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Research Article

CONVENTIONAL THROMBOLYTICS NEED TO REFINE AT MOLECULAR LEVEL FOR SAFE AND EFFICIENT MANAGEMENT OF CEREBROVASCULAR DISORDERS- AN OVERVIEW

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ABSTRACT

The cardio and cerebral disorder are the leading cause of vascular traumas which led to many lives threatening consequences and mortality worldwide. In the last few decades numerous artificial molecules have been implemented in clinical application for management and to overcome with devastating vascular traumas. The management of cardiovascular disorders has made remarkable land mark in the health care system with potential thrombolytic agents. Many of artificial fibrinolytic molecules have been used clinically and many more are in refinement phases to tackle the problem. In this study we have composed evolution of thrombolytics from various sources and refinement with technologies. Though these molecules are quite effective for cardiovascular management but often get fail in case of cerebral traumas. The aim of current study is to have an overview on external fibrinolytic molecules their development and clinical applications. Further we have emphasized on the cerebral vascular disorder and their management with currently available thrombolytic molecules. Further we have summarized toxicity offered by conventional thrombolytic in management of cerebral vascular disorder. Finally we have concluded naval approaches which could be further for management of cerebral vascular traumas.

Keywords: Thrombolytic, Cerebral, Streptokinase, Staphylokinase, Plasminogen activator, Earthworm Fibrinolytic Enzyme.

INTRODUCTION

While biological evolution especially in multicellular animals nature has equipped then with additional protection for vital organs especially brain and other part of central nervous system. These protections often called as biological barrier like Blood Brain Barrier (BBB) offers suitable environment for functioning of such delicate organ. Additionally biological barriers provide a line of control against invading pathogens and toxic chemicals often used as drugs as part of therapeutics [1]. Vascular disorder, cerebral and cordial has become major cause of mortality across the globe and especially in developing country due to lack of proper treatments [2]. The condition become more chronic in case of cerebrovascular disorder where majority of clinically approved artificial thrombolytic often get fail as biological barrier protecting these vital organs [3].

As per WHO report cerebrovascular and cardiovascular disorders are the leading threats for mortality followed by cancer and infectious diseases [4]. The cause of enhanced mortality often point out efficiency of available therapeutics and their clinical implications. The blood plasma contains enormous proteins and biological mediator regulates physiology of clot formation and dissolution [5]. A healthy homoeostatic system governs both aggressive and defensive factor to equilibrate blood clotting mechanism. Thrombus or blood clot is failure of our own homeostatic system leading to over activation of aggressive component over defensive [6]. These changes led to clot formation in vascular pipeline causing serious complications to cerebral and cordial system such as pulmonary and cerebral embolism, deep vein and cerebral thrombosis, myocardial infraction and cerebral hemorrhage and death [7]. Eventually, it was found often clots formed distal part of tissue start flow with systemic circulation called as thrombus. These insoluble slugs of fibrin matrix can limit or block blood supply completely and led chromic consequences as stock in cerebral veins [8]. The management of cerebral vascular disorder is much complicated over cordial vascular disorder as additional protection layers which limit diffusion of therapeutics into cerebral fluid and tissue [9].

Molecular Mechanism

As described earlier, thrombus or blood clot formation is natural and essential physiological event under control of various proteins and mediators in blood plasma [10]. The site of clots formation defines its fate as physiological need or consequence of failure of homeostatic system. Blood clots are the fibrin matrixes which convert into insoluble form soluble fibrinogen through cascade of blood clotting factors [11]. The clot formation initiated by blood platelets cellular component of blood which activates soluble factors in plasma. Plasma component through a series of reaction led generation of fibrin network [12].

The clot dissolution runs simultaneously by plasmin-catalyzed lysis of the fibrin network. Circulating tissue plasminogen activator (t-PA) initiate process of clot dissolution, later plasminogen bind to the surface of a fibrin clot, where t-PA cleaves plasminogen to generate the enzyme plasmin and initiate fibrinolysis [13]. Fibrinolysis is highly controlled process which led to production of small soluble fibrin fragments after cleavage of specific peptide bonds [14]. Further, the process of fibrinolysis accelerated by exposure of C-terminal lysine residues of fibrin matrix; serve as additional binding sites for plasmin [15]. Under the circumstance of thrombus or clot in systemic circulation, it is essential to dissolve clot significantly which is achieved naturally and through artificial agents called as thrombolytic [16]. Most of artificial agents as thrombolytic given externally are basically tissue plasminogen activators which refine system by activating circulating inactive plasminogen into plasmin, catalyze fibrinolysis. Recently, earthworm fibrinolytic enzymes have shown dual fibrinolysis as tissue plasminogen activator and direct on fibrin [17].

Thrombolytic therapeutics

The history of artificial thrombolytic in clinical implication starts in late 1960s with molecular characterization of fibrinolytic activity from Streptococcus species. Further several molecules have been identified and produced exhibiting fibrinolytic activity. In the year 1983, Mihara, et. al., have identified and isolated fibrinolytic protease component from earthworm species *Lumbricus rubellus* [18]. Among these molecules majority of them were isolated from microbial sources are tissue plasminogen activators (t-PA). These molecules Streptokinase (SK), Urokinase (UK), Recombinant tissue-type plasminogen activator (rt-PA), Lumbrokinase, Acylated plasminogen streptokinase activator complex (APSAC) and single chain urokinase-type plasminogen activator (scu-PA) [19]. The available molecules are efficient and have extensively used for management of cardiovascular disorders [20].

Streptokinase (SK)

Streptokinase (SK), plasminogen activator, is produced by various strains of β-hemolytic Streptococci strains is one of oldest thrombolytic molecule [21]. The fibrinolytic activity of SK was first described in 1933 and was crude analysis [22]. Streptokinase molecular mechanism has shown its no specificity towards fibrin which exerts its fibrinolytic action indirectly by activating the

circulatory plasminogen [23]. Proteomics investigations have confirmed molecular weight of 47 KD and made up of single-chain polypeptide of 414 amino acid residues [24]. Further proteomics investigations have revealed, SK is composed of three distinct domains, denoted as α (residues 1–150), β (residues 151–287), and γ (residues 288–414). Despite other thrombolytics like EFE and SAK, SK does not offer enzymatic activity therefore does not exhibit plasmin activity by proteolytic cleavage of plasminogen. Moreover, SK binds non-covalently to plasminogen in stoichiometric ratio 1:1, thereby confers plasmin activity [25]. The SK-Plasminogen complex start processes of fibrinolysis by targeting other plasminogen subsequently generate plasmin which further facilitates the thrombolysis of half-life 30 minute approximately [26].

