Chapter 5

Thrombolytic/Fibrinolytic Mechanism of Natural Products

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1. Introduction

Morphological and angiographic studies have demonstrated that the formation of thrombi at sites of atherosclerotic lesions is the major cause of the development of clinical complications of atherosclerosis, which are leading contributors to morbidity and mortality throughout the industrialized world [1]. Thrombogenicity of the atherosclerotic plaque is determined mainly by the stability of a fibrous cap and contents of tissue factor in its core, which activates the coagulation cascade when exposed to flowing blood. These elements interact with each other and with the blood vessel wall and under physiological conditions the blood flow to tissues is unimpaired by clotting [2]. Under pathophysiological conditions, activation of blood coagulation occurs primary through interaction of platelets, vessel wall and plasma proteins (so-called primary haemostasis). In this sense, there is evidence in the cardiology literature that the combination of thrombolysis with antiplatelet agents speeds and augments thrombolysis and seems to improve survival [3]. Moreover, epidemiologic studies have provided evidence that foods (fruit and vegetables) with the experimentally proven thrombolytic/fibrinolytic effect could reduce the risk of thrombosis [4].

This chapter discusses the involvement of coagulation and fibrinolytic system components in thrombosis, and possible mechanisms of thrombolytic/fibrinolytic effects of natural products.

2. Coagulation and fibrinolytic system components in thrombosis

Thrombosis is associated with activation of several enzymatic cascades, including the coagulation, fibrinolysis, complement, and kinin systems. Thus, plasma markers of coagulation and

fibrinolysis have proven to be sensitive in the initial diagnosis of acute deep venous thrombosis [5]. Nowadays, the use of oral anticoagulants in secondary prevention is widely reported, but inconveniences arising from the need for its stringent control and the thin line between good therapy and incorrect therapy necessitate the search for new anticoagulants with higher specificity and with no need for such strict controls and follow up.

Coagulation components. Coagulation is the process by which blood forms clots. It is an important part of haemostasis; this begins almost immediately after an injury to the blood vessel that has damaged the endothelium lining the vessel. The cessation of blood loss from a damaged vessel begins with the junction of platelet to the subendothelial matrix and subsequent activation of the coagulation system which stabilizes the platelet-rich clot and fibrin. This process fails to stop the bleeding and begins the process of repairing a damaged vessel. Disorders in coagulation can lead to an increased risk of bleeding (hemorrhage) or obstructive clotting (thrombosis). For this reason there must be different mechanisms of regulation of this phenomenon, for example serine protease inhibitors. A major class of serine protease inhibitors regulating procoagulant enzymes is the serpin superfamily [6].

The principal inhibitor of procoagulant enzymes such as thrombin and factor Xa is the serpin antithrombin. There are, however, other serpins that act to control coagulation enzymes, such as heparin cofactor II (HCII), protease nexin I (PN1) and protein C inhibitor (PCI) [6]. Some serpins act to control the action of anticoagulant enzymes, such as activated protein C. Many of the serpins that control enzymes in the coagulation system are under the control of glycosaminoglycans such as heparin, heparan sulfate and dermatan sulfate which have been found to significantly accelerate the interaction between serpins and coagulation proteases, usually increasing the reaction rates from values that are not relevant under physiological conditions to rates that are relevant [5]. Another mechanism involving a serpin is the protein Z/Z-dependent protease inhibitor (PZ/ZPI) system that inhibits activated factors X, XI and IX by different mechanisms. ZPI is catalytically activated by PZ and in that way regulates the function of Xa factor on the surface of the membrane. PZ joins to a binding site which is located in the region of G helix [7]. For example, the ZPI inhibits prothrombinase activity (factor Xa complex) in the presence of phospholipids and calcium ions, the presence of PZ enhances this process 1000 times, but it also directly inhibits coagulation factor Xia [8]. In this same context, it has been recently demonstrated that residues of the C and D helices of ZPI are key to the interactions with heparin and modulate inhibitory function of serpins [9].

It has been recently demonstrated that coagulation systems may be regulated by MicroRNAs (miRNAs) that are an abundant class of small non-coding RNAs which are regulators in a growing number of physiological and pathological processes. However, their role in haemostasis, a complex physiological process involving a multitude of effectors, is just beginning to be characterized. For example miR-19, miR-20, and miR-106b regulate the tissue factor expression or it has been determined that there is an inverse correlation observed between miR-18a and miR-19b levels with antithrombin mRNA and miR-10b that regulate the expression of heparin. miR-15a, miR-21, miR-23b, miR-29c regulate de TFPI expression. The potential target of these miRNAs suggests that certain miRNAs may be involved in the regulation of selected haemostatic proteins and thereby regulate the clotting system [10, 11].

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Fibrinolytic components. Fibrinolysis is a process by which fibrin is eliminated through activation of a blood protease cascade, and plasmin is responsible for such degradation. This process starts with the proteolitic cleavage of the plasminogen zymogen to convert it in plasmin through its tissue plasminogen activator (tPA) and urokinase plasminogen activator(uPA) [5]. Plasmin acts catalytically over fibrin exposing lysine residues of the new carboxyterminals which join to plasminogen and its activator in order to amplify fibrinolysis. Thrombin activatable fibrinolysis inhibitor (TAFI) is a key element in this process as it is able to control the plasmin activity by removing lysine residues in carboxyterminal region thereby preventing positive feedback of the system. In order for TAFI to exert its action over its substrate; it must be activated by the thrombin-thrombomodulin complex [12]. Very recently, it has been reported that the renin-angiotensin-aldosterone system is implied in fibrinolysis regulation; this effect is carried out through the angiotensin receptor type 1. The results of this study demonstrate that angiotensin-1-9 favours the development of venous thrombosis in rats decreasing the levels of plasminogen activator and increasing the levels of Plasminogen activator inhibitor 1 (PAI-1) [13]. PAI-1 also called serpin E1 is the main inhibitor of the plasminogen activator and it has been widely reported in the scientific literature as being responsible for thrombotic events and recurrent foetal loss [14]. The activation of the fibrinolytic system is essential to eliminate intravascular deposits of fibrin resulting from the physiological or pathological activation of the coagulation system, but the proper functioning of this system depends on its regulation. The fibrinolytic system is important not only in physiological processes but also in pathological ones, such as inflammation, tumour invasion or cardiovascular diseases.

