Review Article

Pharmacological Progresses of Puerarin in the Prevention and Treatment of Atherosclerosis

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Atherosclerosis is an independent risk factor for cardiovascular diseases, which is related to dyslipidemia, endothelial injury, inflammation, thrombosis, dysfunction of vascular smooth muscle cells and macrophages, etc. Puerarin, a natural monomer from Chinese herb *Pueraria lobata*, presents multiple cardiovascular protective activities. The paper firstly reviewed the risk factors and drug of atherosclerosis. And then, extraction and synthesis of puerarin were summarized. Especially, it focused on the latest advances in anti-atherosclerosis activities of puerarin from six aspects including lipid-regulating, hyperglycemic, anti-inflammatory, inhibiting thrombosis, promoting microcirculation, improving functions of vascular smooth muscle cells and macrophages. Some novel puerarin derivatives also exhibited favorable anti-atherosclerosis activity. The review would provide new molecular skeletons and lead compounds for anti-atherosclerosis new drug development based on puerarin.

Key words: Puerarin, atherosclerosis, hyperglycemic, anti-inflammatory, pharmacological activity

ATHEROSCLEROSIS (AS)

Cardiovascular Disease (CVD) is one of the major causes of death in the world[1]. According to the statistics of the World Health Organization, and according to the statistics of annual report of CVD in 2021, 17.9 million people died of CVD, accounting for 32 % of the global deaths. The number of CVD patients in China is 330 million, the number of new cases is 12.3411 million, and the death rate is 4.5843 million. The prevalence rate increased from 4235.43/10 million in 1990 to 8460.08/10 million in 2022[2]. AS is the main pathological basis of cardio-cerebrovascular disease[3].

Risk factors of AS:

The disorder of lipid metabolism results in the yellow atherosclerotic appearance of lipid accumulation in the intima of arteries, so it is called AS. AS is a chronic, continuous and complicated inflammatory vascular disease characterized by lipid deposition in the intima of large and middle arteries, atherosclerotic plaque formation, increased fibrous tissue, and AS[4]. The early stage of AS is affected by many factors, that are closely related to dyslipidemia, endothelial injury, the release of inflammatory factors[5], immune dysfunction and hemodynamic changes[6].

Dyslipidemia: When the lipid metabolism is abnormal, the excessive lipid is engulfed and transformed into foam cells, which accumulate and deposit in the artery intima, and then lead to the formation of plaque successively. After lipid metabolism disorder, macrophages phagocytosis and oxidation of low-density lipoprotein and cholesterol further cause lipid accumulation and accelerate plaque formation[7].

Endothelial injury: When the vascular endothelium is damaged and its permeability is enhanced, low-density lipoprotein enters the endothelium, and after oxidation, it is engulfed by macrophages or Vascular Smooth Muscle Cells (VSMCs) and transformed into

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foam cells. The increase of Reactive Oxygen Species (ROS) will cause cell apoptosis and endothelial injury caused by Oxidative Stress (OS), and the Endothelial Cells (EC) will release inflammatory factors when activated[8]. When the endothelium is damaged or ruptured, platelets will adhere to the EC with dysfunction and damage, further forming platelet-rich thrombus[9].

Inflammation: AS is a process of chronic inflammatory reaction. Inflammatory cells can induce the activation of white blood cells and the proliferation and differentiation of VSMCs and macrophages. Inflammatory cells can promote the movement of lipids to plaque, thus increasing the permeability of the endothelial layer or promoting the differentiation VSMCs or macrophages, thus responsible for the encapsulation and transport of lipoproteins[10,11].

Thrombosis: When blood transportation is blocked, thrombosis will occur, resulting in a series of high-risk cardio-cerebrovascular diseases, such as myocardial ischemia and stroke[12-14]. Platelets will rapidly adhere to and gather at the damaged site when blood flows, and fibrin will stimulate and activate them, while releasing Thromboxane A2 (TXA2), to further cause thrombosis, thus accelerating the process of AS. A load of plaque will increase with the growth of age, and the pathogenic factors of AS are various and complex[11].

