The role of plasminogen activators in stroke treatment: fibrinolysis and beyond

Audrey M Thiebaut, Maxime Gauberti, Carine Ali, Sara Martinez De Lizarrondo, Denis Vivien, Manuel Yepes, Benoit D Roussel

Although recent technical advances in thrombectomy have revolutionised acute stroke treatment, prevalence of disability and death related to stroke remain high. Therefore, plasminogen activators—eukaryotic, bacterial, or engineered forms that can promote fibrinolysis by converting plasminogen into active plasmin and facilitate clot breakdown—are still commonly used in the acute treatment of ischaemic stroke. Hence, plasminogen activators have become a crucial area for clinical investigation for their ability to recanalise occluded arteries in ischaemic stroke and to accelerate haematoma clearance in haemorrhagic stroke. However, inconsistent results, insufficient evidence of efficacy, or reports of side-effects in trial settings might reduce the use of plasminogen activators in clinical practice. Additionally, the mechanism of action for plasminogen activators could extend beyond the vessel lumen and involve plasminogen-independent processes, which would suggest that plasminogen activators have also non-fibrinolytic roles. Understanding the complex mechanisms of action of plasminogen activators can guide future directions for therapeutic interventions in patients with stroke.

Lancet Neurol 2018; 17: 1121–32

Normandie Université, UNICAEN, INSERM, INSERM UMR-S U1237, Physiopathology and Imaging of Neurological Disorders, Cyceron, Caen, France (A M Thiebaut MSc, M Gauberti PhD, Prof C Ali PhD, S Martinez De Lizarrondo PhD, Prof D Vivien PhD, B D Roussel PhD)**; Department of Neurology and Center for Neurodegenerative Disease, Emory University School of Medicine, Division of Neuropharmacology and Neurologic Diseases, Yerkes National Primate Research Center, and Department of Neurology, Veterans Affairs Medical Center, Atlanta, GA, USA** (Prof M Yepes MD)**; and Clinical Research Department, University Hospital Caen-Normandy, Caen, France** (Prof D Vivien)

Introduction

The fibrinolytic effect of various types of plasminogen activators for the acute treatment of ischaemic stroke has been explored by many clinical studies. However, tissue-type plasminogen activator (tPA) is the most commonly used plasminogen activator for treatment of patients with acute ischaemic stroke.[1] Thrombolysis with tPA after acute ischaemic stroke has been limited by the short recommended treatment window (time since onset of symptoms <4·5 h), although the 2018 Efficacy and Safety of MRI-based Thrombolysis in Wake-Up Stroke (WAKE-UP) trial[2] showed that an ischaemic lesion that is visible on diffusion-weighted MRI without brain parenchymal hyperintensity on fluid-attenuated inversion recovery can be used to successfully identify patients with acute stroke who would benefit from treatment with tPA even if the time of symptom onset is unknown.

To appreciate the clinical importance of plasminogen activators in treating brain ischaemia, it is pivotal to understand the neurovascular unit—a highly dynamic system composed of endothelial cells surrounded by pericytes and a basement membrane encircled by astrocytic endfeet processes that enter in contact with axonal projections from neighbouring neurons. Cerebral ischaemia induces a rapid release of plasminogen activators from the endothelial cells into the intravascular space, from perivascular astrocytes into the endothelial cell-basement membrane-astrocyte interphase, and from the presynaptic terminal into the synaptic cleft. The main role of plasminogen activators in the intravascular space is to maintain the patency of the blood vessel by promoting plasmin-induced lysis of occluding clots. In the endothelial cell-basement membrane-astrocyte interphase, plasminogen activators regulate the permeability of the blood–brain barrier (BBB),[3] and modulate the synaptic response to the ischaemic injury in the synaptic cleft.[4] Thus far, the rationale to treat patients with ischaemic stroke with plasminogen activators is based on their intravascular fibrinolytic effect. Paradoxically, the fibrinolytic properties of plasminogen activators are also used to clear clotted blood from the brain to treat haemorrhagic stroke. Historically, plasminogen activators were used to remove blood clots from the brain parenchyma, as blood signals were observed radiographically and found to persist for months in patients with intraventricular haemorrhage. However, no studies clearly describe the consequences on plasminogen-activator injection on the brain parenchyma, learning and memory, or neurotoxicity after

Panel 1: Ischaemic stroke and urokinase plasminogen activator

Urokinase plasminogen activator (uPA) is a serine protease with fibrinolytic properties, first identified in human urine.[6] uPA has been used to treat patients with acute limb ischaemia, pulmonary embolism, myocardial infarction, ischaemic stroke, and intracranial haemorrhage. In the rat brain, uPA expression is mostly neuronal[7] but it can also be found in astrocytes and oligodendrocytes.[8] uPA is synthesised as a single-chain pro-enzyme, and secreted in the extracellular space where it is cleaved by plasmin, kallikreins, and stromelysin into fully active two-chain uPA.[9] The cell surface receptor of uPA promotes the local activation of plasminogen into plasmin, but uPA receptor activation can also recruit signalling pathways, induce neuroprotection, neurogenesis, neuritogenesis, axonal growth, and neuronal migration, and promote dendritic spine recovery after ischaemic stroke (figure 1 and appendix).[10–12] The endogenous uPA–uPA receptor system has been shown to be involved in stroke pathophysiology. The bioactive soluble form of the uPA receptor (suPAR) has been described as a strong biomarker of carotid plaques burden and ischaemic stroke occurrence.[13] Elevated plasmatic levels of suPAR are also a predictor of 5-year mortality in patients with ischaemic stroke.[14] Observations in uPA receptor-knock-out and uPA-knock-out mice report that endothelial uPA receptors could be responsible for ischaemia-mediated brain damages independently of uPA,[15] suggesting the existence of another ligand for uPA receptor during cerebral ischaemia. The removal of uPA or uPA receptors (by using knock-out animals) does not influence the size of the ischaemic lesion but improves functional recovery.[16] Alternatively, another hypothesis is that uPA is released by injured neurons and that uPA receptors are recruited to the astrocytic cell surface to activate extracellular signal-regulated kinases (Erk) and signal transducer and activator of transcription (STAT), independently from plasmin generation, leading to astrocytic activation and synaptic recovery.[11]

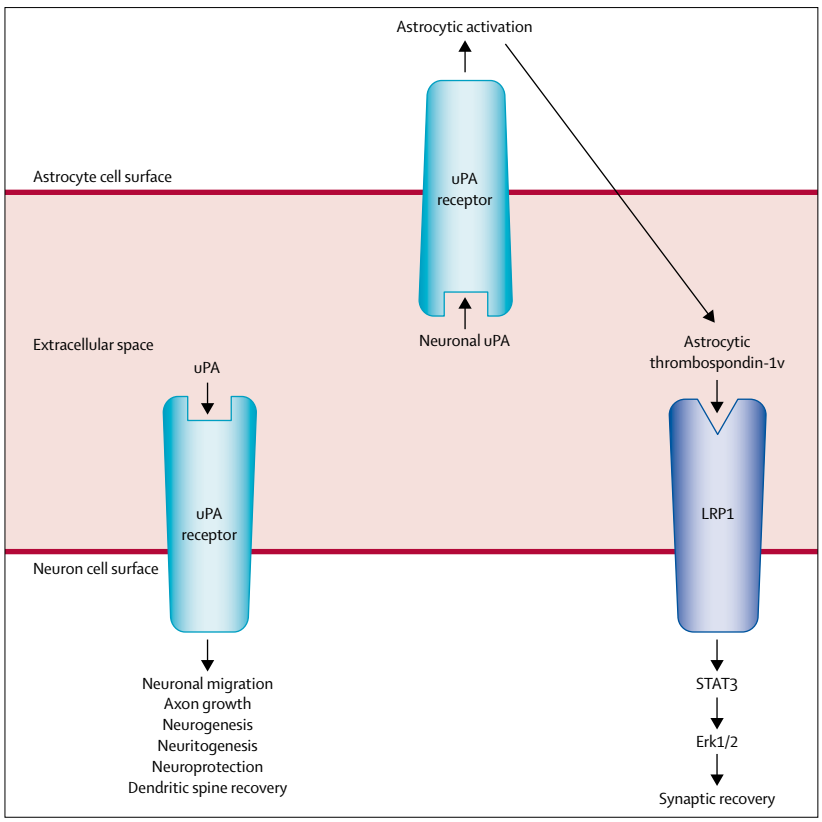

Figure 1: **Pathophysiology of urokinase plasminogen activators from in-vitro studies and animal models**
Urokinase plasminogen activator (uPA) can induce multiple effects in neurons through its receptor: neuronal migration, axon growth, neurogenesis, neuritogenesis, neuroprotection, and dendritic spine recovery.[7,10,16] uPA released by neurons can activate its receptor on the astrocyte cell surface, leading to astrocytic activation, which in turn releases astrocytic thrombospondin-1v into the extracellular space, which then interacts with LRP1 on dendritic spines to promote synaptic recovery via reorganisation of the actin cytoskeleton in the postsynaptic terminal.[11] Erk1/2=extracellular signal-regulated kinases 1 and 2. LRP1=LDL receptor-related protein 1. STAT=signal transducer and activator of transcription.

Correspondence to:
Dr B D Roussel, Normandie Université, UNICAEN, INSERM, INSERM UMR-S U1237, Physiopathology and Imaging of Neurological Disorders, Cyceron, 14000 Caen, France
broussel@cyceron.fr

See Online for appendix

blood-clot removal, despite a clear benefit of the technique for patients.[5]

In this Review, we describe the the pathophysiological effects of plasminogen activators and how, in the future, their non-fibrinolytic functions could be used for the treatment of patients with acute ischaemic and haemorrhagic stroke.