3. Platelets and thrombolysis

The recognition of arterial thrombosis as the major causative factor in acute coronary syndromes, in particular acute myocardial infarction, was a major advance in cardiology in the 1980s [15]. Stroke is considered an independent entity by the World Health Organization classification, the current gold standard treatment of which is the intravenous application of thrombolytic therapy within a 4.5 h time window from the onset of stroke symptoms [16].

The main elements of thrombus include fibrin, thrombin, and platelets [17]. Current techniques to dissolve clot focus on the fibrin and prothrombin activation, fail to address the important effects of platelets; thrombolysis may dissolve the fibrin component of the clot but may have no effect on the platelet portion [18]. Fibrinolytic therapy has a potent platelet aggregating effect as does the exposure of the ruptured atherosclerotic plaque [19].

The use of thrombolytic agents, such as the recombinant tissue-type plasminogen activator (rt-PA), is well established in the strategy for treatment of acute myocardial infarction [20]. The insoluble fibrin fibre is hydrolyzed into fibrin degradation products by plasmin, which is generated from plasminogen by plasminogen activators, such as t-PA, urokinase, Hageman factor, and streptokinase plasminogen complex [21, 22].

However, larger thrombi have notoriously proven to be resistant to intravenous tPA lysis with recanalization rates in the range of only 13% to 20% [23]. Even if endovascular treatment of ischemic stroke is proven to improve clinical outcomes, there will still be many patients with residual partial or complete occlusion after intravenous tPA alone suffering ischemia, whereas waiting for catheter rescue [24]. Thrombolysis resistance has also been demonstrated in platelet-rich thrombi, as seen in postmortem microscopic examination of serial sections of coronary thrombus of patients with acute myocardial infarction and sudden death revealing that thrombus formed at the plaque fissure is very rich in platelets, whereas proximal and distal extensions of the thrombus are composed of erythrocyte-rich material [25].

Platelet activation plays a central role in thrombus formation and can be inhibited by many agents [26], even a weak antiplatelet agent such as aspirin is beneficial when given alone or in conjunction with reperfusion, such therapy may improve early coronary flow rates as well as stabilize or maintain subsequent perfusion and provide an incremental improvement in clinical outcomes [27]. Therefore, development of combination therapies for acute ischemic stroke that can be delivered quickly in the emergency setting is crucial. Ongoing strategies that are in either phase II or III clinical trials include thrombin-inhibition, sonothrombolysis, and platelet-inhibition [28].

With platelet activation, there is high affinity and binding to fibrinogen and von Willebrand factor; in addition, there is up-regulation of further platelet activation. Activated platelets can also facilitate thrombin generation by providing a catalytic surface and by releasing an activated form of factor V [29], resulting in more fibrin production. In addition, exposure of clot-bound thrombin by lytics converts more fibrinogen to fibrin, causing rethrombosis [30].

The active glycoprotein (GP) IIb/IIIa receptors bind fibrinogen, and this forms links between platelets causing aggregation. Hence GP IIb/IIIa antagonists such as abciximab are potent inhibitors of platelet aggregation. There is evidence in the cardiology literature that the combination of thrombolysis with a GP IIb/IIIa antagonist speeds and augments thrombolysis and seems to improve survival [31].

The $\alpha_{IIb}\beta_3$ integrin (GP IIb/IIIa) is found exclusively on platelets and megakaryocytes, with 70,000 to 90,000 receptors expressed on each platelet in the resting state. These heterodimeric molecules have large extracellular regions for cation-facilitated ligand binding and small intracytoplasmic tails mediating intracellular signal transduction [32]. Integrin binding affinity is dynamic and dependent on the receptor's conformational status. In the resting state, affinity for fibrinogen binding is low, platelet agonists, via "inside-to-outside" signals; trigger a change in the receptor's structure, transforming it to a high-affinity state [33, 34].

For platelet inhibition the most commonly used antiplatelet agent is aspirin, which inhibits platelet cyclooxygenase-1, and also, two distinct classes of antiplatelet agents with distinct mechanisms of action, glycoprotein IIb-IIIa antagonists (e.g., abciximab, eptifibatide) and antagonists of the platelet ADP receptor $P2Y_{12}$ (e.g., clopidogrel, prasugrel), have been used in acute coronary syndromes [35].

The advantage of blocking the GP IIb/IIIa receptor is that platelet to platelet binding through fibrinogen or von Willebrand factor is prevented, but platelet binding to the subendothelial

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elements, that is, the surface of the damaged vessel, remains intact. An initial layer of platelets is formed, resulting in hemostasis but not aggregation that can lead to local thrombosis or downstream embolization to the distal microcirculation. These drugs prevent not only local thrombosis attributable to platelet aggregation but also damage to the distal vascular bed by platelet embolization [36].

