Urokinase Plasminogen Activator: A Potential Thrombolytic Agent for Ischaemic Stroke

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Abstract

Stroke continues to be one of the leading causes of mortality and morbidity worldwide. Restoration of cerebral blood flow by recombinant plasminogen activator (rtPA) with or without mechanical thrombectomy is considered the most effective therapy for rescuing brain tissue from ischaemic damage, but this requires advanced facilities and highly skilled professionals, entailing high costs, thus in resource-limited contexts urokinase plasminogen activator (uPA) is commonly used as an alternative. This literature review summarises the existing studies relating to the potential clinical application of uPA in ischaemic stroke patients. In translational studies of ischaemic stroke, uPA has been shown to promote nerve regeneration and reduce infarct volume and neurological deficits. Clinical trials employing uPA as a thrombolytic agent have replicated these favourable outcomes and reported consistent increases in recanalisation, functional improvement, and cerebral haemorrhage rates, similar to those observed with rtPA. Single-chain zymogen pro-urokinase (pro-uPA) and rtPA appear to be complementary and synergistic in their action, suggesting that their co-administration may improve the efficacy of thrombolysis without affecting the overall risk of haemorrhage. Large clinical trials examining the efficacy of uPA or the combination of pro-uPA and rtPA are desperately required to unravel whether either therapeutic approach may be a safe first-line treatment option for patients with ischaemic stroke. In light of the existing limited data, thrombolysis with uPA appears to be a potential alternative to rtPA-mediated reperfusive treatment due to its beneficial effects on the promotion of revascularisation and nerve regeneration.

Keywords: urokinase plasminogen activator; ischaemic injury; reperfusive therapy; stroke; rtPA; thrombolysis

1. Introduction

Stroke is defined as an acute neurological dysfunction lasting more than 24 hours, attributed to cerebrovascular aetiology. In general, it is classified into ischaemic and haemorrhagic types. Ischaemic stroke is a focal cerebral infarction causing an episode of neurological deterioration, which represents around 85% of stroke incidents. Haemorrhagic stroke is defined as the rapid development of neurological dysfunction, attributed to focal collection of blood in brain parenchyma or ventricular system that is not caused by trauma, which accounts for the remaining 15% of strokes (Hisham and Bayraktutan 2013; Sacco et al. 2013).

The latest Global Burden Disease (GBD) data showed that stroke is the third leading cause of death and disability worldwide (Kyu et al. 2018; Roth 2018). In the UK, more than 100,000 people suffer from stroke each year, and nearly two-thirds of stroke survivors leave hospital with a disability (Stroke Association 2018). In addition, stroke costs almost £9 billion every year in health and societal care, accounting for about 5% of net UK National Health Service costs (Saka et al. 2009).

Recombinant tissue plasminogen activator (rtPA) has been approved by US Food and Drug Administration (FDA) and European Medicines Agency (EMEA) as the only thrombolytic drug that can be used in ischaemic stroke. However, due to high costs, extremely short therapeutic window (<4.5 hours), and increased risk of haemorrhage beyond this time point, less than 2% of all ischaemic stroke patients globally benefit from this drug (Hacke et al. 2008; Powers et al. 2018). Recently, mechanical thrombectomy has made its way into clinical practice and extended the therapeutic time window for reperfusion therapy (Turc et al. 2019). However, mechanical thrombectomy is only effective in ischaemic stroke stemming from large vessel occlusion and can only be performed in clinical units equipped with advanced facilities, technical resources, and highly specialised skills in neurology, interventional neuroradiology,

and anaesthesiology. Consequently, only very limited number of patients receive this treatment (Chia et al. 2016; Leischner et al. 2019).

In contrast to rtPA, urokinase plasminogen activator (uPA) has received considerably less attention in stroke medicine due to its lack of specificity for fibrin, and associated with high risk for haemorrhage (Adivitiya and Khasa 2017). Even so, a limited number of studies performed with uPA have reported marked improvements in recanalisation and neurological outcomes similar to those achieved with treatments with rtPA (Ogawa et al. 2007; Wang et al. 2017). Furthermore, as uPA is significantly cheaper than rtPA, it is widely used in developing countries as the mainstay thrombolytic agent for acute ischaemic stroke (Dong et al. 2017; Lee et al. 2012; Misra et al. 2011). Therefore, further investigation is urgently needed to reveal the actual benefit and safety profile of uPA. If uPA is beneficial and safe for ischaemic stroke patients, its use should be disseminated and promoted worldwide. Conversely, if it is harmful, the current application of uPA as a cheap stop-gap in resource-scarce healthcare systems should be stopped.

