

Chinese herbal remedies affecting thrombosis and hemostasis: A review

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ABSTRACT

Acute coronary syndrome, stroke and other ischemic events continue to be the most common causes of mortality and morbidity in the world, and their incidence is rapidly increasing in the developing nations. These cardiovascular disorders clinically manifest as acute atherothrombotic events. Application of oral antiplatelet drugs is a milestone in the therapy of cardiovascular diseases. However, the limited efficacy of these drugs in the setting of arterial thrombosis, their unfavorable side effects, cost-to-benefit issues and the drug resistance phenomenon substantiate the need for the development of new and more efficacious antithrombotic drugs. In recent years, with the progress in the study of the Chinese medicine pharmacology, many Chinese herbs and formulas, as well as active constituents have been reported to possess not only effects on platelet aggregation and activation but also beneficial roles in vascular functions. Compared with currently used antithrombotic agents, herb remedies exert antithrombotic effects in a multi-pathway and multi-target manner. This paper will cover the progress in research on the ameliorating effects of herbal remedies on thrombosis, with focusing on their protection of vascular endothelial cells and inhibition of platelet activation.

Key words: Chinese herbs, thrombosis, endothelium, platelet

1. INTRODUCTION

Acute coronary syndrome, stroke and other ischemic events remain a challenge for human health worldwide. Although the incidence of these cardiovascular diseases has decreased in industrialized countries, but is increasing in developing nations^[1]. These cardiovascular disorders stem from acute atherothrombotic events. Arterial thrombosis is a pathological process in which the hemostatic system is overly active causing development of platelet aggregates that abate normal blood circulation in coronary, cerebral or peripheral arteries. Obstruction of the blood circulation in these vessels may lead to myocardial infarction, ischemic stroke or limb gangrene.

Thrombosis occurs in four well-defined steps: endothelial activation, platelet tethering and rolling, platelet activation and firm adhesion, in which platelet-endothelium interactions play a critical role^[2]. Under normal conditions, platelets circulate in a quiescent state, and do not interact with the endothelial cells that cover the vascular wall. The inflammation resulting from mechanical injury, chemical agents or pathological conditions leads to the exposure of the subendothelial extracellular matrix which provokes the adhesion and subsequent activation of platelets, thereby initiating thrombosis. The activated platelets and endothelial cells release a range of prothrombotic substances near the injured area that favor the recruitment of more platelets to form a stable hemostatic plug. This platelet plug is then stabilized via a fibrin network, forming the final product of the coagulation cascade.

Importantly, thrombus formation may occur at an unstable surface, and the hemostatic plug may embolize and occlude vessels downstream of its original location.

The widely used antithrombotic drugs at present include anticoagulant drugs (such as heparin and warfarin), anti-platelet drugs (aspirin) and fibrinolysis drugs (streptokinase), which decrease the risk of thrombus formation and are relatively well tolerated by patients. However, the limited efficacy of these drugs in the setting of arterial thrombosis, their adverse side effects, cost-to-benefit issues and the drug resistance phenomenon substantiate the need for the development of new and more efficacious antithrombotic drugs.

The use of plants as remedies for various ailments has formed the basis of our modern medicinal sciences. According to the World Health Organization (2008) approximately 80% of Asia and Africa's population use traditional medicine as a form of healthcare for treatment of diseases including blood disorders^[3]. In recent years, with the development of the Chinese medicine pharmacology, many Chinese herbs and formulas, as well as active constituents have been reported to have not only effects on platelet aggregation and activation but also beneficial effects on vascular functions. Compared with currently used antithrombotic agents, herbal remedies exert antithrombotic effects via multiple pathways acting at multiple targets. This review will discuss recent advances in the research on the ameliorating effects of herbal remedies on thrombosis, with focus on their role in

protection of vascular endothelial cells and inhibiting platelet activation.

2. EFFECT OF HERBS ON VASCULAR ENDOTHELIAL CELLS

The endothelium plays a crucial role in maintaining hemostatic balance. Under physiological conditions, endothelial cells prevent thrombosis through inhibition of platelet aggregation and blood coagulation, as well as through the activation of fibrinolysis by tissue plasminogen activator. Endothelial cells can be activated by a diversity of conditions, such as hypertension, hypercholesterolemia, atherosclerosis, hypoxia and chronic heart failure, exogenous or endogenous substances including lipopolysaccharide (LPS), tumor necrosis factor alpha (TNF- α), Interleukin (IL)-1 and viral infections^[4-6]. Activated endothelial cells express adhesion molecules that promote platelet and leukocyte adhesion to the endothelium, releasing cytokines and procoagulant factors that assist in thrombosis. Obviously, agent that acts at any of the factors may be expected to modulate thrombosis. Increasing Chinese herbs or their active compounds have revealed potential to interfere in thrombosis by acting at endothelial cells via different mechanisms.

