotic state.<sup>41</sup> Moreover, NO produced by endothelial cells is also an important short-acting platelet inhibitor, and the regulation of NO by diosgenin and diosgenin may have a positive role in platelet aggregation and inhibition of thrombosis

To sum up, diosgenin and its analogs are pleiotropic drugs, which can regulate endothelial function, reduce endothelial dysfunction and inhibit atherosclerosis by regulating nitric oxide metabolism, monocyte adhesion, redox balance, leukocyte adhesion, hemostasis and thrombotic balance.

## Regulation of the Function of Vascular Smooth Muscle

### Inhibit Intimal Hyperplasia/Vascular Smooth Muscle Cell Proliferation and Migration

When stimulated by inflammatory factors, chemokines, and growth factors, VSMCs with contractile phenotype are transformed to cells with synthetic phenotype, which have more invasive, synthetic, and proliferative features. These cells migrate into the damaged vascular intima, where they proliferate and secrete a large amount of extracellular matrix and inflammatory factors (such as IL-1 and TNF- $\alpha$ ), participating in the formation and development of fibrous caps in the early stage of atherosclerosis.<sup>70</sup> Smooth muscle cells (SMCs), together with the interstitial collagen and elastin, contribute to plaque stability to a certain extent. However, with the proliferation of SMC, the atherosclerotic plaque thickens can cause coronary artery stenosis and lead to a series of ischemic syndromes, such as hypertension, ischemic stroke, and renal impairment. Studies have suggested that diosgenin 10 $\mu$ M can inhibit the migration of VSMC by 45%, contraction by 25%, improve cell viability and calcium homeostasis, and further improve VSMC function.<sup>71</sup> Intimal hyperplasia is one of the key factors causing restenosis and atherosclerosis after percutaneous coronary angioplasty. Studies have shown that diosgenin and PD can inhibit proliferation, migration, and phenotypic transformation of VSMC and inhibit intima thickening in rat carotid artery balloon injury model through the inhibition of ERK1/2, FOXM1, and ADAM15 expression.<sup>72,73</sup> Therefore, Diosgenin is considered as a promising drug for the treatment of arteriosclerosis and restenosis after PCI.

## Regulation of Vascular Calcification

Focal calcification gradually develops in atherosclerotic plaques with age, promoting plaque rupture.<sup>74</sup> Senescent apoptotic VSMCs are not easily removed and become the main source of calcification matrix, which promotes vascular calcification through conversion to osteoblast phenotype.<sup>75</sup> H<sub>2</sub>O<sub>2</sub> can promote VSMC calcification, which is associated with increased activity and expression of Runx-related transcription factor 2 (Runx2).<sup>76</sup> Furthermore, Runx2 promotes the

differentiation and maturation of osteoblast and chondrocytes, thereby increasing vascular calcification.<sup>77</sup> Studies have shown that diosgenin can inhibit aortic VSMC phenotype changes and vascular calcification induced by renal failure via regulation of oxidative stress and reduction of the activity and expression of Runx2 by reducing H<sub>2</sub>O<sub>2</sub>.<sup>37,51</sup> Therefore, diosgenin may have an important role in vascular calcification. Above all, diosgenin and its analogs can inhibit the progression of atherosclerosis by inhibiting the proliferation, migration, and calcification of VSMCs.

## Regulation of Lipid Metabolism

Dyslipidemia has an important role in atherosclerosis. The infiltration of plasma lipoproteins, free cholesterol (FC), cholesterol ester (CE), triglycerides (TG), phospholipids, and apolipoproteins, may all lead to atherosclerosis. Low-density lipoprotein cholesterol (LDL-C) is the most important and accepted risk factor for atherosclerosis.<sup>78</sup> On the other hand, high-density lipoprotein (HDL) promotes atherosclerotic plaque regression by promoting cholesterol outflow in foam cells and is negatively correlated with the occurrence and progression of atherosclerosis.<sup>79</sup>

## Improvement of Hyperlipidemia

Numerous studies have shown that diosgenin, dioscin, tomatidine, dioscorea nipponica Makino, and *Sanyaku* containing diosgenin can induce total serum cholesterol (TC), very-low-density lipoprotein cholesterol (VLDL-C), LDL-C, TG, FTC, TTC and promote HDL-C levels in rats and mice<sup>52,53,61,80–85</sup> This mechanism is related to the up-regulation of peroxisome proliferation-activated receptor  $\gamma$  (PPAR $\gamma$ ), cholesterol acyltransferase (LCAT), pancreatic lipase (PL), hepatic proteinase (HL), PGC1 $\alpha$  -mediated lipoprotein lipase (LPL) and estrogen receptor  $\alpha$  (ER $\alpha$ ).<sup>49,52,80–82,86</sup>