1.1. Thrombus formation

Thrombus formation involves a highly complex process, including breach of endothelial cells, activation of platelets, and the coagulation system. In response to endothelial injury, collagen and tissue factor are exposed to blood flow (Figure 1). Exposed collagen binds to circulating von Willebrand factor (vWF), which subsequently binds to platelet through GP1b-IX-V receptor, leading to the formation of the collagen-vWF-GP1b-IX-V axis, a key step for the initial attachment of platelets (Furie and Furie 2008). However, this axis does not generate stable platelet adhesion, rather it decreases the velocity of the flowing platelets. Consequently, this initial platelet adhesion provides specific collagen receptors on the platelet surface (Glycoprotein VI, GPVI), which induce cellular activation and subsequent release of various platelet agonists, notably thromboxane (T_xA_2) and adenosine diphosphate (ADP), which then

generate thrombin (Nieswandt et al. 2011). Thrombin in turn activates protease-activated protein (PAR) to further activate platelets (De Candia 2012). Platelet activation signalling events trigger functional upregulation of GPIIb/IIIa, which subsequently mediates mass aggregation of platelets through binding to fibrinogen and vWF, thereby cross-linking the activated platelet and stabilising the growing platelet plug (Thomas and Storey 2015).

Alongside this, tissue factor induced by endothelial cell injury triggers a secondary pathway whereby tissue factor binds to circulating FVIIa, forming tissue factor-FVIIa complex (also called binary complex), which in turn activates FX to FXa, whereby it converts FV into FVa. In the presence of Ca^{2+}, FVa and FXa convert prothrombin to thrombin. As mentioned above, thrombin plays a central step in thrombus formation by stabilising the platelet plugs and by converting fibrinogen to fibrin monomers (Chapin and Hajjar 2015; Riddel et al. 2007).

1.2. Thrombolytics

Since thrombolytics degrade cross-links between fibrin networks and restore blood flow to penumbra, they represent the primary drug option for ischaemic stroke patients (Ramos-Cabrer et al. 2011). According to their fibrin specificity, thrombolytics are divided into two categories, namely fibrin-specific agents, which prevent systemic fibrinolysis; and nonfibrin-specific drugs. Table 1 summarises the characteristics of such drugs.

2. Urokinase-plasminogen activator

uPA is secreted as an inactive single chain precursor (pro-uPA) with high fibrin specificity. Proteolytic cleavage by plasmin or plasma kallikrein at Lys158-Ile159 peptide bond converts pro-uPA into high molecular weight uPA (54kDa) which in turn is converted to low molecular weight uPA (32kDa) by sustained proteolytic activity by plasmin (Figure 1) (Poliakov et al. 2001). Once generated, uPA, a member of the serine protease protein family, converts plasminogen into plasmin by binding to its receptor (uPAR) on the cell surface (Blasi and

Carmeliet 2002). Other than being instrumental in plasminogen activation, the uPA-uPAR binding also promotes the degradation of the extracellular matrix, a major event that underlies tumour progression and metastasis (Mahmood et al. 2018). Recent studies proving soluble uPAR as an important independent predictor for cardiovascular events in patients with first AMI and all-cause mortality at five-year after ischaemic strokes, indicating the importance of this particular receptor and the overall uPA activity in ischaemic vascular events (Onatsu et al. 2017; Wlazel et al. 2019).

In contrast to tPA, uPA has lower affinity for plasminogen and does not require fibrin as a cofactor. Similar to tPA, uPA is rapidly neutralised in circulation by plasminogen activator inhibitors (Chapin and Hajjar 2015). It is noteworthy that pro-uPA can directly activate plasminogen without being transformed into uPA (Pannell and Gurewich 1986). However, as plasmin-mediated conversion of pro-uPA to uPA negates its fibrin-specificity, a single site mutant (M5) of native pro-uPA, resistant to conversion into uPA, has been designed (Tomasi et al. 2011).

