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tPA as a therapeutic target in stroke

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Abstract

Background—Ischemic stroke is a leading cause of morbidity and mortality worldwide and recombinant human tissue-type Plasminogen Activator (tPA) is the prominent among very few therapeutics used in its treatment. Due to complications attributed to the drug, most notably transformation of ischemia to hemorrhage, tPA is used only in a small number of ischemic stroke cases, albeit significantly more often in specialized stroke centers.

Objective—What are the mechanisms of tPA action and side-effects in ischemic stroke and can the knowledge about these mechanisms aid in making tPA a more efficacious and safe therapeutic or in developing alternative therapeutics?

Methods—tPA use and alternative/combination therapies in acute ischemic stroke treatment are summarized. The review focuses on literature concerning tPA neurotoxicity and its implications for further development of tPA as a stroke therapeutic.

Results/Conclusion—Exogenously administered recombinant tPA and endogenous tPA have both turned into promising therapeutic targets for the stroke patient.

Keywords

ischemia; tissue plasminogen activator; hemorrhagic conversion; neuroserpin

Introduction

Stroke is the 3rd most common cause of death in the US after heart disease and cancer and a major source of disability. According to the World Health Organization, 15 million people suffer stroke worldwide each year. Of these, 5 million die and another 5 million are permanently disabled. 80% of stroke cases are of the ischemic type, in which a blood clot occludes a blood vessel supplying the brain [1]. The clot may form locally (*thrombotic* stroke) or it may form elsewhere in the body and then travel to the brain vasculature (*embolic* stroke). The sensitive brain tissue is quickly damaged from the reduced blood flow and oxygenation, unless the clot is removed or dissolved and blood flow is restored. Tissue type Plasminogen Activator is a blood factor/protein orchestrating the breakdown of blood clots and it is exogenously administered in a recombinant form in ischemic stroke patients to aid the endogenous fibrinolytic processes in dissolving the clot. Various modifications have been made to the recombinant form of tPA to enhance its pharmacokinetic and pharmacodynamic properties, primarily to increase its short half-life in the systemic circulation and increase its fibrin specificity, accordingly. Thus far, these modifications have not reduced the possibility of side-effects, primarily the hemorrhagic conversion that takes place leading to intracranial hemorrhages, which limit the use of tPA in ischemic stroke. The

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biology of tPA toxicity on the neurovascular unit and the brain parenchyma has been studied for more than a decade and may suggest ways to reduce tPA side-effects while maintaining its thrombolytic efficacy. This review briefly overviews tPA as a means of stroke treatment and examines how it has evolved into a target of stroke therapy.

The biology of tPA in the fibrinolytic system

Blood clots are formed from the aggregation of activated platelets onto fibrin meshes. The breakdown of the fibrin meshes is achieved by plasmin, a broad spectrum protease found in the blood as an inactive zymogen, plasminogen. Plasmin cleaves fibrin thus breaking down the meshwork of the clot, and it is extremely short lived; it is quickly inactivated by α_2-antiplasmin, an abundant inhibitor that restricts the action of plasmin to the vicinity of the clot (reviewed in [2] and [3]). Successful fibrinolysis is achieved by adequate generation of plasmin from plasminogen by one of two Plasminogen Activators (PAs), the tissue-type Plasminogen Activator (tPA) and the urokinase-type Plasminogen Activator (uPA). The two are thought to have redundant functions in the fibrinolytic system, since genetic deletion of either one is not accompanied by major fibrinolytic defects, and only tPA/uPA doubly deficient mice display phenotypes similar to plasminogen deficient ones [4, 5]. As opposed to uPA, the activity of tPA is itself regulated by binding to fibrin, which increases its catalytic efficiency [6, 7]. Thus, the formation of the clot initiates, with the generation of fibrin, the fibrinolytic/ clot dissolving cascade. The catalytic activity of tPA in the bloodstream is terminated with the binding of (a) protein inhibitor(s), primarily the Plasminogen Activator Inhibitor 1 (PAI 1) [8]. The inactive PAI 1-bound tPA is cleared from the circulation by the liver via a scavenger receptor, the LDL Receptor Related Protein 1 (LRP1) [9]. LRP1 also binds catalytically active tPA, but with lower affinity [10]. A neuronal-specific inhibitor of tPA, neuroserpin, is the primary modifier of tPA activity in the nervous system [11, 12]. Inhibited tPA-neuroserpin complexes are internalized by LRP1, similarly to tPA-PAI 1 complexes [13]. The half-life of tPA in the bloodstream is rather short, 5-10 minutes in humans, as a result of PAI-1-mediated inhibition and LRP1-mediated liver uptake [14]. Carbohydrate side chains added to the backbone of tPA protein also play important roles in the clearance of tPA from the bloodstream and forms of tPA with longer half lives have been generated by mutating the glycosylation sites of tPA (reviewed in [15]).

tPA is a secreted serine protease consisting of a single polypeptide chain with 3 or 4 glycosylation sites and numerous disulfide bonds in its secondary structure [16, 17]. The action of plasmin cleaves tPA into an N-terminal light chain and a C-terminal heavy chain still held together by disulfide bonds [18]. Two-chain tPA has higher catalytic efficiency compared to the single chain form and is essentially constitutively fully active, contrary to the single chain form which only becomes fully active upon binding to fibrin [19]. The catalytic domain of tPA lies towards its C-terminal end and comprises the light chain of the protease [20, 21]. The protein also contains the following domains: an N-terminal fibronectin type III finger domain, an epidermal growth factor-like domain and two kringle domains [16, 22]. Plasminogen binds to the second tPA kringle domain and fibrin binds to the finger domain and the second kringle domain. Finally, inhibition by PAI 1 is achieved by covalent binding of PAI 1 to the catalytic domain and the formation of a complex [21]. The presence of the additional domains beyond the catalytic one not only allows for regulation of the catalytic function, but also suggests hitherto unknown interactions and potentially functions. Indeed, both fibronectin finger and growth factor domains are known modalities involved in protein-protein interactions.

tPA as a thrombolytic in stroke

The use of tPA in ischemic stroke has been the subject of numerous recent reviews, so that only key concepts are being mentioned here and the interested reader is referred to such reviews for more information [23, 24, 25, 26, 27, 28].

Recombinant human tPA produced in mammalian cell lines was introduced as a thrombolytic agent in selected cases of stroke following the results of the National Institute of Neurological Diseases and Stroke (NINDS) study in 1995 [29]. This study consisted of two independently powered trials that both showed clinical benefit from the use of tPA. A European study conducted around the same time also showed clinical improvement, albeit more modest, and the use of tPA was not recommended, for fear that eligible patients could not be easily identified [30]. A second European and Australian study showed no benefit from tPA [31], a fact which delayed the use of the protease in Europe until 2002 when a license was granted, provided that an observational safety study was conducted to assess the safety profile of tPA in routine clinical practice. The results of this study were recently published and confirmed the positive effect of tPA [32], thus further relieving reservations concerning its use as a thrombolytic in stroke. However, tPA is still used only in a small number of ischemic stroke cases (3-8%, [33]), but this percentage greatly increases in specialized stroke centers [28].

The rationale behind the use of tPA in ischemic stroke is that by breaking down the clot, recanalization of the occluded blood vessel occurs. The restoration of blood vessel patency is meaningful, however, only if the brain tissue of the ischemic area is still viable. Unfortunately, brain tissue is metabolically very active and thus very sensitive to ischemia. The core of the area serviced by the occluded vessel becomes necrotic very quickly, whereas the periphery, which receives some perfusion by adjacent non-ischemic regions, is still salvageable within a reasonable time frame of a few hours, and is referred to as the penumbra. Thrombolysis by tPA resulting in successful recanalization potentially saves this ischemic but still viable penumbra, but it has to be administered quickly. The positive effect of tPA was clearly demonstrated in the two trials of the NINDS study [29], in which the cut-off time from symptoms onset to initiation of thrombolysis was set at three hours. The effect was more moderate in the ECASS study (30), where treatment was initiated within 6h from stroke onset, but clearer in the subgroup of ECASS patients treated within the first three hours [34]. Along the same lines tPA showed moderate benefit upon administration between 3 and 5 hours from stroke onset [35]. To date the initial recommendation remains that tPA should be administered within three hours from stroke onset [29, 36, 32]. This is a narrow time window since the patient needs to be transferred to the health care unit, and at minimum a CT scan has to be performed, primarily to exclude the possibility of hemorrhage and to assess the size of the infarction. Since potentially positive effects have also been reported with administration of tPA between 3 and 5 hours from stroke onset [35, 36], clinical trials are under way to further extend the time window for tPA thrombolysis [37, 38].