Other: Recent studies have shown that the development of AS may be affected by bad habits such as staying up late, drinking and smoking, as well as by the lack of some common trace elements. Not only is the regulation of blood flow distribution in various parts of the body affected, but when AS also occurs in the aorta and its branches, it can lead to the destruction of the elastic and muscular layer of the arterial wall, as well as affecting the elasticity of the vessel wall, making it thin and brittle. AS is a chronic degenerative disease that mostly occurs at an advanced age. With increasing of our population aging, cardiovascular and cerebrovascular-related diseases caused by AS have become one of the main factors causing

AS therapeutic agents:

Lipid-regulating agents: Lipid-lowering drugs statins, such as atorvastatin and rivastatin, are currently the most common in the clinical treatment of cardiovascular and cerebrovascular diseases. It mainly inhibits the production of Total Cholesterol (TC) and Low-Density Lipoprotein Cholesterol (LDL-C) in the body by inhibiting 3-Hydroxy-3 Methylglutaryl Coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme for early cholesterol synthesis, to lower lipids, slow down the development of vascular lipid infiltration, and stabilize atheromatous plaques. Statins reduce LDL-C and TG levels by approximately 50 % and 30 %[13], thus reducing plaque lipid composition and inflammatory infiltration. However, it is indicated for high-risk patients without drug contraindications, that can trigger some liver function injury, muscle toxicity, muscle pain, even rhabdomyolysis in severe cases, cognitive impairment and so on, which limited their clinical applications[14].

Anti-platelet aggregating drugs: Platelet hyper activation is a key factor in thrombosis, and when platelets are impaired with aggregation, TXA2, synthesis is blocked by closing the receptors on the platelet membrane[15]. Clopidogrel, aspirin and tegrel are commonly used antiplatelet drugs. Clopidogrel is an adenosine diphosphate inhibitor, it can activate the platelet glycoprotein receptor by combining with the adenosine triphosphate receptor on the platelet surface, and reduce the platelet aggregation rate to stabilize plaque and prevent thrombosis. It also stimulates the gastric mucosa and tends to cause bleeding. Thrombolytic drugs streptokinase, urokinase, and anticoagulant drugs heparin can also cause bleeding or ischemia for patients with vascular obstruction[16]. Some patients with acute atherosclerotic cerebral infarction who take aspirin and clopidogrel regularly for a long time still have a recurrence, which may be related to the occurrence of resistance to aspirin and clopidogrel *in vivo*[16].

Anti-hypertensive drugs: Calcium Channel Blockers (CCBs) can prevent extracellular Ca^{2+} entering VSMCs through L-type calcium channels, reduce intracellular Ca^{2+} concentration, relax vascular smooth muscle, and then lower blood pressure. Meanwhile, CCBs can also inhibit lipid peroxidation, VSMCs proliferation, and platelet aggregation. The overactivation of the Renin-Angiotensin-Aldosterone (RASS) system and receptors are closely related to hypertension. Telmisartan, an inhibitor of Angiotensin II Receptor 1 (AT1), exerts antihypertensive effects by competitively binding AT1 to inhibit the activity of angiotensin II to block the excessive activation of the RASS system[17].

Antioxidants: Antioxidants can slow down the occurrence of AS by increasing the level of Superoxide Dismutase (SOD), reducing the level of Malonaldehyde (MDA), thus antagonizing the oxidative stress reaction produced by ox-LDL. Ox-LDL can increase Ca^{2+} concentration under reactive oxygen species, thus inducing apoptosis and inflammation[18]. Vitamin C can increase the elasticity of blood vessels, improve endothelial damage and remove oxygen free radicals in the body. Vitamin E can improve AS by inhibiting VSMCs proliferation and platelet adhesion and aggregation[19].

EXTRACTION OF PUERARIN

The traditional extraction method of *Pueraria lobata* (*P. lobata*) is extraction, but the amount of extraction is small. At present, the commonly used extraction methods include solvent extraction, microwave-assisted extraction, ultrasonic-assisted extraction, etc. Generally, a single factor test, 4 factors, and 3 levels orthogonal test are used to find its optimal extraction process parameters. The advantages and disadvantages of extract methods for puerarin are summarized in Table 1.

