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*Review*

# Antithrombotic Natural Products That Inhibit Plasminogen Activator Inhibitor 1 (PAI-1)

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**Plasminogen activator inhibitor 1 (PAI-1) stabilizes the thrombus by suppressing the activation of plasminogen to plasmin at the site where the thrombus is formed, thereby inhibiting fibrinolytic reaction. Because inhibition of PAI-1 production or activity facilitates fibrin degradation and eliminates unnecessary thrombus, it is believed that suppression of PAI-1 production or activity prevent thrombosis. Some natural substances that inhibit PAI-1 production and activity were found from medicinal plants, health foods, and purified natural substances and they have potential for realistic use in clinical field or as functional foods. Here, we reviewed these natural products that inhibit PAI-1 production and activity. In addition, we described the potential applications of these substances in clinical field or as functional foods.**

**Key words** thrombosis, fibrinolysis, medical plants, functional foods

## INTRODUCTION

Many components are complexly involved in the reaction that participate in the hemostatic process.[1,2] These are platelets, coagulation factors and fibrinolytic factors, and blood vessels and endothelial cells which undercoat blood vessels. When a blood vessel is damaged, platelets adhere and aggregate to the wound. Then, a blood coagulation cascade reaction proceeds on the activated platelet phospholipid membrane and the resulting thrombin converts fibrinogen to fibrin, that comprises fiver network structure that stabilizes the thrombus. Thereafter, when the damaged blood vessel is repaired and the thrombus that has stopped bleeding is no longer necessary, the activated fibrinolytic system remove the unnecessary thrombus. Like the coagulation cascade, fibrinolyic system is also controlled by a series of cofactors, inhibitors, and receptors.[3] If fibrinolytic system or anticoagulant system are abnormal, or if endothelial cells inside the blood vessel are damaged for some reason, an unnecessary thrombus is formed and the blood flow is blocked, and thereafter, peripheral tissue necrosis occurs. This is called thrombosis and it is associated to scary condition that can kill human lives, such as cerebral infarction, myocardial infarction, and deep vein thrombosis called economy class syndrome.[2]

Various physiologically active and useful natural products are found from various sources such as plants, foods and herbal medicine, and the possibility of food functional or clinical use of them has been proposed. In these natural products, various compounds that have anithrombotic properties from medicinal plants had also been reported. In this article, we focused on the natural products that inhibit plasminogen activator inhibitor 1 (PAI-1) production or activity, a key protease inhibitor of fibrinolysis, that stabilizes the thrombus by suppressing the fibrinolytic activity, and their potential applications to clinical field or functional foods.

## PLASMINOGEN ACTIVATOR INHIBITOR 1 (PAI-1)

Fibrinolysis regulates the extent of clot formation, maintains vascular patency as part of the hemostatic system, and is tightly controlled by a series of cofactors, inhibitors, and receptors like the coagulation cascade.[3] The formation of thrombus induces many mechanisms to modulate fibrinolysis. The activation of plasminogen to the key fibrinolytic enzyme plasmin is mediated by tissue-type (tPA) and urokinase-type (uPA) plasminogen activators that are mostly involved in the dissolution of fibrin in the circulation and pericellular proteolysis during tissue remodeling or tumor invasion, respectively.[4] Both activators are regulated by the circulating serine protease inhibitor (serpin) plasminogen activator inhibitor-1 (PAI-1), which is a 45kDa protein belongs to serpin superfamily.[4] This inhibitor stabilizes thrombus by suppressing plasminogen activation to plasmin at sites of thrombus formation, and consequently inhibits the fibrinolytic reaction (Fig. 1). The conformational flexibility of renders PAI-1 unique among serpins; it is secreted as an active protein that forms covalent complexes and thereby inhibits both uPA and tPA . Active PAI-1 is unstable and under normal physiological conditions spontaneously converts to a stable non-reactive (latent) conformation with an apparent half-life of 1–2 hours.[4] Vascular endothelial cells in various organs[4] and adipocytes[5] produce PAI-1. Elevated PAI-1 concentrations comprise an established risk factor for coronary artery disease.[6] Substantially increased PAI-1 as an acute-phase protein largely contributes to prothrombotic states in inflammation and infection. The expression of PAI-1 is also increased in persons with lifestyle-related diseases such