Time is not the only parameter to be taken into account in the decision to initiate tPA thrombolysis; novel imaging technologies may aid in the identification of thrombolysis candidates at late time points. Diffusion-weighted and perfusion-weighted MRIs are being employed to identify patients with large diffusion-perfusion mismatches [39, 40], i.e. patients with significant brain areas that still receive some oxygen and nutrients from adjacent non-ischemic areas by diffusion, although they do not receive any blood flow directly. These patients are predicted to benefit the most from recanalization which would restore blood flow to viable, salvageable tissue even after a relatively long time from stroke onset [40]. Perfusion CTs (PCT) and CT angiography source image (CTA-SI) analysis are

CT modalities that could substitute for MRIs in cases where an MRI is contraindicated or not available [41]. However, it should be noted that the usefulness of imaging techniques in detecting thrombolysis candidates at late time points after stroke onset has not yet been unequivocally proven. The results of the DEFUSE study [40, 42] are expected to shed light on this issue.

The need for strict criteria and time-consuming diagnostic procedures prior to the administration of tPA results from the adverse side-effects of tPA, most notably that of intracerebral hemorrhagic conversion [43], even though such conversion occurs only in 6-7% of patients. This is considered a result of systemic plasminemia/fibrinolytic activation, i.e. the tipping of the coagulation/fibrinolysis balance towards the latter side in the systemic circulation which is thought to disrupt microscopic plugs that physiologically prevent hemorrhages at transiently injured spots on vessel walls [43, 44]. Although this lytic state can manifest itself as hemorrhage in different organs, intracranial hemorrhage is the most dramatic manifestation and can complicate thrombolysis taking place in the body, such as during myocardial infarction where brain vasculature is otherwise healthy, although not necessarily. However, it is much more common in ischemic stroke, because brain vasculature and the Blood Brain Barrier (BBB) are already compromised from the ischemia. In general, the greater the severity of the ischemia and the longer the time from stroke onset, the higher the likelihood of hemorrhage ensuing when recanalization of the occluded vessel occurs in the course of thrombolysis [30]. Moreover, tPA may also directly damage the cerebrovascular endothelium and disrupt the BBB, as suggested by animal studies discussed below although evidence from human trials is lacking.

One important issue in the use of tPA in stroke thrombolysis is whether efficient and permanent recanalization of the occluded vessel occurs. This may be the case with as little as 10% to 25% of cases [45], as tPA only works on the surface of the clot. Therefore larger size clots would take a long time to break down. Moreover, tPA is rapidly removed from the systemic circulation and its thrombolytic effect is quickly lost after termination of its IV infusion. This short half-life results in inability to act on subsequent and continued vessel occlusions that occur both in bigger and smaller blood vessels. Attempts at increasing the dose are hampered by increased incidence of hemorrhagic complications and a potential solution would be to alter the tPA protein, so that favorable properties (such as fibrin specificity) are maintained or enhanced and undesirable ones (such as short half life) are modified or abolished. Mutant forms of tPA exhibiting longer half lives and increased fibrin specificity have been generated and their specific mutations and properties have recently been reviewed elsewhere [15]. Although these tPA mutants have been evaluated as thrombolytics for myocardial infarctions, their use for ischemic stroke is still under investigation [46, 47, 48, 49]. Another solution would be to locally deliver tPA via an arterial catheter in the vicinity of the clot (an approach that seems to extend the therapeutic window) rather than intravenously, so that higher local concentrations and hence higher fibrinolytic efficiency are achieved. Unfortunately, it appears that systemic plasminemia and the risk of intracranial hemorrhage are not avoided this way [50]. This method of intra-arterial tPA administration has been used in combination with the intravenous infusion and is referred to as bridging therapy [45, 51]. Its disadvantages are that it can only be used in large artery occlusions, and that it is more interventional, thus requiring more specialized personnel and equipment for its successful implementation. The appearance of specialized stroke units circumvents this problem, although the cost remains considerable.

Combination and alternative therapies in stroke

tPA is not the sole plasminogen activator used as a thrombolytic therapeutically. Streptokinase, a bacterial enzyme derived from species of Streptococcus, is widely used for

myocardial infarction thrombolysis. Clinical trials using streptokinase in stroke were terminated before completion, as the use of the thrombolytic was associated with higher mortality over placebo, most often due to intracranial hemorrhage [52, 53, 54]. An important point is that streptokinase was most often administered within 6 hours from stroke onset, a fairly late time point associated with higher incidence of hemorrhagic conversion as discussed earlier for tPA. Indeed in one study [52], when analysis was restricted to patients receiving treatment within three hours from stroke onset, moderate benefit from streptokinase administration was observed, although the number of patients was probably not high enough for the result to reach statistical significance. Importantly, streptokinase administration was followed by worse clinical outcomes compared to placebo at the later time points, while at the same time points tPA showed no benefit over placebo at worst. As a result, attempts at introducing streptokinase in stroke thrombolysis were abandoned.

Urokinase plasminogen activator (uPA) was tested in the PROACT II study and it resulted in no improvement in mortality and higher incidence of intracracial hemorrhage [55, 56], for which reasons it was not introduced in clinical practice. Staphylokinase, another bacterial enzyme derived from Staphylococcus species is a highly fibrin-specific plasminogen activator that does not induce systemic plasminemia [57]. Derivatization with polyethyleneglycol (PEG) has helped increase its half-life [58], but staphylokinase has yet to be tried clinically in stroke. Desmoteplase (DSPA) is a plasminogen activator derived from the saliva of the vampire bat Desmodus rotundus [59, 60]. It has a much higher fibrin specificity compared to tPA [61] and does not share neurotoxic properties attributed to tPA in mice [62]. Desmoteplase evoked great excitement, but upon being tested in clinical trials it has yet to prove safe and efficient. More specifically, it has failed to demonstrate any effect in terms of survival and neurological improvement, although it appeared to produce a lower rate of intracerebral hemorrhages [63, 64, 65]. A rediscovered thrombolytic is plasmin itself, which is highly ineffective upon systemic administration due to rapid inhibition from α_2-antiplasmin, as mentioned above. With the advent of intra-arterial administration of thrombolytics, plasmin reemerged as an attractive thrombolytic candidate, since it only acts locally in the vicinity of the clot where it is delivered and does not induce any systemic plasminemia. The downside is that it necessitates highly specialized health care units and thus increases the cost of thrombolysis [66]. Microplasmin, a mutant form of plasmin lacking the five kringle domains, cannot be inactivated by α_2-antiplasmin, thus making it suitable for intravenous administration. Microplasmin did not induce intracerebral hemorrhages compared to tPA in animal models of stroke [67, 68]. A small clinical trial is underway to investigate the safety and efficacy of microplasmin in stroke thrombolysis [69].

A different concept lies behind the use of ancrod [70, 71] and batroxobin (defibrase) [72]. These two agents cleave fibrinogen, the precursor of fibrin, and essentially tip the balance of coagulation and fibrinolysis towards the breakdown of clots, since the clot cannot grow in the absence of its substrate. Defibrinogenating agents are also thought to decrease blood viscosity, thus increasing blood flow through not completely occluded blood vessels. Ancrod, derived from pit viper venom is thought to have additional modes of action in preventing clot formation. However, clinical trial data have not supported the use of fibrinogen depleting agents in the treatment of stroke [71].

Heparin is an anticoagulant essentially exerting an indirect fibrinolytic effect since it prevents further clot formation. Despite being tried in many clinical trials (e.g. [73], reviewed in [74]) it has failed to demonstrate any clinical benefit, but continues to be tried in the management of stroke [75].

Drugs preventing platelet activation (antiplatelets) are being tested as monotherapies or, more frequently, in combination with tPA or other thrombolytics. These include traditional

antiplatelets, such as aspirin [73, 76] or combinations of tPA with more novel antiplatelets, such as glycoprotein IIb/IIIa inhibitors (abciximab [77, 78] and eptifibatide [79]). Combinations of tPA with glycoprotein IIb/IIa inhibitors have not yet shown any clinical benefit; on the contrary, the AbESTT II trial employing abciximab was stopped prematurely due to excessive incidence of intracerebral hemorrhages. However, clinical trials employing abciximab and eptifibatide are still ongoing [80, 81, 82]. Finally, another combination under study is that of argatroban, a thrombin inhibitor, and tPA [83]. An earlier study did not show increased risk or clinical benefit from the use of argatroban as monotherapy [84]. Similar to ancrod, batroxobin and heparin, antiplatelets and argatroban act as indirect thrombolytics, since they interfere with further clot formation.