Solvent extraction method:

The solvent extraction method is based on the principle of "similar phase dissolution", and selects the appropriate solvent to extract the effective chemical components from the principle. Zhang *et al.*[20] studied the effects of different extraction methods on the purity, extraction rate, and extract rate of puerarin in *P. lobata* by High Performance Liquid Chromatography (HPLC). The results showed that the total amount and purity of puerarin extracted by the ethanol heating reflux extraction method were higher, and the transfer rate and extraction rate were the highest among the four methods[32]. The best experimental conditions for extraction are the ethanol heating reflux method; ethanol volume fraction is 60 %, extraction temperature is 80°, solution dosage is 40 ml, extraction time is 60 min, the solid-liquid ratio is 1:8, extraction filtration, and three times of filtration. Pan *et al.*[21] used the low-temperature extraction method to optimize the extraction process based on the single factor test and the orthogonal test. The experimental results showed that the best conditions were; the ratio of material to liquid was 1:16, the extraction temperature was 60°, the extraction time was 2.5 h, the mass concentration of Polyvinylpyrrolidone (PVP) was 45 mg/ml, and the extraction rate of puerarin was 1.60 %. It can be used to extract puerarin from *P. lobata*, and adding cosolvent PVP can improve the antioxidant activity of its extraction[20]. The solvent extraction method has the advantages of a higher extraction rate, simple operation, harmless to the environment, simple equipment, and low cost, but its solvent consumption is large, time-consuming, high energy consumption and high required temperature, which is suitable for mass production[22].

Ultrasonic assisted extraction:

The ultrasonic-assisted extraction method uses the mechanical vibration and cavitation effect of ultrasound to destroy the structure of plant cells, accelerate the solvent entering into cells, accelerate the dissolution of substances in cells, and improve the yield[23]. Based on single factor test, Chen *et al*[24]. analyzed the results using the response surface method according to the design principle of the star point test, and found that the best extraction process conditions were; extraction temperature 57°, ethanol concentration 69 %, ultrasonic time 45 min, liquid-solid ratio 21:1.

TABLE 1: ADVANTAGES AND DISADVANTAGES OF KUDZU EXTRACT METHODS

Name	Condition	Advantages	Disadvantages
Solvent extraction	Proper solvent and temperature, repeated extraction	Simple operation, simple equipment and low cost[22,23]	High solvent consumption, long time consumption, high energy consumption, and high temperature[23]
Ultrasonic-assisted extraction	Extraction of plant active ingredients. Frequency (20~50 kHz), temperature (40°-60°)	High efficiency, short time consumption, low solvent consumption, simple operation, no heating, green environmental protection, wide application range[22]	Not-easy controlled extraction time[24]
Microwave-assisted extraction	Polar solvent	Time-saving, efficient and green initiative[26]	Not-easy controlled extraction time[24]

Under this process condition, the predicted value of puerarin extraction rate is 3.53 %, %, and the verified value is 3.52 %. The ultrasonic method has the characteristics of high efficiency, short time consumption, low solvent consumption, simple operation, green and environmental protection, but it needs to control the time to reduce the impact of this method[22].

Microwave-assisted extraction:

The microwave-assisted extraction method is an extraction method that accepts the thermal effect generated by the microwave through the conductivity effect of the ions in the solution and the effect of the molecules due to polarization and improves the solute entering the solvent. The microwave extraction method mainly uses ions to realize the direct heating of substances. Its main characteristics are short action time, fast speed, high extraction efficiency and energy saving[23].

Li *et al.*[25] extracted total flavonoids from *P. lobata* by microwave-assisted extraction with the aqueous solution of 1-butyl-3-methylimidazole bromide as solvent. The optimum extraction conditions were obtained by single factor experiment and response surface experiment; solid-liquid ratio 1:20, extraction time 9 min and extraction temperature 76°. It was verified that this method improved the extraction rate of total flavonoids in *P. lobata*. Wang *et al.*[26] applied microwave-assisted hydrolysis based on the ionic liquid to the hydrolysis of puerarin in *P. lobata* extract, using sulfite as the catalyst and optimizing the hydrolysis parameters using response surface methodology, and the optimal conditions were obtained as follows; 0.82 mol/l sulfite as catalyst, 400 W microwave power and 7 min microwave time. Compared with the traditional acid catalyst, it shows higher efficiency, which indicates that microwave-assisted water makes the process faster and more efficient. Liu *et al.*[27] optimized the microwave-assisted extraction by response surface method, determined the content of puerarin by HPLC fluorescence method, soaked the sample with 70 % methanol (1:15, v/v) for 30 min, and then optimized the extraction procedure by microwave irradiation for 11 min at 600 W power, which is more accurate and efficient than the method in Chinese Pharmacopoeia, and has a better linear relationship. The microwave extraction method has the advantages of high efficiency, low cost, fast, efficient, and simple extraction method, low pollution to the environment, and has broad development prospects in puerarin extraction[23,26].

