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Research Progress on the Mechanism of Salvia Miltiorrhiza Extract Inhibiting Platelet Aggregation

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Abstract: *Thrombotic diseases have high disability rates, high mortality rates, and seriously endanger human health and safety. Platelets play an important role in the process of thrombus formation. Salvia miltiorrhiza has been widely recognized in clinical practice both domestically and internationally for its ability to promote blood circulation, remove blood stasis, antioxidant, and anti-platelet aggregation, and anti-atherosclerosis effects. Due to the multi-component, multi-pathway, and multi-target characteristics of the anti-platelet aggregation mechanism of Salvia miltiorrhiza, the specific signal transduction pathway has become a hot and difficult research topic in studying the pharmacological mechanism of Salvia miltiorrhiza. Based on the latest literature, this article reviews the targets and pathways of the modern pharmacological mechanism of Salvia miltiorrhiza extract in anti-platelet aggregation, providing new ideas for the research of safer and more effective anti-platelet aggregation drugs.*

Keywords: *thrombotic diseases; salvia miltiorrhiza extract; anti-platelet aggregation; thrombosis; signaling pathway*

1. Background

Thrombotic diseases are a group of diseases characterized by the formation of blood clots, including coronary artery thrombosis, ischemic stroke, deep vein thrombosis (DVT), and other conditions. Various causes lead to vascular endothelial damage, increased blood coagulability, decreased antifibrinolytic activity, hemodynamic changes, enhanced platelet activity and quantity, and activation of coagulation factors[1]. Thrombotic diseases are major causes of death and disability worldwide and pose a serious threat to people's health[2]. Therefore, effective prevention and treatment of thrombotic diseases are particularly important. Currently, the treatment of thrombotic diseases mainly involves medication and surgery. In the acute phase, surgery is the primary treatment with medication as a secondary option. In the prevention and maintenance phase, medication is the primary treatment, including anticoagulants and antiplatelet drugs. Commonly used antiplatelet agents in clinical practice include cyclooxygenase inhibitors and adenosine diphosphate P2Y12 receptor blockers. Aspirin, a classic antiplatelet drug, has drawbacks such as low drug selectivity, significant gastrointestinal reactions, and a high risk of bleeding. Similarly, the platelet glycoprotein IIb/IIIa (GPIIb/IIIa) receptor antagonist tirofiban has limited clinical application due to severe adverse reactions such as platelet reduction and bleeding[3].

In the field of antiplatelet pharmacology research, traditional Chinese medicine has also made significant progress. Salvia miltiorrhiza, a plant in the Lamiaceae family, has been used as a medicinal herb in China for nearly 2000 years. It was first recorded in the "Shennong Bencao Jing" and is known as "Danshen"[4]. Its root and rhizome residues have therapeutic effects such as promoting blood circulation, removing blood stasis, improving hemodynamics, anti-atherosclerosis, antiplatelet aggregation, lipid regulation, antioxidant, and anti-inflammatory effects[5]. The main active component

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of Danshen is a compound called tanshinone, which has various pharmacological effects such as anti-inflammatory, antiplatelet aggregation, and antioxidant activities. It is widely used in the treatment of cardiovascular and cerebrovascular diseases, and can prevent and treat conditions such as coronary heart disease, angina pectoris, myocardial infarction, and stroke.

2. Danshen Active Ingredients

The chemical composition is the foundation of the pharmacological effects of each drug. Danshen's therapeutic effects on multiple targets and systems depend on its various pharmacological components[6], mainly including two types of compounds. One is water-soluble phenolic acids, including salvianolic acid, methyl rosmarinate, isorosmarinic acid, salvianolic acid A, B, C, K, protocatechuic acid, rosmarinic acid, caffeic acid, and isorosmarinic acid. The other is lipid-soluble diterpenoid quinone compounds, mainly including salvianol B, salvianone I, cryptotanshinone, 15,16-dihydrotanshinone I, tanshinone IIA, tanshinone IIB, tanshinone VI, hydroxytanshinone IIA, methyl salvianate, salvianol A, salvianol C, purple tanshinone A, and danshenol. Each component can exert different biological effects, and studying their pharmacological mechanisms and signaling pathways is of great significance [7].