Mechanical removal of the clot responsible for the occlusion has successfully been combined with tPA thrombolysis and one such device, the MERCI retrieval device, has been approved by the FDA for clinical use [85, 86, 87]. Many other devices have been developed and are being tried with the same rationale [27]. They all require arterial catheterization to allow the retrieval device to reach the clot. This is also the case for a combination of tPA with ultrasound alone [88] or combined with micro- or nano-bubbles [89]. The ultrasound aids in the breakdown rather than the removal of the clot. The bubbles increase the effectiveness of the ultrasound-mediated clot breakdown.

Finally, tPA thrombolysis is being combined at the experimental level with many different neuroprotective agents, e.g. drugs that inhibit excitotoxicity and oxygen free radical generation [90, 91, 92]. Excitotoxicity, which implies neuronal dysfunction and death from overt excitation, and oxidative stress are two mechanisms thought to underlie the gradual demise of the ischemic penumbra. Since tPA is essentially the sole therapeutic used in ischemic stroke, any candidate drug or therapeutic method is or will be combined with tPA in clinical trials to examine potentially more favorable outcomes with the combination compared to tPA alone and to attempt to extend the therapeutic window between stroke onset and treatment.

The neurotoxicity of tPA

Several animal studies over the last 10-15 years have suggested that tPA has neurotoxic properties, a fact which may provide some insight into the incidence of side effects associated with its use clinically.

Tissue culture experiments have attempted to elucidate the beneficial or detrimental role of tPA in *in vitro* models of ischemia or neurotoxicity in general. tPA was added to cultures of cerebrocortical or hippoccampal neurons usually subjected to oxygen-glucose deprivation [93, 94], but also to hemoglobin toxicity [95], microglial conditioned medium [96] or zinc [97, 98]. Results have been contradictory with some studies reporting neuroprotection and others demonstrating neurotoxicity of tPA. Clearly, differences in the injury model and the manner in which the neuronal cultures were prepared and maintained could very well affect the final outcome of tPA treatment.

A more physiologically relevant way to address the general question whether tPA affects stroke severity, progression and prognosis, is to compare transgenic mice lacking the protease with wild type animals of the same genetic background in murine experimental models of stroke. Along these lines, it was shown that tPA deficient animals were more resistant to excitotoxicity induced by intracerebral injection of kainate, a glutamate analog, the injection of which mimics the abundance of glutamate observed in the course of a stroke. These high levels of glutamate are thought to compromise neurons by overt excitation [99]. The resistance to excitotoxicity was lost when tPA was infused into the brain prior to the kainate injection, thus further confirming the neurotoxic role of tPA in the acute phase of the

injury [100]. tPA was similarly shown to exacerbate toxicity after intrastriatal injection of NMDA, another glutamate analog [101].

In models that mimic an ischemic stroke more closely, genetic absence of tPA was accompanied by contradictory results. Wang et al [102] observed less severe outcomes in tPA deficient animals, in accord with the aforementioned results, but Tabrizi et al [103] reported the exact opposite result. Several other studies employing recombinant tPA in small animal models of ischemic stroke reported variable effects (positive [104, 105], neutral [105, 106] or negative [107, 108, 109]). Although the disagreement between the two studies using tPA deficient mice has been attributed to potential differences in genetic background between the animals used in the two instances, a more realistic explanation focuses on the model of ischemia employed in each case: Wang et al [102] used mechanical obstruction of the vessel which could not be modified by the action of endogenous or exogenously administered tPA, whereas Tabrizi et at [103] used thrombi to obstruct the vessel which could be broken down by the action of tPA only in wild type animals. It can be reasonably argued that the obstruction of blood flow was more severe in tPA deficient animals incapable of breaking down the clot, hence the worse outcome. Essentially, Tabrizi et al [103] observed the combined effect of tPA on restoring blood flow by breaking down the clot and on exerting its neurotoxic effect on the brain parenchyma. In agreement with this argument, a recent study confirmed the neurotoxic role of tPA in exacerbating ischemic damage upon mechanical occlusion of the blood vessel, but beneficial effect of tPA in reducing ischemic damage was observed when blood clots were used to obstruct the blood vessel [110]. Finally, it should also be noted that Wang et al [102] made use of a transient ischemia model, in which occlusion of the blood vessel for 2 or 3 hours was followed by reperfusion for 22 or 23 accordingly. A study employing MRI to address the therapeutic efficacy of tPA administered 1 or 4 hours after stroke induction found that tPA was beneficial at the early, but detrimental at the late time point [111]. More recently, Nagai et al [112] found that the size of the infarcted area determines the positive or negative effect of tPA, in that smaller infarcts show better outcomes with the use of tPA, while in larger ones tPA has a detrimental effect. Therefore, it appears that, similarly to what has been observed clinically, parameters such as time from onset of symptoms (duration of ischemia) and size of infarcted area, in other words the severity of the stroke, determine the protective or neurotoxic effect of exogenous recombinant tPA and of endogenous tPA likewise.

The argument as to whether tPA is neurotoxic or neuroprotective could be continued ad infinitum and it could well be the case that it is both: the protective function is undoubted and has prompted its use as a stroke therapeutic; the neurotoxic property would need to be confirmed by identifying (an) actual toxicity mechanism(s).

Many different suggested toxicity mechanisms have actually been proposed and deciphered to an extent. For starters, tPA is thought to affect vessel tone [113, 107]. This has been proposed to induce hemodynamic alterations that ultimately hamper the perfusion of the ischemic area, so that hypoperfusion ensues despite restoration of vessel patency [107]. The effect on the vessel tone is considered a direct one on smooth muscle cells, to which tPA signals via integrin $a_v\beta_3$ in a non-proteolytic fashion to induce vasoconstriction. The signaling is terminated by the binding of PAI 1 on to tPA, the internalization on the integrin-tPA-PAI 1 complex via LRP-mediated endocytosis and the dissociation of the tPA-PAI 1 complex from the integrin followed by its breakdown [114].

tPA was also found to exacerbate brain edema thus leading to worsening of neurological outcomes in two types of brain injury unrelated to ischemia: brain trauma [115] and intracerebral hemorrhage [116, 117]. A potential mechanism to explain the exacerbation of brain edema comes from stroke models and involves the action of tPA on the Blood Brain

Barrier (BBB): it was shown that tPA can disrupt the BBB via LRP-mediated signaling to brain vascular endothelial cells. The signaling does not involve the proteolytic function of tPA, but rather its binding to LRP [118]. Another popular explanation states that tPA activates matrix metalloproteases (MMPs) and more specifically MMP-9 [119, 120]. It also induces MMP-9 expression, which is then activated from its precursor form by the action of tPA [121]. MMPs are established culprits in the opening of the BBB in ischemia and also during the reperfusion injury, when tPA thrombolysis is complicated by intracerebral hemorrhage [122, 123]. Thus, tPA functions both proteolytically to activate MMP-9 and non-proteolytically to induce its expression. In agreement with the proposed mechanism, MMP inhibition reduces infarct size after focal cerebral ischemia and protects against intracerebral hemorrhage as a consequence of tPA thrombolysis [124, 125, 126].

More direct effects of tPA on the brain parenchyma have been considered responsible for its neurotoxic function. tPA was reported to cleave the NR1 subunit of the NMDA receptor and thus increase calcium influx via this subtype of glutamate receptor, an event predicted to exacerbate excitotoxic injury in the ischemic penumbra [101]. This cleavage event was later questioned [127], although the association with the NDMA receptor was not: tPA was instead suggested to interact with the NR2B subunit of the receptor without inducing any proteolytic event, but rather potentially affecting the receptor function [128]. Independently of the actual mechanism, tPA appears to interfere with glutamate receptors, which are important mediators of excitotoxicity in the course of ischemic stroke.