The results of the Combined Approach to Lysis Utilizing Eptifibatide and rt-PA in Acute Ischemic Stroke–Enhanced Regimen (CLEAR-ER) [37] a multicenter, double-blind, randomized phase II safety trial of intravenous tPA versus eptifibatide, sought to estimate the safety and efficacy of combination GP IIb/IIIa+reduced dose of intravenous tPA when delivered to hyperacute ischemic stroke, demonstrating that emergent adjunctive therapies are feasible within the first few hours of stroke onset and need to be further pursued as a means of amplifying the thrombolysis effect of intravenous tPA [38].

Combination therapy with a local fibrinolytic and systemic GP IIb/IIIa receptor inhibitors in the peripheral setting may represent a promising new means to accelerate reperfusion, prevent reocclusion, allow fibrinolytic dose reductions, and improve clinical outcomes [39].

4. Thrombolytic/fibrinolytic mechanisms of natural products

Thrombolytic drugs (tPA, streptokinase (SK), and uPA) have the ability to effectively dissolve blood clots; they differ in their detailed mechanisms in ways that alter their selectivity for fibrin clots. The SK binds equally to circulating and non-circulating plasminogen, produces significant fibrinogenolysis along with clot fibrinolysis [42]. For this reason, tPA is generally preferred as a thrombolytic agent over SK, especially when used for dissolving coronary and cerebral vascular thrombi. Because SK is derived from streptococci, patients who have had recent streptococci infections can require significantly higher doses of SK to produce thrombolysis.

Moreover, these drugs are not used in patients who have undergone surgery or those with a history of nervous lesions, gastrointestinal bleeding or hypertension [42]. The treatment with tPA is limited in platelet-rich thrombi that are highly resistant to lysis by t-PA [25]. Considerable efforts have been directed towards the discovery and development of natural products from various plants which have antiplatelet [43, 44], anticoagulant [45], antithrombotic [46] and thrombolytic activity [4]. Epidemiologic studies have provided evidence that foods with experimentally proven antithrombotic and thrombolytic effects could reduce the risk of thrombosis (Table 1) [47, 48, 49].

Studies from around the world have demonstrated the potent antiplatelet properties of Ginkgo, which inhibits platelet aggregation and thrombin activity [50, 51]. The extract was obtained by a polyphenolic method, the fibrinolytic effects of Streptokinase was compared with those of the Ginkgo extract using a fluorometric method. The study was performed in vitro on a labeled clot; fibrinogen was labeled with the fluorescent agent fluorescein isothiocyanate and precipitated in the presence of Ca^{2+}. The Streptokinase (100 U/mL to 1000 U/mL)

and Ginkgo extract was added to labeled fibrin in a plasma environment. A linear relationship was observed between the Streptokinase and Ginkgo extract [42]. The results indicate that the effects of Ginkgo extract on the fibrinolytic system are similar to those of streptokinase [42]; hence, this herbal extract can be used as a complement to or as a substitute for streptokinase. In this sense, there is evidence that some natural products have fibrinolytic effects.

Other researchers have found that organic extracts of six Bangladeshi plants (Ageratum conyzoides L., Clausena suffruticosa, Leea indica (Burm.f.) Merr., Leucas aspera Willd., Senna sophera L. Roxb., and Solanum torvum Swartz), have thrombolytic activity. An in vitro thrombolytic model was used to check the clot lysis effect of the all these extracts [52]. The venous blood was allowed to form clots which were weighed and treated with the extract to disrupt the clots,the weight of clot before and after treatment provided a percentage of clot lysis. Among the herbs studied *Clausena suffruticosa, Leea indica* and *Leucas aspera* showed a very significant ($p < 0.0001$) percentage (%) of clot lysis compared to the reference drug streptokinase (75.00 ± 3.04%) [4].

Prasad, S. *et al* [53] have tried six herbal preparations (*Tinospora cordifolia*, *Rubia cordifolia*, *Hemidesmus indicus, Glycyrrhiza glabra Linn*, *Fagonia arabica* and *Bacopa monnieri Linn),* that have been used since ancient times for neuroprotection and for curing vascular diseases. For example, *Hemidesmus indicus* was reported to have antithrombotic activity [54] or *Fagonia arabica* is known to have a blood purifying property [55]. When compared with the clot lysis percentage obtained through water (negative control), a significant thrombolytic activity was observed after treating the clots with *Fagonia arabica* and *Bacopa monnieri* 75.6% and 41.8% clot lysis was obtained respectively (p value < 0.0001 &=0.0023 respectively). Chourasia, S.R. *et al* [55], found the same clot lysis percentage by streptokinase as well as *F. arabica*.

Yamada, K. *et al* [56] analyzed ten onion varieties, the antithrombotic activity of which was assessed in vivo by using a laser-induced thrombosis test in mice. Toyohira, showed significant antithrombotic activity both in vitro and in vivo. Toyohira showed thrombolytic activity in addition to the antiplatelet effect. Superkitamomiji, 2935A, and K83211 showed only thrombolytic activity.

Natto-extracts is soybeans fermented with Bacillus subtilis, Suzuki *et al* [57], investigated the effects of dietary supplementation with natto-extracts on neointima formation and on thrombolysis at the site of endothelial injury. In control animals, thrombolysis started from the center of the thrombus and mural thrombus remained attached on vessel wall. A supplementation with natto-extracts seems to have modulated the process of thrombolysis, which started from near the vessel walls and then thrombi detached from them.

Rajput, M.S. *et al* [58], explored the fibrinolytic potential of the methanolic extract of the fruits of *Lagenaria siceraria* (bottle gourd), the fibrinolytic activity was expressed as percentage of plasma clot liquefaction and was determined by plasma clot lysis at 37°C in 24 h. Treatment of plasma clot combined with methanolic extract showed a reduction by 54.72% which was significant when compared to the control (saline – 3.68%; $p < 0.01$).