2.1 Effect of herbs on adhesion molecules expression in vascular endothelium

Pathological conditions such as inflammation might not compromise the integrity of the vessel, but can damage and activate the vascular endothelial cells. Activated endothelial cells express many adhesion proteins such as von Willebrand factor (vWF), P- and E-selectins, intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1). Endothelial vWF interacts with platelet GPIb, while endothelial P- and E-selectins interacts with platelet P-selectin glycoprotein ligand-1. ICAM-1 can bind fibrinogen, which accumulates on the endothelial cell surface as deposits and mediates α IIB β 3-dependent platelet adhesion. The close attachment to the damaged area activates platelet outside-in intracellular signals that promote thrombus formation. Many Chinese herbs and active compounds have been reported to inhibit stress-induced expression of adhesion molecules on vascular endothelial cells. Curcumin and *Morus alba* extract^[7], *Flos lonicerae* extracts and chlorogenic acid^[8] were reported to inhibit significantly P-selectin expression induced by inflammation in endothelial cells. Acacetin^[9] was reported to inhibit the expression of E-selectin induced by TNF- α in part by regulating the p38 mitogen activated protein kinase (MAPK) signaling pathway and the nuclear transcription factor-kappa B (NF- κ B). Evidence showed that caffeic acid^[10], licorice isoliquiritigenin^[11], Pine bark extract enzogenol^[12], plumericin^[13] are able to abolish TNF- α -induced expression of VCAM-1, E-selectin and/or ICAM-1 in endothelial cells by inhibiting NF- κ B activation. In addition, Dao-Tan decoction^[14], *Ginkgo biloba* extract^[15], Grape seed proanthocyanidin extracts^[16, 17], *Toona sinensis* extract and gallic acid^[18], *Panax notoginseng*^[19], *Platycodon grandiflorum*^[20],

astragaloside IV^[21], magnolol^[22], paeonol^[23], phloretin^[24], protocatechuic aldehyde^[25], salvianolic acid B^[26] were reported to prevent TNF- α induced up-regulation of VCAM-1, ICAM-1, and E-selectin in endothelial cells by blocking MAPK and/or NF- κ B pathway.

2.2 Effect of herbs on antithrombotic factors of endothelium

Endothelial cells are hermetically bound together by tight junctions to prevent extracellular matrix exposure. They are also equipped with a negatively charged glycocalyx on their apical surface that repel circulating platelets and display antithrombotic properties^[4]. The most important inhibitors of platelet activation generated by endothelial cells are nitric oxide (NO) and prostacyclin (PGI₂). NO and PGI₂ are constitutively synthesized by non-activated endothelial cells, and their production is elevated by mechanical stimuli such as shear stress and cyclic stretch^[27]. These substances inhibit the activation of circulating platelets by stimulation of inhibitory cyclic guanosine monophosphate (cGMP) - and cyclic adenosine monophosphate (cAMP) - dependent pathways inside these cells^[28]. Research has found that saponins derived from the roots of *Platycodon grandiflorum* stimulate eNOS phosphorylation and NO production in human endothelial cells via activation of phosphoinositide 3-kinases (PI3K) /Akt, p38/MAPK, adenosine monophosphate activated protein kinase (AMPK), and calcium/calmodulin-dependent protein kinase II (CaMK II)^[20]. Active compounds from Chinese herbal medicines such as andrographolide^[29], aristolochic acid^[30], crocetin^[31], cyclovirobuxine D^[32], resveratrol^[33], sesamol^[34], were reported to enhance NO release from endothelial cells or decrease inactivation of NO, exhibiting superior antithrombotic effect. Honokiol is a bioactive compound extracted from *Magnolia officinalis*. Research shows that it inhibits arterial thrombosis through endothelial cell protection and stimulation of prostacyclin^[35], and its effect on PGI₂ generation attributes to up-regulation of prostacyclin synthase expression^[36].

