Thrombolytics in Acute Ischaemic Stroke: Historical Perspective and Future Opportunities

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Key Words

Thrombolytics · Ischaemic stroke · Tenecteplase · Desmoteplase

Abstract

The discovery of thrombolytic agents goes back to the 1930s, when it was shown that substances derived from bacteria (streptokinase, staphylokinase), tissue (fibrinokinase), urine (urokinase) or bat saliva could activate the fibrinolytic system. The potential to treat arterial thrombosis with plasmin was recognized, but it was not until 1958 that its first use in acute ischaemic stroke (AIS) was described. However, since computer tomography (CT) was not available until the mid 1970s, optimal selection of patients was not possible. Early studies with streptokinase in AIS showed an increased risk of intracranial haemorrhage and lack of efficacy, which was associated with low fibrin specificity. The search for new agents with a better risk-benefit profile continued until 1979 when tissue plasminogen activator (t-PA) was discovered. In 1983 it became possible to produce recombinant t-PA (rt-PA) by expression of a cloned gene which enabled clinical trials to be started, mainly for coronary thrombolysis. In 1995, the National Institute of Neurological Disorders and Stroke study

showed that rt-PA was an effective treatment for AIS, nowadays for use up to 4.5 h after onset. However, rt-PA still often fails in achieving rapid reperfusion, has relatively low recanalization rates and is associated with an increased bleeding risk. Several attempts have been made to develop thrombolytics with a better risk-benefit profile than rt-PA, but no real impact on clinical practice was observed. In 1994, it was shown that tenecteplase (rt-PA-TNK) had a higher fibrin specificity than rt-PA, but its clinical use in AIS was described only in 2005. The recently reported results of a small phase 2B trial showed significantly better reperfusion and clinical outcome with rt-PA-TNK compared to rt-PA; patients were selected by CT perfusion and angiography, and treated within 6 h after stroke onset. Currently, a phase 3 trial of rt-PA-TNK versus rt-PA is being planned in patients at an onset up to 4.5 h. The most fibrin-specific recombinant plasminogen activator desmoteplase originates from 1991, and its clinical development in AIS started in 2005. Desmoteplase is in phase 3 development for the treatment of AIS between 3 and 9 h after onset in AIS patients presenting with occlusion or high-grade stenosis.

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Introduction

Stroke is a major cause of death and morbidity [1]. More than 80% of the strokes are acute ischaemic strokes (AIS) requiring quick restoration of the blood flow to brain areas blocked by a vessel occlusion. Although thrombolytic agents were identified in the 1930s, their development in AIS was hampered by the lack of diagnostic opportunities to exclude intracerebral haemorrhage (ICH), and their high risk of haemorrhagic complications and low efficacy. However, these early studies triggered interest in developing fibrin-specific agents for thrombolytic therapy [2]. Around 1980, tissue plasminogen activator (t-PA) was purified by Rijken and Collen [3] and Collen and Lijnen [4].

Recombinant t-PA (rt-PA) was approved for AIS in 1996 after the positive National Institute of Neurological Disorders and Stroke (NINDS)-2 trial, and it is still the only established acute thrombolytic treatment option in AIS [5]. Progress has been made throughout the years in other areas, for example, with the development of better imaging techniques (to visualize ischaemic core and penumbra, to exclude ICH) and the introduction of stroke care units [1]. However, still many patients are left untreated due to the narrow time window of 4.5 h, unknown onset, lack of awareness, and a high number of exclusion criteria for currently approved treatment.

The current unmet need for new thrombolytics with a benefit-risk profile better than that of rt-PA is high. Accelerated effort is needed for the faster development of new compounds which can bring hope to more AIS patients [6]. Especially, in low- and middle-income countries with the greatest burden of stroke, it must also be easy to deliver [7].

Early Development of Thrombolytics

The history of thrombolytic therapy begins in 1933 with the discovery by Tillett and Garner [8] showing that certain strains of *Streptococcus* could dissolve fibrin clots [2, 9]. This finding was in fact through serendipity: streptococci agglutinated in human serum but not in plasma, hypothesizing the role of fibrinogen [10]. However, it lasted until 1955 when Tillett et al. [11] reported on the clinical effectiveness of streptokinase (SK) in patients with extracranial occluding vascular thrombi. In the 1940s, Macfarlane and Pilling [12] described the fibrinolytic potential of human urine, leading to extraction of urokinase (UK), a potent activator of plasminogen to

Fig. 1. The first publication with a thrombolytic agent in stroke (JAMA, 1958) [14]. Reproduced with permission by the publisher.

form plasmin. Staphylokinase was isolated by Lack [13] from *Staphylococcus* strains. In 1958, Sussman and Fitch [14] were the first to report the use of a thrombolytic agent for AIS. They treated 3 patients with intravenous plasmin (fibrinolysin) daily for 4–6 days; 1 patient showed clinical improvement (fig. 1).