Torres-Urrutia, C. *et al* [59] studied samples of 19 fruits and 26 vegetables. The extracts prepared from each sample included an aqueous (juice or pressed solubles) and/or methanol-

soluble fraction. The extracts were evaluated for antiplatelet, anticoagulant, and fibrinolytic activity in vitro at a final concentration of 1 mg/ml, the fibrinolytic effect was determined with the euglobin clot lysis time and fibrin plate methods. Out of all fruits and vegetables the fibrinolytic activity was observed only in raspberries.

Bordia, A. *et al* [60], determined the effects of a preparation of dried garlic powder (Sapec) in 12 healthy subjects on fibrinolysis and platelet aggregation. Total euglobulin fibrinolytic activity and t-PA activity were significantly higher 4 and 6 h after garlic and placebo ingestion.

Ginger (Zingiber officinale) is a popular food spice and it is reported to contain antihistaminic and antioxidant factors. Verna, S.K. *et al* [61] studied the effect of ginger on fibrinolytic activity on 30 healthy adult with high fat diet. The ginger increased fibrinolysis activity by 31.5% in these patients compared with the placebo.

Morozova, E.N. *et al* [62]found a high fibrinolytic activity in *Flammulina velutipes* (also known as the golden needle mushroom), this was compared with those of *Aspergillus terricola* and *Streptomyces griseusproteinases*. Then Park, S.E. *et al* [63] purified a fibrinolytic enzyme from the culture supernatant by ion exchange and gel filtration chromatographies. This was the first study of fibrinolytic enzyme from mushrooms and their application as therapeutic agents. Other researchers also have isolated different enzymes with fibrinolytic activity from mushrooms [64, 65]. Also Kim *et al* [66], found fibrinolitic activity on *Cordyceps militaris* a medicinal mushroom, their results for the fibrinolysis pattern showed that enzyme rapidly hydrolyzed the fibrin and fibrinogen chains.

Choi, H.S *et al* [67], also isolated a protease with fibrinolitic properties from a Chinese herb (Spirodela polyrhiza), the homogenate of this herb was filtered and centrifuged, the supernatant was concentrated by ultrafiltration. The protease hydrolyzed not only fibrin but also fibrinogen, cleaving Aalpha and Bbeta without affecting the gamma chain of fibrinogen. The fibrinolytic activity was measured in the fibrin plate assay [68].

Another plant extract/product which has been identified to have fibrinolytic activity is *Ananas comosus,* this has a proteolytic enzyme called bromelain, which has displayed anti-inflammatory and analgesic properties in human and laboratory studies. It has been shown to increase fibrinolytic activity [69, 70].

Plant-derived medicines have a long history of use for the prevention and treatment of human diseases. Advances in phytochemistry and identification of plant compounds to cure certain diseases have renewed the interest in herbal medicines; about 30% of pharmaceuticals are prepared from plants worldwide. Some of these plant products are modified further with recombinant technology [71] to make them more effective and site specific. They may even be incorporated as a thrombolytic agent for the improvement of the patients suffering from atherothrombotic diseases [72, 73, 74, 75]. There are several thrombolytic drugs that have be reported to have adverse side effects,, sometimes the patients died due to bleeding and embolism [77, 78, 79, 80]. In this context, on the basis of the beneficial effects of clot dissolving properties of plant extracts/products, these agents should be considered as a complement to or as a substitute for thrombolytic drugs.

Name of Compound	Source	Effect	Mechanism of Action	References
Polyphenols	*Ginkgo biloba*	Antithrombotic	Fibrinolytic	[42]
Terpene lactones	*Ginkgo biloba*	Antithrombotic	Fibrinolytic	[81]
Sterols	*Bacopa monnieri Linn*	Antithrombotic	Fibrinolytic	[82]
Steroidal sapogenins	*Lagenaria siceraria*	Antithrombotic	Fibrinolytic	[83]
Hydroxycinnamic acid	*Ananas comosus*	Antithrombotic	Fibrinolytic	[84]

Table 1. Natural bioactive compound with antithrombotic and fibrinolytic activities.

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References

[1] Libby, P.; Ridker, P.M.; Hansson, G.K. Progress and challenges in translating the biology of atherosclerosis. *Nature* 2011, *473*, 317-325.

[2] Ananyeva, N.M.; Kouiavskaia, D.V.; Shima, M.; Saenko, E.L. Intrinsic pathway of blood coagulation contributes to thrombogenicity of atherosclerotic plaque. *Blood* 2002, *99*, 4475-4485.

[3] Zinkstok, S.M.; Vermeulen, M.; Stam, J.; de Haan, R.J.; Roos, Y.B. Antiplatelet therapy in combination with rt-PA thrombolysis in ischemic stroke (ARTIS): rationale and design of a randomized controlled trial. *Cerebrovasc Dis* 2010, *29*, 79-81.

[4] Rahman, M.A.; Sultana, R.; Bin Emran, T.; Islam, M.S.; Chakma, J.S.; Rashid, H.U.; Hasan, C.M. Effects of organic extracts of six Bangladeshi plants on in vitro thrombolysis and cytotoxicity. *BMC Complement Altern Med* 2013, *13*, 25.

[5] Palomo I, F.P., Pereira J Sistyema de la coagulación y sistema fibrinolítico en Hematología fisiopatología y diagnóstico. *Editorial Universidad de Talca* 2005, 493-514.

[6] Huntington, J.A. Thrombin inhibition by the serpins. *J Thromb Haemost* 2013, *11 Suppl 1*, 254-264.