2.3 Effect of herbs on fibrinolysis and anticoagulation of endothelium

Thrombosis and fibrinolysis are kept in balance in physiological condition by thrombotic and fibrinolytic proteins. The proteins involved in this balance include thrombomodulin, plasminogen activator inhibitor-1 (PAI-1), and tissue-type plasminogen activator (t-PA). Thrombomodulin is a membrane-anchored glycoprotein, which functions in anticoagulation by association with thrombin. The resulted complex can effectively activate protein C, catalyzing the proteolytic inactivation of blood coagulation factors Va and VIIIa, which in turn lead to the down-regulation of the blood coagulation cascade. Studies revealed that the anticoagulant effect of the thrombin/thrombomodulin activated protein C is impaired in parallel with the impairment of endothelial functions. The improvement of the anticoagulant mechanism may reduce the risk of cardiovascular events^[37]. Endothelial cells constitutively release t-PA to the blood stream, which, by

binding to fibrin deposits, activates the transformation of plasminogen into plasmin by proteolytic cleavage. The protease plasmin subsequently catalyzes the degradation of fibrin fibrils and disassembles the fibrin network^[38]. PAI-1 is the primary inhibitor of t-PA, which, together with t-PA, constitutes the major mediators for balancing the fibrinolytic system *in vivo*^[39]. Coronary heart disease and deep-vein thrombosis were reported as vascular disorders with increased PAI or decreased t-PA activity^[40]. Research shows that salvianolic acid B^[37] can increase the fibrinolytic and anticoagulant potential of endothelial cells by up-regulating the expression of t-PA and thrombomodulin and down-regulating the expression of PAI-1. The same effects have been reported for astragaloside IV^[41], baicalin^[42], icariin^[43], oroxylin A^[44] and withaferin A^[45]. He et al. reported that Xiang-Qi-Tang, a Chinese herbal formula containing *Cyperus rotundus*, *Astragalus membranaceus* and *Andrographis paniculata* with alpha-Cyperone (CYP), astragaloside IV and andrographolide as the major active components, inhibited the expression of inflammatory and coagulant mediators via MAPKs and NF- κ B signaling pathways in rat cardiac microvascular endothelial cells^[46].

In addition, membrane-bound tissue factor (TF) expressed on vascular endothelial cells acts as a receptor for activated factor VII (VIIa). The TF/VIIa complex triggers the coagulation cascade and the formation of activated factor X (Xa), ultimately resulting in thrombin formation, which in turn cleaves protease-activated receptors on the platelet surface, boosting platelet activation and clot formation^[47]. In this regard, Chinese herbs display beneficial role as well, and a number of Chinese medicine formula, herbs and their active compounds were found to have inhibitory effect on TF expression and activity, such as Xiang-Qi-Tang^[46], *Polygonatum odoratum*^[48], α -linolenic acid^[47], berberine^[49], *Holothurian glycosaminoglycan*^[50], guggulsterone^[51], ligustrazine^[52], oleanolic acid^[53].

3. EFFECT OF HERBS ON PLATELET

Platelets are blood cells specialized in thrombosis and hemostasis in mammals. When vascular wall is injured and extracellular cell matrix (ECM) exposed, platelets will bind to vWF and collagen via GPIb-IX-V and GPVI, respectively, which are responsible for the initial adhesion and activation of platelets. Platelet activation involving GPIb-IX-V or GPVI leads to secretion of platelet agonists, such as ADP, which functions via the G protein-coupled receptors, P2 and P2Y, to reinforce α IIB β 3-dependent platelet aggregation and thrombosis. Essentially the same events occur in the thrombotic diseases such as heart attack and stroke^[54]. Identification of the factors involved in platelet activation and clarification of the relevant mechanisms lead to the development of antiplatelet agents that results in a decline of the death rates of heart disease and stroke by about 25%^[55]. Commonly used oral antiplatelet drugs include cyclooxygenase inhibitor aspirin, the glycoprotein IIb/IIIa inhibitor ReoPro, and the P2Y12 inhibitor clopidogrel, among others.

However, the limited efficiency and adverse side effects of these antiplatelet drugs make it appeal to develop novel strategy to combat thrombotic disease. Increasing evidence suggests Chinese herbs as a potential resource for this purpose. A number of herbs used in traditional Chinese medicine have been reported to inhibit platelet activation with different mechanisms.

