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Fibrinolytic Enzyme: Restoration Tool for Obliteration of Blood Clots

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Abstract

Body interrelated complex system maintain body hemostasis but severe vascular injury reprehensible for thrombus formation. Fibrinogen is a prime protein in fibrinolysis. Complex cascade pathway involves in conversion of plasminogen to plasmin to perform fibrinolysis and plasminogen activating inhibitor (PAI) cease this pathway. The genetic and acquired factors influence clot formation while it curbs by factor VIII. This review aims to illustrate significance of fibrinolytic enzymes, Nattokinase, Urokinase, Staphylokinase and streptokinase as therapeutic agent to control myocardial infraction, venous stroke and pulmonary clotting.

Keywords: Fibrinolysis; Thrombus; Plasminogen; Nattokinase; Myocardial infraction

Abbreviations: PAI-1: Plasminogen-Activator Inhibitor Type 1; t-PA: Tissue Plasminogen Activator; u-PA: Urinary-Type Plasminogen Activator; RA: Rheumatoid Arthritis; ARDS: Adult Respiratory Distress Syndrome; C4BP: C4B-Binding Protein; MI: Myocardial Infarction; CNS: Central Nervous System

Introduction

Interrelated complex system exists in body just to maintain fluidity in vascular system and allow the formation of solid blood clot in case blood vessel injury [1]. These interrelated complex systems, a key to maintain homeostasis. In result of vascular injury, intravascular thrombus formed. In thrombolysis, Fibrinogen, a prime protein causes fibrinolysis. It is soluble, complex multifunctional 340 kDa of 45 nm in length [2].

The process of fibrinolysis follows a cascade pathway, where plasminogen is converted into plasmin [3]. Certain inhibitors of plasminogen activators like plasminogen activator inhibitor-1 (PAI) cease the formation of thrombus [4]. Aggregation of fibrin fibers is controlled by VIIIa factor [5].

Unusual structural conformation of fibrin along with the Alteration in binding of tPA results in abnormal fibrin clots [6,7], In order to determine structure and formation of fibrin clot genetic and acquired factors are important. But fibrin clot also influenced by pH, temperature, chloride and calcium concentration [8]. Abnormal clots result in various pathological problems which lead to blockage of vessels and contribute to rheumatoid arthritis (RA), ischemic strokes, myocardial infarction (MI), Alzheimer disease and adult respiratory distress syndrome (ARDS) [9]. In order to restore normal blood flow or to dissolve blood clot plasmin performs a diverse role [10].

Other roles of plasmin are in wound healing, angiogenesis cell migration and vascular remolding. Plasmin role is in tissue homeostasis and another role is in acts as modulator of immune response [11]. Cardiovascular diseases (CVDs) is a leading cause of death. An estimation of WHO in 2008, 17.3 million people died of CVDs and the figure would be 23 million in 2030. Thrombogenicity mainly determined by fibrous cap stability of plaque, when come in contact of free blood, activates cascade coagulation. In order to control haemostasis its mandatory to eject blood clot and fibrin deposit mostly done by enzymatic process in which fibrinolytic enzyme activate plasminogen by its proteolytic cleavage into its active form, plasmin.

Activation is due to presence of C-terminal lysine residues in fibrin that permit plasminogen to orient itself in order to be convertibly cleaved by tissue-type plasminogen activator (t-PA) [12]. Fibrinolytic enzyme is mostly obtained from many microbial sources, animals and plants. Traditionally, thrombolytic agents like plasminogen activator (t-PA), urokinase plasminogen activator (u-PA) and streptokinase used to treat CVDs, being expensive, research continue and result in Nattokinase, a fibrinolytic enzyme, isolated from *Bacillus natto* [13].

The oral administration of Natto enhance release of plasminogen activators in humans. Another novel fibrinolytic metalloprotease extracted from edible mushroom. These thrombolytic agents are used to treat thrombolysis mostly fibrinolytic enzyme as having no side effects. It has dual effect, not only deteriorate fibrin but also liberate tissue plasminogen activator which cause fibrin decay [14].

Literature Review

Formation of fibrin clot

In vascular injury, platelets activate, exposing cell to anionic phospholipid, modus in assembly of procoagulant proteins [1]. Platelet activation initiates coagulation cascade, a seriene protease mediated cleavage, activating thrombin from zymogen prothrombin.

Thrombin generation results in conversion of fibrinogen to fibrin a soluble protein consists of two sets of three polypeptide chains (Aα, Bβ and γ) linked by 24 di-sulfide bonds [3].