[7] Huang, X.; Yan, Y.; Tu, Y.; Gatti, J.; Broze, G.J., Jr.; Zhou, A.; Olson, S.T. Structural basis for catalytic activation of protein Z-dependent protease inhibitor (ZPI) by protein Z. *Blood* 2012, *120*, 1726-1733.

[8] Karimi, Z.; Falsafi-Zade, S.; Galehdari, H. The role of Ca(2+) ions in the complex assembling of protein Z and Z-dependent protease inhibitor: A structure and dynamics investigation. *Bioinformation* 2012, *8*, 407-411.

[9] Yang, L.; Ding, Q.; Huang, X.; Olson, S.T.; Rezaie, A.R. Characterization of the heparin-binding site of the protein z-dependent protease inhibitor. *Biochemistry* 2012, *51*, 4078-4085.

[10] Camaioni, C.; Gustapane, M.; Cialdella, P.; Della Bona, R.; Biasucci, L.M. Microparticles and microRNAs: new players in the complex field of coagulation. *Intern Emerg Med* 2013, *8*, 291-296.

[11] Stavik, B.; Skretting, G.; Olstad, O.K.; Sletten, M.; Dehli Vigeland, M.; Sandset, P.M.; Iversen, N. TFPI alpha and beta regulate mRNAs and microRNAs involved in cancer biology and in the immune system in breast cancer cells. *PloS one* 2012, *7*, e47184.

[12] Antovic, J.P.; Blomback, M. Thrombin-activatable fibrinolysis inhibitor antigen and TAFI activity in patients with APC resistance caused by factor V Leiden mutation. *Thromb Res* 2002, *106*, 59-62.

[13] Mogielnicki, A.; Kramkowski, K.; Hermanowicz, J.; Leszczynska, A.; Przyborowski, K.; Buczko, W. Angiotensin-(1-9) enhances stasis-induced venous thrombosis in the rat because of the impairment of fibrinolysis. *Journal of the renin-angiotensin-aldosterone system : JRAAS* 2013.

[14] Su, M.T.; Lin, S.H.; Chen, Y.C.; Kuo, P.L. Genetic association studies of ACE and PAI-1 genes in women with recurrent pregnancy loss: a systematic review and meta-analysis. *Thromb Haemost* 2013, *109*, 8-15.

[15] Skinner, M.P. Thrombosis and thrombolysis: platelet membrane glycoproteins. *Heart Lung Circ* 2007, *16*, 176-179.

[16] Hacke, W.; Kaste, M.; Bluhmki, E.; Brozman, M.; Davalos, A.; Guidetti, D.; Larrue, V.; Lees, K.R.; Medeghri, Z.; Machnig, T.; Schneider, D.; von Kummer, R.; Wahlgren, N.; Toni, D. Thrombolysis with alteplase 3 to 4.5 hours after acute ischemic stroke. *N Engl J Med* 2008, *359*, 1317-1329.

[17] Moliterno, D.J.; Topol, E.J. Conjunctive use of platelet glycoprotein IIb/IIIa antagonists and thrombolytic therapy for acute myocardial infarction. *Thrombosis and haemostasis* 1997, *78*, 214-219.

[18] Cannon, C.P. Overcoming thrombolytic resistance: rationale and initial clinical experience combining thrombolytic therapy and glycoprotein IIb/IIIa receptor inhibition for acute myocardial infarction. *J Am Coll Cardiol* 1999, *34*, 1395-1402.

[19] Combining thrombolysis with the platelet glycoprotein IIb/IIIa inhibitor lamifiban: results of the Platelet Aggregation Receptor Antagonist Dose Investigation and Reperfusion Gain in Myocardial Infarction (PARADIGM) trial. *J Am Coll Cardiol* 1998, *32*, 2003-2010.

[20] Huang, T.C.; Jordan, R.E.; Hantgan, R.R.; Alevriadou, B.R. Differential effects of c7E3 Fab on thrombus formation and rt-PA-Mediated thrombolysis under flow conditions. *Thrombosis research* 2001, *102*, 411-425.

[21] Kim, J.S.; Kim, J.E.; Choi, B.S.; Park, S.E.; Sapkota, K.; Kim, S.; Lee, H.H.; Kim, C.S.; Park, Y.; Kim, M.K.; Kim, Y.S.; Kim, S.J. Purification and characterization of fibrinolytic metalloprotease from Perenniporia fraxinea mycelia. *Mycol Res* 2008, *112*, 990-998.

[22] Bhargavi, P.L.; Prakasham, R.S. A fibrinolytic, alkaline and thermostable metalloprotease from the newly isolated Serratia sp RSPB11. *Int J Biol Macromol* 2013.

[23] Alexandrov, A.V.; Demchuk, A.M.; Burgin, W.S.; Robinson, D.J.; Grotta, J.C. Ultrasound-enhanced thrombolysis for acute ischemic stroke: phase I. Findings of the CLOTBUST trial. *J Neuroimaging* 2004, *14*, 113-117.

[24] Barreto, A.D.; Pedroza, C.; Grotta, J.C. Adjunctive Medical Therapies for Acute Stroke Thrombolysis: Is There a CLEAR-ER Choice? *Stroke* 2013, *44*, 2377-2379.

[25] Jang, I.K.; Gold, H.K.; Ziskind, A.A.; Fallon, J.T.; Holt, R.E.; Leinbach, R.C.; May, J.W.; Collen, D. Differential sensitivity of erythrocyte-rich and platelet-rich arterial thrombi to lysis with recombinant tissue-type plasminogen activator. A possible explanation for resistance to coronary thrombolysis. *Circulation* 1989, *79*, 920-928.