3.1. Inhibition of Platelet Aggregation

Platelet aggregation, a process of the clumping together of platelets in the blood, plays a key role in the hemostasia and pathogenesis of atherothrombosis. The presence of platelet agonists on the injured vascular wall or in blood serum induces platelet activation through stimulation of their plasma membrane receptors. Platelet activation comprises reorganization of the cytoskeleton and shape change, activation of Ca²⁺ - dependent and independent signaling pathways and activation of adhesion proteins exposed on their surface, increasing their adhesion among platelets and between platelet and ECM, leading to the formation of platelet aggregates. The triggers of platelet aggregation may be either a chemical agent such as ADP, collagen, thrombin, arachidonic acid and platelet activating factor (PAF), or shear stress. Platelet aggregation rate is used as a marker for evaluation of antiplatelet efficacy of a regime. Studies show that a wide range of Chinese herb and formulas can reduce the aggregation rate in patients or animal models with thromboembolic diseases. The Chinese medicine formulas that have been reported to exhibit potential of antiplatelet activation include Buyang Huanwu Decoction^[56], Dan Shen Di Wan^[57], Dang-Gui-Shao-Yao-San^[58, 59], Jia-Wei-Xiao-Yao-San^[59], SiWu decoction^[60, 61], Xiao-Chai-Hu-Tang^[62], Xue-Fu-Zhu-Yu-Tang^[63], Mailuoning injection^[64], *Anemarrhena asphodeloides*^[65], *Attractylodes lanceae* Rhizoma and *Poria*^[59], *Artemisia princeps* Pampanini^[66], *Cyperus rotundus*^[67], *Hippophae rhamnoides* L^[68], *Ilex pubescens*^[69], *Ocimum basilicum* L^[70], *Panax notoginseng*^[71], *Persicaria Semen*^[72, 73] and *Carthami Flos*^[73], *Trigonella foenum-graecum*^[74], *Umbilicaria esculenta*^[75], *Usnea longissima*^[76] and *Veratrum patulum* L^[77]. Of notice, most of these formulas or herbs have long been used as treatment of diseases that are related to disordered platelet function. A number of active components from herbs show activity to inhibit the platelet aggregation as well, such as brazilin^[78], curdione^[79], diosgenin^[80], hydroxysafflor yellow A^[81], marchantinquinone^[82], morusinol^[83], neferine^[84], obovatol^[85], paeoniflorin and senkyunolide I^[61], protocatechuic aldehyde^[86], protocatechuic acid^[87], quercetin and 3',4'-dihydroxyflavonol^[88], salvianolic acids^[89, 90], tetramethylpyrazine and salvianolic acid B^[91], timosaponin B-II^[92] and Z-ligustilide^[93].

3.2. Inhibition of platelet granule secretion

Prothrombotic and antithrombotic factors stored in α -granule and dense granule of platelet are released in response to activation including P-selectin (CD62p), glycoprotein (GP) IIb/IIIa, CD40 ligand (CD40L), platelet factor 4 (PF-4), β -thromboglobulin (β -TG), and Ca²⁺. Controlling platelet

granule secretion is considered an effective strategy to dampen thrombosis and prevent atherosclerosis.

3.2.1 P-selectin

P-selectin is a transmembrane protein that resides within the α -granule membrane of unstimulated platelets. Upon stimulation, P-selectin is phosphorylated and translocated to the plasma membrane, which is generally applied as a gold marker of platelet activation^[94], and used as an indication of effectiveness of a Chinese herb or its active compound in antiplatelet. By evaluation of the membrane expression of P-selectin, the following preparations have been revealed to have antiplatelet activity *in vivo* and/or *in vitro*: Quyu Xiaoban capsules^[95], salviolate^[96], 5-caffeoylquinic acid and caffeic acid^[97], anthocyanins^[98], delphinidin-3-glucoside^[99], resveratrol^[100], salicylic acid, p-coumaric acid, ferulic acid, 4-hydroxyphenylpropionyl glycine, 5-methoxy-salicylic acid, and catechol^[101].

3.2.2 CD40L

As a member of the tumor necrosis factor- α family, CD40L has been identified as a proinflammatory mediator and risk factor for cardiovascular events on activated platelets. It can bind to and activate platelet $\alpha_{IIb}\beta_3$ in thrombosis. It is also able to activate endothelial cells. Soluble CD40L (sCD40L) is generated by shedding of the surface-bound CD40L. High level of sCD40L significantly increases platelet activation and aggregation, while blockade of this pathway with anti-CD40L antibodies can prevent or delay atheroinflammation progression. Researchers have found that Xinfeng capsules^[102], *Allium macrostemon* Bunge^[103], parthenolide^[104], delphinidin-3-glucoside^[99] and guanosine from *Solanum lycopersicum*^[105] can significantly inhibit the expression of CD40L on the membrane of activated platelets or reduce the levels of sCD40L to dampen undesirable platelet activation.