3. Platelet Activation Response

In the occurrence of cardiovascular and cerebrovascular diseases, the endothelium of arterial blood vessels is damaged under the stimulation of inflammatory factors, lipid deposition, oxidative stress, and mechanical injury [8]. Collagen fibers beneath the endothelium are exposed, promoting the secretion of vWF and ADP, causing platelets to transition from a resting state under intact endothelium to an activated state. The expression of the activated platelet surface glycoprotein GPIb-IX-V receptor increases, binding with collagen and integrin αIIbβ3, initiating signal transduction mechanisms, promoting changes in platelet morphology, causing platelet degranulation, releasing lysosomes, dense granules, α-granules, and arachidonic acid. The above substances, through the thromboxane A2 (TXA2)-mediated cyclooxygenase (COX)-TXA pathway, promote platelet aggregation and participate in hemostatic response [9]. In recent years, more and more studies have shown that Danshen has the effect of blocking platelet aggregation and mediating platelet activation pathways. Its effective components in anti-platelet aggregation have been a hot research direction in recent years [10].

4. Platelet Activation Pathways

4.1 P2Y12 Receptor Signaling Pathway

Platelets express the P2Y12 receptor on their surface, which is a G protein-coupled receptor. When the endothelium of blood vessels is damaged, collagen fibers are exposed, leading to the binding of the platelet surface receptor P2Y12 with ADP secreted by platelets, causing a change in platelet conformation and triggering platelet aggregation response[11]. In studies on the role of P2Y12 receptor signaling in the process of cardiac remodeling in mice, it was found that compared to wild-type mice, mice with P2Y12 gene knockout had significantly prolonged bleeding time and impaired platelet aggregation[12]. Research indicates that the P2Y12 receptor on the surface of platelets can promote platelet activation and is an important molecule in initiating platelet activation.

4.2 Protease-Activated Receptor Signaling Pathway

Anticoagulant therapy plays an important role in the treatment of thrombotic diseases, mainly due to the involvement of thrombin in platelet aggregation reactions, acting as an activator during platelet aggregation. The molecular mechanism involves the activation of platelets mediated by the protease-activated receptor (PAR) signaling system, which consists of two G-protein-coupled receptors, PAR1 and PAR4[13]. The activation of platelets occurs when PAR1 and PAR4 receptors are cleaved by proteases, exposing their N-terminus and triggering transmembrane signaling, leading to platelet aggregation reactions[14]. It is worth noting that PAR1 alone can induce platelet adhesion by thrombin, and even low concentrations of thrombin can activate PAR1, resulting in transient and rapid effects. PAR4, on the other hand, requires the presence of PAR1 and a thrombin concentration more than 10 times its own to exert its effects, resulting in a relatively prolonged effect. Therefore, by understanding

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the different concentrations and functional characteristics of PAR1 and PAR4, novel antiplatelet aggregation drugs that act quickly and have stable effects can be developed.

4.3 Reactive Oxygen Species-Mediated Activation Pathway

Reactive oxygen species (ROS) are activated oxygen components produced after platelet activation, which regulate the crosslinking between platelets and collagen fibers and participate in thrombus formation. Oxidative stress is an important risk factor in the process of atherosclerosis, as it can promote endothelial damage, leading to vascular stenosis, hemodynamic changes, and thrombus formation[15]. In the pathway of ROS generation, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX) is mainly involved in the reaction process [16], with NOX2 being the primary component regulating platelet activation. Platelets lacking NOX2 gene knockout do not produce ROS [17].

In summary, platelet activation is mediated by multiple pathways and factors. Understanding the pathways of platelet aggregation and activation is an important foundation for the treatment of thrombotic diseases. Based on their molecular mechanisms, the development of effective and safe drugs that inhibit platelet aggregation is a key area of research and attention.

5. Signal pathway of antiplatelet aggregation mediated by Danshen extract

5.1 Danshensu B

Danshensu B has good water solubility and has the functions of vasodilation, anti-thrombosis, protection of cardiac microvascular endothelial cells, and antioxidant effects. It is widely used in the treatment of coronary heart disease in clinical practice[18,19]. Liu et al. [20]added Danshensu B to 67 patients with chest pain in standard dual antiplatelet therapy to study the antiplatelet effects and safety of Danshensu B. The study showed that compared with the control group, the experimental group with Danshensu B showed significantly reduced expression of CD62P on platelets, inhibition of PDE and P2Y12 pathways, inhibition of platelet activation, without increasing the risk of bleeding. CD62P belongs to the selectin family. When platelets are activated, α-granules release CD62P, causing platelet adhesion and thrombus formation [21]. This indicates that Danshensu B can inhibit the expression of CD62P, enhance the antiplatelet aggregation effect, and does not increase the risk of bleeding. Danshensu B can participate in the activation process of platelets through multiple pathways. Danshensu B inhibits ADP-induced platelet aggregation and GPIIb/IIIa activation; inhibits thrombin, arachidonic acid, and collagen-induced platelet aggregation; inhibits platelet spreading; increases cAMP levels in resting and ADP-stimulated platelets; enhances VASP phosphorylation in ADP-stimulated platelets.