2.1. uPA in neuroreparation

Incomplete neurological recovery of uPA- ($uPA^{-/-}$) or uPAR- ($uPAR^{-/-}$) deficient mice subjected to middle cerebral artery occlusion (MCAo) confirms the pivotal role of uPA-uPAR pathway in neuroreparation. Restoration of neurological recovery by immediate intra-arterial administration of recombinant uPA to mice deficient in $uPA^{-/-}$, but not $uPAR^{-/-}$, indicates that the presence (and therefore binding) of uPA to uPAR is a prerequisite for functional improvement after a cerebral ischaemic injury (Wu et al. 2014). In support of this notion, uPA-uPAR binding has been coupled to successful repair of damage to actin cytoskeleton in post-synaptic compartment, and therefore is implicated in the recovery of synaptic contacts destructed by ischaemic stroke (Merino et al. 2018). In addition to this, attribution of synaptic recovery in ischaemic brain to a crosstalk between neuronal uPA and astrocytic uPAR has

further corroborated the reparative role of uPA-uPAR pathway (Diaz et al. 2017). Enhanced expression of uPAR and its association with uPA in injured axons has been shown to promote axonal regeneration, by triggering membrane recruitment and activation of β1 integrin through a mechanisms involving small GTPase Rac1, suggesting that the uPA-uPAR-Rac1 system may be a potential target for induction of axonal recovery in ischaemic stroke (Merino et al. 2017; Semina et al. 2016).

Contrary to these restorative effects, increased activity of uPA in various experimental settings mimicking ischaemic stroke appears to impair the integrity and function of an *in vitro* model of human blood-brain barrier (BBB). Since BBB disruption represents the main cause of death within the first week after stroke (Giraud et al. 2015; Jha 2003), its association with uPA-uPAR system is of high clinical importance (Lasek-Bal et al. 2019). To this end, increased availability and activation of calcium and protein kinase C-α have been shown to account for ischaemia-induced uPA release, which in turn evokes BBB dysfunction through cytoskeletal reorganisation of BBB-forming cells and concomitant activations of NADPH oxidase and MMP-2 (a basement membrane-degrading enzyme). Changes in cellular architecture induced by neuroinflammatory cytokine TNF-α or agents that rearrange actin- or tubulin-based cytoskeleton have also increased uPA and uPAR expression in other cell lines (Abdullah and Bayraktutan 2014; Abdullah and Bayraktutan 2016; Bayraktutan and Jones 1995). Although this subject remains to be explored, the concentration of uPA may determine the nature of response (i.e., restorative vs. disruptive) subsequently attained.

2.2. Translational studies

Treatment with uPA has shown promising results in various animal models of ischaemic stroke, including MCAo baboon and cynomolgus monkey model of thromboembolic stroke, as evidenced by significant improvements in functional outcome and reductions in infarct

volumes compared to placebo-treated animals (Del Zoppo et al. 1986; Susumu et al. 2006). As suggested above, the dose of uPA appears to play an important role in its response, in that while improving functional outcome, higher doses of uPA also enhance the risk of intracranial haemorrhage (ICH). Lower doses of uPA, on the other hand, reduced the occurrence of ICH while presenting markedly attenuated (albeit still significant) neurological improvements, compared to placebo-treated animals (Shuaib et al. 1998). In addition to these findings, the presence of uPA in animal model of myocardial infarction and hind limb muscle enhanced angiogenesis, resulting in increased blood flow and tissue protection (Traktuev et al. 2007). Similar to uPA, intravenous administration of recombinant pro-uPA to animal models of ischaemic stroke also dramatically improved neurological function, comparable to the effects generated by rtPA (Hao et al. 2018a; Hao et al. 2018b).