3.2.3 PF-4 and β -TG

PF-4 and β -TG are platelet α -granule proteins, the high plasma levels of which are the specific indicators of platelet activation and secretion and are commonly present in thromboembolic disease and prethrombotic state. PF-4 enhances the metabolism of membrane phospholipid and arachidonic acid to produce thromboxane (TXA₂). Also, PF-4 can promote precipitation and polymerization of fibrin monomer thus accelerate platelet aggregation. β -TG has been reported to inhibit PGI₂ production by endothelial cells and enhance the aggregation of platelet. Research has found that QiShen YiQi Dropping Pill and naringin can reduce the β -TG^[106] or PF-4^[107] concentration obviously to inhibit platelet aggregation in hyperlipidemic rabbits.

3.2.4 Ca²⁺

A central event in platelet activation is the increase of its cytoplasmic Ca²⁺ concentration, which acts as an integrator of all signaling pathways triggered by the different platelet agonist receptors.

Studies have indicated that some Chinese herbs play a role as calcium channel antagonists and thereby inhibit platelet aggregation and activation, including *Salvia Miltiorrhiza*^[57], *Bulnesia sarmienti*^[108], *Phellinus baumii*^[109], *Pistacia chinensis*^[110], and *Viscum Coloratum*^[111], and some active constituents of herbs such as 15,16-dihydrotanshinone I^[112], andrographolide^[29], aristolochic acid^[30], cordycepin^[113], curdione^[79], epigallocatechin gallate^[114], ginsenoside-Rp1^[115], hydroxychavicol^[116], marchantinquinone^[82], naringin^[107], resveratrol^[33], salvanolic acid B^[117], sanguinarine^[118], and sesamol^[119].

3.3. Effect on platelet arachidonic acid metabolism

Both TXA₂ and PGI₂ are the metabolites of arachidonic acid. TXA₂ is produced by platelets as a potent vasoconstrictor, while PGI₂ is generated in vascular endothelium with a function of vasodilator. TXA₂ is synthesized and released by platelet microsome and quickly degrades to the TXB₂. TXA₂ promotes the release of Ca²⁺ in density tube system to make dense bodies constrict and release adenosine diphosphate glucose pyrophospheralase (ADP) and 5-hydroxytryptamine (5-HT), which result in platelet aggregation. Whereas, PGI₂ inhibits platelet aggregation by virtue of stimulation of platelet adenylyl cyclase. A balance between formation and release of PGI₂ and TXA₂ in circulation is of utmost importance for the control of intra-arterial thrombi formation and plays a role in the pathogenesis of atherosclerosis. Study shows that DanQi pill can down-regulate the TXB₂ and up-regulate the PGI₂ in diverse way, suggesting its potential as an antithrombotic therapy by improving the balance of TXA₂/PGI₂^[120]. Similar results have been found for the following herb and active components: alditols and monosaccharides extracted from sorghum vinegar^[121], aristolochic acid^[30], brazilin^[78], hesperetin^[122], phloroglucinol^[123], *Pistacia chinensis*^[110], *Salix matsudana*^[124].

3.4. Effect on the signal transduction in platelet activation

Platelet activation has long been recognized as critical for the formation of haemostatic plugs and thrombosis. Over the past decade, using gene-knockout studies and multiple receptor antagonists, the details of the process of platelet activation have become much clearer. It is known that platelet activation requires agonist induction. Because most agonists function synergistically in platelet aggregation, the signaling pathway of platelet activation is complicated. For example, many platelet agonists bind more than one receptor (e.g. von Willebrand factor binds both GPIb and $\alpha_{IIb}\beta_3$, collagen binds to GPVI and $\alpha_2\beta_1$, thrombin interacts with protease-activated receptors and GPIb, and ADP binds to at least two ADP receptors on platelets). In addition, activated platelets themselves rapidly secrete additional agonists (e.g. TXA₂, ADP, serotonin and ATP), which act as positive feedback mediators that amplify the initial signals to ensure the rapid activation and recruitment of platelets into a growing thrombus. In terms of the signaling pathways involved in platelet activation, the possible antiplatelet targets of Chinese herbs involve (i) agonist receptors; (ii) cAMP/cGMP; and (iii) enzymatic cascades.