In early studies of thrombolytic therapy for AIS using either SK or UK, ICH was a leading cause of death, and no clear benefit was seen [15]. The risk of ICH and lack of efficacy in these studies partially reflected the limitations in study design and technology. For these reasons, thrombolytic therapy for AIS was abandoned since a proper diagnosis could not be made.

A limited number of pilot studies were performed using angiography as a diagnostic method. A placebo-controlled pilot study was conducted in 1963 by Meyer et al. [16] administering intravenous plasmin or placebo over 4 h daily for 3 days in 40 patients with middle cerebral artery occlusion. No differences between treatment groups were observed. A subsequent trial in 73 patients with progressive stroke of SK plus heparin showed a higher mortality and ICH rate than heparin alone [17]. It should be noted that computed tomography (CT) was not available until the mid 1970s; thus, ICH could already have been present upon admission. However, the MAST-I and MAST-E trials performed in the mid 1990s con-

firmed a high ICH rate on SK, and it was subsequently abandoned for the treatment of AIS [18, 19].

Advances in neuro-imaging assessment by CT scanning, combined with expanding knowledge about AIS, led to the initiation of new investigations in the 1980s. Fujishima et al. [20] reported on 143 patients treated with either UK or a combination of UK and dextran sulphate. Clinical improvement and safety of 74% was reported for UK and 84% for the combination. Due to lack of progress, in 1992 (34 years after the first patients being treated by Sussman and Fitch), Wardlaw and Warlow [21] still urged the stroke community to start randomized trials in AIS to answer the simple question: 'Does it work?'

Tissue Plasminogen Activator

t-PA is a naturally occurring fibrinolytic agent produced by endothelial cells. In 1979, it was purified by Colen and coworkers in sufficient amounts from the Bowes melanoma cell line [3, 4]. The first study with t-PA in patients with acute myocardial infarction was performed in 1983, leading to recanalization in 6 out of 7 patients [22]. Its breakthrough came later in that year when rt-PA was obtained by expression of the cloned gene in a mammalian cell system [23]. Large-scale use of rt-PA in acute myocardial infarction started after the publication of the GUSTO-I trial, a study that randomized over 40,000 patients to combinations of SK or rt-PA with heparin [24]. In this trial, rt-PA appeared to be superior to SK.

The results of the first rt-PA trial in AIS were published in 1990 by Terashi et al. [25], who treated 364 patients with either rt-PA or UK. No differences were observed between the two groups. Two pilot studies, sponsored by the NINDS, were performed in 1992 using escalating doses of 0.35–1.08 mg/kg rt-PA in a total of 94 patients and a time window up to 3 h after onset [26, 27]. The subsequent major NINDS-2 trial, published in 1995, showed that 0.9 mg/kg i.v. rt-PA, administered within 3 h of symptom onset to patients with AIS (measurable deficit and CT excluding haemorrhage) resulted in improved clinical outcome at 3 months compared to patients who received placebo, in spite of a risk of symptomatic ICH of 6.4% [5]. In 1996, the Food and Drug Administration approved rt-PA in AIS patients, mainly based on the outcome of the NINDS-2. The safety and efficacy of rt-PA in AIS was further investigated between 1998 and 2008 in the ECASS-1/2/3, ATLANTIS-A/B and EPI-THET trials [28–33], of which only the ECASS-3 showed conclusive benefit. A pooled analysis, performed by Lees

et al. [34] in 2010, showed that rt-PA is moderately beneficial between 3 and 4.5 h, with the greater benefit with earlier treatment. This was confirmed by the findings in a Canadian rt-PA registry [35, 36].