Other direct effects of tPA on the brain parenchyma have been proposed in the kainate excitotoxicity model: tPA was shown to exert its toxicity in both proteolytic (plasmin-mediated) [129] and non-proteolytic [130, 131, 132] fashions. By generating plasmin as a protease, tPA could be cleaving laminin and other important components of the brain extracellular matrix and inducing neuronal anoikis (apoptosis by loss of pro survival signaling from contacts with the extracellular matrix) [133]. Plasmin could also be activating MCP-1, which is a chemokine recruiting monocytes and also microglia, the brain resident immune cells, to the site of injury [134]. In agreement with a role of plasmin in recruitment of inflammatory cells, chemotaxis defects have been observed in palsminogen deficient mice [135]. Microglia have been implicated as important mediators of cell death in kainate excitotoxicity [130] and, interestingly, tPA functions in a non-proteolytic fashion to activate microglia in the same model [131, 132] and to cause monocytes to adhere to sites of inflammation [136]. Administration of tPA directly into sites of intracerebral hemorrhage to aid in the breakdown of the hematoma is accompanied by significant infiltration by inflammatory cells [137] in addition to exacerbating the edema, as mentioned before [116, 117]. This could be a result of microglial activation (mediated by tPA) and microglial chemotaxis (mediated by plasmin) followed by inflammatory cell recruitment from the bloodstream or a direct chemotactic effect of plasmin and/or adhesion effect of tPA on circulating monocytes. Finally, MMP-9 activity has been implicated as an important pathophysiologic factor in kainate excitotoxicity [138] and MMP inhibition was shown to protect from kainate toxicity and associated leukocyte recruitment [139]. MMP-9 upregulation and activation are non-proteolytic and proteolytic functions of tPA accordingly, as mentioned above.

Elucidation of the neurotoxicity mechanisms of tPA affords the potential to modify our therapeutic approach to stroke in different ways. Identification of downstream mediators of tPA toxicity could allow selective inhibition of these with combination therapy, so that the direct fibrinolytic effect remains intact while toxicity is abolished. Combination of tPA with MMP inhibitors could, for example, prevent the neurotoxic sequelae of MMP activation and BBB breakdown that occur in the course of tPA thrombolysis [124, 125, 126]. Prevention of MMP-9 upregulation could potentially be achieved, on the other hand, by statin treatment

[140]. Alternatively, toxicity attributed to tPA functions unrelated to its proteolytic one could be avoided by engineering the protein, so that it no longer exerts these functions, provided that the proteolytic one remains intact. As an example, microglial activation by tPA has been mapped primarily to the N-terminal fibronectin finger domain of tPA [132]. Deletion of the whole domain is predicted to decrease the fibrin specificity of tPA, an undesirable effect. Single aminoacid substitutions or even deletion of smaller regions could, however, prevent microglial activation from happening while preserving fibrin specificity. Importantly, many of the neurotoxic functions of tPA seem to be non-proteolytic in nature. In addition to microglial activation mentioned already, these functions include effects on vessel tone [107, 113, 114] and LRP-mediated opening of the BBB [118] and upregulation of MMP-9 [121]. It is also conceivable that the binding of inhibitors to tPA does more than to render the protease inactive. The tPA/inhibitor complex could have functions not shared by tPA or the inhibitor separately, so that the termination of the proteolytic activity allows these non-proteolytic, potentially neurotoxic functions to come into play. Interestingly, it was recently shown that a small peptide known to prevent the binding of PAI1 to tPA, but not affecting the half-life of tPA or its fibrinolytic efficiency, is capable of reducing the neurotoxicity of exogenously administered tPA [110].

Finally, a portion of the neurotoxicity of tPA is undoubtedly proteolytic in nature, mediated potentially by tPA itself [101] or by generation of plasmin [129, 133, 134] and/or MMPs [119, 120]. However, this toxicity still allows room for intervention, as it may be distinguished from the desirable fibrinolytic activity temporally, spatially and/or functionally. In other words, toxicity ensues when tPA is administered late in the course of ischemia, when it leaks through a compromised BBB to the brain parenchyma and/or when it acts on cells/systems other than the fibrinolytic one. In accord with such dissociation between fibrinolytic and other proteolytic/neurotoxic functions of tPA, the neuronal-specific tPA inhibitor neuroserpin has been reported to have neuroprotective properties when administered in ischemia on its own [141, 142] or in combination with tPA [143]. However, the exact mechanism(s) of neuroserpin protection has(ve) not been elucidated yet, and it was not addressed whether the fibrinolytic effect of tPA was compromised from the co-administration of neuroserpin.

Expert Opinion

The prevalence of ischemic stroke, the morbidity and mortality associated with it and the limited therapeutic means available for its treatment explain and justify the intense research devoted to the development of stroke therapeutics. Brain tissue is highly sensitive to ischemia and the temporal window available for treatment before it becomes irreversibly compromised or deleterious side-effects ensue in the course of therapy is small.

A lot has and can be said about stroke prevention, both primary in the general population and secondary after a history of stroke or a Transient Ischemic Attack (TIA). Control of hypertension, hypercholesterolemia, diabetes mellitus, smoking and obesity, adoption of a less stressful, less sedentary lifestyle and a healthier diet, appropriate use of pharmacotherapy can all help prevent atherosclerosis, the major culprit behind ischemic stroke, and clot formation at predisposed vascular sites.

Acute and complete occlusion of a blood vessel by a clot is the initiating event behind the clinical presentation of a stroke, which is why thrombolytics are and will continue to be a major area of stroke research. Despite its side-effects and limitations, tPA holds a unique position among them. Although the advantage of tPA over alternative thrombolytics (primarily streptokinase) has been criticized, these have yet to demonstrate efficacy and safety comparable to that of tPA in clinical trials. On the other hand, tPA has repeatedly

demonstrated efficacy and safety in treating stoke patients, so that its use should not only continue but also be expanded within the suggested indications. Clearly, the search for novel thrombolytics should continue. An attractive avenue of research is the modification of the tPA protein, to augment desirable properties such as thrombolytic efficiency, fibrin specificity and delayed inhibition and clearance from the bloodstream. Hopefully this avenue of research will also continue with a different objective, that is the removal of proposed undesirable side effects, such as hemodynamic perturbations, disruption of the BBB and the extracellular matrix, induction of edema and recruitment and activation of inflammatory cells, both microglia and cells of the systemic circulation. Although these side effects come from in vitro and animal studies and have not been confirmed in human studies, they offer plausible explanations for the clinically observed tPA toxicity and, more importantly, suggest ways that this toxicity could be minimized or completely avoided. The ultimate test and objective is of course the emergence of safer stroke thrombolytics, be it tPA mutants or altogether different molecules.

The pathophysiology of stroke is quite complex and many different mechanisms leading to compromise of neurological function are at play at the same time. Research into these mechanisms promises to identify important therapeutic targets and lead to more successful therapeutic regimens employing combinations of different agents. Research into the biology of tPA and its neurotoxicity in brain injury in general and ischemic stroke more specifically is important for two reasons: first, tPA will and should continue to be increasingly used as a thrombolytic in stroke in the foreseeable future, so that understanding of its neurotoxicity may help prevent deleterious side effects by modifying the protein itself or by introducing other agents in the treatment, which help prevent or limit the severity of these side effects; second, such neurotoxicity is still operating in the absence of exogenous tPA administration albeit in a smaller scale due to the presence of endogenous tPA and its prevention can have its place in the management of stroke, most probably as part of a combination therapy.

The field of ischemic stroke is undoubtedly in need of more effective and safer therapeutics. tPA research holds and will continue to hold an important place in our attempts to more effectively treat ischemic stroke.

Conclusion

tPA has long been the single available therapeutic to treat ischemic stroke. Significant side effects have limited its use to a small number of stroke cases, but enthusiasm about its use is increasing. Modified tPA versions and altogether different thrombolytics are being tested in an attempt to increase both efficacy and safety. Combination therapies targeting different aspects of stroke pathophysiology are constantly being discovered and tried. In parallel to these avenues of action, research into the biology of tPA holds significant promise to improve our stroke therapy regimens by turning tPA from a therapeutic means into a key therapeutic target.