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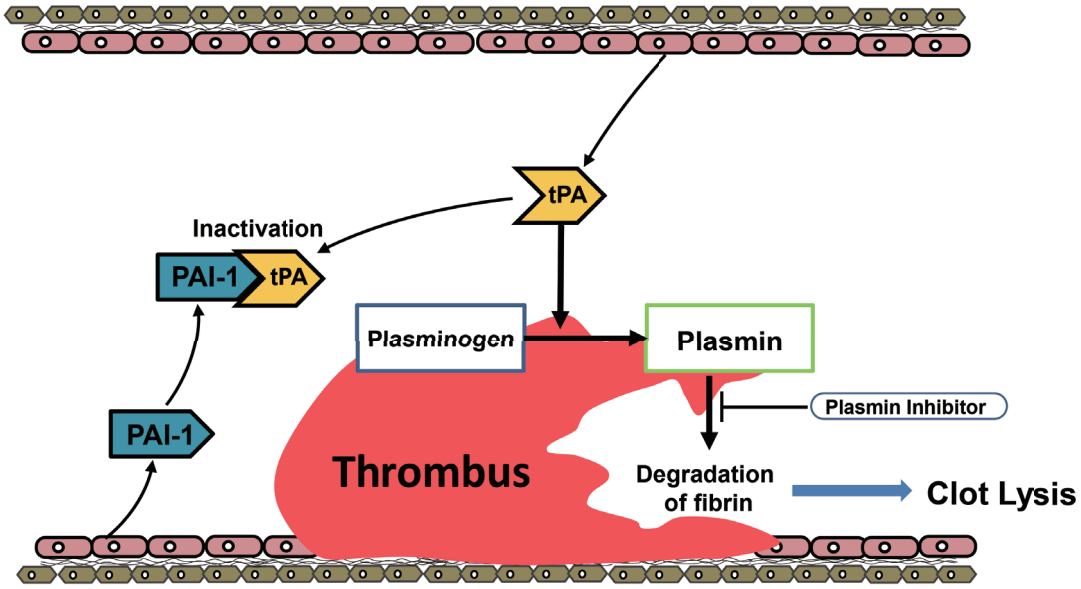


**Fig. 1.** Mechanism of Thrombus Degradation by Fibrinolytic System

**Table 1.** Natural Substances That Inhibit PAI-1 Production (Cultured Cell Experiments)

| Natural substances | Cell type | Stimulant | Effective concentration | References |
|---|---|---|---|---|
| Baicalein | HUVECs | Thrombin<br>Thrombin receptor agonist | 10 μM | Kimura *et al*., 1997 [10] |
| Salvianolic acid B | HUVECs | TNF-α | 0.05 μM | Zhou *et al*., 2005 [14] |
| Xanthoangelols | HUVECs | TNF-α | 10-30 μM | Ohkura *et al*., 2011 [22] |
| Chrysin | HUVECs | TNF-α | 20 μM | Ohkura *et al.,* 2012 [24] |
| (-)-Epigallocatechin gallate (EGCG), | HUVECs | TNF-α | 10 μM | Cao *et al*., 2013 [15] |
| Scolymoside | HUVECs | TNF-α | 5 μM | Yoon *et al*., 2015 [12] |
| Hesperetin and its sulfate and glucuronide metabolites | HAECs | TNF-α | 0.1-10 μM | Giménez-Bastida *et al*., 2016 [13] |
| Astragaloside IV | HUVECs | None | 12 μM | Zhang *et al*., 1997 [11] |
| Salvianolic acid B | HUVECs | None | 70 μM | Shi *et al*., 2007 [17] |
| Grape seed extract | Human fibroblast cells | None | 10 mg/mL | Sandra *et al*., 2007 [19] |
| Green tea polyphenols | BAECs | None | 40 μg/mL | Liu *et al*., 2009 [16] |
| Salvia miltiorrhiza (water-soluble components) | Rat mesangial cells | Angiotensin II | 37.5 mg/mL | Yuan *et al*., 2008 [20] |
| (-)-Epigallocatechin gallate | MDA-MB-231 cells | None | 30 μM | Shin *et al*., 2018 [21] |

HUVECs; human umbilical endothelial cell
HAECs; human aortic vein endothelial cell
BACEs; bovine aortic endothelial cells

as obesity, insulin resistance and dyslipidemia.[5,7,8] In addition to its traditional role in fibrinolysis, PAI-1 plays important roles in metabolic syndrome, obesity, diabetes, and acute respiratory distress syndromes, including coronavirus disease (COVID)-19.[9]

Suppressing PAI-1 production facilitates fibrin degradation and eliminates unnecessary thrombus.[4] Therefore, attempts to actively prevent thrombosis using natural products that inhibit PAI-1 production and activity should be focused as a new strategy for preventing thrombosis.