Acrylated plasminogen streptokinase complex

APSAC (APSAC; antistreplase) an inactive, non-covalent synthetic construct of SK and plasminogen in equimolar ratio [27]**.** After infusion into systemic circulation rapid deacylation of the catalytic center occurs, activating the complex led to thrombolysis begins [28]. The objective for constructing APSAC was controlled enzymatic activity of the plasmin (ogen)-SK complex through specific reversible chemical, protecting its catalytic center [29]. Rather than SK, APSAC exhibits extended biological half-life in circulation and immediately get activated in systemic circulation, rapidly combines with plasminogen for activation additionally given single dosage as intravenous injection [30]. The clinical data have shown APSAC construct exhibits extended plasma half-life of 70 minutes compared with 25 minutes in case SK via SK-Plasminogen complex auto-generated in-vivo after the administration of SK. Though construct APSAC offer extended half-life but antigenicity often remain is major drawback of designed molecule [30]. Despite all these clinical interpretations, more attention in progressive research is running mainly concern on relative safety and effectiveness of APSAC in contrast to other thrombolytic agents [31].

Staphylokinase (SAK)

Despite Streptokinase, Staphylokinase (SAK) a fibrin specific clot dissolving agent has been used for myocardial infraction. SAK, an extracellular protein produced by *Staphylococcus aureus* strain after lysogenic conversion with bacteriophages [32]. Beside Streptokinase, Staphylokinase molecule, single polypeptide chain of 136 amino acids which lacks any disulfide bridges of molecular weight is approximately 16 KD [33]. With the difference in molecular arrangement, SAK differs in molecular mechanism of activation tissue plasminogen activators [34]. Despite streptokinase, SAK forms staphylokinase-plasmin complex rather than stoichiometric complex with plasminogen in case of SK. Another difference in molecular mechanism of SAK, the SAK-Plasmin complex is inactive and requires further conversion to staphylokinase: Plasmin complex to expose its active site and led to plasminogen activation [35].

Recombinant tissue plasminogen activator (t-PA)

Recombinant plasminogen activator (Reteplase, r-PA) is single chain deletion variant of alteplase expressed in *Escherichia coli*. Reteplase possess deletion mutation at 176-527(deletion of Val 4-Glu175): without damage of plasmin cleavage site at Arg275-Ile276 [36]. Reteplase, a non-glycosylated 355 amino acid single chain protein of 39KD molecular weight which lack fibronectin finger region and epidermal growth factor domain. Though, it works in absence of fibrin but lack of fibronectin finger region because 5 fold reductions in fibrin binding [37]. Since kringle 2 domain a part of reteplase essentially required for stimulation to protease in the presence of fibrin, which is stimulated to a lower extent than alteplase in case of Reteplase. These finding suggest significance of fibronectin finger which directly involved in the stimulation of the protease [38]. Additionally, due to deletion of the finger domain, epidermal growth factor domain and kringle 1domain with carbohydrate side chain, led to reduction in hepatic clearance of reteplase [39]. These modifications additionally provide extended plasma half-life 14–18 minutes comparatively alteplase half-life of 3–4 minutes. A significant reduction in hepatic clearance and enhanced biological half-life allows reteplase to be administered as boli against as an initial bolus followed by an infusion [40].

Another transform version of t-PA, Tenecteplase multiple (three) point mutation of alteplase exhibits difference with retplase which is deletion mutant. The design of Tenecteplase is based on to have an extended half-life, allowing convenient single bolus dosing, increased fibrin specificity and patency [41]. More precisely, substitutions of Asn-117 with Gln delete the glycosylation site in kringle 1 domain additionally replacement of Thr-103 with Asn reintroduced a new glycosylation site at different locus. These change in amino acids level reduces renal clearance of Tenecteplase hence prolongs half-life by 20 minute [42]. Further the substitution of amino acids Lys-296, His-297; Arg-298 and Arg-299 are each replaced with Ala which increases resistance approximately 80 times to inhibition by PA-1 [44]. Despite these features, Tenecteplase also possess similarity with native t-PA towards fibrin specificity with enhanced fibrin specificity 14-fold higher [45].

Earthworm fibrinolytic Enzyme (EFE)

Earthworm fibrinolytic Enzyme (EFE) is group of six enzymes exist in isoforms as serine protease naturally founds in alimentary canal of earthworm [46]. Though it was first isolated and characterized in earthworm species *Lumbricus rubellus* by Mihara in 1993, hence named Lumbrokinase. Later many other species of earthworm were explored for isolation and characterization of fibrinolytic components [47]. Since, EFE exist in different isoforms hence molecular weight and fibrinolytic activity varies with individual component. The most potent component of EFE, molecular weight 25.4KD exists in ubiquitously in many species of earthworm. Other fibrinolytic component ranges in molecular weight from 24.6KD to 33 KD with different fibrinolytic activity [48]. The EFE has some unique features over other artificial agent as thrombolytic such as stability against temperature, pH and various chemicals [49].

EFE is only artificial thrombolytic effectually absorb from intestinal mucosa as complete molecule with intact activity. The ability of EFE to resist against chemical and temperature led design of dosage for oral administration to avoid bleeding on injection site often noticed with other external fibrinolytic thrombolytic [50]. Moreover, EFE protection against cerebral ischemia, anti-thrombosis and anti-apoptosis was confirmed in the year 2008. In a study carried out by Hongrui Ji, has described molecular mechanism for anti- ischemic role of EFE. In the current study EFE has shown anti-ischemic activity by potentiating activity of adenylate cyclase (AC), which led increase in c-AMP level. These modulations in the c-AMP inhibit expression of Glycoprotein IIB/IIIA (GPIIB/IIIA) and P-selectin essential responsible for the cerebral traumas [51].