In addition, sterol response element-binding proteins (SREBPs) are important lipid-derived transcription factors involved in cholesterol metabolism and lipid production.<sup>87</sup> Phosphorylation of AMPK inhibits lipid production and improves hepatic steatosis and atherosclerosis by inhibiting cleavage, nuclear translocation, and transcriptional activity of SREBP-1C and SREBP2.<sup>88</sup> Studies have shown that dioscin and diosgenin could inhibit the accumulation of fatty acids and triglycerides in HepG2 cells, AML12 cells, LO2 cells, 3T3-L1 cells and mouse liver and plasma by regulating the miR-125a-5p/STAT3 signaling pathway, the expression levels of AMPK, SREBP-1C, and downstream proteins related to lipid metabolisms, such as SCD, CPT, FAS, FoxO1, FASN, ACC, and ATGL, thereby reducing lipids improving the lipid profile.<sup>89–95</sup> Also, MPD, PD, and PPD can reduce the expression of genes related to triglyceride and cholesterol synthesis by inhibiting the levels of SREBP1c, SREBP2, and microRNA33a/b.<sup>96,97</sup> In addition, PPD has a significant therapeutic effect on estrogen-deficient atherosclerosis, and its therapeutic effect is comparable to that of 17 $\beta$ -estradiol.<sup>98</sup>

Liver X receptor (LXRs) is also an important factor regulating lipid metabolism, mediating the activation of SREBP-1c.<sup>99</sup> Diosgenin and yamogenin (dienantiomer of Diosgenin) have LXR- $\alpha$  antagonist effect; they can inhibit the upregulation of SREBP-1C induced by LXR- $\alpha$  agonist and LXR- $\alpha$  -mediated SREBP-1c induced by high-fat diet, and inhibit the accumulation of fat in plasma and liver cells. However, the activation effect of yamogenin on LXR is lower than that of diosgenin.<sup>94,100,101</sup> Also, LXRs may mediate the regulation of diosgenin on cholesterol homeostasis, yet, further studies are needed to confirm this data.

The proprotein convertase subtilisin-like/kexin type 9 (PCSK9) promotes LDLR degradation and has an important role in cholesterol homeostasis.<sup>102</sup> Studies have shown that MPD can promote the up-regulation of LDLR, reduce LDL-C level and improve atherosclerosis by inhibiting PCSK9.<sup>96</sup> Human acyl-CoA:cholesterol acyltransferase (ACAT), the only intracellular cholesterol esterification enzyme, is involved in the absorption, transport, and storage of cholesterol, and its overexpression is closely related to atherosclerosis.<sup>103,104</sup> Tomatidine can reduce serum LDL and cholesterol ester by inhibiting ACAT, inhibiting foam cell formation and alleviating atherosclerosis.<sup>105</sup>

Diosgenin can also promote the synthesis rate of liver cholesterol and improve hypercholesterolemia in rats via inhibition of the absorption of intestinal cholesterol by reducing intestinal surface area and reducing liver and plasma cholesterol.<sup>106,107</sup> The increased rate of liver cholesterol synthesis is partially due to the increased activity of HMG-CoA reductase.<sup>15,52,85,108</sup> In addition, compared with atorvastatin treatment alone, the combination of diosgenin resulted in a higher rate of cholesterol reduction and an increase in neutral sterol excretion in the liver and other tissues of rats.<sup>15,109</sup> To sum up, diosgenin combined with statins may have a greater role in improving plasma cholesterol levels.

## Inhibition of Plaque/Foam Cell Formation

The large lipid nuclei formed by foam cells are the hallmark feature of atherosclerosis. After being recruited to the intima, monocytes differentiate into macrophages, which ingest modified lipids through various pathways and become foam cells.<sup>110</sup> Foam cells not only secrete pro-inflammatory mediators but also lead to the formation of the necrotic core caused by macrophage apoptosis, which further promotes arterial wall inflammation and monocyte recruitment in advanced lesions, further aggravating atherosclerosis.<sup>111</sup> Therefore, inhibition of foam cell-mediated plaque formation is important in treating atherosclerosis.