2.3. Clinical trials

The majority of clinical trials using uPA for ischaemic stroke are non-randomised controlled trials (i.e., single-arm trials), which inevitably cannot attribute the variations observed in recanalisation rate, neurological improvement, and incidence of haemorrhagic transformation to treatments with uPA, placebo, or natural history of the disease. This wide variation is most likely due to factors including the dose of uPA, time window, outcome assessment grading system, route of administration, application of mechanical thrombectomy, and differences in patients' baseline demographic and neurological characteristics (e.g., age and neurological status at admission). For example, mechanical thrombectomy followed by administration of uPA to patients within six hours of symptom onset led to successful recanalisation of patients (94.7%) and improved functional outcome (57.9%). Conversely, application of uPA within 7.5 hours of symptom onset without mechanical thrombectomy led to less than half the sample being recanalised (Table 2) (Jahan et al. 1999; Kim et al. 2008).

Intra-arterial administration of recombinant pro-uPA to ischaemic stroke patients within the first six hours of stroke symptoms in Prolyse in Acute Cerebral Thromboembolism II (PROACT II) trial resulted in 40% of patients being classified with a good functional outcome (mRS 0-2) at day 90, whereupon the incidence of symptomatic ICH was not statistically significant between treatment and control group (Furlan et al. 1999). This result not only showed the benefit of recombinant pro-uPA, but also raised the hopes of extending the time window from 4.5 to 6 hours. Despite the premature cessation of uPA due to the approval of rtPA as the only thrombolytic drug for ischaemic stroke in Japan, Middle Cerebral Artery Embolism Local Fibrinolytic Intervention Trial (MELT) also reported excellent functional outcome (mRS 0-1) in uPA-treated versus control group. Good functional outcome was still more frequent in the treatment arm compared to control group, although this did not reach statistical significance (Ogawa et al. 2007). In addition, a recent single-centre prospective study revealed that intra-arterial administration of uPA also demonstrated an enormous advantage for patients with small branches occlusion, in which the uPA group had higher score of National Institute of Health Stroke Scale (NIHSS) on the second day and more frequent good functional outcome after 3 months with no increase in rates of ICH. This study is remarkably important as intra-arterial reperfusion known to be effective only for large vessel occlusion (Shang et al. 2018).

A meta-analysis involving PROACT, PROACT II, and MELT trials also confirmed that intra-arterial application of either uPA or pro-uPA is safe and effective in ischaemic stroke patients, despite increasing the risk of symptomatic ICH (Fields et al. 2011). An ongoing phase III clinical trial investigating the therapeutic efficacy of pro-uPA within 4.5-6 hours (NCT03578822) may add further weight to this finding. However, rtPA, at present, remains the only FDA-approved agent for ischaemic stroke.

2.4. Pro-uPA/uPA versus rtPA

The first study to compare rtPA and uPA in ischaemic stroke patients showed comparable effectivity, safety, and recanalisation rates (Terashi et al. 1990). A subsequent comparative analysis of pro-uPA and rtPA in patients with acute myocardial infarction (AMI) demonstrated similar rates of patency, reocclusion, and ICH between both treatment groups (Bar et al. 1997).

Although a retrospective study of patients with vertebrobasilar occlusion showed that rtPA is more effective in achieving complete recanalisation compared to uPA, the incidence of haemorrhage also appeared to be higher in the rtPA group. It is noteworthy that the higher dosages of rtPA and greater volume of thrombus in uPA group may contribute to these results (Schulte-Altedorneburg et al. 2007). A recent clinical trial comparing IV alteplase, IV uPA, and interventional uPA (i.e., uPA injected to the specific vascular occlusion area via a microcatheter) also reported insignificant differences in recanalisation, good prognosis, and mortality rates among the three approaches (Wang et al. 2017). Comparative investigation of the benefits attained by treatments with pro-uPA, uPA, and rtPA within 4.5 hours is being undertaken in a cohort study (NCT02854592, estimated sample size: 4000) and a phase III randomised controlled trial (NCT03541668, estimated sample size: 680).

2.5. Synergism of pro-uPA and rtPA

Increasing evidence suggests that pro-uPA and tPA may be complementary and synergistic in their action, owing to their functional distinction, where tPA acts as an initiator of thrombolysis while pro-uPA finishes it (Gurewich 2016). Indeed, once a thrombus is generated, tPA catalyses the conversion of plasminogen to plasmin, which ultimately facilitates fibrin dissolution, and consequently generates two new plasminogen binding sites on degraded fibrin (Oliver et al. 2005). The first of these, a triple carboxy-terminal lysine binding site on the E-domain of degraded fibrin, promotes a conformational change in plasminogen and allows pro-uPA to execute its fibrinolytic effect on degraded fibrin, leading to the conversion of pro-uPA

to uPA by plasmin. uPA then dissolves the remaining fibrin-bound plasminogen and completes the fibrinolysis (Gurewich 2016; Petersen 1997).