ANTI-AS ACTIVITIES OF PUERARIN

Puerarin is derived from the traditional Chinese herb dried roots of *P. lobata*[28]. The chemical structure of puerarin is 4,7-dihydroxy-8-β-D-glucose isoflavone, the relative molecular weight is 416, melting point is 187° and solubility in water is 6.24 g/l[29]. Flavonoids, puerarin, coumarins, triterpenoids and triterpenoid saponins are the main representative bioactive substances of P. lobata, among which puerarin isoflavones play a major pharmacological role[30-32].

Puerarin exhibits multiple pharmacological activities, such as anti-inflammatory, anti-lipid oxidation, improving microcirculation, improving hemorheology, vasodilating blood vessels, lowering blood lipids, which is widely used to treat cardiovascular diseases[33,34].

Improving AS by lipid-regulating:

The lipid metabolism disorder is the key point to develop AS[30]. Lipid aggregation in macrophages activates the NLRP3 signal pathway and then promotes the secretion of inflammatory factors to participate in the formation and development of AS plaque. Macrophages can phagocytose ox-LDL and cholesterol in the high-fat environment, resulting in a large amount of lipid deposition, further accelerating to form local vascular plaques[35,36].

Cholesterol comes from endogenous synthesis and food intake. After metabolism is blocked, the cholesterol level in the blood cannot maintain normal. Puerarin not only inhibits the production of free radicals but also accelerates their clearance, thus reducing blood lipids. Ox-LDL, an AS independent risk factor, accumulates in endothelial cells to form plaque to make vessels brittle and easy to crack[37]. Lysophosphatidylcholine (LPC) is the main component of ox-LDL to produce oxygen free radicals and cause vascular endothelial damage, which will reduce the endothelium-dependent vasodilation[30]. Puerarin can improve vascular endothelial injury caused by LPC, which is caused by reducing the endothelium-dependent vasodilation of rabbits injured by glycosylated bovine serum albumin, increasing the Nitric oxide (NO) content and SOD activity in vascular tissue, thus playing a protective role on the endothelium[29,36]. The large accumulation of ox-LDL in macrophages by scavenger receptors can induce the endothelial

injury and the inflammatory reaction[37]. Puerarin can effectively reduce lipid peroxidation, protect endothelial function and reduce ischemic damage by improving antioxidant capacity. SOD, an antioxidant marker, can effectively remove superoxide anion free radicals, reduce lipid peroxidation reaction and protect cells from oxidative damage. MDA, a product of the reaction of polyunsaturated fatty acids and reactive oxygen species, reflects the degree of lipid peroxidation[29]. It was found that *P. lobata* extract can effectively reduce the level of blood lipids in hyperlipidemic rats by reducing the degree of lipid peroxidation and improving antioxidant capacity[34]. It was indicated that puerarin could significantly increase the uptake of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) by cells, reduce the release of Lactate Dehydrogenase (LDH), and effectively reduce the effects of MDA and TXA2. It showed that puerarin had a protective effect on endothelial injury caused by lipid peroxidation. At the early stage of cell apoptosis, the cell membrane is intact and the cell structure changes slightly. At this time, the cell function could be reversed by appropriate intervention and treatment with puerarin[38].

Puerarin exerts protective effects on vessels injured by lipid peroxidation from the following ways, provide proton to directly destroy hydrogen peroxide and reduce its damage to cells; improve the activity of endogenous antioxidant enzymes, such as improving the activity of SOD in vivo, and then improve the cell antioxidant capacity[1]; it is directly involved in repairing damaged DNA to protect cells. Puerarin can reduce the lipid peroxidation of brain cells, inhibit the effects of superoxide anion free radicals and thus playing a protective role against free radical damage. The 3-hydroxy group of puerarin plays an important role in the removal of total reactive oxygen species. Puerarin can significantly reduce blood lipid levels and alleviate the hypercoagulable state caused by hyperlipidemia by improving platelet aggregation and blood coagulation system of hyperlipidemia rats, thus playing a role in protecting the cardiovascular system[29].