## NATURAL PRODUCTS THAT INHIBIT PAI-1 PRODUCTION

Many studies have investigated the inhibition of PAI-1 production by natural products (Table 1). The flavonoid, baicalein, isolated from Scutellariae Radix inhibits PAI-1 production in cultured human umbilical vein endothelial cells (HUVECs) induced by thrombin and a thrombin receptor agonist.[10] Astragaloside IV purified from the Chinese herb drug, *Astragalus membranaceus*, which is used in traditional Chinese medicine to treat cardiovascular diseases, decreases basal PAI-1 production from unstimulated HUVECs.[11] Many

**Table 2.** Natural Substances That Inhibit PAI-1 Production (Animal Experiments)

| Natural substances | Animals | Tissue | Induction of PAI-1 | References |
|---|---|---|---|---|
| *Angelica keiskei* yellow exudate | Mice | Plasma | Lipopolysaccharide | Ohkura *et al*., 2011 [22] |
| *Angelica keiskei* yellow exudate | Mice | Plasma | Spontaneously diabetic mice | Ohta *et al*., 2019 [36] |
| *Angelica keiskei* yellow exudate | Mice | Plasma | Aged mice | Ohkura *et al*., 2023 [37] |
| Black vinegar concentrate and Black vinegar mash powder | Mice | Plasma | Lipopolysaccharide | Ohkura *et al*., 2018 [26] |
| Propolis ethanol extract | Mice | Plasma | Lipopolysaccharide | Ohkura *et al*., 2016 [28] |
| Corilagin | Rats, Rabbit | Plasma | Electrically-induced thrombosis | Shen *et al*., 2003 [38] |
| Salvianolic acid B | Rats | Lung | Bleomycin-induced idiopathic pulmonary fibrosis | Zhang *et al*., 2021 [39] |
| Tongqiaohuoxue decoction (water extract of eight medicinal herbs) | Mice | Adipose | High-fat diet induced obesity | Kim *et al*., 2016 [40] |
| Magnolia extract | Mice | Kidney | High-fat diet induced obesity | Cui *et al*., 2013 [41] |

natural compounds suppress PAI-1 production in vascular endothelial cells stimulated with TNF-α. Scolymoside is an active compound in *C. subternata* that also inhibits the PAI-1 increase induced by TNF-α in HUVECs.[12] Hesperetin from citrus fruits and its sulfate and glucuronide metabolites inhibit TNF-α-induced PAI-1 increases in human aortic endothelial cells (HAECs)[13]. Salvianolic acid B from *Salviae miltiorrhizae* inhibits TNF-α-induced PAI-1 mRNA production and protein secretion in HUVECs.[14] Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) and extracellular signal-regulated kinase- activator protein-1 (ERK-AP-1) pathways are possible targets of salvianolic acid B in the regulation of TNF-α-induced PAI-1 production in HUVECs.[14] The major catechin (-)-epigallocatechin gallate derived from green tea reduces the TNF-α-induced PAI-1 increase in HUVECs and phosphorylation of ERK1/2.[15] A mixture of green tea polyphenols reduces PAI-1 production in unstimulated bovine aortic endothelial cells (BAECs) and the PI3K/Akt pathway plays an important role in regulating the fibrinolytic balance in the vascular wall.[16] Natural products that affect other coagulation-related proteins have also been identified. Salvianolic acid B increases the fibrinolytic and anticoagulant potential of cultured HUVECs by upregulating the expression of tPA and thrombomodulin and downregulating that of PAI-1.[17]

Various compounds can inhibit PAI-1 production in cells other than vascular endothelial cells. The Chinese medical plant, *Salvia miltiorrhiza*, inhibits PAI-1 production induced by angiotensin II in renal mesangial cells.[18] Grape seed extract significantly downregulates uPA and PAI-1 expression at the RNA and protein levels[19] and delays cell migration towards wound sites. A water-soluble component of *Salvia miltiorrhiza* inhibits PAI-1 production in rat mesangial cell lines.[20] Derivatives of (-)-epigallocatechin gallate reduce PAI-1 expression, which inhibits the migration, adhesion, and invasion of MDA-MB-231 cells.[21] Natural products with significantly different structures suppress PAI-1 production, and few natural products have been studied to date. Thus, a structure-activity relationship cannot yet be proven. However, while many natural products are effective at concentrations of around tens of μM in cultured cells, salvianolic acid B is notably active at ~ 0.05 μM[14].