Uncontrolled internalization of low-density lipoprotein promotes the formation of foam cells. Scavenger receptors (SRs), including SR-A, CD36, SR-BI, and LOX-1, further mediates the binding and internalization of oxLDL.<sup>112</sup> Diosgenin can prevent dendritic cell activation and atherosclerotic plaque formation by inhibiting oxLDL uptake by inhibiting CD36, SR-A, LOX-1, and P38 MAPK expression.<sup>113</sup> It could also reduce cholesterol uptake of ox-LDL-treated macrophages through LOX-1 and decrease cholesterol levels in cells and aortic tissues, which leads to the inhibition of foam cell formation and atherosclerotic plaques.<sup>83</sup>

Efflux of cholesterol from macrophages is an important defense against foam cell formation, a process also known as reverse cholesterol transport (RCT). High-density lipoproteins promote macrophage cholesterol efflux by binding their apolipoprotein APOA-I to specific transporters of the ATP-binding cassette (ABC) gene family,<sup>114</sup> among which ABCA1 mediates approximately one-third of cholesterol efflux.<sup>115</sup>

In vivo and in vitro experiments demonstrated that diosgenin promotes ABCA1-mediated cholesterol excretion from macrophage foam cells, reduces cholesterol accumulation in macrophages and lipid deposition in the aorta, and alleviates atherosclerosis by inhibiting miR-19b expression.<sup>116</sup> Moreover, MPD, PD, and PPD can promote ABCA1-mediated cholesterol efflux by inhibiting the level of microRNA33a/b.<sup>96,97</sup>

Hence, diosgenin and its analogs can inhibit the formation of atherosclerotic plaques not only by regulating systemic lipid metabolism but also by improving local lipid absorption.

## Inhibition of Intestinal Cholesterol Absorption/Promotion of Bile Cholesterol Excretion

Inducing plaque regression is often the best target for treating severe atherosclerosis. RCT can inhibit plaque progression and induce plaque regression. Besides RCT, the cholesterol transport system composed of the liver and intestine contributes to maintaining systemic cholesterol homeostasis and reducing atherosclerosis.<sup>117</sup> The cholesterol transport system not only inhibits the intestinal absorption of cholesterol but also carries cholesterol from peripheral tissues to the liver, excreting it into bile or forming free cholesterol, which is then excreted through the intestine to the body.<sup>118</sup> Diosgenin can promote fecal cholesterol excretion and inhibit cholesterol absorption in mice and rats,<sup>85,107,119</sup> this effect is related to Niemann-Pick C1-Like 1 (NPC1L1) and ATP-binding cassette G5/8(ABCG5/8). NPC1L1 is a transmembrane protein highly expressed in the intestine and liver, which mediates the excretion of intestinal sterols and hepatic cholesterol.<sup>120</sup> Li et al found that diosgenin could inhibit cholesterol absorption by down-regulating intestinal NPC1L1 expression.<sup>85</sup> However, Temel et al obtained different results, suggesting that diosgenin does not alter the expression of cholesterol transport-related genes (such as NPC1L1) in intestinal epithelial cells but stimulates fecal cholesterol excretion by regulating hepatic cholesterol metabolism.<sup>107</sup> Yet, this contradictory result may be related to different experimental animal models and detection methods.

ABCG5/8, as an important transporter in the intestine and liver, mediates the elimination of most bile cholesterol.<sup>121</sup> Previous studies found that diosgenin increases the cholesterol concentration in the bile of normal and cholesterol-fed rats by 4–15 times but does not affect the levels of bile acids, phospholipids, and bile salts.<sup>15,122–125</sup> Temel et al and Yu et al further suggested that cholesterol secretion is unrelated to liver ABCG5/8 expression.<sup>107,126</sup> These data were further confirmed by Kusters et al;<sup>123</sup> still, they also found no cholesterol secretion in ABCG8-/- mice treated with diosgenin, thus suggesting that ABCG8 activity is indispensable for diosgenin-mediated cholesterol excretion. Further studies showed that diosgenin could increase the transfer of cholesterol to heterodimer and promote bile cholesterol secretion through ABCG5/8 dependent and non-dependent pathways.<sup>127</sup> Besides, Li et al also found that diosgenin could inhibit LXR- $\alpha$  and increase the expression of ABCG5 and ABCG8 in the liver and intestine.<sup>85</sup> However, Kamisako et al found that diosgenin only affects the expression of ABCG5/8 in the liver but has no effect on the expression of ABCG5/8 in the

