

## Chapter 2

# The March of Thrombolytic Therapy for Acute Ischemic Stroke to Clinical Trials: Pre-clinical Thrombolysis and Adjuncts to Thrombolysis Research

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### Abbreviations

|        |  |
|--------|--|
| rt-PA  | Recombinant tissue plasminogen activator |
| SK     | Streptokinase                            |
| UK     | Urokinase                                |
| Pro-UK | Pro-urokinase                            |
| TNK    | Tenecteplase                             |
| MCA    | Middle cerebral artery                   |
| IU     | International units                      |
| ICH    | Intracerebral hemorrhage                 |
| PET    | Positron emission tomography             |
| IV     | Intravenous                              |
| IA     | Intra-arterial                           |
| d:     | Days                                     |

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|       |  |
|-------|--|
| kg:   | Kilograms                                      |
| h     | Hours  |
| min   | Minutes  |
| PAI-1 | Tissue plasminogen activator inhibitor-1       |
| U     | Units  |
| DNA   | Deoxyribonucleic acid                          |
| DSPA  | <i>Desmodus</i> salivary plasminogen activator |
| kDa   | Kilodaltons                                    |
| CBF   | Cerebral blood flow                            |

## Introduction

Prior to definitive human trials, considerable effort had been expended in the laboratory perfecting thrombolytic therapy. The essential literature that provided the experimental impetus for proceeding to human trials is summarized in this chapter. From this experience, two important lessons emerged. First, we must thoroughly explore drug risks and benefits in relevant animal models prior to human trials. Second, although animal models can predict human results, this is true only if the correct models are chosen, and the results are handled rigorously. For example, the experimental data clearly predict the efficacy, and the side effects, of thrombolytic therapy with rt-PA. Furthermore, the excessive risk associated with streptokinase (SK) was predicted by the animal models. More recent work has now been focused on the use of animal models to identify and support new approaches as adjuvants to thrombolysis therapies for future stroke intervention. These and their progression from early work are described below. The progression (or failure of progression) of these adjuncts into clinical trials are discussed in detail. The success (or lack of success) of animal models in predicting clinical trial outcome and the important issues that need to be addressed preclinically to increase translation to the clinic are addressed where appropriate.

## Early Experimental Studies of Thrombolysis for Stroke Using Plasmin, Urokinase, or Streptokinase

The experimental and basic early studies of thrombolytics for ischemic stroke are summarized in Table 2.1. Meyer et al. [1] created pumice emboli with subsequent platelet and thrombi adherence to the regions of damaged endothelium in cats and monkeys. Intravenous (IV) or intra-arterial (IA) injection of either bovine or human plasmin resulted in lysis of thrombi in every experiment. IA infusion caused more rapid clot dissolution and the SK-activated human plasmin was believed to be minimally more effective than the bovine-fibrinolysin. Clot lysis began 4–18 min after IA infusion and 8–30 min after dosing. Hemorrhagic infarction did not appear to be

**Table 2.1** Experimental and basic studies of thrombolytics for ischemic stroke

| Author                 | Year | Urokinase/streptokinase/<br>plasmin animal model | Main results (compared with controls<br>when applicable)   |
|------------------------|------|--|--|
| Whisnant [78]          | 1960 | Clot in Internal Carotid<br>Artery               | No increased risk of hemorrhagic<br>infarction compared with controls  |
| Centero [79]           | 1985 | Rabbit autologous clot                           | Variable and no statistical differences<br>with controls, no gross ICH   |
| Del Zoppo [2]          | 1986 | Baboon MCA balloon                               | Improved neurologic function,<br>reduced infarct size,<br>no macroscopic ICH   |
| Hirschberg<br>[80, 81] | 1987 | Dog MCA occlusion                                | Thrombolysis, no ICH 5 petechial<br>hemorrhages  |
| Slivka [12]            | 1987 | Rabbit CCA/MCA<br>occlusion                      | 2 gross ICH; petechial hemorrhage<br>same as controls  |
| Deley [82]             | 1988 | Dog MCA clot                                     | Thrombolysis without improved<br>tissue perfusion or infarct<br>reduction if treatment 30 min<br>after 3-h-old clot injected.<br>If treatment within 5 min of insult:<br>normalization of cerebral blood<br>flow and salvaged tissue |
| Clark [15]             | 1989 | Rabbit autologous clot                           | >50 % hemorrhage   |

*Abbreviations: ICH intracranial hemorrhage, MCA Middle Cerebral Artery, CCA Common Carotid Artery*

increased by fibrinolytic therapy. However, 2–4 h post-fibrinolysis infusion the thrombus usually started to reform and propagate. This did not occur if heparin was given ½–1 h before fibrinolytics. Distal emboli resulting from the parent clot dissolution was documented in five experiments. These smaller emboli were then also dissolved.