[26] Kessel, D.O.; Patel, J.V. Current trends in thrombolysis: implications for diagnostic and interventional radiology. *Clin Radiol* 2005, *60*, 413-424.

[27] Alexander, J.H.; Harrington, R.A. Adjunctive Antiplatelet Therapy in Acute Myocardial Infarction: The Road to Improved Infarct-Related Artery Patency. *J Thromb Thrombolysis* 1997, *4*, 353-355.

[28] Barreto, A.D.; Alexandrov, A.V. Adjunctive and alternative approaches to current reperfusion therapy. *Stroke* 2012, *43*, 591-598.

[29] Reverter, J.C.; Beguin, S.; Kessels, H.; Kumar, R.; Hemker, H.C.; Coller, B.S. Inhibition of platelet-mediated, tissue factor-induced thrombin generation by the mouse/human chimeric 7E3 antibody. Potential implications for the effect of c7E3 Fab treatment on acute thrombosis and "clinical restenosis". *J Clin Invest* 1996, *98*, 863-874.

[30] Becker, R.C. Thrombosis and the role of the platelet. *Am J Cardiol* 1999, *83*, 3E-6E.

[31] Ohman, E.M.; Kleiman, N.S.; Gacioch, G.; Worley, S.J.; Navetta, F.I.; Talley, J.D.; Anderson, H.V.; Ellis, S.G.; Cohen, M.D.; Spriggs, D.; Miller, M.; Kereiakes, D.; Yakubov, S.; Kitt, M.M.; Sigmon, K.N.; Califf, R.M.; Krucoff, M.W.; Topol, E.J. Combined accelerated tissue-plasminogen activator and platelet glycoprotein IIb/IIIa integrin receptor blockade with Integrilin in acute myocardial infarction. Results of a randomized, placebo-controlled, dose-ranging trial. IMPACT-AMI Investigators. *Circulation* 1997, *95*, 846-854.

[32] Wagner, C.L.; Mascelli, M.A.; Neblock, D.S.; Weisman, H.F.; Coller, B.S.; Jordan, R.E. Analysis of GPIIb/IIIa receptor number by quantification of 7E3 binding to human platelets. *Blood* 1996, *88*, 907-914.

[33] Chew, D.P.; Moliterno, D.J. A critical appraisal of platelet glycoprotein IIb/IIIa inhibition. *J Am Coll Cardiol* 2000, *36*, 2028-2035.

[34] Cierniewski, C.S.; Byzova, T.; Papierak, M.; Haas, T.A.; Niewiarowska, J.; Zhang, L.; Cieslak, M.; Plow, E.F. Peptide ligands can bind to distinct sites in integrin alphaIIb-beta3 and elicit different functional responses. *J Biol Chem* 1999, *274*, 16923-16932.

[35] Michelson, A.D. Advances in antiplatelet therapy. *Hematology Am Soc Hematol Educ Program* 2011, *2011*, 62-69.

[36] Topol, E.J.; Yadav, J.S. Recognition of the importance of embolization in atherosclerotic vascular disease. *Circulation* 2000, *101*, 570-580.

[37] Pancioli, A.M.; Adeoye, O.; Schmit, P.A.; Khoury, J.; Levine, S.R.; Tomsick, T.A.; Sucharew, H.; Brooks, C.E.; Crocco, T.J.; Gutmann, L.; Hemmen, T.M.; Kasner, S.E.; Kleindorfer, D.; Knight, W.A.; Martini, S.; McKinney, J.S.; Meurer, W.J.; Meyer, B.C.; Schneider, A.; Scott, P.A.; Starkman, S.; Warach, S.; Broderick, J.P. Combined Approach to Lysis Utilizing Eptifibatide and Recombinant Tissue Plasminogen Activator in Acute Ischemic Stroke-Enhanced Regimen Stroke Trial. *Stroke* 2013, *44*, 2381-2387.

[38] Pancioli, A.M.; Broderick, J.; Brott, T.; Tomsick, T.; Khoury, J.; Bean, J.; del Zoppo, G.; Kleindorfer, D.; Woo, D.; Khatri, P.; Castaldo, J.; Frey, J.; Gebel, J., Jr.; Kasner, S.; Kidwell, C.; Kwiatkowski, T.; Libman, R.; Mackenzie, R.; Scott, P.; Starkman, S.; Thurman, R.J. The combined approach to lysis utilizing eptifibatide and rt-PA in acute ischemic stroke: the CLEAR stroke trial. *Stroke* 2008, *39*, 3268-3276.

[39] Shlansky-Goldberg, R. Combination therapy in peripheral vascular disease: the rationale of using both thrombolytic and antiplatelet drugs. *J Am Coll Surg* 2002, *194*, S103-113.

[40] Palomo, I.; Fardella, P.; Pereira, J. Sistema de la Coagulación y Sistema Fibrinolítico. In *Hematología: Fisiopatología y Diagnóstico*; Palomo, I.; Pereira, J.; Palma, J., Eds.; Talca, 2005.

[41] Collen, D. The plasminogen (fibrinolytic) system. *Thromb Haemost* 1999, *82*, 259-270.

[42] Naderi, G.A.; Asgary, S.; Jafarian, A.; Askari, N.; Behagh, A.; Aghdam, R.H. Fibrinolytic effects of Ginkgo biloba extract. *Exp Clin Cardiol* 2005, *10*, 85-87.

[43] Demrow, H.S.; Slane, P.R.; Folts, J.D. Administration of wine and grape juice inhibits in vivo platelet activity and thrombosis in stenosed canine coronary arteries. *Circulation* 1995, *91*, 1182-1188.