3.4.1 Receptor antagonists

Upon agonist stimulation, specific membrane receptor of platelet undergoes conformational change to activate the key enzymes, which produce or release the signal molecules leading to platelet adhesion, aggregation, and ultimately thrombosis. Thus, application of receptor antagonists is potentially a strategy to prevent platelet aggregation. To this end, several TXA2 receptor antagonists have been shown feasibility in the treatment of cardiovascular diseases. Recent reports suggest that antagonists of TXA2 receptors may be able to restrict vascular inflammation in atherosclerotic vessels. Studies have found that active constituent of Chinese herb, such as carnosol^[125] and piperlongumine^[126, 127] can inhibit rabbit platelet activation by antagonizing TXA2 receptor.

ADP induces integrin activation and platelet aggregation through its receptors P2Y1 and P2Y12. P2Y12 plays a critical role in platelet activation and thrombosis. Research showed that SalB can inhibit human platelet activation by inhibiting phosphodiesterase and antagonizing P2Y12 receptor^[96]. Other study indicated that SalB can inhibit rat platelet adhesion to immobilized collagen by interfering with the collagen receptor $\alpha 2\beta 1$ ^[128], suggesting the beneficial role of SalB in thrombosis is multifaceted.

Platelets also express chemokine receptors such as chemotaxis growth factor receptor – 4 (CXCR4), stromal-derived factor-1/chemokine CXCL 12 (CXCL12), chemokine receptor (CCR)-4, CCR1 and CCR2 on their cell surface, which mediate weak platelet responses. Research shows that tetramethylpyrazine significantly down-regulates the expression of CXCR4 in platelets and inhibits rat platelet aggregation^[129].

3.4.2 Regulation of GPIIb/IIIa

The GPIIb/IIIa complex, a member of integrin family, is a heterodimeric adhesive protein receptor, located on the surface of resting platelets. Platelet activation induces a calcium-dependent conformational change in GPIIb/IIIa exposing a ligand binding site. The binding of fibrinogen to the activated GPIIb/IIIa receptor is required for platelet aggregation. Pro-caspase activating compound 1 (PAC-1) is a monoclonal IgM specific for the recognition site within GPIIb/IIIa on activated platelets. The detection of PAC-1 is used as a sensitive measurement of platelet activation.

Salvia miltiorrhiza (SM) has been applied for thousands of years in China and some other Asian countries to treat atherothrombotic diseases. Research shows that *Salvia*olate, an aqueous extract from SM consisting of three ingredients purified from SM, can reduce ADP-induced PAC-1 binding and P-selectin expression on platelets in patients with acute coronary syndrome^[96]. Other studies found that *Spatholobus suberectus*^[130], 2,3,5,4'-tetrahydroxystilbene-2-O- β -D-glucoside, a water-soluble component of the rhizome extract from *Polygonum multiflorum*^[131], phloretin^[24], quercetin and 3',4'-dihydroxyflavonol^[132] can diminish agonist-stimulated expression of the activated form of the GPIIb/IIIa complex, which provide experimental evidences that these compounds improve blood flow following arterial injury in part by attenuating platelet granule exocytosis.

3.4.3 Regulation of cAMP/cGMP

cAMP and cGMP play a pivotal role in platelet regulation. cAMP is synthesized by adenylyl cyclase. Platelet activators, such as ADP and thrombin, block adenylyl cyclase function through inhibitory Ga-i proteins, resulting in a drop in cAMP levels during platelet activation. cGMP production in platelets depends on a single enzyme, the soluble NO-sensitive guanylyl cyclase. Endothelial release of NO is linked to cGMP-dependent platelet inhibition. cAMP elevation and/or cGMP elevation have shown clinical benefit as platelet inhibitors. Recently, a group of Chinese herbs or compounds have been shown to reduce thrombus formation in animal models by modulating cAMP/cGMP metabolism, including QiShen YiQi Dropping Pill^[106], *Pistacia chinensis*^[110], 8-Prenylnaringenin^[133], cordycepin^[113], curdione^[79], ent-16 β ,17-dihydroxy-kauran-19-oic acid^[134], hydroxysafflor yellow A^[81], ginsenoside-Rp1^[115], salvianolic acid A^[135], sanguinarine^[118], sesamol^[34], and sulforaphane^[136].