Tissue-type plasminogen activator

tPA and urokinase-type plasminogen activator (uPA; panel 1, figure 1) are the two main mammalian plasminogen activators that convert plasminogen into active plasmin, which can then degrade fibrin, although others (eg, desmoteplase) have been tested in humans and animals. Recombinant forms of plasminogen activators (native or engineered) such as tenecteplase and reteplase have been used to treat patients with thrombotic diseases such as acute ischaemic stroke[17] or myocardial infarction (figure 2 and appendix).[19] However, only tPA administration is approved for the treatment of patients with acute ischaemic stroke.[1]

Physiological functions of tPA

tPA was first identified in the blood and later in the brain.[20] It is synthesised as a single-chain protein and, in vitro, is converted into a two-chain form by plasmin or kallikreins,[21] although this conversion does not affect fibrinolytic efficacy of tPA.[22] In vivo, tPA is synthesised and released by endothelial cells in the blood-stream,[23] but it is also produced by brain cells such as neurons[24] and glial cells;[25] in vitro studies have shown tPA synaptic release[26] and endocytosis by astrocytes.[27] tPA controls many cerebral physiological events, such as neuronal migration,[28] neurite outgrowth,[29] glutamatergic neurotransmission,[30] long-term potentiation,[31] synaptic plasticity,[32] neurovascular coupling,[33] and anxiety,[34] through interactions with various receptors (figure 3).

Contrasting neurotoxic and neuroprotective effects of tPA

Since the pioneering work of Tsirka and colleagues[42] in 1995, several animal studies have supported the possibility that tPA might have a pro-neurotoxic effect under pathological conditions. Nowadays, this possibility remains uncertain because, in animal studies, tPA has been shown to promote both neuronal survival and death. The opposing roles of tPA in neuronal survival could depend on tPA dose, the type of neurons, or the experimental models of neuronal death, or a combination of these factors. For example, tPA increases NMDA-receptor (NMDAR) signalling to toxic levels in vitro.[30] Even though this finding was initially controversial,[43] it is now accepted by the research community. However, this toxic effect of NMDAR signalling could require a coreceptor such as LDL receptor-related protein 1 (LRP1).[37] In contrast with the studies that showed a neurotoxic effect of tPA, other animal studies indicate that tPA might promote synaptic adaptation to metabolic stress,[35] might induce homoeostatic plasticity,[4] and might protect the postsynaptic density from the harmful effects of ischaemic injury.[44] Proteostasis (ie, equilibrium between protein synthesis and degradation) is also emerging as a potential signalling pathway modulated by tPA during stroke. Indeed, an animal study[45] has shown that co-treatment with a proteasome inhibitor enhances the beneficial effects of tPA. These results are consistent with recent findings in mice[41] that inhibition by tPA of endoplasmic reticulum stress protects neurons from oxygen and glucose deprivation in vitro. Furthermore, other animal studies[35,46] have shown that tPA also has a neuroprotective effect via the activation of the mammalian target of rapamycin (mTOR), which is an inhibitor of autophagy.

Several questions relating to neurotoxicity remain: how strongly does the tPA–NMDAR interaction affect the pathophysiology of stroke? Is the stimulation of NMDAR sufficient to trigger the negative effects of tPA in stroke? Does tPA promote any effect in ischaemic brain tissue independent of its enzymatic activity? In oxygen and

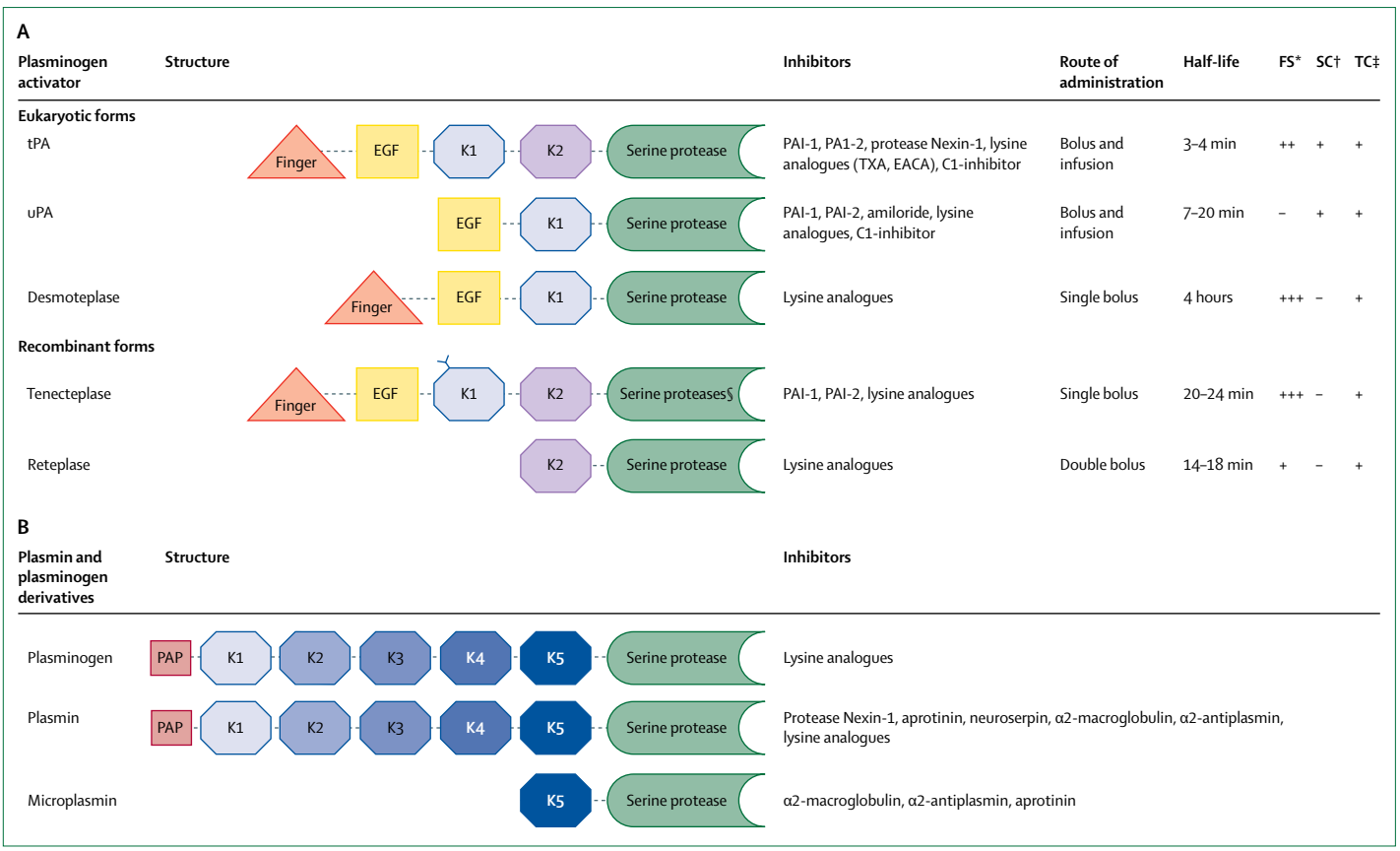

Figure 2: **Plasminogen activators and plasmin or plasminogen derivatives**
(A) Plasminogen activators are classified into eukaryotic forms and recombinant forms. They have various structural domains, inhibitors, and different specificities to fibrin; thus, their route of administration and half-life vary. Plasminogen activators can have a SC form, a TC form, or both.[18] (B) Plasmin or plasminogen derivatives before and after cleavage by plasminogen activators. Plasminogen activators convert plasminogen into active plasmin, which can then degrade fibrin. EACA=ε-aminocaproic acid. EGF=epithelial growth factor. Finger=finger domain. FN=fibronectin domain. FS=fibrin specificity. K=Kringle domain. PAI=plasminogen-activator inhibitor. PAP=pan apple domain. SC=single chain. TC=two chain. tPA=tissue-type plasminogen activator. TXA=tranexamic acid. uPA=urokinase plasminogen activator. *Plasminogen activator has no (-), low (+) moderate (++), or high (+++) fibrin specificity.[18] †Plasminogen activator has (+) or does not have (-) a SC form. ‡Plasminogen activator has (+) or does not have (-) a TC form. §Amino acid substitution in the serine protease domain: Lys296Ala, Hys297Ala, Arg298Ala, Arg299Ala.

glucose deprivation experiments in vitro, tPA protects neurons from death,[35,41] whereas in a mouse model[47] of thromboembolic stroke with tPA-mediated recanalisation, the injection of a specific antibody into circulation to inhibit the interaction between tPA and NMDAR is beneficial. However, an effect of the antibody on the neurovascular unit or the endothelial cells cannot be excluded, as suggested by the effect of this antibody in a mouse model of multiple sclerosis.[48]

Although these animal models provide valuable mechanistic information, clinical support for these models remains poor. One case-control registry study[49] suggested that thrombolysis using tPA was associated with epileptic seizures within 7 days of treatment (28 [1%] of 2327 patients with stroke compared with 100 controls with stroke who did not have seizures), independently of recanalisation or haemorrhagic transformation. However, neither a retrospective analysis[50] of 302 patients with stroke nor a meta-analysis[51] of 4362 participants who had a stroke identified an association between tPA and seizures after stroke. Nevertheless, a multicentre analysis[52] of 1004 patients who had a stroke and were treated with thrombolysis reported that people injected with a higher ratio of single-chain tPA than two-chain tPA are more likely to develop seizures soon after treatment, although functional outcome was not affected. One explanation is that the conflicting results regarding the benefits and adverse effects of tPA might arise from the differences between studies in animal models of stroke and clinical studies, in which the large benefits of reperfusion in humans might conceal the adverse effects of tPA.