5.2 Tanshinone IIA

Tanshinone IIA (TIIA) is a diterpenoid quinone compound extracted from Danshen. It is a cell lipid peroxide inhibitor that can interact with DNA, alleviate cell toxicity, and has antiplatelet aggregation, anti-thrombus formation, antioxidant, microcirculation improvement, anti-inflammatory, neuroprotection, and immune-enhancing effects [22]. In order to elucidate the molecular mechanism of TIIA's antiplatelet aggregation, Francesco et al. [23] used male Wistar rats and found that TIIA inhibited collagen and ADP-induced platelet aggregation by regulating microtubule protein acetylation and inhibiting Erk-2 phosphorylation. The inhibitory effect was concentration-dependent. Acetylation of microtubule proteins changes the platelet morphology, causing platelet activation and aggregation. The study demonstrated that TIIA selectively inhibited Erk-2 phosphorylation in the MAPKs signaling pathway at different time points in vitro, and increased bleeding time. The mechanism of action of TIIA in vivo is still unclear, but it may be related to reducing platelet adhesion to blood vessel walls.

5.3 Rosmarinic Acid

Rosmarinic acid is a water-soluble phenolic compound in the chemical composition of Salvia miltiorrhiza, which has biological characteristics such as antioxidant, anti-tumor, and anti-anxiety properties[24]. ERp57 is a type of thiol oxidoreductase in the protein disulfide isomerase family, which can regulate cell apoptosis and anti-tumor responses. Recent studies have shown that ERp57 is expressed on the surface of platelets along with protein disulfide isomerase (PDI), and they have highly

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similar structures[25]. Previous studies have shown that PDI inhibitors can significantly inhibit platelet aggregation and thrombus formation, suggesting that ERp57 may also be involved in the process of platelet aggregation and thrombus formation[26]. Zhou et al. [27]used virtual screening technology to study the binding reaction between rosmarinic acid and ERp57 and its impact on platelet function through transmittance aggregation and insulin reduction methods. The results showed that rosmarinic acid forms hydrogen bonds with the Ser312, Lys366, Asp440, and Val441 structures of ERp57, and exhibits concentration and time-dependent inhibition. The authors demonstrated through in vitro stimulation experiments that rosmarinic acid significantly inhibits platelet aggregation induced by arachidonic acid and ADP. It is worth noting that there is no significant difference in the inhibitory results of platelet aggregation between rosmarinic acid and hydrogen chloride picotamide sulfate. The study showed that ERp57 antibody inhibited the expression of integrin αIIbβ3, reducing the platelet aggregation response. The mechanism may be that ERp57 reduces platelet activity by inhibiting the calcium signaling of integrin αIIbβ3 and fibrinogen. This speculation needs further experimental confirmation[28].

5.4 Tanshinone

Danshen diterpenoids are the main water-soluble components of Danshen extract, and tanshinone is the most important substance among them. Tanshinone has pharmacological effects such as anti-platelet aggregation, anti-atherosclerosis, anti-inflammatory, and antioxidant effects[29]. Cui et al. [30]used thrombin-induced platelet activation to study the inhibitory mechanism and targets of tanshinone. The results showed that tanshinone can inhibit platelet aggregation through the interaction between the protein disulfide isomerase homolog ERp57 and integrin αIIbβ3. Interestingly, coagulation factor 7 is also involved in the signaling pathway of tanshinone's anti-platelet aggregation effect. Tanshinone may exert its inhibitory effect on ERp57 and integrin αIIbβ3 by acting on coagulation factor 7, but this mechanism is still unclear. The latest research shows that tanshinone induces the expression of SIRT1, reduces the generation of reactive oxygen species (ROS), prevents the release of mitochondrial DNA, and inhibits the dendritic cell-dependent non-integrin activation of platelets[31].

5.5 Protocatechuic acid

PrG is a type of antioxidant extracted from Chinese herbal medicine in the 1980s, which is of great significance in the treatment of coronary heart disease and ischemic cerebrovascular diseases[32]. In the study, it was found that PrG is a stimulant for platelet granule release, which can reduce the release of TXA2 and inhibit platelet aggregation[33]. Huang et al. [34]found through molecularly imprinted polymer component analysis that the protocatechuic acid extracted from Salvia miltiorrhiza has similar biological activities to PrG. In order to verify the pharmacological effects of protocatechuic acid, the authors introduced the original solution of Salvia miltiorrhiza into the MIP-SPE extraction column, eluted with methanol-acetic acid, and introduced into the LC system to observe mass spectrometry peaks. The results showed that protocatechuic acid can bind to the same specific site as PrG, and it was proven that they share the same benzoic acid core structure. Benzoic acid is the molecular active site of polyphenolic acids, with o-2-hydroxybenzoic acid having the strongest effect, and protocatechuic acid is a type of o-2-hydroxybenzoic acid substance extracted from Salvia miltiorrhiza. Therefore, protocatechuic acid may play a role in inhibiting platelet aggregation by blocking the metabolism of arachidonic acid to TXA2 through COX inhibition.