3.4.4 Inhibition of enzymatic cascades

The platelet signaling pathways are still not completely understood, and their exploration presents an important objective for basic cell biology as well as for the development of both new drugs and diagnosis methods in hemostasis disorders. Most of these G-protein-dependent phosphorylation pathways end in the activation of phospholipase C (PLC) β 1-3 isoforms, while integrins and immunoreceptor tyrosine-based activation motif (ITAM)-containing receptors mainly activate PLC γ 2 isoforms. Another activation pathway involves phospholipase A2 (PLA2) that is induced by G protein coupled receptor (GPCRs). Activated PLA2 generates arachidonate following platelet activation, which is used by cyclooxygenase (COX) enzyme as a substrate to generate TXA2 and to activate platelets further. Finally, PI3K-dependent signaling pathways have been reported to play an important role in platelet activation. PI3K can be activated by GPCRs, ITAM-containing receptors and integrins, which results in increased adhesive functions of integrins. In addition, the presence and activation of 3 members of the MAPK family, p38, extracellular stimuli-responsive kinase (ERK), and c-Jun NH2-terminal kinase (JNK) have been demonstrated in platelets.

In recent years, increasing study has been conducted to address the signaling pathway involved in the inhibitory effect of Chinese herb and formulas on platelet activation. For example, salvianolic acid A was reported to attenuate mouse arterial thrombus formation *in vivo* by inhibiting platelet activation via downregulating PI3K^[137]. Tanshinone IIA and ginsenoside Rg1 inhibit rat or human platelet activation via ERK signaling pathway^[138, 139]. The antiplatelet activities of arsenic trioxide^[140], epigallocatechin gallate^[114], hesperetin^[122], *Peganum harmala*^[141], and sulforaphane^[136] have been reported to be mediated by inhibiting PLC γ 2 pathway. *Bulnesia sarmienti*^[108], ginsenoside-Rp1^[115], *Phellinus baumii*^[109], phloroglucinol^[123] and *Pistacia chinensis*^[110] inhibit platelet activation, granule secretion, aggregation, and

thrombus formation most likely by inhibition of P38, ERK2 or JNK1 phosphorylation.

Because an herb usually contains numerous active constituents, its antiplatelet and antithrombotic activity is commonly mediated by multiple mechanisms involving multiple pathways. Research shows that Andrographolide possesses a potent antiplatelet activity, the underlying mechanism involves the activation of the eNOS-NO/cyclic GMP pathway, resulting in the inhibition of the PI3K/Akt-p38 MAPK and PLC γ 2-PKC cascades, thereby leading to inhibition of human platelet aggregation^[29]. Andrographolide may also increase cGMP/PKG activity, followed by inhibition of the p38 MAPK/(●)HO-NF- κ B-ERK2 cascade in activated human platelets^[142]. Another study shows that antiplatelet activity of sesamol^[34] may involve activation of the cAMP-eNOS/NO-cGMP pathway, resulting in inhibition of the PLC γ 2-PKC-p38 MAPK-TXA2 cascade, and, finally, inhibition of human platelet aggregation. Sesamol is also reported to activate cAMP-PKA signaling, followed by inhibition of the NF- κ B-PLC-PKC cascade, thereby leading to inhibition of [Ca²⁺] i mobilization and human platelet aggregation^[119]. It has been reported that the inhibitory effects of aristolochic acid^[30] or resveratrol^[33] on human platelet activation possibly involve (i) inhibition of the p38MAPK-cytosolic phospholipase A2 arachidonic acid-TXA2-[Ca²⁺] i cascade and (ii) activation of NO/cyclic GMP, resulting in inhibition of phospholipase C and/or PKC activation.

Proteomics-based studies have in recent years shed considerable light on platelet activation mechanisms by identification of novel proteins involved in platelet signaling pathways. Studies have examined signal cascades in rat platelet after salvianolic acid B^[117] or notoginsenosides^[143] treatment using deferential proteomics of platelet, contributing significantly towards understanding platelet regulation of Chinese herb and discovering potential therapeutic targets.

4. EFFECT OF HERBS ON THROMBOSIS *IN VIVO*

Although *in vitro* experiments proved successful in both identifying new receptors and pathways and developing potent and selective antithrombotic drugs, but cannot mimic the myriad hemodynamic and spatiotemporal cellular and molecular interactions that occur during the generation and propagation of thrombi *in vivo*. Animal models, with the availability of modern intravital imaging techniques, have opened new ways to identify both individual roles and the interplay of platelet proteins in complex *in vivo* settings. A large number of experimental models have been established to allow *in vivo* observation of thrombus formation. Thrombosis can be induced in large arteries or veins or in arterioles or venules of the microcirculation, often by damaging the vessel. The injury can be applied from the “outside” by puncture, through column laser injury, suture ligation, or the external application of ferric chloride. Injury can also come from the “inside” by systemic administration of inflammatory agents, such as LPS; by generating reactive oxygen