Ischaemic stroke and tPA

tPA administration is combined with endovascular thrombectomy[53] in patients with large vessel occlusion (panel 2). There have been 27 trials of the use of thrombolytic agents in the treatment of ischaemic stroke according to the latest Cochrane database systematic review.[60] Four of these trials administered streptokinase

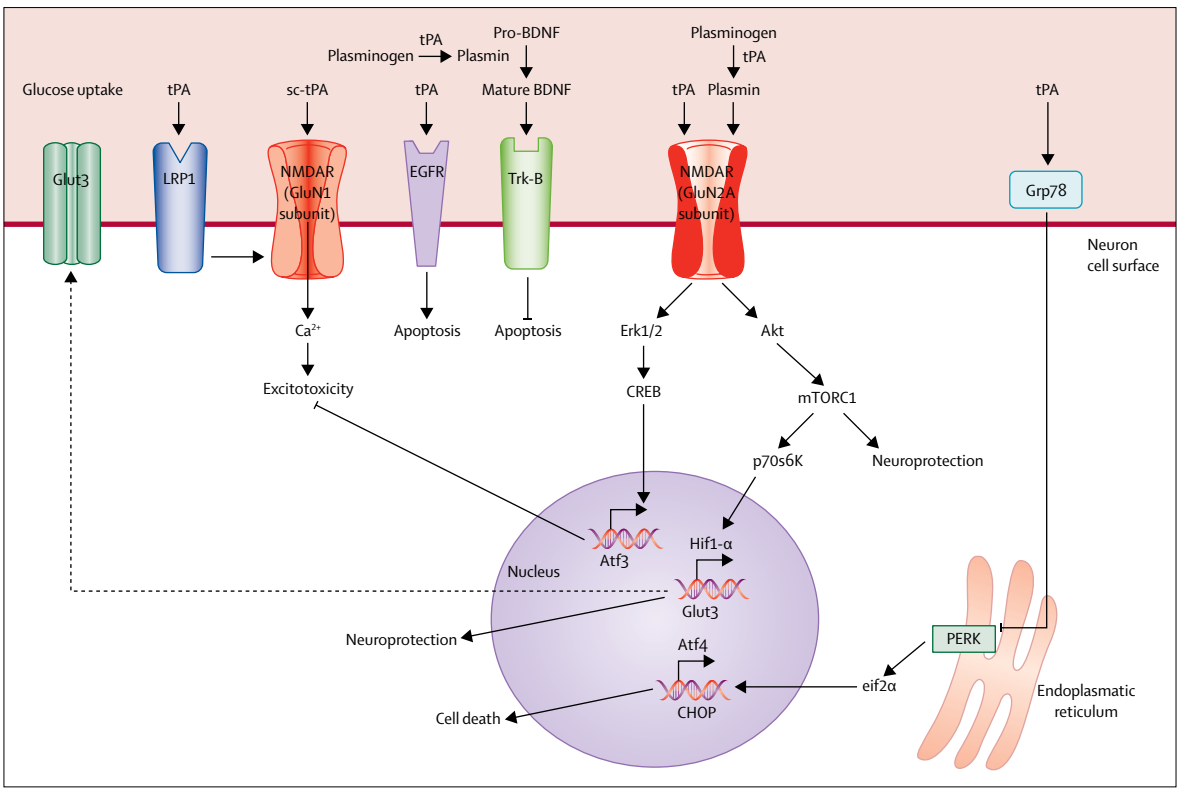

Figure 3: **Pathophysiology of tissue-type plasminogen activator from in-vitro studies and animal models**
At the neuronal cell surface, tissue-type plasminogen activator (tPA) interacts directly with NMDARs with opposite results reported in the scientific literature. When it interacts with the GluN2A subunit, tPA activates either the Akt/mTOR/p70S6K/HIF1α signalling pathway, leading to neuroprotection and to an increase of glucose uptake,[35] or the Erk1/2/CREB/ATF3 signalling pathway to decrease excitotoxicity.[36] sc-tPA also interacts with the GluN1 subunit of NMDAR either directly[30] or through LRP1,[37,38] and promotes excitotoxicity. This neurotoxic effect is mediated by the single-chain form of tPA, while both sc-tPA and tc-tPA interact with EGFR to inhibit apoptosis.[39] tPA also cleaves plasminogen into plasmin, leading to the cleavage of NMDAR GluN2A subunit and supporting cell survival, and activation of pro-BDNF into BDNF, which in turn activates Trk-B and protects from apoptosis.[40] Finally, the interaction of tPA and Grp78 is involved in decreasing endoplasmic reticulum stress through an inhibition of PERK/Eif2alpha/CHOP/Atf4 pathways.[41] Genes activated by each pathway are indicated above the arrows on the DNA segments. Akt=protein kinase B. Atf4=activating transcription factor 4. BDNF=brain-derived neurotrophic factor. CHOP=CCAAT-enhancer-binding protein homologous protein. CREB=C-AMP response element-binding protein. EGFR=EGF receptor. eif2α=eukaryotic translation initiation factor 2 α. Erk1/2=extracellular signal-regulated 1/2. Glut3=glucose transporter 3. Grp78= glucose-regulated protein 78 kDa. Hif1-α=hypoxia inducible factor 1. JAK2=JANUS kinase 2. LRP1=LDL receptor-related protein 1. mTORC1=mammalian target of rapamicyn complex 1. NMDAR= NMDA receptor. p70s6K=ribosomal protein S6 kinase beta-1. PERK=protein kinase RNA-like endoplasmic reticulum kinase. sc-tPA=single-chain tPA. STAT=signal transducer and activator of transcription. tc-tPA=two-chain tPA. Trk-B=tropomyosin receptor kinase B.

(at a dose of 1·5 MU), 12 administered recombinant tPA (at doses from 0·9 mg/kg to 1·1 mg/kg), six administered urokinase (at a dose of either 1·5 MU or 1·0 MU), two administered pro-urokinase (at doses of 6 mg and 9 mg), and three administered desmoteplase (at doses between 62 µg and 125 µg per kg of bodyweight).[60] These trials identified the main limitations of the use of tPA in ischaemic stroke to be the narrow therapeutic window (4·5 h after stroke symptom onset) of tPA,[61] its low rate of recanalisation, and the risk of symptomatic parenchymal haemorrhage.[17]

Rate of recanalisation and improved thrombolysis

The rate of acute recanalisation within 2 h after tPA administration remains low (<35% of patients had acute recanalisation, figure 4), according to a review[62] of the Calgary Stroke Program (2002–09), which included 1341 patients. Multiple variables, such as prolonged time to treatment, poor collaterals,[63] and thrombus size greater than 8 mm[64] are major predictive factors for unsuccessful tPA treatment. The low rate of recanalisation observed in patients treated with tPA prompted the ongoing investigation of the effect of different mechanical devices in removing the occluding clot from the intravascular space (panel 2).

Since acute recanalisation is a major prognostic factor for good functional outcome, therapeutic strategies that aim to improve the rate of recanalisation with intravenous thrombolysis remain of great clinical interest. Increasing the efficiency of tPA in degrading fibrin is one potential approach to improve the rate of recanalisation. There are different potential strategies to improve the efficiency of tPA, based either on the prevention of tPA inhibition by endogenous factors or on increasing the proteolytic

activity of tPA. One such strategy consists of bio-engineering tPA through targeted mutagenesis to increase its resistance to inhibitors. This approach led to the development of tenecteplase (figure 2), a recombinant tPA that has increased resistance to plasminogen-activator inhibitor 1 (PAI-1), improved fibrin specificity, and enhanced half-life. A phase 3 randomised, open-label, blinded-endpoint trial[65] assessed functional outcome in 1107 patients enrolled within 4·5 h of onset of symptoms in 13 stroke units in Norway, 3 months after treatment with either tenecteplase or alteplase (tPA). These investigators found no difference in either mortality or functional outcome between groups.[65] By contrast, a subsequent multicentre, randomised, open-label, blinded-outcome trial[66] with 202 patients enrolled within 4·5 h of onset of stroke symptoms and treated with tenecteplase or alteplase showed reperfusion greater than 50% in 22 (22%) of 101 patients who received tenecteplase versus 10 (10%) of 101 treated with alteplase, and a better functional outcome at 90 days in tenecteplase-treated patients than in alteplase-treated patients.

Another strategy to increase the efficacy of tPA proposes to use a diabody (ie, an engineered antibody targeting two proteins) to block two inhibitors of the fibrinolytic process—PAI-1 and thrombin-activatable fibrinolysis inhibitor (TAFI)—to promote thrombolysis.[67] In a mouse model of thromboembolic stroke, co-administration of a diabody improved the efficacy of tPA, without promoting bleeding.[67] TAFI plasma concentrations affected the rate of recanalisation after tPA administration in 136 patients with stroke[68] and correlated with stroke severity and outcome[69] in 109 patients with stroke. Additionally, another study of 139 patients with ischaemic stroke[70] has shown that the presence of functional polymorphism on the genes that encode for TAFI and PAI-1 influenced tPA-induced recanalisation. An inhibitor of TAFIa is currently being tested in a placebo-controlled phase 1b/2 study (ClinicalTrials.gov, NCT02586233) of patients with ischaemic stroke who are not eligible to be administered tPA (>4·5 h after ischaemic stroke symptom onset). Numerous molecules which inhibit PAI-1 have been described, including antibodies, nanobodies, and small molecules.[71,72] Although promising results have been obtained in animal models, no PAI-1-inhibition molecule has been tested in patients with ischaemic stroke thus far. Boosting plasminogen activation has been shown to be achievable in rodents by injecting the soluble form of annexin A2 into the bloodstream. Annexin A2 forms complexes with both tPA and plasminogen, leading to an accelerated kinetics of plasmin formation. In a mouse model of cerebral ischaemia, both the fibrinolytic activity of tPA and its therapeutic window were increased by the injection of annexin A2.[73]

Another approach to increase efficacy of thrombolysis is to degrade thrombus constituents other than fibrin, which represents only a part of the thrombus volume. To test this approach, several predictive characteristics of the thrombus have been identified. For example, a higher proportion of red blood-cells in samples from retrieved thrombi is associated with an increased response to tPA and to improved clinical outcomes

Panel 2: Endovascular thrombectomy

The Mechanical Embolus Removal in Cerebral Ischaemia (MERCI) trial,[54] a prospective, single arm, multicentre study with 151 patients with large vessel stroke showed that a mechanical embolectomy device can safely restore vascular patency in patients presenting within 8 h of onset of an acute ischaemic stroke. More remarkably, this study showed a rate of recanalisation of 46% with the MERCI device alone, and 60·8% when combined with intra-arterial tissue-type plasminogen activator (tPA).[54] Despite these encouraging results, enthusiasm for the use of thrombectomy was temporarily diminished by the findings from several randomised controlled trials[55,56] that did not show improved efficacy of endovascular clot retrieval compared with intravenous tPA administration. However, important technical advances that allowed the use of new-generation stent-retriever devices led to several randomised controlled trials[53,57] that reported a consistent superiority of endovascular clot retrieval over standard medical care alone. Importantly, a post-hoc analysis[58] of 291 patients from two large multicentre prospective trials treated with mechanical thrombectomy or intravenous tPA, or both, found no benefit from intravenous thrombolysis in patients with acute ischaemic stroke undergoing mechanical thrombectomy. Finally, a multicentre, randomised, open-label study[59] with 206 patients who had occlusion of the intracranial internal carotid artery or proximal middle cerebral artery showed improved outcomes for disability at 90 days in patients with 6–24 h onset of symptoms treated with thrombectomy in addition to standard care (5 of whom were treated with tPA) compared with patients who received standard care alone (13 of whom received tPA).