Improving AS by hyperglycemic effects:

The disorder of blood glucose metabolism is closely related to AS. Metabolic Syndrome (MS) is the aggregation of multiple risk factors such as abnormal glucose metabolism, elevated blood pressure and abnormal lipid metabolism. The healthy ECs with dysglycemia can maintain the normal functioning of the vascular system[39,40]. AS is the main cause of death in patients with type II diabetes. The disorder of glucose metabolism is one of the risk factors for AS in patients with diabetes. Hyperglycemia can directly participate in vascular endothelial damage and promote the formation of AS plaque. The significant increase of serum markers of vascular endothelial dysfunction, such as E-selectin and Vascular Adhesion Molecule-1 (VCAM-1), and the oxidative damage of vascular endothelial dysfunction caused by OS are the key factors leading to the structural damage and dysfunction of EC. The increase of soluble lectin-like Oxidized LDL receptor-1 (sLOX-1) further promotes the uptake of oxLDL by cells, thus participating in the formation of Advanced Glycation End Products (AGEs) induced foam cells. High concentrations of glucose or oxLDL can promote the increase of calcium in EC, thus activating calcium dependent endonuclease to cleave DNA and induce apoptosis of EC. Dunn *et al.*[7] found that hyperglycemia can promote the binding of kinase A Anchoring Protein (AKAP) and Protein Kinase A (PKA) of VSMCs, thus activating L-type Ca^{2+} channels. The binding of AGEs and their receptors can increase the expression of B7.1, B7.2, and Cluster of Differentiation (CD) 40 molecules on the surface of monocytes and combine with the corresponding ligands on the surface of T cells to produce inflammatory factors, leading to inflammatory injury of blood vessels. Puerarin can reduce blood sugar levels and improve glucose, and lipid metabolism by vasodilating blood vessels and reducing blood viscosity. The phenolic hydroxyl in the puerarin structure can combine with oxygen free radicals to maintain the stability of the cell membrane. It can also increase the activity of nitric oxide synthase, thereby increasing the level of NO expression to improve AS[41,42].

OS leads to the increase of ROS and Reactive Nitrogen Species (RNS) and the production of lipid peroxidation products, resulting in a long-term imbalance between the body's antioxidant defense and ROS levels[37,43]. Persistent high ROS levels lead to vascular endothelial remodeling, vascular barrier destruction, and tissue damage and affect vascular regulation, further activate inflammatory reaction, and finally develop AS[44]. Valerie *et al.*[45] fed low-density lipoprotein receptor-deficient (LDLR−/−) mice with a high-fat diet for 22 w, found that the expression level of glucose transporter GLUT1 in arterial VSMCs near atherosclerotic

lesions increased, and SMC glucose metabolism and MS had a significant synergistic effect in accelerating the progression of AS[63]. The increase of intracellular glucose level is realized through the increase of cellular metabolic pathways (such as the mitochondrial electron transport system), which may lead to the excessive production of ROS. In addition, glucose metabolites can be activated by protein kinase Cβ and aldose reductase to induce proinflammatory reactions[46].

Improving AS by anti-inflammation:

Early AS is accompanied by the occurrence of vascular endothelial inflammation. When ECs are activated, they express inflammatory factors, attract lymphocytes and monocyte biomolecules that bind with ECs, and infiltrate the arterial wall to induce inflammation[8]. Relevant inflammatory factors and endothelial cell macrophages are important inflammatory effector cells in the body. Under the stimulation of inflammatory factors, resting macrophages undergo M1 polarization and release a large amount of lipopolysaccharide, Interleukin (IL-6), Tumor Necrosis Factor-Alpha (TNF-α), inducible Nitric Oxide Synthase (iNOS), and other inflammatory factors to combat exogenous infection[47], AS is a chronic vasculitis disease and the inflammation is the inducing factor for the occurrence and development of AS. A large amount of ox-LDL deposite on the inner wall of blood vessels. Macrophages or VSMCs engulf them and convert it into foam cells[48]. The formation of foam cells is an important indicator for the early pathological changes of AS[49].