Table 1: List of available thrombolytic

Thrombolytic	Molecular Weight (KD)	Half life (Minute)	Mechanism	Source	Plasmin Specificity	Antigenicity
Streptokinase	47	30	Indirect	Streptococcus	No	Yes
Staphylokinase	16.5	6	Indirect	Staphylococcus	Yes	Yes
Tissue Plasminogen activator (t-PA)	72	4	Direct	Human	Yes	No
Earthworm Fibrinolytic Enzyme (EFE)	24-33KD	30	Direct & Indirect	Earthworm celomic fluid	Yes	No
Reteplase	40	20	Indirect	Synthetic /Recombinant	No	No
Antistreplase	131	90-110	Indirect	Synthetic/Recombinant	No	No
Urokinase	55	15	Direct	Urine	No	No
Altreplase (rt-PA)	70	70	Indirect	Synthetic/ Recombinant	Yes	No

Clinically approved molecules

Though the history of thrombolytics much old and several molecules have been isolated and produced recombinant way. Among then majority of molecules subjected to clinical trials study for evaluation of potency of drug in human model [52]. Despite all the very few molecules got approved by FDA for therapeutic applications. The streptokinase and its recombinant variant have been approved for management of cordial vascular disorder [53]. The novel variant of recombinant tissue plasminogen activator was approved by FDA in 1996 in the name of alteplase. The clinical application of alteplase was performed later on and was found satisfactory for cordial complication but failed in cerebral ischemia [54]. The EFE has shown promising result in lab model while tackle cerebral ischemia but its clinical trials studies are still going on. Though many molecules approved by FDA and most of them for cardiovascular disorder not for cerebral. More practically till now none of thrombolytic has been approved especially for managing cerebral ischemic stroke, proposed reason majority of conventional thrombolytic are neurotoxic and led to loss of neuronal tissue permanently [55].

Table 2: Commercially available thrombolytic with brand name

S. No.	Trade Name	Manufacture
1.	Heberkinase	Heber Biotech SA, Cuba
2.	Striptease	Kyung Dong Pharm Co. Ltd, Korea
3.	Streptokinase TTK	BBT Biotech, Germany
4.	Eskinase	BBT Biotech, Germany
5.	Recombinant Streptokinase	Shanghai SIIC SMU Biotech Co. Ltd,
6.	SK-PDC	Aventis Behring GmbH Germany
7.	Bolouk	Netriceuticals, CA , USA
8.	Kabikinase	Pharmacia Upjohn, Sweden
9.	Treptase	Se Jong Pharm, Korea
10.	Thrombosolv	Korea United Pharm Inc., Korea

Challenges with conventional thrombolytics

Numbers of artificial thrombolytic agent have been used in management of vascular disorder since last two decades. These agents have shown promising results to overcome vascular trauma with many complications. Complications often noticed with these agents such as systemic bleeding, short half-life, rapid reoclusions, antigenicity, low perfusion rate, high bypass, and lack of plasmin specificity, route of administration and cost factor [56]. For an ideal fibrinolytic agent it would achieve 100% patency in a short span time period and have minimal bleeding complications. Further this agent would be easily administered as a bolus, have a prolonged half-life and slow renal clearance [57]. Additionally, it must have fibrin specificity with little or no fibrinogen depletion with improved micro-vascular function and flow. Despite these challenges management of cerebral disorder such as cerebral ischemia is another primer challenge for conventional thrombolytic often cause toxicity in significant levels [58].

Cerebral Disorders

Despite, the cardiovascular disorders cerebral ischemia is major cause of mortality in concern with cerebral problems. Cerebral ischemia is most often brought about by interruption of the blood supply to a part of the brain [59]. Embolic or atherothrombotic occlusion of the cerebral artery is a primary event that occasionally progresses to cerebral infarction in humans. Focal ischemia due to middle cerebral artery (MCA) occlusion encompasses a densely ischemic core of tissue where CBF decreases remarkably and a marginally better perfused area, the so-called "ischemic penumbra [60]. The management of cerebral vascular disorder and its importance we can understand by cerebral metabolism and blood flow. Essentially, Neuronal function and cerebral metabolism are critically dependent on sufficient oxygen and glucose supply led energy production via phosphorylation of ADP into ATP [61]. It is quite obvious for cerebral tissue to have adequate cerebral blood flow (CBF) for metabolic processes. The requirements of blood supply depend on the metabolic demand which is regulated in feedback mechanism. The requirement of blood concurrently glucose and oxygen is much more than any other organ as per body weight approximately brains contribute only 2% of body weight which consume 25% of oxygen supplied by systemic circulation [62].

Additionally, brain does not store energy, any interruption of the blood supplies easily and quickly results in neuronal dysfunction and neuronal damage [63]. Now it's obvious the importance of thrombolytics and their efficiency which reflects functioning of brain and cerebral tissue. The ischemic tissue start expression of stress proteins which modulate the functioning of cellular protein network helps to tackle under stressed conditions [64]. In a study it was found, mice under ischemic stress led to over expression of BCL2, a protein modulate programed cell death led to abnormality in process of control cell death [65].

Thrombolytic for cerebral Traumas

Over last one decade several clinical trials have been performed for emergency treatment for intracranial thromboembolic occlusions by triggered thrombolysis with infusion of streptokinase and recombinant tissue plasminogen activator intra-arterially or intravenously [66]. Clinical interpretation collected from the studies carried out so far, it appears that the risk of intracranial hemorrhage via clinical deterioration is not increased by thrombolysis if thrombolysis is performed during the first 8 hours after symptom onset [67]. In another study it was founds on successful recanalization of occlusions in the anterior or posterior circulation is associated with a decrease of mortality and morbidity [68]. Hence it was predicted from the data of clinical implementation, Intra-arterial application of the thrombolytic agent seems to be more effective than intravenous application in mice model. Still the studies carried out for management of cerebral vascular disorder with conventional thrombolytics molecules are not sufficient and their results were not found satisfactory [69]. Despite all these outcomes, there is still a lack of controlled studies that could exclusively prove which thrombolytic agent and which mode of application is the most effective with regard to arterial recanalization and clinical outcome [70].