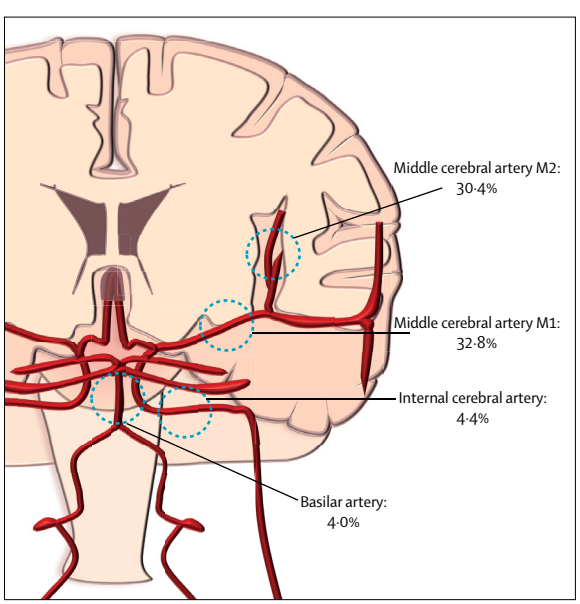

Figure 4: **Rates of tissue-type plasminogen activator-mediated recanalisation**
Rates of recanalisation within 2 h of tissue-type plasminogen activator (tPA) administration vary according to thrombus location. Data come from a review[62] of the Calgary Stroke Program (2002–09), which included 1341 patients with acute ischaemic stroke secondary to proximal vessel occlusions who received intravenous recombinant tPA. Acute rates of recanalisation were assessed using CT angiography. M1 and M2 are two of the four segments the middle cerebral artery is divided in.

compared with a lower proportion of red blood-cells.[74] Since red blood cell-rich thrombi modify the MRI signal (ie, presence of the susceptibility vessel sign), this biomarker enables the use of imaging for the prediction of tPA efficacy. Thrombi containing neutrophil extracellular traps—DNA extracellular networks produced by activated neutrophils—are also more resistant to mechanical or pharmacological reperfusion.[75] An analysis of 108 thrombi from patients with stroke found that neutrophil extracellular traps are present in all thrombi and might be responsible for reperfusion resistance.[75] Ex-vivo degradation of the thrombus is accelerated by the co-administration of tPA and DNase1. These findings have prompted a clinical trial of DNase1 (ClinicalTrials.gov, NCT02900833). The low efficacy of tPA on platelet-rich thrombi might also prevent recanalisation. However, clinical trials[76,77] investigating co-administration of tPA and an antiplatelet drug (eg, aspirin, anti-GpIIb/IIIa) showed low efficacy and increased bleeding risk. In animal studies,[78,79] platelet aggregation in arteries has been shown to involve specific receptors and proteins that are not essential for haemostasis in the brain, such as von Willebrand factor (VWF) and the glycoprotein GpIb-α. Therefore, strategies that specifically target platelet aggregates formed under conditions of arterial shear stress can induce arterial recanalisation without substantially increasing the risk of haemorrhagic transformation.[78] Thus, pharmacological strategies that target VWF with a disintegrin and metalloprotease with thrombospondin type-I motif, member-13 (ADAMTS-13)[80] or with N-acetylcysteine[81] are also promising, especially since intracranial thrombi in patients with stroke contain large amounts of VWF,[80] and low plasma concentrations of ADAMTS-13 might be a marker for unsuccessful treatment with tPA.[82]

Furthermore, clinical deterioration—possibly due to re-occlusion—occurs in at least 15% of patients treated with tPA. Thus, other strategies that aim to increase the speed of recanalisation and prevent re-occlusion to reduce the extent of infarction have been studied. In the ARTSS-2 randomised exploratory study[83] of 90 patients with stroke treated with tPA, adjunctive argatroban (a direct thrombin inhibitor) was not associated with an increased risk of symptomatic intracerebral haemorrhage. This finding provides the basis for a future efficacy trial combining tPA with an antigocoagulant.

tPA-mediated haemorrhagic transformation

A meta-analysis[84] of 27 trials with 10 239 patients with stroke treated with streptokinase, tPA, urokinase, or desmoteplase found a four-time increase in the incidence of symptomatic intracerebral haemorrhage among patients with stroke treated with any of these plasminogen activators. Remarkably, when these analyses were limited to 12 trials using tPA, there were 60 additional symptomatic intracerebral haemorrhages per 1000 treated patients. Symptomatic intracerebral haemorrhage has since been the most feared complication of tPA treatment, with a higher incidence within the first 24 h (median time ranging from 5 h to 10 h) than after 24 h, and almost 50% mortality.[85] In contrast to tPA, tenecteplase does not change the incidence of intracerebral haemorrhage.[66]

An international, multicentre, randomised, open-label trial[86] evaluated the incidence of symptomatic intracerebral haemorrhage and the clinical outcome in 3297 predominantly Asian patients with stroke treated within 4·5 h of the onset of symptoms with either low (0·6 mg/kg) or standard (0·9 mg/kg) doses of alteplase. This study showed a difference in haemorrhagic transformation between the low-dose group (1·0%) and the standard-dose group (2·1%), which indicates that the prevalence of haemorrhagic transformation is associated with the dose of tPA. Converesely, time-to-treatment is not significantly associated with haemorrhagic-transformation risk, according to a large meta-analysis.[87,88] By contrast, the TARGET-STROKE initiative reported in a study[89] of 71 169 patients with acute ischaemic stroke treated with tPA that an intervention aiming to reduce the time-to-treatment delay also led to a reduction of the prevalence of haemorrhagic transformation.

Haemorrhagic transformation after tPA administration is a dynamic phenomenon, and its pathophysiology remains unclear. Indeed, animal studies have shown that tPA increases the permeability of the BBB through multiple mechanisms. For instance, tPA can bind to and cleave LRP1 at the astrocytic endfeet, resulting in BBB alterations.[90] The activation of platelet-derived growth factor-CC (PDGF-CC) by tPA in the brain has also been shown to lead to BBB leakage.[91] PDGF-CC plasma concentrations can be used to predict haemorrhagic transformation and severe cerebral oedema in patients with stroke treated with tPA.[92] A pilot study based on this finding has shown that imatinib (a PDGF-receptor inhibitor) might reduce the risk of haemorrhagic transformation in patients treated with tPA.[93]

tPA also induces the activation of matrix metalloproteinases (MMPs),[3] which are involved in the degradation of the extracellular matrix and in the promotion of BBB impairment. Results from an animal study[94] suggest that minocycline, a tetracycline antibiotic and an inhibitor of MMPs, prevents the increase in concentration of plasma MMP-9 associated with tPA administration in a rat model of thromboembolism. A minocycline dose-escalation study[95] (alone or in combination with tPA) was shown to be safe in 60 patients with ischaemic stroke. A meta-analysis of three studies[96] also reported that minocycline was safe, although statistically significant heterogeneity among the trials precludes conclusions about efficacy. Cytokines and growth factors could also counteract tPA-mediated haemorrhagic transformation. However, animal studies of granulocyte-colony stimulating factor have reported conflicting findings that the factor,

co-administrated with tPA during the acute phase of stroke, either protects against haemorrhagic transformation caused by delayed tPA treatment[97] or increases risk of haemorrhagic transformation.[98]

Brain oedema and mortality after tPA treatment

Several studies suggest an increased risk of early mortality with tPA treatment, especially as onset-to-treatment delay increases. In the IST-3 trial,[99] the mortality of tPA-treated patients increased by 60% in the first 7 days, compared with controls. This finding cannot be explained solely by the increase in haemorrhagic transformation[100] and suggests that other mechanisms might be at play. Notably, the prevalence of fatal brain oedema in the IST-3 trial increased in tPA-treated patients with stroke compared with controls, with an odds ratio of 1·89 (95% CI 1·14–3·14).[99] This finding was supported by the case-control ICARO study[101] of 506 patients with occlusion of the internal carotid artery, among whom the prevalence of fatal symptomatic brain oedema was 8·3% in 253 tPA-treated patients versus 3·1% in 253 controls. By comparison, the prevalence of fatal haemorrhagic transformation was only 2·8% in tPA-treated patients, suggesting that the contribution of symptomatic brain oedema to mortality could be greater than haemorrhagic transformation in this subset of patients with occlusion of a proximal artery. By contrast, other trials (ECASS-3,[61] for instance) reported a reduction in prevalence of symptomatic brain oedema after tPA treatment. These discrepancies between studies suggest that tPA has both pro-oedema and anti-oedema effects in patients with acute ischaemic stroke, with the anti-oedema effects being probably associated with the capacity of tPA to promote reperfusion and alleviate ischaemic damage.