The beneficial effect of this combinatory strategy was evident in the PATENT trial, in which over 80% of patients with AMI exhibited complete coronary artery patency within 24 hours after receiving a small bolus of tPA (5mg, 5% of the monotherapy dose), followed by a reduced dose of pro-uPA infusion (40mg/h for 90 minutes, 50% of the monotherapy infusion rate) (Zarich et al. 1995). Interestingly, the patency rate was ~45% in the optimum rtPA trial, GUSTO. The mortality rates for PATENT and GUSTO were 1% and 6.3%, respectively (Zarich et al. 1995) . Although the PATENT trial presented sophisticated results, combination therapy was not approved as the standard treatment, as no follow-up study was provided.

In case of ischaemic stroke, the efficacy of combining pro-uPA and rtPA is only recently being studied in the DUMAS phase II multicentre randomised controlled trial.

3. Future directions

Despite increasing the risk of haemorrhage, uPA is widely used as the main thrombolytic drug for ischaemic stroke in several countries, due particularly to its low cost (Dong et al. 2017; Kleindorfer et al. 2017; Lee et al. 2012; Misra et al. 2011). However, at present there is insufficient evidence for the efficacy and the safety profile of this therapy, necessitating larger clinical trials to support or dismiss the application of uPA for ischaemic stroke. MELT trial remains the largest clinical trial documenting the safety and efficacy of uPA, but in interpreting its results, it is important to remember that patients with relatively mild stroke (median NIHSS=14) who received mechanical thrombectomy and/or thrombolytic therapy during an earlier time window (median time=3.8 hours) were included in this study (Ogawa et al. 2007). It is equally important to remember that rtPA and pro-uPA are complementary and synergistic in their fibrinolytic action, and their simultaneous application at lower doses is more effective

in recanalising occluded vessels in AMI patients than monotherapy, without increasing the risk of haemorrhage (Zarich et al. 1995). If replicated, this may be supported as the first-line therapeutic option for ischaemic stroke patients. Nevertheless, the disruptive effect of uPA on cerebral barrier integrity necessitate uPA dose-rate response investigation and should be considered in future trials (Abdullah and Bayraktutan 2016; Rakkar et al. 2014).

4. Conclusion

Pre-clinical studies unanimously confirm that, similar to rtPA, uPA degrades fibrin cross-link network and restores blood flow. Recent evidence shows that activation of uPA/uPAR system contributes to post-ischaemic nerve regeneration through restoration of actin cytoskeleton, recovery of post-synaptic compartment, and promotion of axonal regeneration. While administration of uPA has been shown to reduce infarct volume and neurological deficit in clinical settings, it also appeared to elevate haemorrhagic transformation, a set of findings documented with rtPA. Hence, comparable safety profiles and functional clinical outcomes have been reported in studies employing rtPA and uPA after ischaemic strokes.

Despite increases in data revealing the benefits obtained with uPA treatment, health authorities in developed countries are reluctant to consider it as an alternative thrombolytic agent to rtPA. However, in developing countries, uPA continues to be used as an alternative agent for ischaemic stroke. Therefore, large-scale clinical trials are urgently needed to investigate whether uPA is safe and of clinical benefit. If that is the case, the health authorities should perhaps consider its use as an alternative around the world enabling an increasing proportion of patients receiving early thrombolysis. If not, the current application of uPA as a cheap stop-gap in resource-scarce healthcare systems ought to be proscribed.