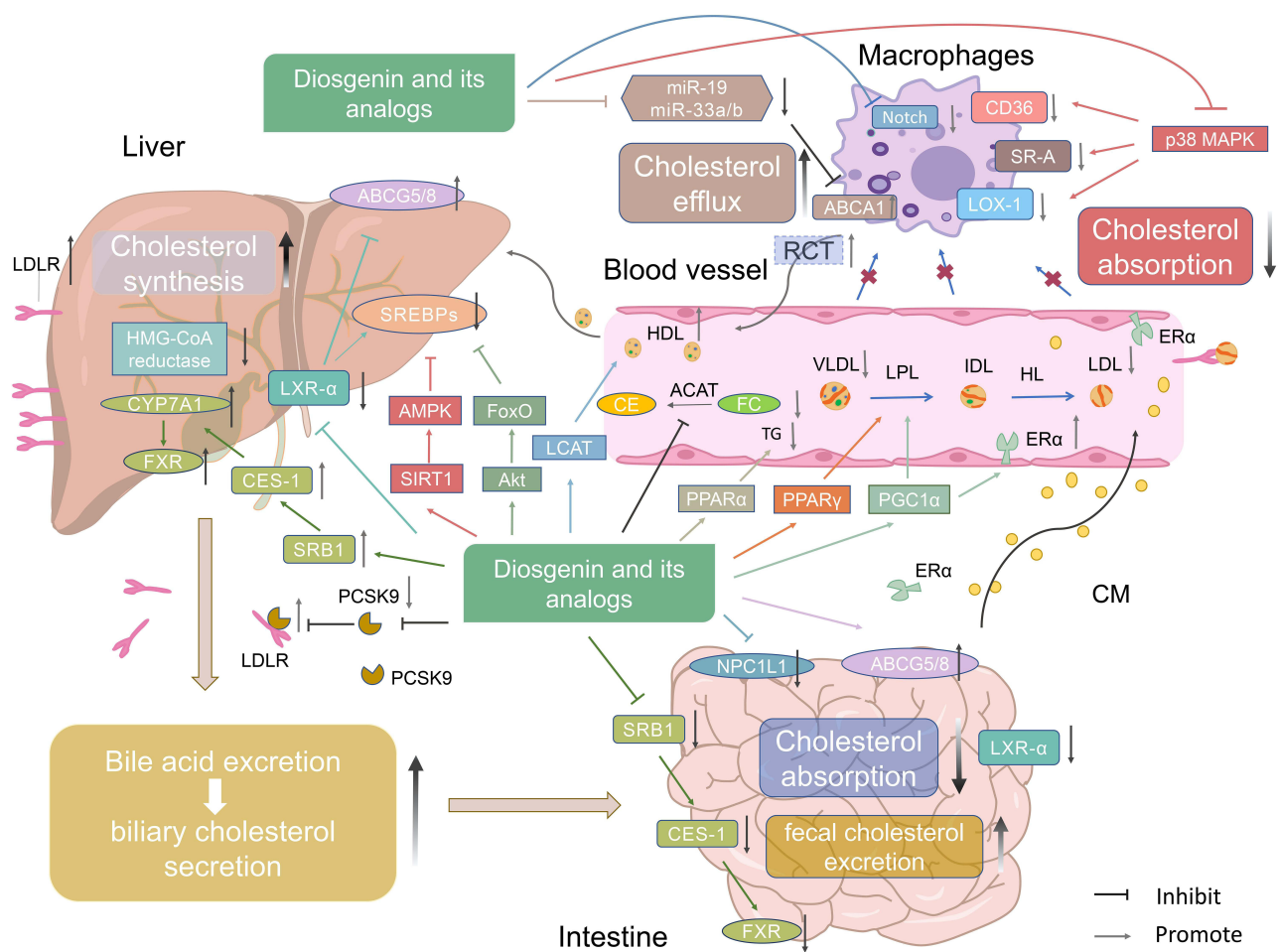


intestine.<sup>128</sup> In addition, diosgenin enhances the transport of peripheral cholesterol to the liver by promoting the expression of SRB1 and CES-1 and promoting the conversion of cholesterol into bile acid by stimulating the expression of CYP7A1 and FXR. It can also inhibit cholesterol absorption by increasing intestinal SRB1 and CES-1.<sup>129</sup>

Thus, it has been concluded that diosgenin and its analogs are effective cholesterol absorption inhibitors, affecting lipid metabolism through various pathways and alleviating hypercholesterolemia and atherosclerosis. Its mechanism is related to the regulation of lipase activity (LPL, PL, and HL), the expression of transcription factors related to lipid metabolism, the promotion of cholesterol outflow, the inhibition of intestinal absorption of cholesterol, and the increase of cholesterol secretion into bile. (As shown in Figure 2)

## Inhibition of Inflammation

There is no doubt that the progressive narrowing of arterial lumen caused by hyperlipidemia is causally related to atherosclerosis. However, recent studies have proved that atherosclerosis may be characterized as chronic artery wall inflammation.<sup>130</sup>