The mechanisms by which tPA promotes infarct swelling remain poorly understood, although tPA promoted brain oedema in a mouse model of ischaemic stroke through activation of the contact phase,[102] which is a biochemical cascade responsible for the generation of bradykinin (a short pro-inflammatory peptide that increases vascular permeability). Furthermore, plasmatic concentration of bradykinin is increased in patients with ischaemic stroke after tPA administration.[103] It is believed that tPA-mediated generation of plasmin activates factor XII (a blood coagulation factor), which can in turn activate plasma kallikrein, the main enzyme responsible for bradykinin generation by cleavage of its precursor (ie, high molecular-weight kininogen).[102] Therefore, factor XII, plasma kallikrein, or bradykinin receptors are all potential therapeutic targets to prevent tPA-induced infarct swelling.[104] These clinical and animal data demonstrate that the effect of tPA administration in patients with stroke is not limited to the degradation of the fibrin clot. The diversity of effects offers the opportunity to improve the safety and efficacy of tPA treatment.

Haemorrhagic stroke

Haemorrhage is a devastating form of stroke, as it is associated with high mortality and long-term disability. Spontaneous haemorrhagic strokes are usually divided into intracerebral haemorrhage and subarachnoid haemorrhage, both of which are potentially associated with intraventricular haemorrhage.[105] In patients with haemorrhagic stroke, the mass effect of the haematoma and—in the case of subarachnoid or intraventricular bleeding—the interruption of CSF circulation are responsible for an increase in intracranial pressure and high mortality. Plasminogen activators could be used to mitigate the deleterious consequences of intracranial bleeding by promoting the dissolution of the haematoma and restoring CSF circulation.

Intracerebral haematoma

Intracerebral haemorrhage is characterised by parenchymal bleeding and accounts for approximately 10% of all strokes.[105] Since haematoma volume is a key prognostic factor after intracerebral haemorrhage, the development of surgical procedures for the evacuation of the haematoma is an ongoing effort. Open surgical evacuation of supratentorial haematoma—one of the first strategies to have been developed—has not shown any benefit in large clinical trials,[106] possibly because it is associated with iatrogenic tissue injury during surgery. Simple aspiration has been abandoned because it could only remove small amounts of the coagulated blood. To overcome this limitation, stereotactic aspiration of the liquefied haematoma after in-situ instillation of plasminogen activators has been investigated. The phase 2 MISTIE 2 trial[107] randomly assigned 96 patients to either standard care or minimally invasive haematoma aspiration using tPA instillation after documented interruption of haematoma growth. The trial confirmed the safety of thrombolytic aspiration despite an increased risk of asymptomatic bleeding in the treated group. When adjusted to baseline severity, the increase in the proportion of patients achieving a good outcome was significant, but these results should be interpreted with caution since this trial was not powered to show efficacy.[107] A phase 3 trial is ongoing (MISTIE 3; ClinicalTrials.gov, NCT01827046) and the results are expected soon.

During minimally invasive drainage of intracerebral haemorrhage, a potential concern is the effect of in situ instilled tPA on and around the BBB. In a porcine model of intracerebral haemorrhage,[108] in-situ fibrinolytic therapy with tPA without aspiration was associated with an increase in the perihaematomal oedema, as measured by MRI. In another study in pigs, this effect was mitigated by the co-administration of dizocilpine (MK-801), suggesting an NMDAR-dependent mechanism.[109] Accordingly, administration of PAI-1 reduces cerebral oedema following tPA treatment in the same model.[110] This noxious effect of tPA was confirmed in a rat model of intracerebral haemorrhage,[111] in which

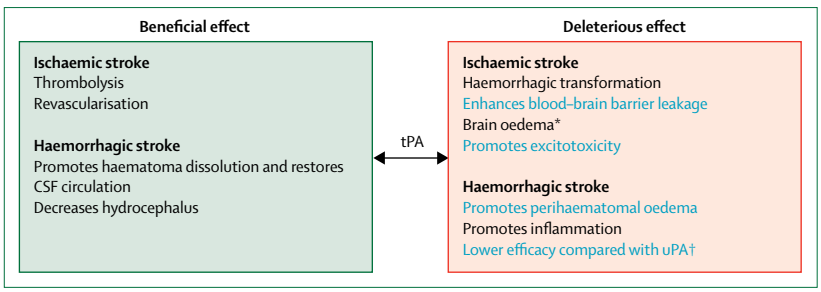

Figure 5: **Effects of tissue-type plasminogen activator in ischaemic and haemorrhagic stroke**
Beneficial[17,114,116,121] (green box) and deleterious[17,30,85,90,91,99,101,102,108,114,119,122] (red box) effects of tPA in both ischaemic and haemorrhagic stroke. Effects shown in clinical studies are indicated in black, and effects shown in animal models are indicated in blue. tPA=tissue-type plasminogen activator. uPA=urokinase plasminogen activator. *It is unclear whether brain oedema is present following thrombolysis by tPA in humans. †A preclinical study[114] has shown that uPA is better than tPA for the fibrinolysis of intraventricular haemorrhage.

administration of optimised tPA (an engineered non-proneurotoxic tPA variant) decreased perihaematomal neuronal death and subsequent oedema progression compared with administration of tPA. These animal data are contradicted by a secondary analysis of the MISTIE 2 trial,[112] which shows that instillation of tPA combined with stereotactic aspiration significantly reduces perihaematomal oedema. Moreover, a retrospective study[113] reported that uPA and tPA present similar efficacy and safety profiles for the drainage of basal ganglia haematoma, even though animal studies of uPA have not shown the same pro-excitotoxic effects as tPA.[114] Thus, there is no clinical evidence supporting a neurotoxic role for tPA in patients with intracerebral haemorrhage.

Intracerebral haemorrhage associated with intraventricular haemorrhage

Extension of haemorrhage to the ventricles occurs in approximately 25% of cases of non-traumatic intracerebral haemorrhage and promotes the obstruction of CSF outflow and, hence, the development of hydrocephalus.[115] Thus, intraventricular administration of plasminogen activators could prevent obstruction of the flow of CSF and accelerate clearance of the intraventricular blood. The phase 2 CLEAR IVH trial[116] in 48 patients reported preliminary results for safety and clot removal efficacy of recombinant tPA compared with placebo, which prompted the CLEAR 3 trial.[117] In this phase 3 trial, 500 patients were randomly assigned to thrombolytic removal of intraventricular haemorrhage using either alteplase or placement of an extraventricular drain. The proportion of patients in the alteplase group (117 [48%] of 246) who achieved good functional outcome was similar to the placebo group (110 [45%] of 245) at 6 months (risk ratio 1·06, 95% CI 0·88–1·28; p=0·554). The mortality rate was lower in the tPA group (46 [18%]) than in the placebo group (73 [29%]), but patients had higher disability rates in the tPA group (42 [17%]) than in the placebo group (21 [9%]).[117] Greater intraventricular clot removal was associated with improved outcome, but only 82 patients (33%) in the tPA group reached the objective of 80% clot dissolution (as assessed by CT scan), which might have reduced the clinical benefit of intraventricular thrombolysis. A randomised trial of 87 patients,[118] which investigated the efficacy of a strategy that combined intraventricular fibrinolysis with lumbar drain versus intraventricular fibrinolysis alone, showed that dual therapy significantly reduced the occurrence of permanent shunt dependency for post-intracerebral-haemorrhage hydrocephalus. Overall, these results suggest that there is still room to improve the intraventricular thrombolysis procedure in patients with intraventricular haemorrhage.

Another explanation for the neutral results of the CLEAR 3 study could be related to side-effects of tPA. Indeed, a mouse study[114] investigated drainage of intraventricular haematoma using tPA compared with uPA and reported that, although both fibrinolytics reduced hydrocephalus, only uPA improved functional recovery significantly, confirming the results of a meta-analysis[115] of intraventricular fibrinolysis in patients with intraventricular haemorrhage that found an improved clinical efficacy of uPA compared with tPA. These results could be explained by the fact that tPA promotes inflammation after intraventricular injection in both mice[114] and humans.[119] We cannot exclude that direct detrimental effects of tPA on the BBB are masked by the beneficial effects of haematoma drainage.

Subarachnoid haemorrhage

Besides restoring CSF outflow, the main aim of fibrinolytics as a potential therapy for subarachnoid haemorrhage is to reduce the risk and occurrence of delayed cerebral ischaemia by accelerating blood clearance from the subarachnoid space. A meta-analysis of 652 patients[120] that included predominantly non-randomised studies suggests a significant beneficial effect of intracisternal fibrinolysis in patients with subarachnoid haemorrhage. It has also been shown in a phase 2 randomised trial of 60 patients[121] that intraventricular fibrinolysis in combination with low-frequency head-rotation is a safe and effective way to accelerate haematoma clearance, although the prevalence of delayed cerebral ischaemia was not significantly reduced and functional outcome was not significantly improved. A phase 3 trial to study the efficacy of intraventricular fibrinolysis using tPA in subarachnoid haemorrhage is ongoing (ClinicalTrials.gov, NCT03187405). A small randomised study of 6 patients[122] showed that intraventricular tPA administration produces a transient local inflammatory response, which correlates with the degree of fibrinolysis, raising the possibility that the release of haematoma breakdown products, rather than tPA itself, is responsible for the pro-inflammatory effects. Overall, while plasminogen activators seem to have an acceptable safety profile to accelerate the clearance of intracranial haematoma in

Search strategy and selection criteria

We searched PubMed for all indexed articles published from Jan 1, 2013, to Aug 1, 2018, with the terms ("tPA" OR "uPA") AND ("ischemic stroke" OR "haemorrhagic stroke"). We included only articles published in English. Titles and abstracts were screened, and relevant papers were selected for detailed assessment. The bibliographies of the selected articles were also screened for additional sources. Whenever possible, priority was given to articles published in the past 5 years, except for landmark and historical papers or clinical trials. Additionally, the ClinicalTrials.gov database was screened to include ongoing trials regarding use of plasminogen activators in ischaemic or haemorrhagic stroke, with a specific focus on phases 3 and 4 trials.

some subtypes of haemorrhagic stroke, their efficacy remains uncertain.