References

1. Rosamond W, Flegal K, Friday G, et al. American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics--2007 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Circulation. 2007; 115:e69–171. [PubMed: 17194875]
2. Aoki N. Discovery of α_2-plasmin inhibitor and its congenital deficiency. J Thromb Haemost. 2005; 3:623–631. [PubMed: 15842343]
3. Gallimore MJ. More on: discovery of α_2-plasmin inhibitor and its congenital deficiency. J Thromb Haemost. 2006; 4:284–285. [PubMed: 16409492]

4. Carmeliet P, Schoonjans L, Kieckens L, et al. Physiological consequences of loss of plasminogen activator gene function in mice. Nature. 1994; 368:419–424. [PubMed: 8133887]

5. Bugge TH, Flick MJ, Daugherty CC, Degen JL. Plasminogen deficiency causes severe thrombosis but is compatible with development and reproduction. Genes Dev. 1995; 9:794–907. [PubMed: 7705657]

6. Hoylaerts M, Rijken DC, Lijnen HR, Collen D. Kinetics of the activation of plasminogen by human tissue plasminogen activator. Role of fibrin. J Biol Chem. 1982; 257:2912–2919. [PubMed: 7199524]

7. Ranby M. Studies on the kinetics of plasminogen activation by tissue plasminogen activator. Biochim Biophys Acta. 1982; 704:461–469. [PubMed: 6214279]

8. Van Mourik JA, Lawrence DA, Loskutoff DJ. Purification of an inhibitor of plasminogen activator (antiactivator) synthesized by endothelial cells. J Biol Chem. 1984; 259:14914–14921. [PubMed: 6438106]

9. Orth K, Madison EL, Gething MJ, et al. Complexes of tissue-type plasminogen activator and its serpin inhibitor plasminogen-activator inhibitor type 1 are internalized by means of the low density lipoprotein receptor-related protein/alpha 2-macroglobulin receptor. Proc Natl Acad Sci U S A. 1992; 89(16):7422–6. [PubMed: 1502153]

10. Bu G, Williams S, Strickland DK, Schwartz AL. Low density lipoprotein receptor-related protein/alpha 2-macroglobulin receptor is an hepatic receptor for tissue-type plasminogen activator. Proc Natl Acad Sci U S A. 1992; 89:7427–31. [PubMed: 1502154]

11. Osterwalder T, Contartese J, Stoeckli ET, et al. Neuroserpin, an axonally secreted serine protease inhibitor. EMBO J. 1996; 15:2944–2953. [PubMed: 8670795]

12. Krueger SR, Ghisu GP, Cinelli P, et al. Expression of neuroserpin, an inhibitor of tissue plasminogen activator, in the developing and adult nervous system of the mouse. J Neurosci. 1997; 17:8984–8996. [PubMed: 9364046]

13. Makarova A, Mikhailenko I, Bugge TH, et al. The low density lipoprotein receptor-related protein modulates protease activity in the brain by mediating the cellular internalization of both neuroserpin and neuroserpin-tissue-type plasminogen activator complexes. J Biol Chem. 2003; 278:50250–50258. [PubMed: 14522960]

14. Verstraete M, Bounameaux H, De Cock F, et al. Pharmacokinetics and systemic fibrinogenolytic effects of recombinant human tissue-type plasminogen activator (rt-PA) in humans. J Pharmacol Exp Ther. 1985; 235:506–512. [PubMed: 4057083]

•15. Collen D, Lijnen HR. Thrombolytic agents. Thromb Haemost. 2005; 93:627–630. Thorough description of tPA mutants engineered to date. [PubMed: 15841305]

16. Pennica D, Holmes WE, Kohr WJ, et al. Cloning and expression of human tissue-type plasminogen activator cDNA in E. coli. Nature. 1983; 301:214–221. [PubMed: 6337343]

17. Pohl G, Kallstrom M, Bergsdorf N, et al. Tissue plasminogen activator: peptide analyses confirm an indirectly derived amino acid sequence, identify the active site serine residue, establish glycosylation sites, and localize variant differences. Biochemistry. 1984; 23:3701–3707. [PubMed: 6433976]

18. Rijken DC, Collen D. Purification and characterization of the plasminogen activator secreted by human melanoma cells in culture. J Biol Chem. 1981; 256:7035–7041. [PubMed: 6787058]

19. Loscalzo J. Structural and kinetic comparison of recombinant human single- and two-chain tissue plasminogen activator. J Clin Invest. 1988; 82:1391–1397. [PubMed: 3139714]

20. Rijken DC, Groeneveld E. Isolation and functional characterization of the heavy and light chains of human tissue-type plasminogen activator. J Biol Chem. 1986; 261:3098–3102. [PubMed: 3081501]

21. Van Zonneveld AJ, Veerman H, Macdonald ME, et al. Structure and function of human tissue-type plasminogen activator (t-PA). J Cell Biochem. 1986; 32:169–178. [PubMed: 3097031]

22. Banyai L, Varadi A, Patthy L. Common evolutionary origin of the fibrin-binding structures of fibronectin and tissue-type plasminogen activator. FEBS Lett. 1983; 163:37–41. [PubMed: 6685059]

23. Durukan A, Tatlisumak T. Acute ischemic stroke: overview of major experimental rodent models, pathophysiology, and therapy of focal cerebral ischemia. Pharmacol Biochem Behav. 2007; 87:179–197. [PubMed: 17521716]

•24. Mehdiratta M, Caplan LR. Acute [corrected] stroke thrombolysis: an update [corrected]. Prog Cardiovasc Dis. 2007; 49:430–438. Erratum in: Prog Cardiovasc Dis. 2007; 50:81 Good overview of original clinical trials leading to introduction of tPA in stroke thrombolysis and of introduction of novel imaging techniques and intra-arterial thrombolysis in stroke management. [PubMed: 17498523]

••25. Khaja AM, Grotta JC. Established treatments for acute ischaemic stroke. Lancet. 2007; 369:319–330. Comprehensive overview of current stroke management. [PubMed: 17258672]

••26. Suwanwela N, Koroshetz WJ. Acute ischemic stroke: overview of recent therapeutic developments. Annu Rev Med. 2007; 58:89–106. This review describes all recent developments that are starting and will continue to impact on stroke management. [PubMed: 17217327]

••27. Sacco RL, Chong JY, Prabhakaran S, Elkind MS. Experimental treatments for acute ischaemic stroke. Lancet. 2007; 369:331–341. Excellent overview of experimental treatments and current clinical trials. [PubMed: 17258673]

••28. Martínez-Sánchez P, Díez-Tejedor E, Fuentes B, et al. Systemic reperfusion therapy in acute ischemic stroke. Cerebrovasc Dis. 2007; 24(Suppl 1):143–152. Description of current status of stroke thrombolysis and thrombolysis methods in clinical development. [PubMed: 17971650]

•29. 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. This was the first study based on the results of which tPA was introduced as a thrombolytic in ischemic stroke. [PubMed: 7477192]

30. Hacke W, Kaste M, Fieschi C, et al. Intravenous thrombolysis with recombinant tissue plasminogen activator for acute hemispheric stroke. The European Cooperative Acute Stroke Study (ECASS). JAMA. 1995; 274:1017–1025. [PubMed: 7563451]

31. Hacke W, Kaste M, Fieschi C, et al. Randomised double-blind placebo-controlled trial of thrombolytic therapy with intravenous alteplase in acute ischaemic stroke (ECASS II). Lancet. 1998; 352:1245–1251. [PubMed: 9788453]

32. Wahlgren N, Ahmed N, Davalos A, et al. SITS-MOST investigators. 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. [PubMed: 17258667]

33. Reeves MJ, Arora S, Broderick JP, et al. Paul Coverdell Prototype Registries Writing Group: Acute stroke care in the US: results from the 4 pilot prototypes of the Paul Coverdell National Acute Stroke Registry. Stroke. 2005; 36:1232–1240. [PubMed: 15890989]

34. Steiner T, Bluhmki E, Kaste M, et al. The ECASS 3-hour cohort. Secondary analysis of ECASS data by time stratification. ECASS Study Group. European Cooperative Acute Stroke Study. Cerebrovasc Dis. 1998; 8:198–203. [PubMed: 9684058]

35. Clark WM, Wissman S, Albers GW, et al. Recombinant tissue-type plasminogen activator (alteplase) for ischemic stroke 3 to 5 hours after symptom onset. The ATLANTIS Study: A randomized controlled trial. Alteplase Thrombolysis for Acute Noninterventional Therapy in Ischemic Stroke. JAMA. 1999; 282:2019–2026. [PubMed: 10591384]

36. ATLANTIS, ECASS, and NINDS rt-PA Study Group Investigators. Association of outcome with early stroke treatment. Pooled analysis of the ATLANTIS, ECASS, and NINDS stroke trials. Lancet. 2004; 363:768–774. [PubMed: 15016487]

37. [7 Dec 2007] ECASS-III: Placebo controlled trial of alteplase (rt-PA) in acute ischemic hemispheric stroke where thrombolysis is initiated between 3 and 4 hours after stroke onset. http://www.strokecenter.org/trials/TrialDetail.aspx?tid=475

38. [7 Dec 2007] IST-3: Third International Stroke Trial. http://www.strokecenter.org/trials/TrialDetail.aspx?tid=81

39. Staroselskaya IA, Chaves L, Silver B, et al. Relationship between magnetic resonance arterial patency and perfusion-diffusion mismatch in acute ischemic stroke and its potential clinical use. Arch Neurol. 2001; 58:1069–1074. [PubMed: 11448295]