In the history of past 50 years, thrombolytic agents who have been infused with the aim of recanalizing occluded coronary vessels, and later on, applied in the setting of acute ischemic stroke [71]. These external thrombolytic agents act on plasminogen-plasmin transformation, improving the natural process of fibrinolysis. With the advancement in technologies third generation thrombolytic agents with refined fibrin selectivity and biological half-life have improved both recanalization rates and hemorrhagic complications, inside and outside the CNS [72]. In case of intra-arterial (IA) administration of fibrinolytic agents improves delivery of the drug to the thrombus at a higher concentration with smaller quantities and hence lowers systemic exposure [73]. Additionally, mechanical methods for thrombus dissolution allows for drug delivery to larger surface area of the thrombus. To overcome IV infusion based complication open combinations options of IA-IV treatments have been studied [74]. Till date, there are no direct comparative clinical trials to show that endovascular administration is more efficacious or carries a lower risk of hemorrhagic complications than IV tissue plasminogen activator [75].

While the management of acute ischemic stroke, there is need of immediate recanalization of the occluded artery by optimal means for restoring blood flow and rescuing the ischemic brain from complete infarction and neuronal death [76]. Subsequently, intravenous or intra-arterial infusion of local thrombolytic therapy with streptokinase or tissue-plasminogen activator (t-PA) has often in practice for acute ischemic stroke, but unfortunately the efficiency of these clinical manifestations has not established till date preciously [77]. Moreover route of administration of thrombolytic is more important than thrombolytics molecules in management of cerebral vascular disorders [78].

Toxicity offered by Conventional Thrombolytic

The major challenges with conventional therapeutics are neuronal toxicity which causes serious complications to cerebral tissue. Intravenous infusion of tissue plasminogen activator (t-PA) is used to treat acute stroke because of its thrombolytic activity and its ability to restore circulation to the brain [79]. However, this protease also promotes neuro-degeneration after intra-cerebral injection of excitotoxins such as glutamate, and neuronal damage after a cerebral infarct is thought to be mediated by excitotoxins [80]. In another study, a comparative analysis was carried out with streptokinase, staphylokinase and tissue plasminogen activator to evaluate efficiency of these molecules. Based on study conclusion were made after infusion of these thrombolytic agent led to increase chance of damage of neuronal tissue and more risk of cerebral ischemia subsequently in mice model [81]. Though, tissue plasminogen activator (t-PA) has been considering better agent for thrombolysis which exposed as much toxic towards neuronal damage. Further, more often larger dose of conventional thrombolytic may damage to blood brain barrier which subsequently led to other serious complications [82]. Moreover, the thrombolytics molecule often led to proteolysis of cerebral tissue and led to apoptosis [83]. With these potential threats to neuronal tissue with conventional thrombolytics led novel options cerebral vascular traumas.

Future prospects of thrombolytics

Moreover, the management of cardiovascular disorder is much easier and convenient against cerebrovascular disorder. Numerous inventions have been made in last one decade to improve existing molecule as suitable agent for cerebral traumas.

Nano-scale Technology and Targeted delivery

In recent time many new technologies have been developed especially for cerebral vascular disorder management. Though streptokinase has been used maximally among available thrombolytics molecules but must be given in larger dosages. Essentially larger dose required for these artificial thrombolytic to overcome complications like liver bypass. Simultaneously, larger dose of thrombolytics by intravenous infusion often cause hemorrhagic complications. Recently, streptokinase loaded liposome has been designed and evaluated its efficiency. [84] The studies have shown, liposomal delivery of streptokinase not only minimize hemorrhagic complications but also improve molecule in concern with targeted delivery and stability in systemic circulation [85].

Improved fibrinolysis

The biological half-life of thrombolytic molecules often led to serious complications in management of vascular disorders. Numerous refinements have been made in the existing molecules to improve half-life for extended thrombolysis and used successfully. Another novel approach in the year 2008, in this a novel micro-mixer using a biological molecular ATP motor was designed and evaluated in-vivo [86]. The Nano-machine was constructed using F0F1-ATPases (arrays of chromatophore-embedded d-free), in which d-free F1 part designed for rotator to mix solutions and the F0 part was driven by light. The constructed micro-mixer works without any physical contact with fibrin was confirmed by microscopic analysis. To understand molecular mechanism of constructed micro-mixer fibrin was labeled with FITC and which further confirm the Nano-mechanical force generated by motor accelerate fibrinolysis by improving solvent movement which facilitates drug kinetics. This study was performed with EFE in lower concentration with statistical analysis of large volume of fibrinolytic often led to hemorrhagic traumas [87].

Protein Engineering

Protein engineering certainly involve in all the aspect while refining biomolecules. Though, we are having enormous number of fibrinolytic molecules but majority of them must refine to acquire desired properties and design of novel one too [88]. The proteomics approaches while design can lead to improved therapeutics such as biological half-life, optimized renal clearance and least toxic effects [89]. Recently, in a study carried out to optimize renal clearance of tissue plasminogen activator (t-PA). The need of such study is important as fast renal clearance required rapid infusion of thrombolytics often cause serious complications. In this study a novel variant of t-PA was designed and introduced intravenous into systemic circulation and found 22 fold improved fibrinolysis [90]. These approaches even become more significant for management of cerebral ischemia and related traumas which required more targeted therapeutics [91]. Altogether, hybridization of these technologies may lead to new era of therapeutics which will offer safe and easy medication.

CONCLUSION

The 20th century had remarkable achievement in medicine and especially managing vascular disorders. The cerebral and cardial disorders management has been challenge for physician and researcher and now it directed to destination. The journey of clinical application of external fibrinolytic which starts in late 1960s with native streptokinase and related molecules is reached to refined, efficient and affordable outcomes [91]. Though, many obstacles are still there which need many attentions to look further refinements in existing therapeutics. In the evolution of thrombolytics explored several molecules from microbial source to higher animal origin. In the late 1980s, many breakthroughs have been made by researcher worldwide for hunting thrombolytics molecules and their characterizations [92]. The earthworm was explored for its therapeutics potential with six thrombolytic protein molecules as outcome.

Despite all these inventions, the existing molecules were subjected to molecular characterization and chemical and biophysical levels. Several novel variants of streptokinase, staphylokinase and t-PA came in clinical practice with improved therapeutically potential [93, 94, 95]. After clinical acknowledgement in cordial disorders, these existing molecules were implemented in the management of cerebral traumas. The refined forms of existing molecules have shown satisfactory outcomes in managing cerebral ischemia. After biophysical characterization of EFE, it has shown ability to tackle cerebral ischemia in-vivo [96]. Despite EFE, novel variant of streptokinase and tissue plasminogen activators (t-PA) have shown promising result in in-vivo system for managing cerebral ischemia. The nano- scale technology further enabled existing variant of t-PA and streptokinase to minimize infusion of larger dosage of these therapeutics [97].