Conclusions and future directions

Endovascular thrombectomy has revolutionised the treatment of patients presenting with occlusion of a proximal intracranial artery. The positive results of endovascular thrombectomy in patients presenting up to 24 h after stroke onset indicate that, if safe, recanalisation can still be beneficial in patients who meet the treatment criteria.[1,2,59] However, reperfusion of smaller arteries is not easily achievable, and many patients remain ineligible for reperfusion therapies. Thus, pharmacological thrombolytic strategies remain relevant for the treatment of patients with ischaemic stroke. The mismatch between the therapeutic windows of endovascular thrombectomy and tPA could reflect the deleterious effects of tPA on the neurovascular unit (figure 5). These deleterious effects should stimulate the development of safer thrombolytic strategies, with increased reperfusion. To this aim, further understanding of the biological effects of tPA on the neurovascular unit is necessary.

In haemorrhagic stroke, large clinical trials[116–118] showed that the use of tPA in intraventricular haemorrhage only slightly reduced mortality (figure 5). Based on the available information, these results are not directly attributed to the deleterious effects of in situ-instilled plasminogen activators on the neurovascular unit. However, consistent results in intraventricular haemorrhage and subarachnoid haemorrhage indicate that tPA could have a pro-inflammatory effect once injected in the ventricles, which might attenuate the beneficial effects of haematoma drainage. Further investigations into the mechanisms underlying this pro-inflammatory effect are needed to design safer thrombolytic strategies. Moreover, refinement of surgical techniques could also improve the efficacy of plasminogen activators to clear intracranial haematoma, especially in patients with intraventricular haemorrhage, in whom greater haematoma clearance is associated with improved functional outcome.

Contributors

All authors contributed equally to literature searches and to drafting and correcting the manuscript.

Declaration of interests

DV holds patents but does not receive royalties on Optimised tPA (licensed US9249406 B2), on Glunomab (issued PCT/WO2014187879) and on the TAFIa/PAI-1 diabody (licensed GB1404879). All other authors declare no competing interests.

Acknowledgments

We thank Thomas Gaberel (Caen University Hospital and INSERM U1237) for sharing his expertise on haemorrhagic stroke.

References

1 Powers WJ, Rabinstein AA, Ackerson T, et al, and the American Heart Association Stroke Council. 2018 guidelines for the early management of patients with acute ischemic stroke: a guideline for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke* 2018; **49:** e46–110.

2 Thomalla G, Simonsen CZ, Boutitie F, et al, and the WAKE-UP Investigators. MRI-guided thrombolysis for stroke with unknown time of onset. *N Engl J Med* 2018; **379:** 611–22.

3 Yepes M, Sandkvist M, Moore EG, Bugge TH, Strickland DK, Lawrence DA. Tissue-type plasminogen activator induces opening of the blood-brain barrier via the LDL receptor-related protein. *J Clin Invest* 2003; **112:** 1533–40.

4 Jeanneret V, Wu F, Merino P, et al. Tissue-type plasminogen activator (tPA) modulates the postsynaptic response of cerebral cortical neurons to the presynaptic release of glutamate. *Front Mol Neurosci* 2016; **9:** 121.

5 Ziai WC, Tuhrim S, Lane K, et al. A multicenter, randomized, double-blinded, placebo-controlled phase III study of Clot Lysis Evaluation of Accelerated Resolution of Intraventricular Hemorrhage (CLEAR III). *Int J Stroke* 2014; **9:** 536–42

6 Appella E, Robinson EA, Ullrich SJ, et al. The receptor-binding sequence of urokinase. A biological function for the growth-factor module of proteases. *J Biol Chem* 1987; **262:** 4437–40.

7 Dent MA, Sumi Y, Morris RJ, Seeley PJ. Urokinase-type plasminogen activator expression by neurons and oligodendrocytes during process outgrowth in developing rat brain. *Eur J Neurosci* 1993; **5:** 633–47.

8 Lahtinen L, Lukasiuk K, Pitkänen A. Increased expression and activity of urokinase-type plasminogen activator during epileptogenesis. *Eur J Neurosci* 2006; **24:** 1935–45.

9 Schmitt M, Goretzki L, Jänicke F, et al. Biological and clinical relevance of the urokinase-type plasminogen activator (uPA) in breast cancer. *Biomed Biochim Acta* 1991; **50:** 731–41.

10 Semina E, Rubina K, Sysoeva V, et al. Urokinase and urokinase receptor participate in regulation of neuronal migration, axon growth and branching. *Eur J Cell Biol* 2016; **95:** 295–310.

11 Diaz A, Merino P, Manrique LG, et al. A cross talk between neuronal urokinase-type plasminogen activator (uPA) and astrocytic uPA receptor (uPAR) promotes astrocytic activation and synaptic recovery in the ischemic brain. *J Neurosci* 2017; **37:** 10310–22.

12 Merino P, Diaz A, Manrique LG, Cheng L, Yepes M. Urokinase-type plasminogen activator (uPA) promotes ezrin-mediated reorganization of the synaptic cytoskeleton in the ischemic brain. *J Biol Chem* 2018; **293:** 9234–47.

13 Persson M, Östling G, Smith G, et al. Soluble urokinase plasminogen activator receptor: a risk factor for carotid plaque, stroke, and coronary artery disease. *Stroke* 2014; **45:** 18–23.

14 Onatsu J, Taina M, Mustonen P, et al. Soluble urokinase-type plasminogen activator receptor predicts all-cause 5-year mortality in ischemic stroke and TIA. *In Vivo* 2017; **31:** 381–86.

15 Nagai N, Okada K, Kawao N, et al. Urokinase-type plasminogen activator receptor (uPAR) augments brain damage in a murine model of ischemic stroke. *Neurosci Lett* 2008; **432:** 46–49.

16 Wu F, Catano M, Echeverry R, et al. Urokinase-type plasminogen activator promotes dendritic spine recovery and improves neurological outcome following ischemic stroke. *J Neurosci* 2014; **34:** 14219–32.

17 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:** 1581–87.