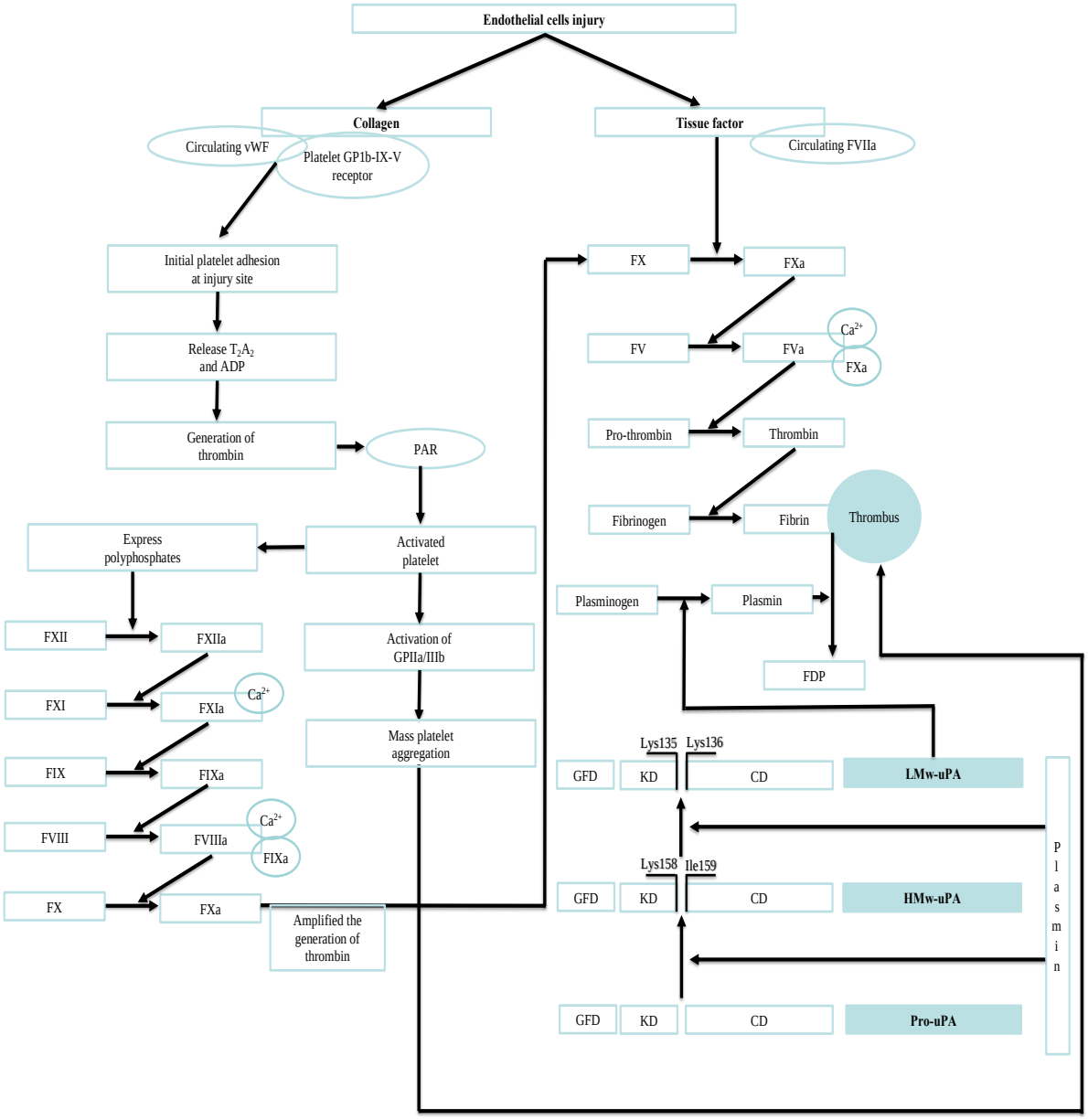

Figure 1. Simplified scheme of thrombus formation and mechanism of action of uPA.

vWF: von Willebrand factor; uPA: urokinase plasminogen activator; pro-uPA: pro-urokinase plasminogen activator; GP1b-IX-V: glycoprotein 1b-IX-V; GPVI: glycoprotein VI; PAR: protease-activated receptor; Ca^{2+}: calcium; PAI: plasminogen activator inhibitor; FDP: fibrin degradation product; GFD: growth factor domain; KD: kringle domain; CD: catalytic serine protease domain; HMw-uPA: high molecular weight uPA; LMw-uPA: low molecular weight uPA.

Table 1. The characteristics of various thrombolytic agents.