Our study also had been found some PAI-1 production inhibitors from natural substances and foodstuff. Our study used assay system that detect the inhibition of TNF-α- induced PAI-1 increase in the culture medium of HUVEC. These are chalcones isolated from *Angelica keiskei* Koidzumi (Ashitaba), a large perennial herb that is native to the Pacific coast of Japan,[22,23] ethanol extract obtained from Brazilian propolis which is the hive product made by honey bees,[24] Chrysin, a kind of flavonoid,[25] and ethanol extract of Kurozu Moromi that is solid residue produced during the brewing of Kurozu, a traditional Japanese black vinegar.[26] Screening study was performed using the inhibitory effect of PAI-1 production from endothelial cells as an index using various compounds derived from crude drugs.[27]

We also performed animal experiments using mice model. We used mice with thrombotic tendency induced by lipopolysaccharide (LPS). When a small amount of LPS is administered to a mouse, the mouse tends to form a thrombus called thrombotic state, and PAI-1 in the blood also increases. We administrated LPS to mice that were fed lyophilized *Angelica keiskei* (Ashitaba) yellow exudate, black vinegar concentrate, black vinegar mash powder or propolis ethanol extract mixed with each food. These mice were found to suppress the plasma PAI-1 increase induced by LPS.[22,26,28] Increased PAI-1 levels are demonstrated to be associated with obesity, insulin resistance, and metabolic syndrome.[29,30] High PAI-1 concentration is associated with an increased risk of thrombotic diseases in individuals in these diseases. Obese and diabetic mice are useful model for studying the effect of PAI-1 increase in these diseases.[31,32] In our study, we used type 2 diabetic model Tsumura, Suzuki, Obese Diabetes mice (TSOD mice)[33-35] to examine the effect naturally-derived substances. As a result of study in TSOD mice fed with diet containing Ashitaba yellow exudate, it was found that the increased blood PAI-1 was significantly reduced in these mice by this diet.[36] Recently, we have shown in aged mice that age-related PAI-1 elevation is suppressed by yellow juice consumption.[37]

There are some animal studies other than us. Shen *et al*. showed that corilagin inhibited plasma PAI-1 activity in the electrically-induced thrombosis model of rats and rabbits.[38] However, other studies showed the inhibition of tissue PAI-1 production by natural products.[39-41] These studies are not directly related to antithrombotic activity (Table 2).

## NATURAL PRODUCTS THAT INHIBIT PAI-1 ACTIVITY

Identification of small molecule PAI-1 activity inhibitors attracted great interest in recent years. These PAI-1 inhibitors have the potential for the prevention and treatment of thrombotic diseases. As PAI-1 inhibitors that are being developed as pharmaceuticals, they are not PAI-1 production inhibitors but are being developed as antagonists of PAI-1 molecules.[42,43] Interestingly, some natural product that inactivates PAI-1 activity by affecting PAI-1 molecule are reported. Jankun *et al*. reported that heaflavin-3'-gallate and theaflavin-3,3'-gallate in black tea inactivate PAI-1.[44] Recently, Pautus *et al*. found a new natural product PAI-1 inhibitor. This molecule is a natural product belonging to the *Annonaceous acetogenins* group specifically found in plants of the Annonaceae family. Annonacinone inhibited formation of PAI-1/tPA complex.[45]

There are no PAI-1 activity inhibitor currently used in clinical situation. However, clinical applications of drugs that inhibit the binding of PAI-1 to t-PA and drugs that inactivate PAI-1 by changing the three-dimensional structure are considered.[46]

## CONCLUSION

In this review, we introduced the possible inhibition of PAI-1 production and activity by natural products. Many natural products have potential to be used as antithrombotic agents. In these natural product, we believe that antithrombotic natural products which are suitable for the prevention of thrombotic diseases for people with high risk of these diseases due to obesity, diabetes, dyslipidemia are PAI-1 production inhibitor or PAI-1 activity inhibitor. However, there will be many problems that must be solved before these natural substances will be used for therapy of thrombotic diseases or dietary supplements or functional foods for the prevention of thrombotic diseases. Possible problems are as follows; (1) Complex Regulatory Mechanisms: PAI-1 is a complex functional protein that regulates the balance between blood coagulation and fibrinolysis, and its regulation is a very subtle process. Adequate suppression of PAI-1 production is important, but excessive suppression may increase the risk of abnormal bleeding. (2) Risk of side effects: Therapies that directly inhibit PAI-1 may also affect other physiological processes in which PAI-1 is involved. This carries the risk of unintended side effects. Side effects that are difficult to predict and manage can limit their practical utility as a treatment. (3) Prioritization of other therapeutics: Currently, there are already more effective methods and agents in the treatment of thrombotic diseases than therapies that directly target PAI-1. These existing therapies tend to be preferred due to their well-established efficacy in reducing thrombus formation and cardiovascular risk, despite the superiority of inhibiting PAI-1.

However, we hope that it will be applied to the clinical fields or health maintenance of the people in the future by taking advantage of the general characteristics of natural product, such as mild action, high safety.

**Conflict of interest** The authors declare no conflict of interest.

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