40. Albers GW, Thijs VN, Wechsler L, et al. DEFUSE Investigators. Magnetic resonance imaging profiles predict clinical response to early reperfusion: the Diffusion and Perfusion Imaging Evaluation for Understanding Stroke Evolution (DEFUSE) study. Ann Neurol. 2006; 60:508–17. [PubMed: 17066483]
41. Schellinger PD, Richter G, Kohrmann M, Dorfler A. Noninvasive angiography (magnetic resonance and computed tomography) in the diagnosis of ischemic cerebrovascular disease. Techniques and clinical applications. Cerebrovasc Dis. 2007; 24(Suppl 1):16–23. [PubMed: 17971635]
42. [7 Dec 2007] DEFUSE: Diffusion-weighted imaging Evaluation For Understanding Stroke Evolution. http://www.strokecenter.org/trials/TrialDetail.aspx?tid=588
43. Gardell SJ. The search for the ideal thrombolytic agent: maximize the benefit and minimize the risk. Toxicol Pathol. 1993; 21:190–198. [PubMed: 8210941]
44. Collen D, Bounameaux H, De Cock F, et al. Analysis of coagulation and fibrinolysis during intravenous infusion of recombinant human tissue-type plasminogen activator in patients with acute myocardial infarction. Circulation. 1986; 73:511–517. [PubMed: 2419009]
45. The IMS Investigators. Combined intravenous and intra-arterial recanalization for acute ischemic stroke: the Interventional Management of Stroke study. Stroke. 2004; 35:904–911. [PubMed: 15017018]
46. Haley EC Jr, Lyden PD, Johnston KC, Hemmen TM. A pilot dose-escalation safety study of tenecteplase in acute ischemic stroke. Stroke. 2005; 36:607–612. [PubMed: 15692126]
47. [7 Dec 2007] TNKilas: Tenecteplase for Ischemic Lacunar Stroke. http://www.strokecenter.org/trials/TrialDetail.aspx?tid=598
48. Qureshi AI, Ali Z, Suri MF, et al. Intra-arterial third-generation recombinant tissue plasminogen activator (reteplase) for acute ischemic stroke. Neurosurgery. 2001; 49:41–50. [PubMed: 11440458]
49. Sugg RM, Noser EA, Shaltoni HM, et al. Intra-arterial reteplase compared to urokinase for thrombolytic recanalization in acute ischemic stroke. AJNR Am J Neuroradiol. 2006; 27:769–773. [PubMed: 16611762]
50. Furlan, AJ.; Higadashi, R.; Katzan, I., et al. Intra-arterial thrombolysis in acute ischemic stroke. In: Lyden, PD., editor. Thrombolytic therapy for acute stroke. 2. Totowa (NJ): Humana press; 2005.
51. Lewandowski CA, Frankel M, Tomsick TA, et al. for the EMS Bridging Trial Investigators. Combined intravenous and intra-arterial r-TPA versus intraarterial therapy of acute ischemic stroke. Emergency Management of Stroke (EMS) Bridging Trial Stroke. 1999; 30:2598–2605.
52. Donnan GA, Davis SM, Chambers BR, et al. Streptokinase for acute ischemic stroke with relationship to time of administration: Australian Streptokinase (ASK) Trial Study Group. JAMA. 1996; 276:961–966. [PubMed: 8805730]
53. MAST-I Group. Randomized controlled trial of streptokinase, aspirin, and combination of both in treatment of acute ischaemic stroke. Multicentre Acute Stroke Trial Lancet. 1995; 346:1509–1514.
54. The Multicenter Acute Stroke Trial-Europe Study Group. Thrombolytic therapy with streptokinase in acute ischemic stroke. N Engl J Med. 1996; 335:145–150. [PubMed: 8657211]
55. Furlan A, Higashida R, Wechsler L, et al. Intra-arterial prourokinase for acute ischemic stroke. The PROACT II study: A randomized controlled trial. JAMA. 1999; 282:2003–2011. [PubMed: 10591382]
56. Kase CS, Furlan AJ, Wechsler L, et al. Cerebral hemorrhage after intra-arterial thrombolysis for ischemic stroke: The PROACT II trial. Neurology. 2001; 57:1603–1610. [PubMed: 11706099]
57. Collen D. Staphylokinase: a potent, uniquely fibrin-selective thrombolytic agent. Nat Med. 1998; 4:279–284. [PubMed: 9500599]
58. Collen D, Sinnaeve P, Demarsin E, et al. Polyethyleneglycol-derivatized cysteine-substitution variants of recombinant staphylokinase for single-bolus treatment of acute myocardial infarction. Circulation. 2000; 102:1766–1772. [PubMed: 11023930]
59. Gardell SJ, Duong LT, Diehl RE, et al. Isolation, characterization, and cDNA cloning of a vampire bat salivary plasminogen activator. J Biol Chem. 1989; 264:17947–17952. [PubMed: 2509450]

60. Kratzschmar J, Haendler B, Langer G, et al. The plasminogen activator family from the salivary gland of the vampire bat Desmodus rotundus: cloning and expression. Gene. 1991; 105:229–237. [PubMed: 1937019]

61. Bergum PW, Gardell SJ. Vampire bat salivary plasminogen activator exhibits a strict and fastidious requirement for polymeric fibrin as its cofactor, unlike human tissue-type plasminogen activator. A kinetic analysis. J Biol Chem. 1992; 267:17726–17731. [PubMed: 1387641]

62. Liberatore GT, Samson A, Bladin C, et al. Vampire bat salivary plasminogen activator (desmoteplase): a unique fibrinolytic enzyme that does not promote neurodegeneration. Stroke. 2003; 34:537–543. [PubMed: 12574572]

63. Hacke W, Albers G, Al-Rawi Y, et al. The Desmoteplase in Acute Ischemic Stroke Trial (DIAS): a phase II MRI based 9-hour window acute stroke thrombolysis trial with intravenous desmoteplase. Stroke. 2005; 36:66–73. [PubMed: 15569863]

64. Furlan AJ, Eyding D, Albers GW, et al. Dose Escalation of Desmoteplase for Acute Ischemic Stroke (DEDAS): evidence of safety and efficacy 3 to 9 hours after stroke onset. Stroke. 2006; 37:1227–1231. [PubMed: 16574922]

65. [7 Dec 2007] DIAS-2: Desmoteplase in Acute Ischemic Stroke-2. http://www.strokecenter.org/trials/TrialDetail.aspx?tid=515

•66. Marder VJ, Stewart D. Towards Safer Thrombolytic Therapy. Semin in Hematol. 2002; 39:206–216. This paper makes a strong argument for the re-introduction of plasmin as a safer thrombolytic compared to Plasminogen Activotors with the advent of highly interventional stroke management methods.

67. Lapchak PA, Araujo DM, Pakola S, et al. Microplasmin: a novel thrombolytic that improves behavioral outcome after embolic strokes in rabbits. Stroke. 2002; 33:2279–2284. [PubMed: 12215599]

68. Suzuki Y, Chen F, Ni Y, Marchal G, Collen D, Nagai N. Microplasmin reduces ischemic brain damage and improves neurological function in a rat stroke model with MRI. Stroke. 2004; 35:2402–2406. [PubMed: 15322296]

69. [7 Dec 2007] MITI-IV: Intravenous Administration of Microplasmin for Treatment of Acute Ischemic Stroke. http://www.strokecenter.org/trials/TrialDetail.aspx?tid=523

70. Sherman DG, Atkinson RP, Chippendale T, et al. Intravenous ancrod for treatment of acute ischemic stroke: the STAT study: a randomized controlled trial. Stroke treatment with Ancrod trial. JAMA. 2000; 283:2395–2403. [PubMed: 10815082]

71. Hennerici MG, Kay R, Bogousslavsky J, et al. ESTAT Investigators. Intravenous ancrod for acute ischaemic stroke in the European Stroke Treatment with Ancrod Trial: a randomised controlled trial. Lancet. 2006; 368:1871–1878. [PubMed: 17126719]

72. The Cooperative Group For Reassessment Of Defibrase. Reassessment of defibrase in treatment of acute cerebral infarction: a multicenter, randomized, double-blind, placebo-controlled trial. Chin Med Sci J. 2005; 20:151–158. [PubMed: 16261883]

73. International Stroke Trial Collaborative Group. The International Stroke Trial (IST): A randomised trial of aspirin, subcutaneous heparin, both, or neither among 19,435 patients with acute ischaemic stroke. Lancet. 1997; 349:1569–1581. [PubMed: 9174558]

74. Sherman DG. Antithrombotic and hypofibrinogenetic therapy in acute ischemic stroke: what is the next step? Cerebrovasc Dis. 2004; 17(Suppl 1):138–143. [PubMed: 14694291]

75. Adams HP Jr. Emergent use of anticoagulation for treatment of patients with ischemic stroke. Stroke. 2002; 33:856–861. [PubMed: 11872915]

76. CAST (Chinese Acute Stroke Trial) Collaborative Group. CAST: Randomised placebo-controlled trial of early aspirin use in 20,000 patients with acute ischaemic stroke. Lancet. 1997; 349:1641–1649. [PubMed: 9186381]

77. The Abciximab In Ischemic Stroke Investigators. Abciximab in acute ischemic stroke: A randomized, double-blind, placebo-controlled, dose-escalation study. Stroke. 2000; 31:601–609. [PubMed: 10700492]

78. Adams HP Jr, Effron MB, Torner J, et al. for the ABESTT-II Investigators. Emergency Administration of Abciximab for Treatment of Patients With Acute Ischemic Stroke: Results of an

International Phase III Trial. Abciximab in Emergency Treatment of Stroke Trial (AbESTT-II). Stroke. 2007 Nov 21. Epub ahead of print.