Mechanism of fibrinolysis

Plasminogen (PLG), zymogen plasma, novice to plasmin through tissue PLG activator (tPA), a urokinase (uPA) by positive feedback mechanism.

The tPA and uPA cleave by plasmin, thus, altering from single chain to two active polypeptides. Fibrin nuisance to both PLG and tPA, intensifying plasmin generation. Once, it forms it cleaves fibrin [4] (Figure 1).

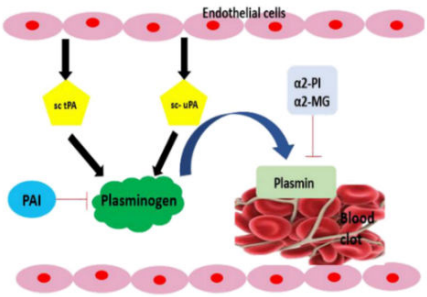

Figure 1: Overview of fibrinolytic system. The plasminogen neophyte to active serine protease, plasmin, by two-chain tissue plasminogen activator (tc-tPA) and urokinase (tc-uPA), commute from endothelial cells. Both tPA and uPA, inhibited by plasminogen activator inhibitor-1 (PAI). The α 2-plasmin inhibitor (α2-PI), cause plasmin inhibition and by α 2-macroglobulin (α2-MG). Once it generated, converts to single chain tPA and uPA. These can rapidly inhibited unless it remains bound to fibrin or to its cell surface receptors.

Fibrin fiber assembly

Fibrin fibers assembly revenue is a stepwise manner, subsist major three steps. 1. Activation, 2. Polymerization, 3. Lateral association. After fibrinopeptide A commutes, fibril dimer formation followed by fibrin monomer. Protofibril formation done by the lateral aggregation of fibrin fibers [5]. Fibrin, cross-linked at lysine residues by factor XIIIa forming fibril aggregates with platelets and red blood cells sustain structural integrity to the growing thrombus [6] **(Figure 2).**

Two factors assist in fibrin polymerization are Turbidity and circulatory flow.

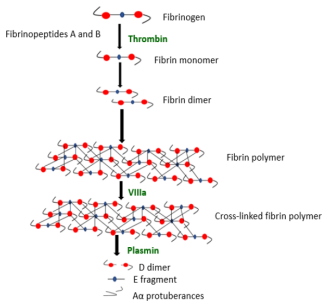

Figure 2: Assembly of fibrin fibers

Abnormal fibrin clot

Heterogenous fibrilization or unusual structural conformation leads to unstable thrombus. Abnormal fibrin network makes thrombus resistant to degradation. Clots with fine fibrin conformation display slower lysis. Alteration in binding of tPA and plasminogen both effect the normal structure of structure of fibrin clot [7].

Factors affecting clot formation

Genetic and acquired factors results in abnormal clot formation are described in detail [8].

Genetic factors

If there is genetic problem then fibrinogen production is inhibited so bleeding problem appears and another case is that abnormal structure and function of fibrinogen is predicted result in fibrinolytic inactivation by plasmin [13].

Fiber thickness and ultrastructure is determined by fibrinogen β-chain while α-chain is associated with fibrin thick fiber which has more cross linkage ability. Polymorphism affects fibrin structure and rigidity. Polymorphism in factor XIII i.e., G changes in T in codon 34 in which valine is replaced with leucine result in less permeable clots and cross linking gets ineffective [15].

Acquired factors

The acquired factors results in abnormal clot formation are blood flow, oxidative stress, as well as abnormal concentration of factor XIII in plasma and thrombin. Effect of blood flow on clot is highly controversial. Forces acting on blood vessels result in changes in fibrin structure. It is reported that fibers which are thinner and have smaller pores are resistant to lysis by plasmin [16].

Oxidation of fibrinogen is 20 times more than albumin. Rate of clot formation reduced when oxidation of fibrinogen occurs by metals, oxygen and myeloperoxidase-derived oxidants. While if oxidation occur by Fe^{3+} ascorbate then clot formation is promoted. Rate of clot stability and thrombin generation is enhanced by Recombinant factor VIIa [17].

In regulating fibrin structure prothrombin concentration play an important role. When level of prothrombin increases then it results in reduction in diameter of fibrin [18]. If there is elevated concentration of thrombin in clot then result in network of thin fibers along with smaller pores. But if reduction in thrombin level occurs then it leads to hemophilia B [19].