[44] Briggs, W.H.; Folts, J.D.; Osman, H.E.; Goldman, I.L. Administration of raw onion inhibits platelet-mediated thrombosis in dogs. *J Nutr* 2001, *131*, 2619-2622.

[45] Leta, G.C.; Mourao, P.A.; Tovar, A.M. Human venous and arterial glycosaminoglycans have similar affinity for plasma low-density lipoproteins. *Biochim Biophys Acta* 2002, *1586*, 243-253.

[46] Rajapakse, N.; Jung, W.K.; Mendis, E.; Moon, S.H.; Kim, S.K. A novel anticoagulant purified from fish protein hydrolysate inhibits factor XIIa and platelet aggregation. *Life Sci* 2005, *76*, 2607-2619.

[47] Bordbar, S.; Anwar, F.; Saari, N. High-value components and bioactives from sea cucumbers for functional foods--a review. *Mar Drugs* 2011, *9*, 1761-1805.

[48] Chakrabarti, S.; Freedman, J.E. Review: Nutriceuticals as antithrombotic agents. *Cardiovasc Ther* 2010, *28*, 227-235.

[49] Phang, M.; Lazarus, S.; Wood, L.G.; Garg, M. Diet and thrombosis risk: nutrients for prevention of thrombotic disease. *Semin Thromb Hemost* 2011, *37*, 199-208.

[50] Kellermann, A.J.; Kloft, C. Is there a risk of bleeding associated with standardized Ginkgo biloba extract therapy? A systematic review and meta-analysis. *Pharmacotherapy* 2011, *31*, 490-502.

[51] Mahady, G.B. Ginkgo biloba for the prevention and treatment of cardiovascular disease: a review of the literature. *J Cardiovasc Nurs* 2002, *16*, 21-32.

[52] Prasad, S.; Kashyap, R.S.; Deopujari, J.Y.; Purohit, H.J.; Taori, G.M.; Daginawala, H.F. Development of an in vitro model to study clot lysis activity of thrombolytic drugs. *Thromb J* 2006, *4*, 14.

[53] Prasad, S.; Kashyap, R.S.; Deopujari, J.Y.; Purohit, H.J.; Taori, G.M.; Daginawala, H.F. Effect of Fagonia Arabica (Dhamasa) on in vitro thrombolysis. *BMC Complement Altern Med* 2007, *7*, 36.

[54] Mary, N.K.; Achuthan, C.R.; Babu, B.H.; Padikkala, J. In vitro antioxidant and antithrombotic activity of Hemidesmus indicus (L) R.Br. *J Ethnopharmacol* 2003, *87*, 187-191.

[55] Chourasia, S.R.; Kashyap, R.S.; Purohit, H.J.; Deopujari, J.Y.; Taori, G.M.; Daginawala, H.F. In-vitro clot lytic potential of Fagonia arabica: a comparative study of two methods. *Blood Coagul Fibrinolysis* 2011, *22*, 288-294.

[56] Yamada, K.; Naemura, A.; Sawashita, N.; Noguchi, Y.; Yamamoto, J. An onion variety has natural antithrombotic effect as assessed by thrombosis/thrombolysis models in rodents. *Thromb Res* 2004, *114*, 213-220.

[57] Suzuki, Y.; Kondo, K.; Matsumoto, Y.; Zhao, B.Q.; Otsuguro, K.; Maeda, T.; Tsukamoto, Y.; Urano, T.; Umemura, K. Dietary supplementation of fermented soybean, natto, suppresses intimal thickening and modulates the lysis of mural thrombi after endothelial injury in rat femoral artery. *Life Sci* 2003, *73*, 1289-1298.

[58] Rajput, M.S.; Mathur, V.; Agrawal, P.; Chandrawanshi, H.K.; Pilaniya, U. Fibrinolytic activity of kaempferol isolated from the fruits of Lagenaria siceraria (Molina) Standley. *Nat Prod Res* 2011, *25*, 1870-1875.

[59] Torres-Urrutia, C.; Guzman, L.; Schmeda-Hirschmann, G.; Moore-Carrasco, R.; Alarcon, M.; Astudillo, L.; Gutierrez, M.; Carrasco, G.; Yuri, J.A.; Aranda, E.; Palomo, I. Antiplatelet, anticoagulant, and fibrinolytic activity in vitro of extracts from selected fruits and vegetables. *Blood Coagul Fibrinolysis* 2011, *22*, 197-205.

[60] Legnani, C.; Frascaro, M.; Guazzaloca, G.; Ludovici, S.; Cesarano, G.; Coccheri, S. Effects of a dried garlic preparation on fibrinolysis and platelet aggregation in healthy subjects. *Arzneimittelforschung* 1993, *43*, 119-122.

[61] Pignon, J.M.; Henni, T.; Amselem, S.; Vidaud, M.; Duquesnoy, P.; Vernant, J.P.; Kuentz, M.; Cordonnier, C.; Rochant, H.; Goossens, M. Frequent detection of minimal residual disease by use of the polymerase chain reaction in long-term survivors after bone marrow transplantation for chronic myeloid leukemia. *Leukemia* 1990, *4*, 83-86.

[62] Morozova, E.N.; Falina, N.N.; Denisova, N.P.; Barkova, L.V.; Psurtseva, N.V. [Analysis of the component constitution and substrate specificity of a fibrinolytic preparation from the fungus Flammulina velutipes]. *Biokhimiia* 1982, *47*, 1181-1185.

[63] Park, S.E.; Li, M.H.; Kim, J.S.; Sapkota, K.; Kim, J.E.; Choi, B.S.; Yoon, Y.H.; Lee, J.C.; Lee, H.H.; Kim, C.S.; Kim, S.J. Purification and characterization of a fibrinolytic protease from a culture supernatant of Flammulina velutipes mycelia. *Biosci Biotechnol Biochem* 2007, *71*, 2214-2222.