Many inflammatory factors, such as monocyte chemoattractant protein-1, IL-8, Intercellular Adhesion Molecule-1 (ICAM-1), VCAM-1, E-selectin, P-selectin, and other key Nuclear Factor-kappa B (NF-κB) as a major transcription factor in the pathogenesis of inflammation, it can mediate the transcription of inflammatory factors after being activated. Up-regulate adhesion factors, adhere to the cell endothelium and migrate to the intima, and degraded extracellular matrix, participate in the whole process of AS to damage tissues[47]. The pattern recognition receptor Toll-Like Receptor 4 (TLR4), highly expressed by macrophages, participates in the occurrence of AS by mediating inflammatory reaction[50]. It was found that puerarin can reduce inflammatory factors and promote the production of M2 type macrophages, targeting to TLR4-NF-κB anti-inflammatory pathway to slow down the chronic inflammatory reaction and plaque formation of AS[51]. Puerarin exerts anti-inflammatory effects by the following ways, inhibiting the expression of related inflammatory factors such as IL-1, IL-6 in vivo; alleviate inflammation by reducing the expression of Matrix Metalloproteinase (MMP) 2 and MMP-9; reduce the production of ICAM-1; and inhibit the effects of inflammatory chemokines, such as Monocyte Chemoattractant Protein-1 (MCP-1) and Transforming Growth Factor-Beta (TGF-β). Puerarin can also activate the Na+-K+-ATPase, Activating Protein-1 (AP-1) β interferon TIR domain adapter protein (TRIF) and Mitogen- Activated Protein Kinase (MAPK) pathway to reduce inflammatory reaction and C-reactive protein level, thus inhibiting the expression of CD36, the key factor of free fatty acid accumulation, and systemic inflammatory reaction[47-51].

Improving AS by inhibiting thrombosis:

The high coagulation state of blood caused by abnormal hemorheology and coagulation dysfunction is a feature of the high-fat state, thus forming, thrombus. It is also an important factor causing AS, and also leading to cardiovascular and cerebrovascular diseases[11,30]. 55 %-65 % coronary artery thrombus are from AS plaque rupture, 30 %-35 % from plaque erosion, and 2 %-7 % from calcified nodules[9]. When the blood viscosity is high, the flow volume will decrease. A proper blood viscosity can ensure the normal transportation and flow of blood. When EC are damaged, platelets will quickly adhere to and gather at the damaged site when blood flows, and the exposed fibrin will be activated, and release TXA2, to cause thrombosis. Prostaglandin I2 (PGI2) synthesized by the vascular wall has a vasodilation effect and can also cause platelet depolymerization. TXA2 and PGI2 have an antagonistic effect, which can destroy the balance between prostaglandin and TXA2 by activating platelet cyclooxygenase, leading to further aggregation of platelets and triggering vasoconstriction, forming thrombus[11].

Liu *et al*.[30] injected puerarin to rats fed with a high-fat diet. After 30 d, the plasma viscosity and whole blood viscosity, TC, TG and LDL-C were all reduced to normal levels. It was found They found that puerarin could reduce blood lipids by improving blood hypercoagulability, improving microcirculation, and reducing TXA2 to increase the ability of the myocardium to resist hypoxia and ischemia, and avoid the occurrence of CVD. Zou *et*

al. used a metabolic spectrum analysis method based on nuclear magnetic resonance to characterize the metabolic characteristics of blood stasis in rats and measured the biomarkers related to blood stasis in the blood and urine of rats in blood stasis model group, puerarin pretreatment group, and control group respectively. It was found that the rats with blood stasis after puerarin administration were effectively improved. The Erythrocyte Aggregation Index (EAI) was significantly lower than that of the model group, which confirmed the protective effect of puerarin on metabolic changes[11]. Xu *et al.*[52] made rats in a state of blood aggregation and viscosity, resulting in microcirculation perfusion disorder and abnormal coagulation function in rats with acute blood stasis took blood under the abdominal aorta after puerarin administration and detected their erythrocyte aggregation index, whole blood viscosity, plasma viscosity, erythrocyte sedimentation rate at 37°. Compared with the model group, the hemorheology indexes of rats in puerarin administration group showed a significant improvement, indicating that puerarin can effectively improve blood viscosity to prevent AS. It was found in clinical studies that puerarin can ensure the smoothness of blood vessels and prevent the formation of thrombus by stabilizing the elevation of vascular EC and inhibiting the aggregation of platelets. Puerarin can effectively reduce blood viscosity and platelet adhesion rate. Adenosine diphosphate is an important inducer of platelet coagulation. Rats with thrombus induced by adenosine diphosphate have obvious improvement after treatment with puerarin[11]. It was proved that puerarin could reduce blood viscosity, platelet aggregation rate and platelet adhesion rate, and inhibit thrombosis, which would provide new idea for prevention and treatment of patients with hyperviscosity and hypercoagulability[11].