Pathophysiological role of abnormal clots

Due to abnormal blood clot structure, emboli form which lodge in organs result in disrupting of blood flow. So, many fatal diseases occur like rheumatoid arthritis (RA), ischemic strokes, myocardial infarction, Alzheimer disease and adult respiratory distress syndrome (ARDS) [20].

Rheumatoid arthritis (RA)

It is a chronic disease which affects synovial joint. It is an autoimmune disease in which immune system attacks joints result in inflammation. It occurs in age 30 and 60 in women and in man it occurs later [21].

Due to inflammation concentration of coagulation factors at synovial fluid increased. RA patients have clot formation faster and more time required for lysis of clot because clot becomes less permeable.

Fibrinogen, t-PA and Pal-1 are influenced by deposition of fibrin. Fibrin deposition at synovial joint is contributed by C4B-binding protein (C4BP) production which is a regulatory factor and its localization among fibrin rich areas. Fibrin structure changes due to inflammation in joints resulting in resistance to plasmin [22,23].

Ischemic strokes

Ischemic strokes results when there is obstruction in supplying blood to brain. Blockage within blood vessel occurs due to fatty deposits in blood vessels [24,25] If there is abnormal fibrin clot formation then it influenced fibrinolysis. So, due to abnormal fibrinolysis there is deficiency of plasminogen as well as plasminogen activator deficiency. It is difficult to break down fibrin clot due to permeability gets lower. In result clot blocks blood vessels and stroke occurs [26].

Myocardial infarction (MI)

Myocardial infarction (MI) is a situation in which blood does not flow to heart results in damaging of heart muscle. This condition is named as heart attack. In this situation, severe chest pain occurs [27]. If ischemia is not treated then it leads to Myocardial infarction (MI).

Rupturing of coronary artery leads to activation as well as aggregation of platelets. Then thrombin formation occur which blocks blood flow to. heart. As blood not supplied to heart so, heart attack occurs [28]

Alzheimer disease

Alzheimer's is dementia type whose effect is loss of memory, thinking and behavior [29]. Fibrinogen not enters in central nervous system (CNS) due to blood brain barrier. But if injury or any kind of vascular disruption occurs then it results in high concentration of fibrinogen. Due to its high concentration disruption in blood brain barrier occurs. So, fibrinogen enters in CNS. Fibrin clots unable to lyse due to high concentration of fibrinogen which decreases blood flow.

Fibrinolysis alters by blocking plasminogen binding with fibrin so, plasmin formation is inhibited. Another way is altering the rate at which fibrinolysis takes place by plasmin

Adult respiratory distress syndrome (ARDS)

It is an acute lung injury and severe hypoxemia. This occurs due to alveolar fibrin deposition. PAI and α2 -plasmin inhibitor levels increase so, there reduction in fibrinolytic activity occurs. It also promotes angiogenesis and along fibrosis due to fibrin deposition [30].

Role of plasmin

FXIIa have role in fibrinolysis by activating plasminogen [31]. Plasminogen is converted into plasmin which is facilitated by fibrin and fibrinogen [1].

In order to degrade fibrin plasminogen is converted into plasmin as well as fibrin protects plasmin from inhibitors such as α2AP. So, fibrin clot breaks down in soluble fragments. In this way blood clot is degraded. But fibrin is resistant to plasmin due to tighter network of thin fibers which are huge in number and porosity gets declined [7] (Figure 3).

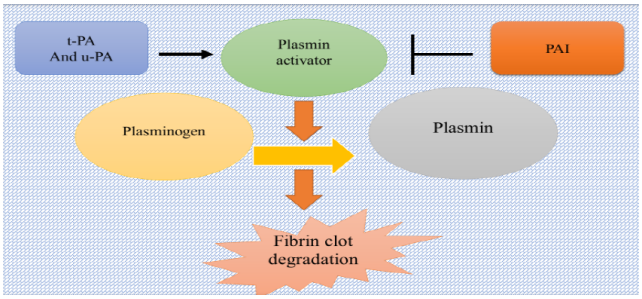

Figure 3: t-PA (Tissue plasminogen activator) and u-PA (Urinary-type plasminogen activator) activates plasminogen while PAI-1 (Plasminogen-activator inhibitor type 1) inhibits this. Palminogen activate once then converted into plasmin and degrade blood clot.