[64] Kim, J.H.; Kim, Y.S. Characterization of a metalloenzyme from a wild mushroom, Tricholoma saponaceum. *Biosci Biotechnol Biochem* 2001, *65*, 356-362.

[65] Shin, H.H.; Choi, H.S. Purification and characterization of cysteine protease from Pleurotus ostreatus. *Biosci Biotechnol Biochem* 1998, *62*, 1416-1418.

[66] Kim, J.S.; Sapkota, K.; Park, S.E.; Choi, B.S.; Kim, S.; Nguyen, T.H.; Kim, C.S.; Choi, H.S.; Kim, M.K.; Chun, H.S.; Park, Y.; Kim, S.J. A fibrinolytic enzyme from the medicinal mushroom Cordyceps militaris. *J Microbiol* 2006, *44*, 622-631.

[67] Choi, H.S.; Sa, Y.S. Fibrinolytic and antithrombotic protease from Spirodela polyrhiza. *Biosci Biotechnol Biochem* 2001, *65*, 781-786.

[68] Jeon O. H.; Moon W. J.; Kim D. S. An anticoagulant/fibrinolytic protease from Lumbricus rubellus. *J. Biochem. Mol. Biol.* 1995, *28*, 138-1452.

[69] Taussig, S.J.; Batkin, S. Bromelain, the enzyme complex of pineapple (Ananas comosus) and its clinical application. An update. *J Ethnopharmacol* 1988, *22*, 191-203.

[70] Ako, H.; Cheung, A.H.; Matsuura, P.K. Isolation of a fibrinolysis enzyme activator from commercial bromelain. *Arch Int Pharmacodyn Ther* 1981, *254*, 157-167.

[71] Kowalski, M.; Brown, G.; Bieniasz, M.; Oszajca, K.; Chabielska, E.; Pietras, T.; Szemraj, Z.; Makandjou-Ola, E.; Bartkowiak, J.; Szemraj, J. Cloning and expression of a new recombinant thrombolytic and anthithrombotic agent-a staphylokinase variant. *Acta Biochim Pol* 2009, *56*, 41-53.

[72] Gillman, M.W.; Cupples, L.A.; Gagnon, D.; Posner, B.M.; Ellison, R.C.; Castelli, W.P.; Wolf, P.A. Protective effect of fruits and vegetables on development of stroke in men. *JAMA* 1995, *273*, 1113-1117.

[73] Joshipura, K.J.; Ascherio, A.; Manson, J.E.; Stampfer, M.J.; Rimm, E.B.; Speizer, F.E.; Hennekens, C.H.; Spiegelman, D.; Willett, W.C. Fruit and vegetable intake in relation to risk of ischemic stroke. *JAMA* 1999, *282*, 1233-1239.

[74] Liu, S.; Manson, J.E.; Lee, I.M.; Cole, S.R.; Hennekens, C.H.; Willett, W.C.; Buring, J.E. Fruit and vegetable intake and risk of cardiovascular disease: the Women's Health Study. *Am J Clin Nutr* 2000, *72*, 922-928.

[75] Bazzano, L.A.; He, J.; Ogden, L.G.; Loria, C.M.; Vupputuri, S.; Myers, L.; Whelton, P.K. Fruit and vegetable intake and risk of cardiovascular disease in US adults: the first National Health and Nutrition Examination Survey Epidemiologic Follow-up Study. *Am J Clin Nutr* 2002, *76*, 93-99.

[76] Baruah, D.B.; Dash, R.N.; Chaudhari, M.R.; Kadam, S.S. Plasminogen activators: a comparison. *Vascul Pharmacol* 2006, *44*, 1-9.

[77] Verstraete, M. Third-generation thrombolytic drugs. *Am J Med* 2000, *109*, 52-58.

[78] Gallus, A.S. Thrombolytic therapy for venous thrombosis and pulmonary embolism. *Baillieres Clin Haematol* 1998, *11*, 663-673.

[79] Sandercock, P.; Berge, E.; Dennis, M.; Forbes, J.; Hand, P.; Kwan, J.; Lewis, S.; Lindley, R.; Neilson, A.; Wardlaw, J. Cost-effectiveness of thrombolysis with recombinant tissue plasminogen activator for acute ischemic stroke assessed by a model based on UK NHS costs. *Stroke* 2004, *35*, 1490-1497.

[80] Capstick, T.; Henry, M.T. Efficacy of thrombolytic agents in the treatment of pulmonary embolism. *Eur Respir J* 2005, *26*, 864-874.

[81] Pietri, S.; Maurelli, E.; Drieu, K.; Culcasi, M. Cardioprotective and anti-oxidant effects of the terpenoid constituents of Ginkgo biloba extract (EGb 761). *J Mol Cell Cardiol* 1997, *29*, 733-742.

[82] Kojima, S.; Soga, W.; Hagiwara, H.; Shimonaka, M.; Saito, Y.; Inada, Y. Visible fibrinolysis by endothelial cells: effect of vitamins and sterols. *Biosci Rep* 1986, *6*, 1029-1033.

[83] Matsuura, H. Saponins in garlic as modifiers of the risk of cardiovascular disease. J Nutr 2001, 131, 1000S-1005S.

[84] Henry, B.L.; Thakkar, J.N.; Martin, E.J.; Brophy, D.F.; Desai, U.R. Characterization of the plasma and blood anticoagulant potential of structurally and mechanistically novel oligomers of 4-hydroxycinnamic acids. Blood Coagul Fibrinolysis 2009, 20, 27-34.

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