5.6 15, 16-Dihydrotanshinone I

During the process of platelet activation, calcium ions are involved in the activation of integrin and the release of TAX2, which are important catalysts for platelet activation and aggregation reactions[35]. 15,16-Dihydrotanshinone I is one of the main components of Danshen extract. Pu et al. [36] used turbidity method and rabbit platelets to study the mechanism of inhibiting platelet aggregation induced by collagen and thrombin by 15,16-Dihydrotanshinone I. The study showed that 15,16-Dihydrotanshinone I inhibited the production of arachidonic acid induced by collagen, reduced platelet aggregation, and inhibited thrombus formation. The authors further used the fluorescent dye Fura-2/AM to label cytoplasmic calcium ions and measure loaded platelets. The experiment showed that 15,16-Dihydrotanshinone I had a significant inhibitory effect on calcium ions, inhibited the release of inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DG) through phospholipase Cb and CY2, and inhibited the entry of calcium ions from the cell into the cytoplasm, thereby inhibiting the activation pathways of ADP and TXA2 and exerting an anti-platelet aggregation effect. This process may be due

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to the inhibitory effect of 15,16-Dihydrotanshinone I on the breakdown of phosphoinositides, which reduces the release of calcium ions. The research results proved that 15,16-Dihydrotanshinone I achieved the therapeutic effect of anti-platelet aggregation by blocking the intracellular calcium pathway, reducing the release of arachidonic acid induced by collagen, and inhibiting the production of TXA2.

5.7 Salvianolic acid A

Salvianolic acid A has anti-neuroinflammatory[37], anti-tumor[38], and anti-leukemia[39] activities. The latest research has proven that salvianolic acid A has pharmacological effects of anti-platelet aggregation. Song et al. [40] prepared a FeCl3 carotid artery injury model and a pulmonary thromboembolism model to detect the mechanism of inhibiting platelets by salvianolic acid A in vivo. The authors found that salvianolic acid A can activate integrin αIIbβ3 by releasing α-granules and dense granules, inhibit platelet spreading and retraction, and prevent thrombus formation. Interestingly, salvianolic acid A has a stronger inhibitory effect on platelet aggregation induced by collagen and thrombin. The study showed that salvianolic acid A can inhibit the phosphorylation of downstream proteins PLCγ2 and PKC substrates in the GPVI signaling pathway. At the same time, the phosphorylation of Akt and the expression of its substrate glycogen synthase kinase 3β (GSK3β) decreased. It is worth noting that Danshenone inhibits the phosphorylation of ERK1/2, but it does not inhibit the phosphorylation of p38, and can even enhance the phosphorylation expression of p38. This may be related to the activation pathway of p38 in the antiplatelet effect of Danshenone. Downstream of the integrin αIIbβ3 signaling, the phosphorylation of FAK and Src is also inhibited by Danshenone, indicating that Danshenone negatively regulates the phosphorylation of the GPVI platelet inhibitory signal pathway. In summary, Danshenone inhibits platelet spreading and contraction, and prevents thrombus formation by affecting the PLCγ2/PKC/ERK1/2, PI3K/Akt, and Src/FAK signaling pathways.

6. Conclusions

Looking ahead, with the growth of the aging population, the incidence of cardiovascular and cerebrovascular diseases is increasing, making them the major causes of death and disability worldwide, seriously endangering people's lives and quality of life. Thrombus formation is an important pathological process, and the antiplatelet aggregation effect plays a significant role in treatment. The commonly used dual antiplatelet aggregation therapy in clinical practice is time-consuming, expensive, and has obvious adverse reactions. The modern pharmacological effects of Danshen have been widely recognized. Different extracts have different targets and pathways to exert antiplatelet aggregation effects. However, the cooperative effects and structure-activity relationships are not yet clear, and there are still deficiencies in clinical treatment applications. Based on the different targets and pathways of Danshen extracts in antiplatelet aggregation, it is possible to develop more safe, effective, and economical antiplatelet aggregation drugs by establishing effective structure-activity relationships.

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