**Figure 2** Mechanism of diosgenin and its analogs in lipid metabolism. Diosgenin and its analogs can affect plasma cholesterol, triglyceride, and fatty acid levels by inhibiting SREBPs and its downstream proteins related to lipid metabolism via regulation of SIRT1/AMPK signaling pathway, Akt/FoxO signaling pathway, LXR signaling pathway and inhibiting lipid metabolism enzymes (LPL, PL, HL). In addition, PPAR signaling pathway and PGC1 $\alpha$ /ER $\alpha$  signaling pathway also play important roles in plasma lipid metabolism. Diosgenin and its analogs can also inhibit hepatic cholesterol synthesis by inhibiting HMG-CoA reductase and increase HDL by improving LCAT. Among these analogs, tomatidine can inhibit cholesterol esterification by inhibiting ACAT, thereby reducing serum LDL and cholesterol esters. During local plaque cholesterol metabolism, diosgenin and its analogs also inhibit foam cell formation by inhibiting Notch signaling pathway and inhibit cholesterol intake by regulating scavenger receptors (SR-A, CD36 and LOX-1) via inhibition of p38 MAPK and promote cholesterol efflux mediated by ABCA1 via inhibition of the expression of miR-19/miR-33a/b. Diosgenin and its analogs can also promote the excretion of liver cholesterol, inhibit the absorption of intestinal cholesterol by promoting the expression of ABCG 5/8 and inhibiting the expression of NPC1L1 in the liver and intestine. In addition, diosgenin can promote the CES-1/SRB1/ CYP7A1/ FXR signaling pathway in the liver, which promote the transformation of cholesterol into bile acids and excretion from feces, and can also decrease CES-1, SRB1 and FXR in the intestine, which inhibits cholesterol absorption. At the same time, MPD and PD promote the up-regulation of LDLR and the transport of circulating cholesterol to the liver by inhibiting PCSK9.

As previously mentioned, endothelial cells are activated by inflammatory signals in the early stage of atherosclerosis. These cells express various adhesion molecules that promote leukocyte adhesion, further stimulating inflammation and plaque progression. Diosgenin and its analogs can inhibit the expression of adhesion molecules, inhibit the adhesion of leukocytes to endothelium, and reduce inflammation. In addition to regulating the expression of adhesion molecules, *in vitro* studies showed that diosgenin can inhibit PA-stimulated HUVEC inflammation (TNF- $\alpha$ , IL-6) through the IKK $\beta$ /NF- $\kappa$ B pathway.<sup>41</sup> It also promotes the expression of adiponectin and PPAR $\gamma$  with anti-inflammatory effects by inhibiting IKK $\beta$ /NF- $\kappa$ B signaling pathway and increasing AMPK activity in PAT, which then inhibits PAT inflammation and alleviates endothelial inflammation through a paracrine or endocrine pathway.<sup>40</sup>

As atherosclerosis progresses, macrophages enter the intima and ingest large amounts of LDL-C, promoting the formation of foam cells and the deposition of lipids on the inner walls of blood vessels. During this process, the pro-inflammatory M1-like macrophages of the disease-modifying macrophages have an important role in inflammation by secreting various chemokines (eg, MCP-1) and cytokines (eg, IL-12, IL-1, TNF $\alpha$ ), which further activate the endothelium and lead to additional rounds of monocyte recruitment.<sup>131,132</sup> In addition, M1 macrophages are the dominant phenotype in the shoulder region of the plaque prone to rupture; they degrade the extracellular matrix in the plaque by increasing the secretion of MMPs, increasing plaque instability and vulnerability to rupture. At the same time, M2 macrophages have a dominant role in scar stability<sup>133</sup> and can promote the alleviation of plaque inflammation by secreting anti-inflammatory cytokines, such as IL-10.

Studies have shown that diosgenin can promote the differentiation of ox-LDL-induced monocytes and monocytes from the aorta into M2 macrophages, as evidenced by the increased expression of M2-specific chemokines (such as MDC, BLC, MIP-1 $\alpha$ , etc.), which, in turn, reduces the expression of inflammatory mediators.<sup>61</sup> High expression of Arg-1 and AMPK promotes M2 differentiation and IL-10 secretion.<sup>134</sup> Diosgenin can also promote the polarization of M2 macrophages by stimulating the expression of Arg-1 and AMPK.<sup>40</sup> It also inhibits the differentiation of macrophages and alleviates atherosclerosis by inhibiting nuclear translocation of the Notch intracellular domain (NICD).<sup>135</sup> In addition to inhibiting the conversion of pro-inflammatory phenotypes, dioscin and diosgenin also inhibit the induction of inflammation in macrophages by pro-inflammatory mediators. For example, dioscin can inhibit the expression of inflammatory cytokines in THP-1 macrophages stimulated by LPS and Pam3CSK4 via downregulation of the TLR2/MyD88/NF- $\kappa$ B signaling pathway.<sup>136</sup> Besides, diosgenin can reduce the expression of inflammatory mediators in LPS/IFN- $\gamma$ -induced RAW264.7 macrophages by inhibiting the activation of NF- $\kappa$ B, CK2, JNK, and AP-1,<sup>137</sup> and also reduce the free fatty acid-induced macrophage cellular inflammation.<sup>138</sup> Similarly, tomatidine can inhibit the expression of proinflammatory enzyme COX-2 and iNOS through suppressing NF- $\kappa$ B and JNK pathways in RAW 264.7 cells, which in turns inhibits macrophage cellular inflammation.<sup>139</sup>