Improving AS by promoting microcirculation:

The changes in microcirculation were related to AS progress. Adrenoceptors are distributed on the effector cell membrane innervated by most of the sympathetic postganglionic fibers, and neurotransmitters and catecholamines β[11]. β-receptors are mainly distributed in the heart, bronchus and VSMCs. β-receptor blockers can effectively relax vessels, prevent endothelial damage caused by stimulation, caused by VSMCs abnormal proliferation, and further reduce the secretion of chemokines and monocyte endothelial adhesion. Puerarin also has β-receptor blocking activity. Puerarin could relax peripheral blood vessels after intravenous injection, which could reduce blood pressure. In rat aortic cells, puerarin could induce NO production in a concentration-dependent manner. The vasodilation effects of puerarin depended on NO/NO-cGMP signal pathway[53,54]. In addition, puerarin could relax rat aortic rings by activating K^+ channels[53]. Puerarin (100 mM) could completely antagonize the contraction of rat aorta induced by PE or KCl and was blocked by the K^+ channel blockers tetraethylamine, 4-aminopyridine, Ba^{2+} and glibenclamide. In the absence of a Ca^{2+} solution, the above vasodilator effects of puerarin completely disappeared, which would be related to the effects of puerarin on the Ca^{2+} influx of vascular endothelial cells[55]. Liu *et al.*[56] measured the relaxation rate of puerarin on rat thoracic aorta at the concentration of 0.01 g/l, 0.05 g/l, and 0.10 g/l by vascular ring tension, which were 18.03 %, 45.73 %, and 66.36 % respectively. Other studies have shown that puerarin (0.1~100 mM) could activate the Ca^{2+}-dependent K^+ channel (BKCa) in a concentration-dependent manner and was blocked by the specific BKCa blocker iberiotoxin[55,57].

The endothelium-dependent mechanism of NO production and the endothelial pathway mediated by K^+ channels can effectively promote vasodilation[3]. It was found that the dysfunction of the vascular endothelial system occurred in the pathological process of atherosclerosis, coronary heart disease, hypertension, etc., and an obvious characteristic was reduced NO secretion to result in decreased vasodilation response. Puerarin could reduce blood pressure and improve endothelial function by increasing NO level in the blood of rats[58,59].

Improving AS by improving functions of macrophages and VSMCs:

VSMCs are the main components of the vascular walls, which is related to vascular remodeling, and determine vascular compliance. The phenotypic transformation, migration, proliferation and apoptosis of VSMCs affect the whole process of AS occurrence[60]. Its excessive migration leaded to pathological intimal thickening. The abnormal proliferation VSMCs infiltrating into the intima would lead to the thickening of the vessel wall, poor elasticity of the vessel, and hypertrophy of the medial wall of the vessel, which became the condition of thrombosis or AS. The formation of thrombus would lead to the occlusion of the blood vessel. At this time,

the migration of proliferative VSMCs would make the intima of the blood vessel thicker[61]. A large number of MMPs and fibrinolytic enzymes at the cell edge could degrade the extracellular matrix and promote the migration of VSMCs. The accumulation of VSMCs in the intima of the vascular wall caused the formation of neointima[60,61].

Homocysteine (Hcy) is a sulfur-containing amino acid and an intermediate product of methionine metabolism. Hcy can induce the proliferation, migration, and phenotype transformation of VSMCs, but the details of these mechanisms are still unclear. The Phosphatidylinositol 3-Kinase/Akt/mammalian Target of Rapamycin (PI3K/Akt/mTOR) signal pathway is involved in a series of cell functions. PI3K/Akt/mTOR pathway is an important biological pathway for the occurrence and development of AS and is involved in the regulation of cell proliferation, differentiation, apoptosis, glucose transport and other cell functions. PI3K can be activated by various signal molecules, leading to the phosphorylation of the downstream target molecule Akt. Phosphorylated Akt (p-Akt) can regulate the expression of the apoptotic protein. Most commonly, p-Akt promotes cell proliferation by inhibiting apoptosis. The down-regulation of the PI3K/Akt/mTOR signaling pathway has been confirmed in many CVD. PI3K/Akt/mTOR has been proven to regulate the phenotypic transformation of VSMCs by regulating the expression of VSMC markers, including L-type calcium channels. Li *et al.*[61] found that puerarin can block the proliferation of smooth muscle and maintain the normal state of vascular intima by effectively inhibiting secretion of MMP-2 and MMP-9. It prevented the abnormal proliferation of VSMCs, endothelial injury from harmful stimulation, reduced secretion and adhesion of chemokines and monocytes. Puerarin could inhibit the secretion of a large number of growth factors from endothelial cells, macrophages, and platelets that were dysfunctional by reducing the level of monocyte chemoattractant protein and preventing the formation of plaque on the inner wall of blood vessels.