Though, the vascular medicine has grown enormously in last few decades with inventions of several potent thrombolytic molecules but still there is much more finding have to make in concern to cerebral traumas [98]. Most challenging area for existing thrombolytics towards cerebral traumas management is their toxicity to neuronal tissue [99]. Current progressive research running at molecular level may take some time but based on the available scientific research literature soon management of cerebral traumas will reach to final destination.

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REFERENCES

1. William H Oldendrof, Blood brain barrier permeability to drugs. Annual review of pharmacology, Vol. 14: 239-248.
2. The National Institute of Neurological Disorders, and Stroke rt-PA Stroke Study Group. Tissue plasminogen activator for acute ischemic stroke.*N Engl J Med*. 1995; 333:1581–1587.
3. The Multicenter Acute Stroke Trial - Europe Study Group. Thrombolytic therapy with streptokinase in acute ischemic stroke. *N Engl J Med*.1996; 335:145–150.
4. Ouriel K.A History of thrombolytic therapy. J Endovasc Ther.Dec 2004; 11 Suppl 2:II 128-33.
5. Collet JP, Allali Y, Lesty C, Tanguy ML, Silvain J, Ankri A, Blanchet B, Dumaine R, Gianetti J,Payot L, Weisel JW, Montalescot G. Altered fibrin architecture is associated with hypofibrinolysis and premature coronary atherothrombosis. Arterioscler Thromb Vasc Biol. 2006; 26(11):2567–2573. [PubMed: 16917107].
6. Rijken DC, Lijnen HR. New insights into the molecular mechanisms of the fibrinolytic system. J Thromb Haemost. 2009; 7(1):4–13. [PubMed: 19017261].
7. Rosamond, W., Flegal, K., Furie, K. et al. Heart Disease and Stroke Statistics– 2008 Update, Circulation, 2008: 117: e25–e146.
8. Cornett, O., Ocava, L.C., Singh, M., Malhotra, S. and Rosenbaum, D.M. Antithrombotic and Thrombolytic Therapy for Ischemic Stroke, Cardiol. Clin., 2008: 26: 251–265.
9. Mackman, N. Triggers, Targets, and Treatments for Thrombosis, Nature, 2008: 451: 914–918.
10. Fatah K, Silveira A, Tornvall P, Karpe F, Blomback M, Hamsten A. Proneness to formation of tight and rigid fibrin gel structures in men with myocardial infarction at a young age. Thromb Haemost.1996; 76(4):535–540.
11. Gailani D, Renné T. Intrinsic pathway of coagulation and arterial thrombosis. Arterioscler. Thromb Vasc Biol 2007;27:2507-13
12. Gorkun OV, Veklich YI, Medved LV, Henschen AH, Weisel JW. Role of the alpha C domains of fibrin in clot formation. Biochemistry. 1994;33(22):6986–6997.
13. Cesarman-Maus G, Hajjar KAMolecular mechanisms of fibrinolysis.. Br J Haematol. 2005 May; 129(3):307-21.
14. Antovic, J.P. & Blomback, M. (2002) Thrombin-activatable fibrinolysis inhibitor antigen and TAFI activity in patients with APC resistance caused by factor V Leiden mutation. Thrombosis Research, 106, 59–62.
15. Aoki, N., Moroi, M. & Tachiya, K. (1978a) Effects of alpha-2-plasmin inhibitor on fibrin clot lysis. Its comparison with alpha-2-macroglobulin. Thrombosis and Haemostasis, 39, 22–31.
16. Bouma, B.N. & Meijers, J.C. (2003) Thrombin-activatable fibrinolysis inhibitor (TAFI, plasma procarboxypeptidase B, procarboxypeptidase R, procarboxypeptidase U). Journal of Thrombosis and Haemostasis, 1, 1566–1574.
17. Fay, W.P. (2004) Plasminogen activator inhibitor 1, fibrin, and the vascular response to injury. Trends in Cardiovascular Medicine, 14, 196–202.
18. H. Mihara, H. Sumi, and K. Akazawa, "Fibrinolytic enzyme extracted from the earthworm," *Thrombosis and Haemostasis*, vol. 50, p. 258, 1983.
19. Kawai C, Hosoda S,Kimata S,Kanmatsuse K,Suzuki S,Motomiya T,et al.Coronary thrombolysis in acute myocardial infarction of E6100(novel modified t-PA) : a multicentre doule blind ,dose – finding study.Jpn Pharmacol Ther 1994;22:3925-50.
20. Wakeham N, Terzyan S, Zhai PZ, Loy J., Tang J, Zhang XC. Effects of deletion of streptokinase residues 48– 59 on plasminogen activation. Protein. Eng. 2002; 15: 753–761.
21. Malke H, Ferretti JJ. Streptokinase: cloning, expression and excretion by Escherichia coli. Proc. Natl. Acad. Sci. (USA). 1984; 81:3557-3561.
22. Lizano1 S and Johnston K H. Structural Diversity of Streptokinase and Activation of Human Plasminogen. American Society for Microbiology 2005; 4451–4453.