In addition to endothelial cells and macrophages, various cells, especially antigen-presenting cells in the adaptive immune response, have an important role in the progression of plaque inflammation. Dendritic cells are key mediators of antigen presentation, which can activate T cells in plaque and promote the progression of plaque inflammation. *In vitro* studies showed that diosgenin inhibits the secretion of pro-inflammatory cytokines IL-6 and IL-12 in dendritic cells and promotes the expression of IL-10. It is speculated that diosgenin may inhibit the activation of T cells, reduce vascular inflammation, and inhibit atherosclerosis by decreasing the maturation of dendritic cells and improving immune function.<sup>113</sup> Other immune cells, such as B cells, T cells, and neutrophils, promote inflammation in plaques; still, the role of diosgenin and its analogs in the immune regulation of atherosclerosis needs to be further examined.

During plaque progression, VSMCs migrate into the intima and secrete adhesion molecules to recruit monocytes into the intima. Diosgenin can inhibit the expression of TNF- $\alpha$  - induced vascular smooth muscle cell adhesion molecules and reduce macrophage adhesion to VSMCs by inhibiting MAPK/Akt/NF- $\kappa$ B signaling pathway.<sup>140</sup> In advanced atherosclerosis, inflammatory mediators such as IL-1 $\beta$  and TNF- $\alpha$  promote the release of matrix metalloproteinases, which degrade extracellular matrix components and contribute to plaque rupture.<sup>141</sup> They also promote tissue factor (TF) expression and thrombogenesis.<sup>142</sup> Diosgenin can inhibit the expression of TF in THP-1 monocytes induced by TNF- $\alpha$  by inhibiting the activation of NF- $\kappa$ B, Akt, and MAPK signaling pathways; it can also inhibit the procoagulant activity of TF, which is expected to prevent inflammation-induced thrombosis in the late stage of atherosclerosis.<sup>143</sup> Furthermore, *in vivo* studies have confirmed that diosgenin and its analogs have positive anti-inflammatory effects. For example, dioscin

and diosgenin can inhibit the nuclear translocation of NF- $\kappa$ B and reduce the expressions of IL-6, IL-12, TNF- $\alpha$ , CYP2E1, COX-2, and HMGB1 in the plasma, liver, and heart of Wistar and SD rats, C57BL/6J mice, and ob/ob mice induced by atherogenic diet, as well as pig models of coronary heart disease.<sup>39,54,83,91</sup> Hence, diosgenin and its analogs are very effective inflammatory inhibitors, acting on various stages of the progression of atherosclerosis. The mechanism is related to the inhibition of the adhesion of pro-inflammatory leukocytes, promoting the phenotypic transformation of anti-inflammatory macrophages, down-regulation of the NF- $\kappa$ B signaling pathway related to inflammation, and inhibition of the expression of inflammatory cytokines.

## Pharmacokinetics and Bioavailability

Although the above data show an exciting anti-atherosclerotic effect of diosgenin and its analogs, scarce information regarding its pharmacokinetics and bioavailability limits its clinical research and development.

Diosgenin is a strong hydrophobic compound with poor pharmacokinetic parameters (LogP; 5.7).<sup>144</sup> Compared with diosgenin, dioscin shows better intestinal permeability but higher instability in gastric and intestinal juices, which results in very low bioavailability;<sup>145</sup> its absolute oral bioavailability is only about 0.2% (vs bioavailability of diosgenin is 6%) with a long half-life (t<sub>1/2</sub>) and weak absorption of drugs by the enterohepatic recycling.<sup>146,147</sup> Both products are mainly excreted through feces.

Recently, several methods have been used to improve its bioavailability. For example, Okawara et al combined diosgenin with  $\beta$ -cyclodextrin ( $\beta$ -CD) to form an inclusion complex, improving the product bioavailability to 45%.<sup>146</sup> Furthermore, the interaction of liquid crystals with  $\beta$ -cyclodextrin further improves the solubility and bioavailability of diosgenin.<sup>148</sup> However, their preparation is time-consuming and requires many excipients, and the drug loading is low. Therefore, another delivery system that is easier to manufacture is needed to improve the drug load. Kim et al improved hydrophobicity, poor pharmacokinetic parameters, and enhanced bioavailability by conjugating diosgenin with a hydrophilic unit, tetraethylene glycol.<sup>149</sup> Therefore, replacing the glycosylated group with small molecular weight polyethylene glycol (PEG) may be a suitable strategy to improve the pharmacokinetic parameters of diosgenin. Moreover, nanocrystalline preparation can be used for drugs with extremely low aqueous solubility and high logP, increasing the adhesion of drugs in the gastrointestinal tract and achieving high drug loading.<sup>150,151</sup> Research indicated that the development of nanocrystals could improve the dissolution rate and oral bioavailability and the biological effects of diosgenin.<sup>152</sup> As mentioned earlier, diosgenin prodrug nanoparticles can prevent thrombosis with no risk of bleeding. However, nanomaterials may lead to the destruction of heat-sensitive components. Pan et al showed that diosgenin content in 50 mesh-size *Dioscorea pseudojaponica* (DP) was higher than in nanoscale DP. They also found a greater lipid-lowering effect, which may be mediated by the AMPK-ACC pathway.<sup>153</sup>