PUERARIN DERIVATIZATION

As one of the representative drugs for the treatment of cardio-cerebrovascular diseases, puerarin has many pharmacological effects, low toxicity, and high safety. Because of its tight hydrogen bond, relatively poor water solubility, and low oral bioavailability, which limited its application. Ethylene glycol and other cosolvents can increase the solubility of puerarin, but it has certain side effects. Therefore, it is necessary to modify its structure to improve its druggability[62]. Under the influence of steric hindrance, the activity of the hydroxyl groups at the position-7 and 8 of puerarin is weaker than that at position-4. Therefore, under alkaline conditions, the puerarin was first salted, and then reacted with double halides, and the halogen elements were retained to continue to react with amines, and finally, the target derivatives were obtained. It was mainly to introduce bromopropyl to the 4-hydroxy group, and then reacted with secondary amine and primary amine to obtain 12 target derivatives to increase their water solubility[62]. The water-soluble 3',5'-hydroxymethyl puerarin and 4'-hydroxyethyl puerarin were obtained by hydroxymethylation reaction and nucleophilic substitution reaction. The water solubility was found to be 10 times and 5 times higher than that of puerarin[63]. To change the lipophilicity of puerarin, the structure of puerarin containing sugar groups was modified by using the method of Atherton-Todd reaction and phosphorylation structure modification, and puerarin-7-phosphate diacetyl was synthesized. With diethyl phosphite and diisopropyl phosphite as the phosphorylation reagent and puerarin as the parent nucleus structure, two phosphorylation products of puerarin, namely 7'-diethyl puerarin phosphate and 7'-diisopropyl puerarin phosphate, were synthesized through the modified AtIlenon-Ted reaction[64]. The acylation modification of the phenolic hydroxyl at C-7 of the puerarin molecule promoted cardiac protection against myocardial ischemia and reperfusion injury, and the derivative formed by replacing the original phenolic hydroxyl in the puerarin molecule increased lipophilicity by inhibiting iNOS activity and increasing the activity of Ca^{2+}-Mg^{2+}-ATPase in the cerebral cortex. Puerarin-7-O-glucuronide, the water-soluble metabolite of puerarin, improved the total SOD activity, glutathione/glutathione disulfide ratio and total anti-oxidation ability of neonatal rat cardiomyocytes induced by xanthine oxidase/xanthine by reducing the level of intracellular superoxide[65]. 3'-methoxy puerarin regulated the dynamic changes of amino acids by inhibiting the levels of aspartic acid, glutamic acid, taurine and GABA[65]. It also increased the clinical therapeutic effects by changing the injection form of puerarin, such as puerarin injection, puerarin sodium chloride injection, and puerarin glucose injection[32].

SUMMARY

AS is the main pathological basis of CVD. It is a complex pathogenesis involved in multiple factors. Ox-LDL enables macrophages to adhere to the endothelium and engulf the lipid under the endothelium to further form foam cells to promote the proliferation, migration, apoptosis and lipid deposition of VSMCs, and finally leads to the formation of AS. It is related to dyslipidemia, endothelial injury, inflammation, thrombosis, dysfunction of VSMCs and macrophages, etc. The exact mechanism of development and progression of AS is unclear, which is worth pursuing.

Puerarin is an effective ingredient of the traditional Chinese herb *P. lobata*, with high safety, low toxicity, and clear efficacy[30], which would provide new molecular skeletons and lead compounds for anti-AS new drug development based on puerarin. Puerarin could improve AS by regulating lipid metabolism, balancing glucose metabolism, improving vascular endothelial inflammation, inhibiting thrombosis, and improving microcirculation and endothelial cell function and so on, which presented favorable druggability. Some novel puerarin derivatives also exhibited favorable anti-AS activity. It is believed that with the deepening of puerarin research, there will be a broader market application prospect in the future.

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Conflict of interest:

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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