79. CLEAR: Combined Approach to Lysis Utilizing Eptifibatide and rt-PA in Acute Ischemic Stroke. http://www.strokecenter.org/trials/TrialDetail.aspx?tid=478
80. ROSIE: ReoPro Retavase Reperfusion of Stroke Safety Study -- Imaging Evaluation. http://www.strokecenter.org/trials/TrialDetail.aspx?tid=462
81. ROSIE-CT: ReoPro Retavase Reperfusion of Stroke Safety Study-Imaging Evaluation with Computed Tomography. http://www.strokecenter.org/trials/TrialDetail.aspx?tid=438
82. ROSIE-2: Reperfusion of stroke Safety study Imaging Evaluation - 2. http://www.strokecenter.org/trials/TrialDetail.aspx?tid=538
83. Sugg RM, Pary JK, Uchino K, et al. Argatroban tPA stroke study: study design and results in the first treated cohort. Arch Neurol. 2006; 63:1057–1062. [PubMed: 16908730]
84. Lamonte MP, Nash ML, Wang DZ, et al. ARGIS-1 Investigators. Argatroban anticoagulation in patients with acute ischemic stroke (ARGIS-1): a randomized, placebo-controlled safety study. Stroke. 2004; 35:1677–1682. [PubMed: 15155959]
85. Gobin YP, Starkman S, Duckwiler GR, et al. MERCI I: a phase 1 study of Mechanical Embolus Removal in Cerebral Ischemia. Stroke. 2004; 35:2848–2854. [PubMed: 15514171]
86. Smith WS, Sung G, Starkman S, et al. Safety and efficacy of mechanical embolectomy in acute ischemic stroke: results of the MERCI trial. Stroke. 2005; 36:1432–1438. [PubMed: 15961709]
87. Smith WS. Safety of mechanical thrombectomy and intravenous tissue plasminogen activator in acute ischemic stroke. Results of the multi Mechanical Embolus Removal in Cerebral Ischemia (MERCI) trial, part I. AJNR Am J Neuroradiol. 2006; 27:1177–1182. [PubMed: 16775259]
88. Alexandrov AV, Molina CA, Grotta JC, et al. for the CLOTBUST Investigators. Ultrasound-enhanced systemic thrombolysis for acute ischemic stroke. N Engl J Med. 2004; 351:2170–2178. [PubMed: 15548777]
89. Molina CA, Ribo M, Rubiera M, et al. Microbubble administration accelerates clot lysis during continuous 2-MHz ultrasound monitoring in stroke patients treated with intravenous tissue plasminogen activator. Stroke. 2006; 37:425–429. [PubMed: 16373632]
90. Ly JV, Zavala JA, Donnan GA. Neuroprotection and thrombolysis: combination therapy in acute ischaemic stroke. Expert Opin Pharmacother. 2006; 7:1571–1581. [PubMed: 16872260]
91. Lees KR, Zivin JA, Ashwood T, et al. Stroke-Acute Ischemic NXY Treatment (SAINT I) Trial Investigators. NXY-059 for acute ischemic stroke. N Engl J Med. 2006; 354:588–600. [PubMed: 16467546]
92. Lapchak PA, Araujo DM. Advances in ischemic stroke treatment: neuroprotective and combination therapies. Expert Opin Emerg Drugs. 2007; 12:97–112. [PubMed: 17355216]
93. Nagai N, Yamamoto S, Tsuboi T, et al. Tissue type plasminogen activator is involved in the process of neuronal death by oxygen-glucose deprivation. J Cereb Blood Flow Metab. 2001; 21:631–634. [PubMed: 11488532]
94. Flavin MP, Zhao G. Tissue plasminogen activator protects hippocampal neurons from oxygen-glucose deprivation injury. J Neurosci Res. 2001; 63:388–394. [PubMed: 11223913]
95. Wang X, Asahi M, Lo EH. Tissue type plasminogen activator amplifies hemoglobin-induced neurotoxicity in rat neuronal cultures. Neurosci Lett. 1999; 274:79–82. [PubMed: 10553942]
96. Flavin MP, Zhao G, Ho LT. Microglial tissue plasminogen activatortriggers apoptosis in vitro. Glia. 2000; 29:347–354. [PubMed: 10652444]
97. Kim YH, Park JH, Hong SH, Koh JY. Nonproteolytic neuroprotection by human recombinant tissue plasminogen activator. Science. 1999; 284:647–650. [PubMed: 10213688]
98. Siddiq MM, Tsirka SE. Modulation of zinc toxicity by tissue plasminogen activator. Mol Cell Neurosci. 2004; 25:162–171. [PubMed: 14962749]
99. Tsirka SE, Gualandris A, Amaral DG, Strickland S. Excitotoxin induced neuronal degeneration and seizure are mediated by tissue plasminogen activator. Nature. 1995; 377:340–344. [PubMed: 7566088]
100. Tsirka SE, Rogove AD, Strickland S. Neuronal cell death and tPA. Nature. 1996; 384:123–124. [PubMed: 8906786]

101. Nicole O, Docagne F, Ali C, et al. The proteolytic activity of tissue-plasminogen activator enhances NMDA receptor-mediated signaling. Nat Med. 2001; 7:59–64. [PubMed: 11135617]

••102. Wang YF, Tsirka SE, Strickland S, et al. Tissue plasminogen activator (t-PA) increases neuronal damage after focal cerebral ischemia in wild-type and t-PA deficient mice. Nat Med. 1998; 4:228–231. This paper suggested neurotoxic role(s) for tPA in cerebral ischemia. [PubMed: 9461198]

••103. Tabrizi P, Wang L, Seeds N, et al. Tissue plasminogen activator (tPA) deficiency exacerbates cerebrovascular fibrin deposition and brain injury in a murine stroke model: studies in tPA-deficient mice and wild-type mice on a matched genetic background. Arterioscler Thromb Vasc Biol. 1999; 19:2801–2806. This paper contradicted the previous one and supported protective role(s) for tPA in cerebral ischemia. [PubMed: 10559029]

104. Kilic E, Hermann DM, Hossmann K-A. Recombinant tissue plasminogen activator reduces infarct size after reversible thread occlusion of middle cerebral artery in mice. Neuroreport. 1999; 10:107–111. [PubMed: 10094143]

105. Meng W, Wang X, Asahi M, et al. Effects of tissue type plasminogen activator in embolic versus mechanical models of focal cerebral ischemia in rats. J Cereb Blood Flow Metab. 1999; 19:1316–1321. [PubMed: 10598935]

106. Klein GM, Li H, Sun P, Buchan AM. Tissue plasminogen activator does not increase neuronal damage in rat models of global and focal ischemia. Neurology. 1999; 52:1381–1384. [PubMed: 10227621]

107. Kilic E, Bahr M, Hermann DM. Effects of recombinant tissue plasminogen activator after intraluminal thread occlusion in mice: role of hemodynamic alterations. Stroke. 2001; 32:2641–2647. [PubMed: 11692029]

108. Kilic E, Kilic U, Bahr M, Hermann DM. Tissue plasminogen activator-induced ischemic injury is reversed by NMDA antagonist MK-801 in vivo. Neurodegener Dis. 2005; 2:49–55. [PubMed: 16909047]