18 Adivitiya, Khasa YP. The evolution of recombinant thrombolytics: current status and future directions. *Bioengineered* 2017; **8:** 331–358.
19 European Myocardial Infarction Project Group. Prehospital thrombolytic therapy in patients with suspected acute myocardial infarction. *N Engl J Med* 1993; **329:** 383–89.
20 Medcalf RL. Fibrinolysis: from blood to the brain. *J Thromb Haemost* 2017; **15:** 2089–98.
21 Rijken DC, Hoylaerts M, Collen D. Fibrinolytic properties of one-chain and two-chain human extrinsic (tissue-type) plasminogen activator. *J Biol Chem* 1982; **257:** 2920–25.
22 Parcq J, Bertrand T, Montagne A, et al. Unveiling an exceptional zymogen: the single-chain form of tPA is a selective activator of NMDA receptor-dependent signaling and neurotoxicity. *Cell Death Differ* 2012; **19:** 1983–91.
23 Angles-Cano E, Balaton A, Le Bonniec B, Genot E, Elion J, Sultan Y. Production of monoclonal antibodies to the high fibrin-affinity, tissue-type plasminogen activator of human plasma. Demonstration of its endothelial origin by immunolocalization. *Blood* 1985; **66:** 913–20.
24 Louessard M, Lacroix A, Martineau M, et al. Tissue plasminogen activator expression is restricted to subsets of excitatory pyramidal glutamatergic neurons. *Mol Neurobiol* 2016; **53:** 5000–12.
25 Correa F, Gauberti M, Parcq J, et al. Tissue plasminogen activator prevents white matter damage following stroke. *J Exp Med* 2011; **208:** 1229–42.
26 Lochner JE, Honigman LS, Grant WF, et al. Activity-dependent release of tissue plasminogen activator from the dendritic spines of hippocampal neurons revealed by live-cell imaging. *J Neurobiol* 2006; **66:** 564–77.
27 Cassé F, Bardou I, Danglot L, et al. Glutamate controls tPA recycling by astrocytes, which in turn influences glutamatergic signals. *J Neurosci* 2012; **32:** 5186–99.
28 Seeds NW, Basham ME, Haffke SP. Neuronal migration is retarded in mice lacking the tissue plasminogen activator gene. *Proc Natl Acad Sci USA* 1999; **96:** 14118–23.
29 Lee SH, Ko HM, Kwon KJ, et al. tPA regulates neurite outgrowth by phosphorylation of LRP5/6 in neural progenitor cells. *Mol Neurobiol* 2014; **49:** 199–215.
30 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.
31 Baranes D, Lederfein D, Huang YY, Chen M, Bailey CH, Kandel ER. Tissue plasminogen activator contributes to the late phase of LTP and to synaptic growth in the hippocampal mossy fiber pathway. *Neuron* 1998; **21:** 813–25.
32 Pang PT, Teng HK, Zaitsev E, et al. Cleavage of proBDNF by tPA/plasmin is essential for long-term hippocampal plasticity. *Science* 2004; **306:** 487–91.
33 Su EJ, Fredriksson L, Schielke GP, Eriksson U, Lawrence DA, and the Su EJ1. Tissue plasminogen activator-mediated PDGF signaling and neurovascular coupling in stroke. *J Thromb Haemost* 2009; **7** (suppl 1)**:** 155–58.
34 Pawlak R, Magarinos AM, Melchor J, McEwen B, Strickland S. Tissue plasminogen activator in the amygdala is critical for stress-induced anxiety-like behavior. *Nat Neurosci* 2003; **6:** 168–74.
35 Wu F, Wu J, Nicholson AD, et al. Tissue-type plasminogen activator regulates the neuronal uptake of glucose in the ischemic brain. *J Neurosci* 2012; **32:** 9848–58.
36 Wu F, Echeverry R, Wu J, et al. Tissue-type plasminogen activator protects neurons from excitotoxin-induced cell death via activation of the ERK1/2-CREB-ATF3 signaling pathway. *Mol Cell Neurosci* 2013; **52:** 9–19.
37 Mantuano E, Lam MS, Gonias SL. LRP1 assembles unique co-receptor systems to initiate cell signaling in response to tissue-type plasminogen activator and myelin-associated glycoprotein. *J Biol Chem* 2013; **288:** 34009–18.
38 Samson AL, Nevin ST, Croucher D, et al. Tissue-type plasminogen activator requires a co-receptor to enhance NMDA receptor function. *J Neurochem* 2008; **107:** 1091–101.
39 Bertrand T, Lesept F, Chevilley A, et al. Conformations of tissue plasminogen activator (tPA) orchestrate neuronal survival by a crosstalk between EGFR and NMDAR. *Cell Death Dis* 2015; **6:** e1924.
40 Echeverry R, Wu J, Haile WB, Guzman J, Yepes M. Tissue-type plasminogen activator is a neuroprotectant in the mouse hippocampus. *J Clin Invest* 2010; **120:** 2194–205.
41 Louessard M, Bardou I, Lemarchand E, et al. Activation of cell surface GRP78 decreases endoplasmic reticulum stress and neuronal death. *Cell Death Differ* 2017; **24:** 1518–29.
42 Tsirka SE, Gualandris A, Amaral DG, Strickland S. Excitotoxin-induced neuronal degeneration and seizure are mediated by tissue plasminogen activator. *Nature* 1995; **377:** 340–44.
43 Matys T, Strickland S. Tissue plasminogen activator and NMDA receptor cleavage. *Nat Med* 2003; **9:** 371–72.
44 Jeanneret V, Ospina JP, Diaz A, et al. Tissue-type plasminogen activator protects the postsynaptic density in the ischemic brain. *J Cereb Blood Flow Metab* 2018; published online March 16. DOI:10.1177/0271678X18764495 (preprint).
45 Zhang L, Zhang ZG, Buller B, et al. Combination treatment with VELCADE and low-dose tissue plasminogen activator provides potent neuroprotection in aged rats after embolic focal ischemia. *Stroke* 2010; **41:** 1001–07.
46 Grummisch JA, Jadavji NM, Smith PD. tPA promotes cortical neuron survival via mTOR-dependent mechanisms. *Mol Cell Neurosci* 2016; **74:** 25–33.
47 Macrez R, Obiang P, Gauberti M, et al. Antibodies preventing the interaction of tissue-type plasminogen activator with N-methyl-D-aspartate receptors reduce stroke damages and extend the therapeutic window of thrombolysis. *Stroke* 2011; **42:** 2315–22.
48 Macrez R, Ortega MC, Bardou I, et al. Neuroendothelial NMDA receptors as therapeutic targets in experimental autoimmune encephalomyelitis. *Brain* 2016; **139:** 2406–19.
49 Alvarez V, Rossetti AO, Papavasileiou V, Michel P. Acute seizures in acute ischemic stroke: does thrombolysis have a role to play? *J Neurol* 2013; **260:** 55–61.
50 Keller L, Hobohm C, Zeynalova S, Classen J, Baum P. Does treatment with t-PA increase the risk of developing epilepsy after stroke? *J Neurol* 2015; **262:** 2364–72.
51 Lekoubou A, Awoumou JJ, Kengne AP. Incidence of seizure in stroke patients treated with recombinant tissue plasminogen activator: a systematic review and meta-analysis. *Int J Stroke* 2017; **12:** 923–31.
52 Leys D, Hommet Y, Jacquet C, et al, and the OPHELIE investigators and the STROKAVENIR network. Proportion of single-chain recombinant tissue plasminogen activator and outcome after stroke. *Neurology* 2016; **87:** 2416–26.
53 Berkhemer OA, Fransen PS, Beumer D, et al, and the MR CLEAN Investigators. A randomized trial of intraarterial treatment for acute ischemic stroke. *N Engl J Med* 2015; **372:** 11–20.
54 Smith WS, Sung G, Saver J, et al. Mechanical thrombectomy for acute ischemic stroke: final results of the Multi MERCI trial. *Stroke* 2008; **39:** 1205–12.
55 Broderick JP, Palesch YY, Demchuk AM, et al. Endovascular therapy after intravenous t-PA versus t-PA alone for stroke. *N Engl J Med* 2013; **368:** 893–903.
56 Ciccone A, Valvassori L. Endovascular treatment for acute ischemic stroke. *N Engl J Med* 2013; **368:** 2433–34.
57 Jovin TG, Chamorro A, Cobo E, et al. Thrombectomy within 8 hours after symptom onset in ischemic stroke. *N Engl J Med* 2015; **372:** 2296–306.
58 Coutinho JM, Liebeskind DS, Slater LA, et al. Combined intravenous thrombolysis and thrombectomy vs thrombectomy alone for acute ischemic stroke: a pooled analysis of the SWIFT and STAR studies. *JAMA Neurol* 2017; **74:** 268–74.
59 Nogueira RG, Jadhav AP, Haussen DC, et al. Thrombectomy 6 to 24 hours after stroke with a mismatch between deficit and infarct. *N Engl J Med* 2018; **378:** 11–21.
60 Wardlaw JM, Murray V, Berge E, del Zoppo GJ. Thrombolysis for acute ischaemic stroke. *Cochrane Database Syst Rev* 2014; **7:** CD000213.
61 Hacke W, Kaste M, Bluhmki E, et al, and the ECASS Investigators. Thrombolysis with alteplase 3 to 4.5 hours after acute ischemic stroke. *N Engl J Med* 2008; **359:** 1317–29.
62 Bhatia R, Hill MD, Shobha N, et al. Low rates of acute recanalization with intravenous recombinant tissue plasminogen activator in ischemic stroke: real-world experience and a call for action. *Stroke* 2010; **41:** 2254–58.
63 Mair G, von Kummer R, Adami A, et al, and the IST-3 Collaborative Group. Arterial Obstruction on Computed Tomographic or Magnetic Resonance Angiography and Response to Intravenous Thrombolytics in Ischemic Stroke. *Stroke* 2017; **48:** 353–60.

64 Riedel CH, Zimmermann P, Jensen-Kondering U, Stingele R, Deuschl G, Jansen O. The importance of size: successful recanalization by intravenous thrombolysis in acute anterior stroke depends on thrombus length. *Stroke* 2011; **42:** 1775–77.

65 Logallo N, Novotny V, Assmus J, et al. Tenecteplase versus alteplase for management of acute ischaemic stroke (NOR-TEST): a phase 3, randomised, open-label, blinded endpoint trial. *Lancet Neurol* 2017; **16:** 781–88.

66 Campbell BCV, Mitchell PJ, Churilov L, et al, and the EXTEND-IA TNK Investigators. Tenecteplase versus Alteplase before Thrombectomy for Ischemic Stroke. *N Engl J Med* 2018; **378:** 1573–82.

67 Wyseure T, Rubio M, Denorme F, et al. Innovative thrombolytic strategy using a heterodimer diabody against TAFI and PAI-1 in mouse models of thrombosis and stroke. *Blood* 2015; **125:** 1325–32.

68 Brouns R, Heylen E, Willemse JL, et al. The decrease in procarboxypeptidase U (TAFI) concentration in acute ischemic stroke correlates with stroke severity, evolution and outcome. *J Thromb Haemost* 2010; **8:** 75–80.

69 Alessi MC, Gaudin C, Grosjean P, et al. Changes in activated thrombin-activatable fibrinolysis inhibitor levels following thrombolytic therapy in ischemic stroke patients correlate with clinical outcome. *Cerebrovasc Dis* 2016; **42:** 404–14.

70 Fernandez-Cadenas I, Alvarez-Sabin J, Ribo M, et al. Influence of thrombin-activatable fibrinolysis inhibitor and plasminogen activator inhibitor-1 gene polymorphisms on tissue-type plasminogen activator-induced recanalization in ischemic stroke patients. *J Thromb Haemost* 2007; **5:** 1862–68.

71 Denorme F, Wyseure T, Peeters M, et al. Inhibition of Thrombin-Activatable Fibrinolysis Inhibitor and Plasminogen Activator Inhibitor-1 Reduces Ischemic Brain Damage in Mice. *Stroke* 2016; **47:** 2419–22.

72 Vaughan DE. PAI-1 antagonists: the promise and the peril. *Trans Am Clin Climatol Assoc* 2011; **122:** 312–25.

73 Zhu H, Fan X, Yu Z, et al. Annexin A2 combined with low-dose tPA improves thrombolytic therapy in a rat model of focal embolic stroke. *J Cereb Blood Flow Metab* 2010; **30:** 1137–46.

74 Choi MH, Park GH, Lee JS, et al. Erythrocyte fraction within retrieved thrombi contributes to thrombolytic response in acute ischemic stroke. *Stroke* 2018; **49:** 652–59.

75 Ducroux C, Di Meglio L, Loyau S, et al. Thrombus neutrophil extracellular traps content impair tPA-Induced thrombolysis in acute ischemic stroke. *Stroke* 2018; **49:** 754–57.

76 Zinkstok SM, Roos YB, and the ARTIS investigators. Early administration of aspirin in patients treated with alteplase for acute ischaemic stroke: a randomised controlled trial. *Lancet* 2012; **380:** 731–37.

77 Adams HP Jr, Effron MB, Torner J, et al. 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* 2008; **39:** 87–99.

78 Le Behot A, Gauberti M, Martinez De Lizarrondo S, et al. GpIbα-VWF blockade restores vessel patency by dissolving platelet aggregates formed under very high shear rate in mice. *Blood* 2014; **123:** 3354–63.

79 Momi S, Tantucci M, Van Roy M, Ulrichts H, Ricci G, Gresele P. Reperfusion of cerebral artery thrombosis by the GPIb-VWF blockade with the Nanobody ALX-0081 reduces brain infarct size in guinea pigs. *Blood* 2013; **121:** 5088–97.

80 Denorme F, Langhauser F, Desender L, et al. ADAMTS13-mediated thrombolysis of t-PA-resistant occlusions in ischemic stroke in mice. *Blood* 2016; **127:** 2337–45.

81 Martinez de Lizarrondo S, Gakuba C, Herbig BA, et al. Potent thrombolytic effect of *N*-Acetylcysteine on arterial thrombi. *Circulation* 2017; **136:** 646–60.

82 Bustamante A, Ning M, García-Berrocoso T, et al. Usefulness of ADAMTS13 to predict response to recanalization therapies in acute ischemic stroke. *Neurology* 2018; **90:** e995–1004.