23. Banerjee A, Chisti Y, Banerjee UC. Streptokinase—a clinically useful thrombolytic agent. Biotechnology Advances. 2004; 22: 287–307.
24. Wu XC, Ye RQ, Duan YJ, Wong SL. Engineering of plasmin-resistant forms of streptokinase and their production in Bacillus subtilis: streptokinase with longer functional half-life. Appl. Environ. Microbiol. 1998; 64: 824–829.
25. Zhang XW, Sun T, Huang XN, Liu X, Gu DX, Tang ZQ. Recombinant streptokinase production by fed batch cultivation of *Escherichia coli*. Enzyme and Microbial Technology. 1999; 24:647-654.
26. Hua LW. Preparation of novel streptokinase mutants as improved thrombolytic agents. 1999; Patent no: US 5876999.
27. Shi GY, Chang BI, Su SW, Young KC, Wu DH, Chang LC, Tsai YS, Wu HL. Preparation of a novel streptokinase mutant with improved stability. Thromb. Hemost. 1998; 79: 992– 997.
28. Torrens I, Ojalvo AG, Seralena A, Hayes O, Fuente de la J. A mutant streptokinase lacking the C-terminal 42 amino acids is less immunogenic. Immunol. Lett. 1999; 70: 213– 218.
29. 40. Pautov VD, Anufrieva EV, Ananeva TD, Saveleva NV, Taratina TM, Krakovyak MG. Structural dynamic and functional properties of native and modified streptokinase. Mol. Biol. 1990; 24: 35 – 41.
30. Delude C. Clot busters- Discovery of thrombolytic therapy for heart attack & stroke. Breakthroughs in Bioscience. Federation of American Societies for Experimental Biology. 2004.
31. Crabbe SJ, Grimm AM, Hopkins LE. Acylated plasminogen-streptokinase activator complex: a new approach to thrombolytic therapy. Pharmacotherapy. 1990; 10 (2):115-26.
32. M.I.Bokarewa, T.Jin, A.Tarkowski.Staphylococcus aureus:Staphylokinase.Int. J.Biochem.Cell Biol. 2006; 38:504-09.
33. Collen D,Schlott B,Engelborghs Y,Van Hoef B,Hartmann M,Lijnen HR,et al.On the mechanism of activation of human plasminogen by recombinant staphylokinase.J.Biol Chem 1993;268:8284-89.
34. Vanderschueren S,Barrio L,Kerdsinchai P etal.for the STAR trail group. A randomized trial of recombinant staphylokinase versus alteplasw for coronary artery patency in acute myocardial infarction. Circulation 1995; 92:2044-49.
35. Armstrong PW, Burton J,Pakola S,Molhoek PG,Betriu A,Tendera M,Bode C,Adgey AA,Bar F,Vahanian A,Van de Werf F.CAPTORS II Investigators. Collaborative Angiographic Patency Trial of Recombinant Staphylokinase (CAPTORS II). Am Heart J. 2003 Sep; 146(3):484-88.
36. Cannon CP, Gibson CM, McCabe CH, et al. TNK-tissue plasminogen activator compared with front-loaded alteplase in acute myocardial infarction: results of the TIMI 10B trial. Circulation 1998; 98:2805–14.
37. ASSENT-2 Investigators. Single-bolus tenecteplase compared with frontloaded alteplase in acute myocardial infarction: the ASSENT-2 double-blind randomised trial. Lancet 1999; 354: 716–22.
38. Nordt TK, Moser M, Kohler B, et al. Pharmacokinetics and pharmacodynamics of lanoteplase (n-PA) Thromb Haemost 1999; 82(suppl):121–23.
39. Suzuki S,Saito M,Suzuki N,et al.Thrombolytic properties of a novel modified human tissue plasminogen activator (E6100): a bolus injection of E6100 has equivalent potency of lysing young and aged canine coronary thrombi.J Cardio Pharmacol 1991;17:738-46.
40. Kawai C, Hosoda S,Kimata S,Kanmatsuse K,Suzuki S,Motomiya T,et al.Coronary thrombolysis in acute myocardial infarction of E6100(novel modified t-PA) : a multicentre doule blind ,dose – finding study.Jpn Pharmacol Ther 1994;22:3925-50.
41. Inoue T,Yaguchi I,Takayangi K,Hayashi T,Moorrks S,Eguchi Y. A new thrombolytic agent,monteplase,is independent of plasminoactivator inhibitor in patients with acute myocardial infarction :comparison with native t-PA.E610 Study group J Am Coll Cardiol 1997;29:1447-53.
42. Yamomto T, Murai K,Tokita Y, Kato K,Iwasaki YK, Sato N,Tajima H,Mizuno K,Tanaka K. Thrombolysis with a novel modified tissue-type plasminogen activator, monteplase, combined with catheter-based treatment for major pulmonary embolism. Circ J. 2009 Jan ;73(1):106-10.
43. Mellott MJ,Stabilito II,Holahan MA,Cuca GC,Wang S,Li P,et al.Vampire bat salivary plasminogen activator promotes rapid and sustained reperfusion without concomitant systemic plasminogen activation in a canine model of arterial thrombosis.Arterioscler Thromb 1992;12:212-21.
44. Liberatore GT,Samson A,Bladin C,Schleuning WD,Medcalf RL,Vampire bat salivary plasminogen activator (desmoteplase): a