109. Mikawa S, Suzuki Y, Kondo K, et al. Essential role of endogenous tissue plasminogen activator through matrix metalloproteinase 9 induction and expression on heparin-produced cerebral hemorrhage after cerebral ischemia in mice. Blood. 2004; 103:2610–2616. [PubMed: 14630814]

110. Armstead WM, Nassar T, Akkawi S, et al. Neutralizing the neurotoxic effects of exogenous and endogenous tPA. Nat Neurosci. 2006; 9:1150–1155. [PubMed: 16936723]

111. Jiang Q, Zhang RL, Zhang ZG, et al. Magnetic resonance imaging indexes of therapeutic efficacy of recombinant tissue plasminogen activator treatment of rat at 1 and 4 hours after embolic stroke. J Cereb Blood Flow Metab. 2000; 20:21–27. [PubMed: 10616789]

112. Nagai N, Zhao BQ, Suzuki Y, et al. Tissue-type plasminogen activator has paradoxical roles in focal cerebral ischemic injury by thrombotic middle cerebral artery occlusion with mild or severe photochemical damage in mice. J Cereb Blood Flow Metab. 2002; 22:648–651. [PubMed: 12045662]

113. Nasser T, Akkawi S, Shina A, et al. In vitro and in vivo effects of tPA and PAI-1 on blood vessel tone. Blood. 2004; 103:897–902. [PubMed: 14512309]

114. Akkawi S, Nassar T, Tarshis M, et al. LRP and αvβ3 mediate tPA activation of smooth muscle cells. Am J Physiol Heart Circ Physiol. 2006; 291:H1351–1359. [PubMed: 16489109]

115. Mori T, Wang X, Kline AE, et al. Reduced cortical injury and edema in tissue plasminogen activator knockout mice after brain trauma. Neuroreport. 2001; 12:4117–4120. [PubMed: 11742249]

116. Thiex R, Kuker W, Muller HD, et al. The long-term effect of recombinant tissue-plasminogen-activator (rt-PA) on edema formation in a large-animal model of intracerebral hemorrhage. Neurol Res. 2003; 25:254–262. [PubMed: 12739233]

117. Thiex R, Mayfrank L, Rohde V, et al. The role of endogenous versus exogenous tPA on edema formation in murine ICH. Exp Neurol. 2004; 189:25–32. [PubMed: 15296833]

••118. Yepes M, Sandkvist M, Moore EG, et al. Tissue-type plasminogen activator induces opening of the blood-brain barrier via the LDL receptor-related protein. J Clin Invest. 2003; 112:1533–1540. This paper suggests non-proteolytic and non-MMP-mediated effects of tPA on BBB integrity. [PubMed: 14617754]

119. Lo EH, Wang X, Cuzner ML. Extracellular proteolysis in brain injury and inflammation: role for plasminogen activators and matrix metalloproteinases. J Neurosci Res. 2002; 69:1–9. [PubMed: 12111810]

120. Lo EH, Broderick JP, Moskowitz MA. Tissue plasminogen activator and proteolysis in the neurovascular unit. Stroke. 2004; 35:354–356. [PubMed: 14757877]

••121. Wang X, Lee SR, Arai K, et al. Lipoprotein receptor-mediated induction of matrix metalloproteinase by tissue plasminogen activator. Nat Med. 2003; 9:1313–1317. This paper suggests proteolytic, MMP-9-mediated effects of tPA on the integrity of the neurovascular unit. [PubMed: 12960961]

122. Kelly MA, Shuaib A, Todd KG. Matrix metalloproteinase activation and blood-brain barrier breakdown following thrombolysis. Exp Neurol. 2006; 200:38–49. [PubMed: 16624294]

123. Sumii T, Lo EH. Involvement of matrix metalloproteinase in thrombolysis-associated hemorrhagic transformation after embolic focal ischemia in rats. Stroke. 2002; 33:831–836. [PubMed: 11872911]

124. Romanic AM, White RF, Arleth AJ, et al. Matrix metalloproteinase expression increases after cerebral focal ischemia in rats: inhibition of matrix metalloproteinase-9 reduces infarct size. Stroke. 1998; 29:1020–1030. [PubMed: 9596253]

125. Lapchak PA, Chapman DF, Zivin JA. Metalloproteinase inhibition reduces thrombolytic (tissue plasminogen activator)-induced haemorrhage after thromboembolic stroke. Stroke. 2000; 31:3034–3040. [PubMed: 11108768]

126. Pfefferkorn T, Rosenberg GA. Closure of the blood-brain barrier by matrix metalloproteinase inhibition reduces rtPA-mediated mortality in cerebral ischemia with delayed reperfusion. Stroke. 2003; 34:2025–2030. [PubMed: 12855824]

127. Matys T, Strickland S. Tissue plasminogen activator and NMDA receptor cleavage. Nature Med. 2003; 9:371–373. [PubMed: 12669038]

128. Pawlak R, Melchor JP, Matys T, et al. Ethanol-withdrawal seizures are controlled by tissue plasminogen activator via modulation of NR2B-containing NMDA receptors. Proc Natl Acad Sci U S A. 2005; 102:443–448. [PubMed: 15630096]

129. Tsirka SE, Rogove AD, Bugge TH, et al. An extracellular proteolytic cascade promotes neuronal degeneration in the mouse hippocampus. J Neurosci. 1997; 17:543–552. [PubMed: 8987777]

130. Rogove AD, Tsirka SE. Neurotoxic responses by microglia elicited by excitotoxic injury in the mouse hippocampus. Curr Biol. 1998; 8:19–25. [PubMed: 9427623]

131. Rogove AD, Siao C, Keyt B, et al. Activation of microglia reveals a non-proteolytic cytokine function for tissue plasminogen activator in the central nervous system. J Cell Sci. 1999; 112:4007–4016. [PubMed: 10547361]

132. Siao CJ, Tsirka SE. Tissue plasminogen activator mediates microglial activation via its finger domain through annexin II. J Neurosci. 2002; 22:3352–3358. [PubMed: 11978811]

133. Chen ZL, Strickland S. Neuronal death in the hippocampus is promoted by plasmin-catalyzed degradation of laminin. Cell. 1997; 91:917–925. [PubMed: 9428515]

134. Sheehan JJ, Zhou C, Gravanis I, et al. Proteolytic activation of monocyte chemoattractant protein-1 by plasmin underlies excitotoxic neurodegeneration in mice. J Neurosci. 2007; 27:1738–1745. [PubMed: 17301181]

135. Ploplis VA, French EL, Carmeliet P, et al. Plasminogen deficiency differentially affects recruitment of inflammatory cell populations in mice. Blood. 1998; 91:2005–2009. [PubMed: 9490683]

136. Cao C, Lawrence DA, Li Y, et al. Endocytic receptor LRP together with tPA and PAI-1coordinates Mac-1-dependent macrophage migration. EMBO J. 2006; 25:1860–1870. [PubMed: 16601674]

137. Thiex R, Kuker W, Jungbluth P, et al. Minor inflammation after surgical evacuation compared with fibrinolytic therapy of experimental intracerebral hemorrhages. Neurol Res. 2005; 27:493–498. [PubMed: 15978174]

138. Jourquin J, Tremblay E, Décanis N, et al. Neuronal activity-dependent increase of net matrix metalloproteinase activity is associated with MMP-9 neurotoxicity after kainate. Eur J Neurosci. 2003; 18:1507–1517. [PubMed: 14511330]

139. Campbell SJ, Finlay M, Clements JM, et al. Reduction of excitotoxicity and associated leukocyte recruitment by a broad-spectrum matrix metalloproteinase inhibitor. J Neurochem. 2004; 89:1378–1386. [PubMed: 15189340]

140. Wang S, Lee SR, Guo SZ, et al. Reduction of tissue plasminogen activator-induced matrix metalloproteinase-9 by simvastatin in astrocytes. Stroke. 2006; 37:1910–1912. [PubMed: 16741180]

141. Yepes M, Sandkvist M, Wong MK, et al. Neuroserpin reduces cerebral infarct volume and protects neurons from ischemia-induced apoptosis. Blood. 2000; 96:569–576. [PubMed: 10887120]

142. Cinelli P, Madani R, Tsuzuki N, et al. Neuroserpin, a neuroprotective factor in focal ischemic stroke. Mol Cell Neurosci. 2001; 18:443–457. [PubMed: 11922137]

143. Zhang Z, Zhang L, Yepes M, et al. Adjuvant treatment with neuroserpin increases the therapeutic window for tissue-type plasminogen activator administration in a rat model of embolic stroke. Circulation. 2002; 106:740–745. [PubMed: 12163437]