	Streptokinase	**Urokinase**	**Pro-urokinase**	**tPA**
Molecular weight (kDA)	47	HMw: 54 LMw: 32	55	70
Fibrin-specific	No	No	Yes	Yes
Production	Streptococcal bacteria	Urine or embryonic kidney cell	Recombinant technology; mammalian cell	Recombinant technology
Half-life (min)	18	6-18	3-6	6
Cost	Low	Moderate	Not known	High
Immunogenicity	Yes	No	No	No
Fibrinogenolytic	Very high	Moderate	Not even at high concentration	Only at high concentration

uPA: urokinase plasminogen activator; pro-uPA: pro-urokinase plasminogen activator; tPA: tissue plasminogen activator; kDA: kilo dalton; HMw: high molecular weight; LMw; low molecular weight.

Table 2. Clinical trials using uPA (*) or pro-uPA (**) as potential therapeutic in ischaemic stroke patients.

Principal Investigator (study name where available)	**Study Design**	**Key Findings**
Mori et al. (1988)*	○ 22 patients ○ Time window: <12 hours ○ Single-arm trial ○ Mechanical disruption: prohibited	○ Immediate recanalisation – 45% of patients, associated with decreased infarct volume ○ Symptomatic ICH – 18% of patients
Jahan et al. (1999)*	○ 21 patients ○ Time window: <7.5 hours ○ Single-arm trial ○ Mechanical disruption: prohibited	○ Successful recanalisation – 42% of patients ○ Good functional outcome – 48% of patients. ○ Symptomatic ICH – 38% of patients.
Gönner et al. (1998)*	○ 43 patients ○ Time window: 6 hours ○ Single-arm trial ○ Mechanical disruption: allowed	○ Favourable functional outcomes on day 90 – 61% of patients ○ Symptomatic haemorrhagic – 4.7% of patients
PROACT (del Zoppo et al. 1998)**	○ 40 patients ○ Time window: <6 hours ○ Randomised-controlled trial ○ Mechanical disruption: prohibited	○ Higher rates of partial and complete recanalisation and haemorrhagic transformation in recombinant pro-uPA vs placebo group

Principal Investigator (study name where available)	Study Design	Key Findings
PROACT II (Furlan et al. 1999)**	○ 180 patients ○ Time window: <6 hours ○ Randomised-controlled trial ○ Mechanical disruption: prohibited	○ Good functional outcome in recombinant pro-uPA versus control group ○ Similar rates of mortality and ICH incidence between the two groups
Arnold et al. (2002)*	○ 100 patients ○ Time window: <6 hours ○ Single-arm trial ○ Mechanical disruption: allowed	○ Successful recanalisation – 76% of patients ○ Good functional outcomes on day 90 – 68% of patients ○ Symptomatic ICH – 7% of patients.
Australian urokinase stroke trial (Macleod et al. 2005)*	○ 16 patients ○ Time window: <24 hours ○ Randomised controlled trial ○ Mechanical disruption: prohibited	○ 4 of 8 patients in the uPA-treated group died ○ 7 of 8 patients in the control group died ○ Good functional outcomes – in all survivors in both groups
Kim et al. (2008)*	○ 19 patients ○ Time window: 3-6 hours ○ Single-arm trial ○ Mechanical disruption: allowed	○ Successful recanalisation – 94.7% of patients ○ Good functional outcome at day 90 – 57.9% of patients ○ symptomatic ICH – 10.5% of patients

Principal Investigator (study name where available)	Study Design	Key Findings
MELT (Ogawa et al. 2007)*	○ 114 patients ○ Time window: <6 hours ○ Randomised controlled trial ○ Mechanical disruption: allowed	○ Better functional outcome at day 90 in uPA versus control group ○ Higher incidence of haemorrhagic transformation in uPA group
Chang et al. (2010)*	○ 25 patients ○ Time window: <6 hours ○ Single-arm trial ○ Mechanical disruption: allowed	○ Successful recanalisation – 68% of patients ○ Favourable outcome (mRS 0-3) – 36% of patients ○ Symptomatic ICH – 12% of patients

uPA: urokinase plasminogen activator; pro-uPA: pro-urokinase plasminogen activator; ICH: intracranial haemorrhage; IV: intravenous; mRS: modified Rankin scale.

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