unique fibrinolytic enzyme that does not promote neurodegradation.Stroke 2003;34:537-43.

45. Sumii T, Singhal, A B, Asahi M, Shimizu-Sasamata M, Suzuki M, Miyata K, Lo EH. Protective effects of pamiteplase, a modified t-PA, in a rat model of embolic stroke. Neuroport 2001; 12(3): 615-18.
46. Mihara, H., Nakajima, N. and Sumi, H. (1993) Characterization of protein fibrinolytic enzyme in earthworm, *Lumbricus rubellus. Biosci. Biotech. Biochem*. **57**, 1726-1731.
47. Hwan Cho, Eui Sung Choi and Hyung Hoan Lee Molecular Cloning, Sequencing, and Expression of a Fibrinolytic Serine-protease Gene from the Earthworm *Lumbricus rubellus Journal of Biochemistry and Molecular Biology*, Vol. 37, No. 5, September 2004, pp. 574-581.
48. N. Nakajima, H. Mihara, and H. Sumi, "Characterization of potent fibrinolytic enzymes in earthworm, *Lumbricus rubellus*," *Bioscience, Biotechnology & Biochemistry*, vol. 57, no. 10, pp. 1726–1730, 1993.
49. Yong Tang,a Jiping Zhang,a Lulu Gui,a Cheng Wu,b Rong Fan,b Wenrui Changa and Dongcai Lianga Crystallization and preliminary X-ray analysis of earthworm fibrinolytic enzyme component A from Eisenia fetida Acta Crystallographica Section D Biological Crystallography Acta Cryst. (2000). D56, 1659±1661.
50. Ning Tao a,b,1, Jie Cheng a,c,1, Jiachang Yue Using F0F1-ATPase motors as micro-mixers accelerates thrombolysis Biochemical and Biophysical Research Communications 377 (2008) 191–194.
51. G. Oster, H.G. Wang, Reverse engineering a protein: the mechanochemistry of ATP synthase, Biochim. Biophys. Acta 1458 (2000) 482–510.
52. Van Zyl W. B, Pretorius G. H. J, Hartmann M, Kotze H. F. Production of a recombinant antithrombotic and fibrinolytic protein, PLATSAK, in Escherichia coli. Thromb Res 1997; 88: 419-26.
53. Szemraj J,Walkowiak B,Kawecka I,Janiszewska,Buczko W,Bartkowiak J,Chabielska E. A new recombinant thrombolytic and antithrombotic agent with higher fibrin affinity-a staphylokinase variant..in vitro study. Thromb Haemost 2005; 3:2156-65.
54. Kowalski M,Brown G, Bieniasz M, Oszajca K, Chabielska E,Pietras T, Szemraj Z,Ola E M, Bartkowiak J ,Szemraj J. Cloning and expression of a new recombinant thrombolytic and anthithrombotic agent — astaphylokinase variant. 2009; 1:41-53.
55. Yamada T,Shimada Y,Kikuchi M. Integrin-specific tissue type plasminogen activator engineered by introduction of the Arg-Gly-Asp sequence. Biochem Biophy Res Commun. 1996; 228:306-11.
56. Wahlgren N, Ahmed N, Davalos A, Ford GA, Grond M, Hacke W, Hennerici MG, Kaste M, Kuelkens S, Larrue V, Lees KR, Roine RO, Soinne L, Toni D, Vanhooren G. Thrombolysis with alteplase for acute ischaemic stroke in the Safe Implementation of Thrombolysis in Stroke-Monitoring Study (SITS-MOST): an observational study. *Lancet*. 2007; 369:275–282.
57. Lee M, Hong KS, Saver JL. Efficacy of intra-arterial fibrinolysis for acute ischemic stroke: meta-analysis of randomized controlled trials. *Stroke*. 2010;41:932–937.
58. Mahon BR, Nesbit GM, Barnwell SL, Clark W, Marotta TR, Weill A, Teal PA, Qureshi AI. North American Clinical Experience with the EKOS MicroLysUS Infusion Catheter for the Treatment of Embolic Stroke. *AJNR Am J Neuroradiol*. 2003; 24:534 –538.
59. Maier CM, Ahern K, Cheng ML, et al. Optimal depth and duration of mild hypothermia in a focal model of transient cerebral ischemia: effects on neurologic outcome, infarct size, apoptosis, and inflammation. Stroke 1998; 29: 2171 -80.
60. Albers GW, Bates VE, Clark WM, et al. Intravenous tissue-type plasminogen activator for treatment of acute stroke: the Standard Treatement wity Alteplase to Reverse Stroke (STARS) study. JAMA 2000; 283: 1145 -50.
61. Daffertshofer M, Gass A, Ringleb P, et al. Transcranial low-frequency ultrasound mediated thrombolysis in brain: increased risk of hemorrhage with combined ultrasound and tissue plasminogen activator: results of a phase II clinical trial. Stroke 2005 ; 36 : 1441 -6.
62. Meixensberger J, Baunach S, Amschler J, et al. Infl uence of body position on tissue-pO2, cerebral perfusion pressure and intracranial pressure in patients with acute brain injury. Neurol Res 1997 ; 19 : 249 -53.
63. Liu M, Counsell C, Zhao XL, Wardlaw J. Fibrinogen depleting agents for acute ischaemic stroke. Cochrane Database Syst Rev 2003; 3 : CD000091.
64. Ouyang YB, Giffard RG. Cellular neuroprotective mechanisms in cerebral ischemia: Bcl-2 family proteins and protection of mitochondrial function. Cell Calcium. 2004 Sep-Oct;36(3-4):303-11.
65. Dena B. Dubal,Paul J. Shughrue,2 Melinda E. Wilson, Istvan Merchenthaler, and Phyllis M. Wise, Estradiol Modulates bcl-2 in Cerebral Ischemia: A Potential Role for Estrogen Receptors The Journal of Neuroscience, August 1, 1999, 19(15):6385–6393.
66. ZHANG Yanbin, XU Changfa Recent Progresses in the Protein Engineering of Thrombolytic Agents, Acta Scicentiarum Naturalum Universitis Pekinesis 2000.
67. Larsen GR, Timony GA, Horgan PG, Barone KM, Henson KS, Angus LB, Stoudemire JB, Protein engineering of novel plasminogen activators with increased thrombolytic potency in rabbits relative to activase. J Biol Chem. 1991 May 5;266 (13):8156-61.
68. Halvorsen H, Huber K. The role of fibrinolysis in the era of primary percutaneous coronary intervention. *ThrombHaemost* 2011:105:390-395.
69. De Luca G, Marino P. Advances in antithrombotic therapy as adjunct to reperfusion therapies for ST segment elevation myocardial infarction. *Thromb Haemost* 2008;100:184–195.
70. Sezer M, Oflaz H, Goren T, et al. Intracoronary streptokinase after primary percutaneous coronary intervention. *N Engl J Med* 2007;356:1823-34.
71. Kidwell CS, Saver JL, Mattiello J, Starkman S, Vinuela F, Duckwiler G, Gobin YP, Jahan R, Vespa P, Kalafut M, Alger JR. Thrombolytic reversal of acute human cerebral ischemic injury shown by diffusion/perfusion magnetic resonance imaging. Ann Neurol. 2000 Apr;47(4):462-9.
72. Padma S, Majaz M.Intra-arterial versus intra-venous thrombolysis within and after the first 3 hours of stroke onset. Arch Med Sci. 2010 Jun 30;6(3):303-15.
73. Zaidat OO, Suarez JI, Santillan C, Sunshine JL, Tarr RW, Paras VH, Selman WR, Landis DM,Response to intra-arterial and combined intravenous and intra-arterial thrombolytic therapy in patients with distal internal carotid artery occlusion. Stroke. 2002 Jul;33(7):1821-6.
74. Wolfe T, Suarez JI, Tarr RW, Welter E, Landis D, Sunshine JL, Zaidat OO.Comparison of combined venous and arterial thrombolysis with primary arterial therapy using recombinant tissue plasminogen activator in acute ischemic stroke. J Stroke Cerebrovasc Dis. 2008 May-Jun;17 (3):121-8.
75. Sen S, Huang DY, Akhavan O, Wilson S, Verro P, Solander S.IV vs. IA TPA in acute ischemic stroke with CT angiographic evidence of major vessel occlusion: a feasibility study. Neurocrit Care. 2009;11 (1):76-81. Epub 2009 Mar 10.
76. Mori E, Yoneda Y, Tabuchi M, Yoshida T, Ohkawa S, Ohsumi Y,Kitano K, Tsutumi A, Yamadori A. Intravenous recombinant tissue plasminogen activator in acute carotid artery territory stroke. Neurology. 1992; 42:976-982.
77. D Gray, Thrombolysis: past, present, and future, Postgrad Med J. 2006 June; 82(968): 372–375.
78. Khatri P, Wechsler LR, Broderick JP. Intracranial hemorrhage associated with revascularization therapies. Stroke 2007;38(2):431–40.
79. Sylaja PN, Cote R, Buchan AM, et al. Thrombolysis in patients older than 80 years with acute ischaemic stroke: Canadian Alteplase for Stroke Effectiveness Study. J Neurol Neurosurg Psychiatry 2006;77(7):826–9.
80. Barber PA, Zhang J, Demchuk AM, et al. Why are stroke patients excluded from TPA therapy? An analysis of patient eligibility. Neurology 2001; 56(8):1015–20.
81. Smith EE, Abdullah AR, Petkovska I, et al. Poor outcomes in patients who do not receive intravenous tissue plasminogen activator because of mild or improving ischemic stroke. Stroke 2005;36(11):2497–9.
82. Gonzales N, Albright K, Noser E, et al. Mild stroke should not exclude patients from thrombolytic therapy. Stroke 2006;37 (2):621.