species with photo-reactive dyes (Rose bengal, Evans blue, or fluorescein isothiocyanate -dextran); or by focused-laser delivered heat to the endothelium. Thrombosis can also be induced in arteriovenous shunts, catheter grafts, or vessels by infusion of platelet agonists^[144–146]. Many Chinese herbs and active compounds have been reported to inhibit thrombus formation in different thrombosis models. Spatholobus suberectus^[130], Umbilicaria esculenta^[75], Usnea longissima^[76], cyclovirobuxine D^[32], diosgenin extract from *Dioscorea zingiberensis* C.H. Wright^[80], and neferine^[84] have been reported to attenuate thrombus formation in collagen-epinephrine-induced acute pulmonary thrombus mouse model or inferior vena cava ligation thrombosis rat model. *Veratrum patulum* L.^[77], crocetin^[31], and honokiol^[35] were reported to prolong the thrombus occlusion time in electrical current-stimulated carotid thrombosis model in rats. Soshiho-tang^[62], epigallocatechin gallate^[114], baicalin^[42], 3',4'-dihydroxyflavonol and quercetin^[132], delphinidin-3-glucoside^[99], ginsenoside Rg1^[139], morusinol^[83], obovatol^[85], protocatechuic acid^[87], and withaferin A^[45] have been reported to reduce thrombus formation in FeCl₃-induced carotid artery or mesenteric arterioles injury in mouse or rat. *Hippophae Rhamnoides* L.^[68], alpha-linolenic acid^[47], guggulsterone^[51], and salvianolic acid A^[137] can prolong arterial occlusion time in photochemical injury-induced arterial thrombosis model. Andrographolide^[29], hydroxychavicol^[116], resveratrol^[33], sesamol^[34], and sulforaphane^[136] exhibited marked antithrombotic effects in ADP-induced acute pulmonary thrombosis or fluorescein sodium-induced platelet thrombi in mesenteric microvessels of mice. *Bulnesia sarmienti*^[108], *Ocimum basilicum* L.^[70], ent-16beta, 17-dihydroxy-kauran-19-oic acid^[134], ginsenoside-Rp1^[115], and Z-ligustilide^[93], significantly reduced thrombus weight in a rat model of arterio-venous shunt.

Our research proved that Cardiogenic pills and its active ingredients 3,4-dihydroxy-phenyl lactic acid and salvianolic acid B can inhibit the formation of a thrombus in the rat mesentery induced by photochemical reaction. This function is related to their antioxidant potential and/or their protective effect against the expression of adhesion molecules in neutrophil and platelet^[147, 148].

5. SUMMARY

The interplay between platelets and the vascular wall is a key episode during thrombosis and hemostasis, wherein the underlying molecular mechanisms orchestrate a complex cross talk between the two cell types. This crosstalk seeks to synchronize their prothrombotic responses by the exchange of chemical messages or factors that stimulate each other and promote thrombus formation. The antithrombotic mechanisms of the vascular system prevent spontaneous initiation of thrombosis under normal conditions. However, they are also active during thrombosis, and disturbances of normal endothelial function are implicated in the initiation and progression of atherothrombotic disease. Platelets are resting circulating cells that constantly enter in contact with these

prothrombotic and antithrombotic factors present in the blood plasma, and integrate their signals. The balance of these signals will determine the prevailing platelet response and therefore, the initiation of thrombus formation. The detailed characterization of the prevailing mechanisms of thrombosis underlying certain vascular diseases will help to design effective antithrombotic therapies.

Recently, rapid progress has been made in the research of antiplatelet and antithrombotic therapy of Chinese herbs and formulas, suggesting Chinese herbs as a promising alternative option for protection and treatment of atherothrombotic diseases. However, there are still some problems in this field. Firstly, most of the experimental researches are conducted using either an *in vivo* or an *in vitro* model, while few studies using both. This limitation in experimental design brings about difficulty in explanation of the result as to the efficiency and mechanism. Secondly, many mechanistic studies focused on one or two constituents of a Chinese herb or formula, though it commonly contains several active compounds. It is well known that thrombosis is a complex, multifactor process, which involves blood cells, vascular endothelium, proteins of the ECM and soluble blood plasma factors. Thus, a systematic study on the anti-thrombotic mechanism of a Chinese herb and formula is preferable. Finally, the safety and efficacy of traditional herbs as anti-thrombotic agents need to be tested in large-scale, multi-center, randomized controlled trials.

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