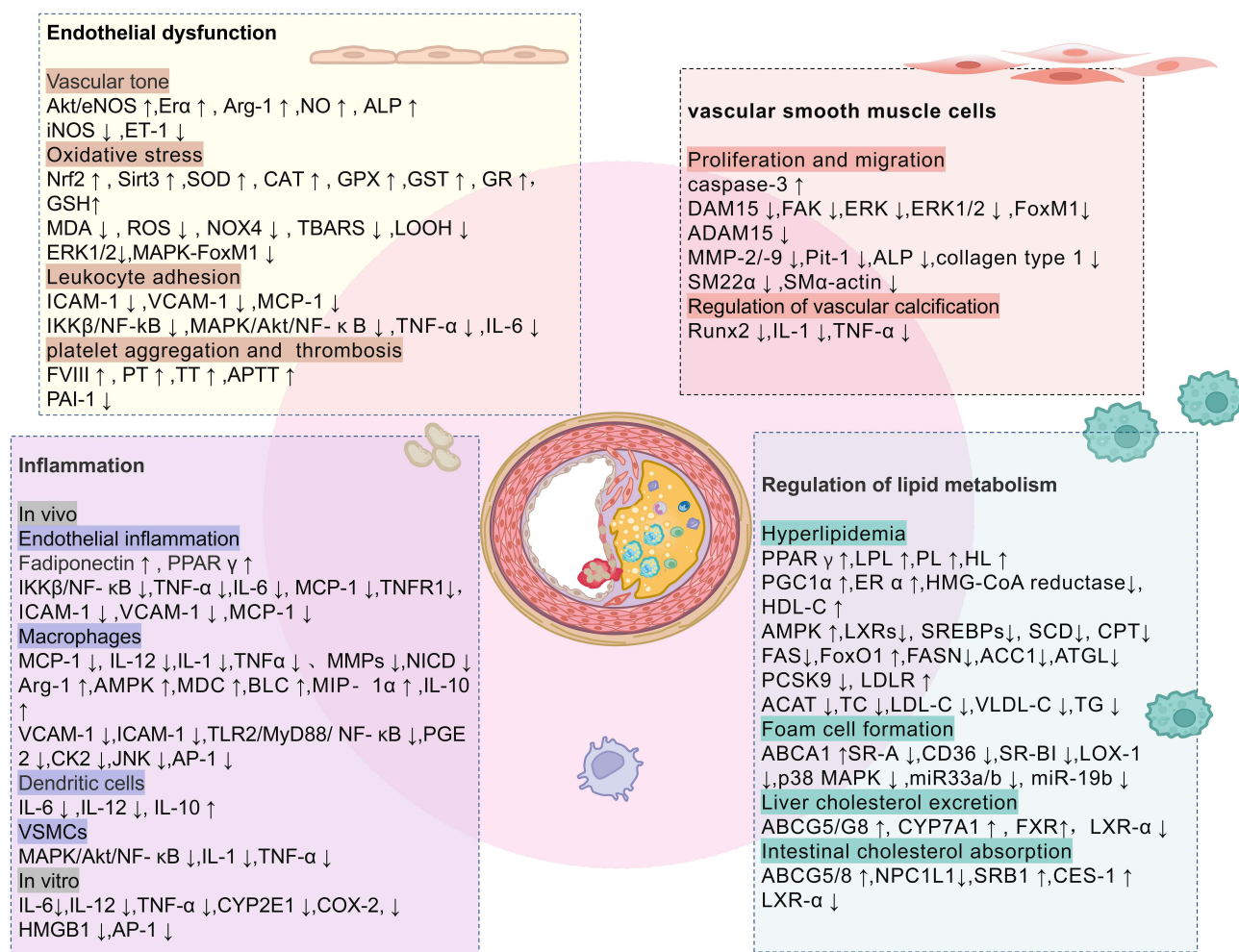
In addition, the development of diosgenin derivatives is beneficial for improving solubility, permeability, and biological activity. For example, compound 5, a diosgenin derivative mentioned earlier, showed better anti-thrombotic properties.<sup>24</sup> Diosgenin derivatives containing primary amine not only enhance the permeability but also inhibit the production of ROS, iNOS, COX-2, and pro-inflammatory cytokines (IL-6, IL-1 $\beta$ , and TNF- $\alpha$ ) in lipopolysaccharide (LPS)-stimulated microglia through NF- $\kappa$ B and JNK MAPK signaling pathways and promote NO synthesis, which has an anti-inflammatory and antioxidant stress role.<sup>154</sup> In addition, the bioactivity of diosgenin can be improved by covalent modification.<sup>155</sup> For example, Laura et al improved the anti-proliferation and anti-oxidation properties of diosgenin by combining thio- and selenoureas at the C-3 position.<sup>156</sup> These data suggest that structural modification of dioscin and diosgenin and the development of new drug delivery systems and derivatives may improve the bioavailability and enhance the anti-atherosclerosis efficacy.