83. The Penumbra Stroke Trial Investigators. The penumbra stroke trial: safety and effectiveness of a new generation of mechanical devices for clot removal in acute ischemic stroke. Stroke 2008;39(2):527–729.
84. Stephen C. Lee Prof. Mark Ruegsegger, Philip D. Barnes, Bryan R. Smith and Mauro Ferrari, Therapeutic Nanodevices, Springer Handbook of Nanotechnology 2004, pp 279-322.
85. Jason R. McCarthy1, Irina Y. Sazonova, S. Sibel Erdem, Tetsuya Hara, Brian D. Thompson1, Purvish Patel, Ion Botnaru, Charles P. Lin, Guy L. Reed, Ralph Weissleder, and Farouc A. Jaffer Multifunctional nanoagent for thrombus-targeted fibrinolyticTherapy Nanomedicine (Lond). 2012 July; 7(7): 1017–1028.
86. Y.H. Zhang, J. Wang, Y.B. Cui, J.C. Yue, X.H. Fang, Rotary torque produced by proton motive force in F0F1 motor, Biochem. Biophys. Res. Commun. 331(2005) 370–374.
87. Geyer, V. Helms, A spatial model of the chromatophore vesicles of Rhodobacter sphaeroides and the position of the Cytochrome bc1 complex, Biophys. J. 91 (2006) 921–926.
88. Paul J. Carter, Introduction to current and future protein therapeutics: A protein engineering perspective. Experimental cell research 317(2011), 1261-1269.
89. Glenn R. Larsen$, Gregg A. Timony, Patricia G. Horgan, Karen M. Barone, Kimberlee S. Henson, Lauren B. Angus, and John B. Stoudemire Protein Engineering of Novel Plasminogen Activators with Increased Thrombolytic Potency in Rabbits Relative to Activase. Journal of Biological Chemistry, Vol. 266, No. 13, Issue of May 5, pp. 8156-8161,1991.
90. Collen D, Gold HK, New developments in thrombolytic therapy. Adv Exp Med Biol. 1990; 281:333-54.
91. M.J. Browne, J.E. Carey, C.G. Chapman, I. Dodd, A.F. Esmail, G.M.P. Lawrence, L. McMurdo, I. Williamson, S. Wilson and J.H. Robinson. Protein engineering and comparative pharmacokinetic analysis of a family of novel recombinant hybrid and mutant plasminogen activators. Fibrinolysis, Volume 7, Issue 6, November 1993, Pages 357–364.
92. Collen D., Designing thrombolytic agents: focus on safety and efficacy. Am J Cardiol. 1992 Jan 3; 69(2):71A-81A.
93. Nelles L., Lijnen H. R., Collen D., and Holmes W. Characterization of a Fusion Protein Consisting of Amino Acids 1 to 263 of Tissue-type Plasminogen Activator and Amino Acids 144 to 411 of Urokinase-type Plasminogen Activator. J. Biol. Chem 1987; 262:10855-62.
94. Mrinmoy Ghosh, KK Pulicherla, VPB Rekha, G. Venkat Rao, KRS Sambasiva Rao, A Review on successive generations of streptokinase based thrombolytic agents, International Journal of Pharmacy and Pharmaceutical Sciences ISSN- 0975-1491 Vol 4, Suppl 3, 2012.
95. Ayant S. Bidkar, Dhanaji DG, Madhuri DB, Ganesh YD. Cardiotonic Activity of Portulaca Oleracea on Isolated Frog Heart. International Journal of Pharmacy and Pharmaceutical Sciences. 2011; 3(5): 118-120.
96. Jin, L., Jin, H., Zhang, G., Xu, G., 2000. Changes in coagulation and tissue plasminogen, activator after the treatment of cerebral infarction with lumbrokinase. Clin. Hemorheol. Microcirc. 23, 213–218.
97. Robinson BR, Houng AK, Reed GL. Catalytic life of activated factor XIII in thrombi Implications for fibrinolytic resistance and thrombus aging. Circulation. 2000; 102:1151–1157. [PubMed: 10973845].
98. Kidwell CS, Latour L, Saver JL, Alger JR, Starkman S, Duckwiler G, Jahan R, Vinuela, Kang DW, Warach S., Thrombolytic toxicity: blood brain barrier disruption in human ischemic stroke. Cerebrovasc Dis. 2008;25(4):338-43. Epub 2008 Feb 27.
99. Latour LL, Kang DW, Ezzeddine MA, Chalela JA and Warach S, Early blood-brain barrier disruption in human focal brain ischemia. Ann Neurol. 2004 Oct;56(4):468-77.