Discussion

Fibrinolytic enzymes as potential therapeutic agents

Nattokinase: Nattokinase is a fibrinolytic enzyme composed of single polypeptide chain having 275 amino acids have intense fibrinolytic activity. It's a formidable clot dissolving protein, obtained from *Bacillus subtilis* and used for myocardial infarction treatment [32], because its naturally have potential to dissolve clots and several other advantages such asorally administration, prolonged stay in body, efficacy and enhance body's yield of clotting agent including plasmin, urokinase, streptokinase etc [33]. NK is supercilious to other clotting agent as develop prolonged effect in bOdy by two ways:

- Dissolve clot
- Averting coagulation.

NT mechanism has been broadly explored than others fibrinolytic agent. It slices fibrin and activate the production of t-PA which activate the plasmin. NK not only enhancing t-PA but also inhibit PAI-1, a typical blocker of fibrinolysis [34]. It was also found that oral administration of Natto repress clotting and regulate dissolution of thrombi [35] (Figure 4 and Table 1).

Staphylokinase: Staphylokinase, a extracellular protein composed of 136 amino acids having no disulfide bond is obtained from *Staphylococcus aureus,* not acts directly on fibrin but forming a SAK complex with plasmin that activate respective plasminogen. Uncompleted plasmin and SAK complex both bounded to fibrin preventing it from repression by α-2-antiplasmin, while unbound part liberated from the clot and repressed the α-2-antiplasmin. Hence, process of thrombolysis by Staphylokinase limited to thrombus, controlling lavish plasmin production, decreases α-2-antiplasmin and fibrinogen deterioration in plasma [34].

It was concluded that SAK act as slow plasminogen activator and platelet aggregation was declined depending upon its dose. Study reveals, a SRK fusion protein not only enhance the thrombolytic property of SRK but it also acts as potential thrombolytic agent [36].

Urokinase: Urokinase, a serine protease has molecular weight of 50 kDa converting inactive plasminogen into active plasmin while standing with in the thrombin and protect it from antiplasmins [34]. Its currently produce from mammalian cell lines attempts also made to obtain it from bacteria, fungi and in mammalian cells [36]. Intravenous administration of pro-UK causes complete fibrinolysis. It used a thrombolytic agent to treat cardiovascular diseases. Currently its marketed as kinlytic serving an important fibrinolytic enzyme to treat myocardial infarction [37].

Streptokinase: Streptokinase, a protein extracted from beta-hemolytic *Streptococci* bacteria although not activator of plasminogen but convert inactive plasminogen into active plasmin by forming a complex with it. Because of fibrinolytic activity, used as thrombolytic agent to treat myocardial infarction, pulmonary clotting and venous stroke [38].

Urokinase: Urokinase is a plasminogen activator.

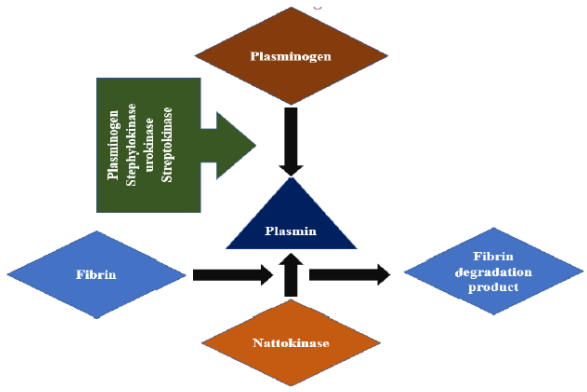

Figure 4: Fibrinolytic enzyme as potential therapeutic agent.

Enzyme/drugs	Classification	Indications	Side effects
Nattokinase	Tissue-type Plasminogen activator (tPA)	Acute myocardial infarction, acute, pulmonary embolism	Bleeding disorders
Staphylokinase	Plasminogen activator	myocardial infarction	-----
Streptokinase	Plasminogen activator	Myocardial infarction, pulmonary embolism, deep vein thrombosis, arterial, thrombosis or embolism	Bleeding, Anaphylaxis
Urokinase	Plasminogen activator	Acute myocardial infarction	Hemorrhage, Anaphylaxis

Table 1: Fibrinolytic enzyme for cardiovascular diseases treatment.

Conclusion

In maintenance of vascular integrity fibrinolysis plays key role. In case of vascular injury, stops bleeding to overcome the threat to life. The enzymes involve in fibrinolysis in spite, thrombus formation show therapeutic potential in treating many diseases like myocardial infraction, venous stroke and pulmonary clotting. In conclusion, by controlling the regulation of fibrinolysis enzymes the formation of clot in deadly diseases could be control. Once, the regulation of these enzymes is fully understood it will be use as treatment of blood clotting diseases and rename the meaning of chemical therapeutic treatment.

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