## Conclusions and Perspectives

In general, diosgenin and its analogs show potential therapeutic effects on atherosclerosis. They can improve endothelial dysfunction by regulating vascular tension, oxidative stress, leukocyte adhesion, platelet aggregation, and thrombosis. They can also inhibit the proliferation, migration, and calcification of VSMCs by regulating VSMC phenotype conversion, improve lipid metabolism by inhibiting foam cell formation, regulating hyperlipidemia, inhibiting intestinal cholesterol absorption and promoting bile cholesterol excretion. These molecules have a positive regulatory effect on

various inflammatory cell types, inflammatory signaling pathways, and inflammatory cytokines involved in various stages of AS. (As shown in Figure 3) Besides, diosgenin and its analogs have also shown promising results in treating hyperlipidemia, diabetes, non-alcoholic fatty liver, obesity, and other metabolic syndromes.<sup>157</sup> We summarized the roles of diosgenin, dioscin, and other analogs of diosgenin in atherosclerosis-related diseases in [Supplementary Tables 1–3](#) respectively respectively.

Statins, a gold standard product for the treatment of atherosclerosis, have also shown some shortcomings in lowering cholesterol. For example, statins can only reduce cholesterol levels by inhibiting its synthesis but have little effect on inhibiting intestinal cholesterol absorption and promoting its excretion.<sup>158</sup> Inflammatory inhibitors against atherosclerosis have also not been commercialized due to their high cost. Diosgenin and its analogs significantly inhibit cholesterol absorption and promote its excretion and reduce hypercholesterolemia. They are also widely available and are low cost. Therefore, diosgenin and its analogs are expected to become a new alternative anti- atherosclerosis drug. Yet, poor pharmacokinetic profile, low bioavailability, low aqueous solubility and instability in the gastrointestinal tract limit their clinical application. Thus, more information on the pharmacokinetics and metabolism of diosgenin and its analogs is required. In addition, it is necessary to develop more efficient drug delivery systems and more safe and effective derivatives to improve their bioavailability.



**Figure 3** Mechanism of diosgenin and its analogs in regulating atherosclerosis. Diosgenin and its analogs improve AS by regulating endothelial dysfunction (eg. vascular tension, oxidative stress, leukocyte adhesion, platelet aggregation, and thrombosis), inhibiting proliferation, migration, and calcification of VSMCs, regulating lipid metabolism (eg. ameliorating hyperlipidemia, inhibiting foam cell formation, promoting liver cholesterol excretion, and inhibiting intestinal cholesterol absorption), and inhibiting inflammatory processes.

Studies on diosgenin and its analogs in atherosclerosis and their effect on immune regulation are still lacking, while studies reporting on other analogs are just in the infancy stage. Parallel comparisons of biological activities, pharmacodynamic properties, and content differences between species among multiple structural analogs are also necessary. What's more, although some clinical trials show that extractive preparations rich in diosgenin and its analogs, such as wild yam,<sup>159</sup> fenugreek,<sup>160,161</sup> Di'ao Xin Xue Kang capsule,<sup>162</sup> etc., have a positive effect on atherosclerosis, clinical trials diosgenin and its analogs are still lacking. In addition, it should be noted that whether these drugs exert their effects through diosgenin or other monomeric analogs alone or drug-drug interactions needs to be further explored. The translation of diosgenin and its analogs to the clinic needs to be strengthened in the future. Research on efficacy, dosage, adverse reactions, and drug interactions is also necessary.

Looking into the future, we believe that natural medicines will provide a broader space for new drug development. In 2015, Dr. Youyou Tu won the Nobel Prize in Physiology or Medicine for the extraction of artemisinin from *Artemisia annua*, a Chinese herbal medicine, which once again demonstrated the appeal and feasibility of treating patients with active ingredients derived from natural products. Therefore, we believe that researcher will recognize great potential of diosgenin and its analogs in developing new agents against atherosclerosis, which are also expected to have an important role in treating cardiovascular diseases in the future.

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## Disclosure

All the authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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