To date, rt-PA is still the only licensed thrombolytic agent for AIS; a dose of 0.9 mg/kg is administered starting with an intravenous bolus of 10% of dose, followed by intravenous infusion of the rest of the dose over 60 min, according to the NINDS study criteria [5]. In 2009, the advice from the American Heart Association Stroke Council was published, recommending that patients can be treated with rt-PA within the time period of 3–4.5 h after onset of ischaemic stroke when additional criteria are taken into account [37]. This advice was mainly based on the results of the ECASS-3 trial [30], the outcome of the pooled analysis performed by Lees et al [34], and the publications from the SITS-ISTR registry [38, 39]. In 2011, these findings led to the approval of rt-PA by the European Medical Agency for its use up to 4.5 h after onset, but still excluded patients older than 80 years. However, there is recent evidence supporting the use of intravenous thrombolysis in patients aged over 80 years as well as in patients with diabetes and prior stroke if they otherwise fulfil treatment criteria [40–43]. The Food and Drug Administration has not extended the license time window beyond 3 h. In Japan, rt-PA (0.6 mg/kg i.v.) was approved in 2005 for use up to 3 h after onset [44], extended to 4.5 h in 2012.

Limitations of Tissue Plasminogen Activator

In clinical practice, rt-PA is not always beneficial, since it often fails in achieving rapid reperfusion, delivers poor recanalization rates in some vessels, and is associated with bleeding risk and potential neurotoxicity [45, 46]. The effect of rt-PA on blood-brain barrier permeability, and its interaction with the N-methyl-D-aspartic acid receptor, may potentially result in its neurotoxic effects [45]. Moreover, its short half-life of about 5 min requires a continuous infusion rather than an intravenous bolus injection [47].

The failure of rt-PA to achieve rapid reperfusion and its risk of bleeding prompted the development of new thrombolytics with greater fibrin specificity and a better benefit-risk profile [48]. Although there is a high unmet need, many efforts to develop an 'improved' thrombolytic have not led to a license for treatment of AIS. Based on their high fibrin selectivity compared to rt-PA, both tenecteplase (rt-PA-TNK) and desmoteplase can be regarded as promising new agents (fig. 2) [49, 50].

Fig. 2. From serendipitous discoveries to fibrin-selective thrombolytic therapy. ^a Rate of plasminogen activation in the presence of fibrin/rate of plasminogen activation in the presence of fibrinogen [estimated for SK and (pro-)UK].

The Search for New Thrombolytic Agents

The PROACT-II trial demonstrated an improved clinical outcome following intra-arterial thrombolysis with pro-UK [51]. However, regulatory authorities did not approve pro-UK for AIS, most probably since it was based on a single trial involving only 180 patients.

Over the past 2 decades, various t-PA variants (reteplase, alteplase, pamiteplase, lanoteplase, altimeprase) were developed without a real impact on clinical practice in AIS [52]. The only variant showing the combination of a higher fibrin selectivity and an extended plasma half-life compared to rt-PA was rt-PA-TNK [47]. Although this phenomenon was already known in 1995 [53], it lasted until 2005 when Haley et al. [54] showed that rt-PA-TNK can safely be given in AIS as an intravenous bolus injection up to 0.4 mg/kg. This trial, funded by the NINDS, stopped prematurely when an increased incidence of symptomatic ICH was observed on 0.5 mg/kg. Parsons et al. [55] compared the safety and efficacy of 0.1 mg/kg i.v. rt-PA-TNK (given 3–6 h after onset) versus 0.9 mg/kg i.v. rt-PA (given <3 h) in a pilot trial. Compared to rt-PA, rt-PA-TNK treatment led to better reperfusion, recanalization and National Institutes of Health Stroke Scale change at 24 h, together with no safety concern [55, 56]. This finding was confirmed in a subsequent phase 2B trial treating patients (selected by advanced CT imaging) within 6 h [57]. A dose of 0.25 mg/kg rt-PA-TNK was found to be superior to rt-PA for efficacy outcomes at both 24 h and 90 days,

and no significant differences in intracerebral bleeding or other safety parameters were observed. The subsequent TASTE phase 3 trial is currently being planned, comparing rt-PA-TNK with rt-PA up to 4.5 h after the onset of AIS.