83 Barreto AD, Ford GA, Shen L, et al, and the ARTSS-2 Investigators. Randomized, multicenter trial of ARTSS-2 (argatroban with recombinant tissue plasminogen activator for acute stroke). *Stroke* 2017; **48:** 1608–16.

84 Wardlaw JM. Overview of Cochrane thrombolysis meta-analysis. *Neurology* 2001; **57:** S69-76.

85 Yaghi S, Willey JZ, Cucchiara B, et al. Treatment and outcome of hemorrhagic transformation after intravenous alteplase in acute ischemic stroke: a scientific statement for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke* 2017; **48:** e343–61.

86 Anderson CS, Robinson T, Lindley RI, et al. Low-dose versus standard-dose intravenous alteplase in acute ischemic stroke. *N Engl J Med* 2016; **374:** 2313–23.

87 Messé SR, Pervez MA, Smith EE, et al. Lipid profile, lipid-lowering medications, and intracerebral hemorrhage after tPA in get with the guidelines-stroke. *Stroke* 2013; **44:** 1354–59.

88 Demchuk AM, Dowlatshahi D, Rodriguez-Luna D, et al. Prediction of haematoma growth and outcome in patients with intracerebral haemorrhage using the CT-angiography spot sign (PREDICT): a prospective observational study *Lancet Neurol* 2012; **11:** 307–14.

89 Fonarow GC, Zhao X, Smith EE, et al. Door-to-needle times for tissue plasminogen activator administration and clinical outcomes in acute ischemic stroke before and after a quality improvement initiative. *JAMA* 2014; **311:** 1632–40.

90 An J, Zhang C, Polavarapu R, Zhang X, Zhang X, Yepes M. Tissue-type plasminogen activator and the low-density lipoprotein receptor-related protein induce Akt phosphorylation in the ischemic brain. *Blood* 2008; **112:** 2787–94.

91 Su EJ, Fredriksson L, Geyer M, et al. Activation of PDGF-CC by tissue plasminogen activator impairs blood-brain barrier integrity during ischemic stroke. *Nat Med* 2008; **14:** 731–37.

92 Rodríguez-González R, Blanco M, Rodríguez-Yáñez M, Moldes O, Castillo J, Sobrino T. Platelet derived growth factor-CC isoform is associated with hemorrhagic transformation in ischemic stroke patients treated with tissue plasminogen activator. *Atherosclerosis* 2013; **226:** 165–71.

93 Wahlgren N, Thorén M, Höjeberg B, et al. Randomized assessment of imatinib in patients with acute ischaemic stroke treated with intravenous thrombolysis. *J Intern Med* 2017; **281:** 273–83.

94 Murata Y, Rosell A, Scannevin RH, Rhodes KJ, Wang X, Lo EH. Extension of the thrombolytic time window with minocycline in experimental stroke. *Stroke* 2008; **39:** 3372–77.

95 Fagan SC, Waller JL, Nichols FT, et al. Minocycline to improve neurologic outcome in stroke (MINOS): a dose-finding study. *Stroke* 2010; **41:** 2283–87.

96 Kohler E, Prentice DA, Bates TR, et al. Intravenous minocycline in acute stroke: a randomized, controlled pilot study and meta-analysis. *Stroke* 2013; **44:** 2493–99.

97 dela Peña IC, Yoo A, Tajiri N, et al. Granulocyte colony-stimulating factor attenuates delayed tPA-induced hemorrhagic transformation in ischemic stroke rats by enhancing angiogenesis and vasculogenesis. *J Cereb Blood Flow Metab* 2015; **35:** 338–46.

98 Gautier S, Ouk T, Tagzirt M, et al. Impact of the neutrophil response to granulocyte colony-stimulating factor on the risk of hemorrhage when used in combination with tissue plasminogen activator during the acute phase of experimental stroke. *J Neuroinflammation* 2014; **11:** 96.

99 Sandercock P, Wardlaw JM, Lindley RI, et al, and the IST-3 collaborative group. The benefits and harms of intravenous thrombolysis with recombinant tissue plasminogen activator within 6 h of acute ischaemic stroke (the third international stroke trial [IST-3]): a randomised controlled trial. *Lancet* 2012; **379:** 2352–63.

100 Lees KR, Bluhmki E, von Kummer R, et al. Time to treatment with intravenous alteplase and outcome in stroke: an updated pooled analysis of ECASS, ATLANTIS, NINDS, and EPITHET trials. *Lancet* 2010; **375:** 1695–703.

101 Paciaroni M, Balucani C, Agnelli G, et al. Systemic thrombolysis in patients with acute ischemic stroke and Internal Carotid ARtery Occlusion: the ICARO study. *Stroke* 2012; **43:** 125–30.

102 Simão F, Ustunkaya T, Clermont AC, Feener EP. Plasma kallikrein mediates brain hemorrhage and edema caused by tissue plasminogen activator therapy in mice after stroke. *Blood* 2017; **129:** 2280–90.

103 Marcos-Contreras OA, Martinez de Lizarrondo S, Bardou I, et al. Hyperfibrinolysis increases blood-brain barrier permeability by a plasmin- and bradykinin-dependent mechanism. *Blood* 2016; **128:** 2423–34.

104 Gauberti M, Potzeha F, Vivien D, Martinez de Lizarrondo S. Impact of Bradykinin Generation During Thrombolysis in Ischemic Stroke. *Front Med (Lausanne)* 2018; **5:** 195.

105 Sonni S, Lioutas VA, Selim MH. New avenues for treatment of intracranial hemorrhage. *Curr Treat Options Cardiovasc Med* 2014; **16:** 277.

106 Prasad K, Mendelow AD, Gregson B. Surgery for primary supratentorial intracerebral haemorrhage. *Cochrane Database Syst Rev* 2008; (4): CD000200.

107 Hanley DF, Thompson RE, Muschelli J, et al, and the MISTIE Investigators. Safety and efficacy of minimally invasive surgery plus alteplase in intracerebral haemorrhage evacuation (MISTIE): a randomised, controlled, open-label, phase 2 trial. *Lancet Neurol* 2016; **15:** 1228–37.

108 Rohde V, Rohde I, Thiex R, et al. Fibrinolysis therapy achieved with tissue plasminogen activator and aspiration of the liquefied clot after experimental intracerebral hemorrhage: rapid reduction in hematoma volume but intensification of delayed edema formation. *J Neurosurg* 2002; **97:** 954–62.

109 Thiex R, Weis J, Krings T, et al. Addition of intravenous N-methyl-D-aspartate receptor antagonists to local fibrinolytic therapy for the optimal treatment of experimental intracerebral hemorrhages. *J Neurosurg* 2007; **106:** 314–20.

110 Keric N, Maier GS, Samadani U, et al. Tissue plasminogen activator induced delayed edema in experimental porcine intracranial hemorrhage: reduction with plasminogen activator inhibitor-1 administration. *Transl Stroke Res* 2012; **3:** 88–93.

111 Goulay R, Naveau M, Gaberel T, Vivien D, Parcq J. Optimised tPA: a non-neurotoxic fibrinolytic agent for the drainage of intracerebral hemorrhages. *J Cereb Blood Flow Metab* 2018; **38:** 1180–89.

112 Mould WA, Carhuapoma JR, Muschelli J, et al, and the MISTIE Investigators. Minimally invasive surgery plus recombinant tissue-type plasminogen activator for intracerebral hemorrhage evacuation decreases perihematomal edema. *Stroke* 2013; **44:** 627–34.

113 Li Y, Yang R, Li Z, et al. Urokinase vs Tissue-Type Plasminogen Activator for Thrombolytic Evacuation of Spontaneous Intracerebral Hemorrhage in Basal Ganglia. *Front Neurol* 2017; **8:** 371.

114 Gaberel T, Montagne A, Lesept F, et al. Urokinase versus for intraventricular hemorrhage fibrinolysis. *Neuropharmacology* 2014; **85:** 158–65.

115 Gaberel T, Magheru C, Parienti JJ, Huttner HB, Vivien D, Emery E. Intraventricular fibrinolysis versus external ventricular drainage alone in intraventricular hemorrhage: a meta-analysis. *Stroke* 2011; **42:** 2776–81.

116 Naff N, Williams MA, Keyl PM, et al. Low-dose recombinant tissue-type plasminogen activator enhances clot resolution in brain hemorrhage: the intraventricular hemorrhage thrombolysis trial. *Stroke* 2011; **42:** 3009–16.

117 Hanley DF, Lane K, McBee N, et al, and the CLEAR III Investigators. Thrombolytic removal of intraventricular haemorrhage in treatment of severe stroke: results of the randomised, multicentre, multiregion, placebo-controlled CLEAR III trial. *Lancet* 2017; **389:** 603–11.

118 Staykov D, Kuramatsu JB, Bardutzky J, et al. Efficacy and safety of combined intraventricular fibrinolysis with lumbar drainage for prevention of permanent shunt dependency after intracerebral hemorrhage with severe ventricular involvement: a randomized trial and individual patient data meta-analysis. *Ann Neurol* 2017; **81:** 93–103.

119 Fam MD, Zeineddine HA, Eliyas JK, et al. CSF inflammatory response after intraventricular hemorrhage. *Neurology* 2017; **89:** 1553–60.

120 Amin-Hanjani S, Ogilvy CS, Barker FG 2nd. Does intracisternal thrombolysis prevent vasospasm after aneurysmal subarachnoid hemorrhage? A meta-analysis. *Neurosurgery* 2004; **54:** 326–34, discussion 334–35.

121 Etminan N, Beseoglu K, Eicker SO, Turowski B, Steiger HJ, Hänggi D. Prospective, randomized, open-label phase II trial on concomitant intraventricular fibrinolysis and low-frequency rotation after severe subarachnoid hemorrhage. *Stroke* 2013; **44:** 2162–68.

122 Kramer AH, Jenne CN, Zygun DA, et al. Intraventricular fibrinolysis with tissue plasminogen activator is associated with transient cerebrospinal fluid inflammation: a randomized controlled trial. *J Cereb Blood Flow Metab* 2015; **35:** 1241–48.