Already in 1932, it was known that the saliva of the vampire bat leads to interference with the haemostatic mechanism of the host animal [58]. In 1991, one of four plasminogen activators (DSPAα1) that have been isolated from the saliva of the common vampire bat *Desmodus rotundus* became available as its recombinant version desmoteplase [59]. It has a very high fibrin specificity, rendering it attractive for clinical development in patients with AIS [60, 61]. Desmoteplase activity was 105,000 times higher in the presence of fibrin than without, while rt-PA, owing to its intrinsic, fibrin-independent activity, only displayed a 550-fold increase [62]. Evidence of the safety and efficacy of desmoteplase in patients with AIS was obtained in the Dose Escalation study of Desmoteplase in Acute Ischemic Stroke (DEDAS) and Desmoteplase in Acute Ischemic Stroke (DIAS) trials performed in 2005 [63, 64]. The DIAS-2 trial supported the safety profile of desmoteplase but did not replicate the previous positive efficacy findings [65]. A post hoc analysis on DIAS-2 data by Fiebach et al. [66] showed a clinical response in those patients with cerebral artery occlusion or high-grade stenosis (Thrombolysis in Myocardial Infarction, TIMI, 0–1). Applied to the pooled data from DEDAS, DIAS and DIAS-2, this difference was statistically significant (odds ratio 4.1), in contrast to those presenting with only moderate or no stenosis (TIMI 2–3; odds ratio 1.1) [66]. Thus, patients with vessel occlusion or severe stenosis were more likely to benefit from treatment with desmoteplase. These findings provided the basis for the design of the currently ongoing DIAS-3 and DIAS-4 phase 3 clinical trials, aiming to enrol a total of 880 patients with AIS [67]. The objective of DIAS-3 and DIAS-4 is to evaluate the efficacy and safety of a single intravenous bolus of 90 µg/kg desmoteplase given 3–9 h after onset of ischaemic stroke (National Institutes of Health Stroke Scale score 4–24, age 18–85 years).

Time Window for Intravenous Thrombolytic Treatment

In 1976, an admission to hospital within 24 h and initiation of treatment with UK within 36 h was considered appropriate [15]. In 1995, the NINDS-2 trial led to in-

roduction of a 3-hour time window for thrombolysis with rt-PA [5], extended to 4.5 h in 2011 by many authorities, but not by the Food and Drug Administration [37]. It became clear that the onset-to-door and door-to-needle times should be kept as short as possible. A concept now known as ‘time is brain’ [68], and awareness campaigns were set up for early detection and diagnosis [69]. However, Saver et al. [70] showed that, in a cohort of over 100,000 patients with ischaemic stroke and a documented time of onset, still 40% arrived at a hospital emergency department after more than 3 h. In a recent review on thrombolytic therapy, Donnan et al. [71] made another plea to eliminate pre-hospital and in-hospital delays. Door-to-needle time should be 60 min or less, and even 20 min is possible in a well-organized stroke unit [72].

In the last decade, more and more evidence has been accumulated showing that a rigid time window does not correspond to the individual pathophysiological state [73]. Time alone is not a ‘go/no go’ criterion, and with better diagnostics and multimodal imaging techniques, patients in later time windows could benefit from thrombolysis [74, 75]. Current developments of newer thrombolytic agents and other techniques such as mechanical thrombectomy, and bridging protocols using combined intravenous and stent retrievers show that the window of opportunity depends on individual factors such as collateral pathways [71, 76, 77].

The EPITHET and DEFUSE studies suggest that multimodal imaging techniques yield a valuable pathophysiological characterization of ischaemic stroke, facilitating the selection of patients for thrombolysis up to 6 h after onset [33, 78]. This concept of perfusion- and diffusion-weighted imaging has been advocated widely, and evidence might be given when the results of the EXTEND, ECASS-4 and ITAIS trials are available [79–81]. The results of the International Stroke Trial-3 (IST-3), the largest AIS trial ever, do not significantly support the use of rt-PA beyond 4.5 h in patients selected with plain imaging [43]. In the IST-3, treatment of elderly patients was at least as effective as in younger patients. A meta-analysis of 12 trials of rt-PA including IST-3 (7,012 patients) strengthened previous evidence to treat AIS as early as possible [82].

Beneficial outcome within the 3- to 9-hour time window was observed for desmoteplase in patients presenting with TIMI 0–1, but this post hoc finding has to be confirmed in the ongoing DIAS-3 and DIAS-4 trials [66, 67].

Conclusion

The use of thrombolytics in AIS has a history of about 50 years and despite serendipity, significant progress has been achieved. The current standard thrombolytic therapy for AIS has been established since 1995 when the results of the NINDS-2 became available, and treatment with rt-PA became possible in selected patients in a time window up to 3 h after onset of stroke symptoms. Recent evidence based on pooled analyses of clinical trials and the outcome of stroke registries led to an extension up to 4.5 h after onset. Seventeen years after the introduction of rt-PA, there is still a high unmet need for effective treatment in the majority of AIS patients. Advancements in public awareness of stroke, accurate diagnoses, pre- and in-hospital care and collaboration, and also the development of thrombolytics with a higher fibrin specificity and a better risk-benefit profile than that of rt-PA (e.g. rt-PA-TNK and desmoteplase), will bring new therapeutic opportunities to more patients in need.

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