Del Zoppo et al. [2] demonstrated that after 3 h of reversible eccentric balloon (inflatable silastic placed transorbitally) compression of the baboon MCA proximal to the lenticulostriate arteries, intracarotid urokinase begun 30 min after balloon deflation), improved neurological function and reduced infarct size without evidence of macroscopic ICH compared with untreated animals.

DeLey et al. [3] showed that very early treatment with intra-carotid SK in conjunction with flunarizine prevented the lowering of the cerebral metabolic rate of oxygen as determined by PET scanning in a dog MCA occlusion (autologous blot clot) model.

***Preclinical Trials of rt-PA***

Table 2.2 summarizes experimental and basic studies of rt-PA for ischemic stroke. There was intense interest in establishing the efficacy of thrombolytics, especially tissue rt-PA for experimental cerebral ischemia, primarily using the autologous clot cerebral embolism model in rabbits [4, 5].

If rt-PA is administered immediately after experimental embolic occlusion, significant reduction in neurological damage occurs [4]. rt-PA may reduce neuro-

**Table 2.2** Experimental and basic studies of thrombolytics for ischemic stroke: rt-PA

| Author            | Year | Animal model                      | Treatment onset          | Main results (compared with controls when applicable)  |
|-------------------|------|-----------------------------------|--------------------------|--|
| Zivin [4]         | 1985 | Rabbit autologous clot            | <2 min up to 45 min, 1 h | Neurologic improvement, no large ICH, no protection  |
| Del Zoppo [5]     | 1986 | Baboon reversible MCA occlusion   | 3 h                      | Improved neurological function   |
| Penar [83]        | 1987 | Rat autologous clot               | —                        | No effect on vessel patency, no ICH, less “low flow” regions, untreated groups, no change in fibrinogen                                  |
| Kissel [19]       | 1987 | Rabbit autologous clot            | —                        | Improved cerebral blood flow at 90 min but not at 30 min   |
| Watson [84]       | 1987 | Rat with laser-induced thrombosis | —                        | Segmental recanalization, decreased lesion volume in 6/9, no ICH   |
| Papadopolous [20] | 1987 | Rat with human clot               | 2 h                      | Increased CBF, within 30 min, improved EEG, thrombolysis achieved, no ICH  |
| Slivka [12]       | 1987 | Rabbit CCA/MCA occlusion          | 24 h                     | ¾ ICH  |
| Chehrazai [85]    | 1988 | Rabbit autologous clot            | 30 min, 2 h, 4 h         | Reduced infarct size in zomin treatment on-set group, no ICH   |
| Philipis [17, 18] | 1988 | Rabbit autologous clot            | 15 min                   | Rapid reperfusion, no macroscopic ICH, no difference in infarct extent   |
| Clark [15]        | 1989 | Rabbit autologous clot            | < 60 min, 6 h            | 14 % hemorrhage (37 % in controls)<br>30 hemorrhage (21 in controls)   |
| Lyden [13]        | 1989 | Rabbit embolism                   | 10 min                   | 100 % lysis at 5 mg/kg, no increased ICH   |
| Bednar [86]       | 1990 | Rabbit embolism                   | < 60 min                 | Restored CBF, reduced final infarct size   |
| Benes [16]        | 1990 | Rabbit embolism                   | 30 min                   | Reduced infarct incidence, no ICH  |
| Terashi [87]      | 1990 | Hypertensive rats given           | rt-PA preischemia        | Higher brain ATP and lower lactate I rt-PA than vehicle treated rats, no hemorrhagic lesions, no arterial platelet in rt-PA treated rats |

*Abbreviations:* ICH intracranial hemorrhage, MCA Middle Cerebral Artery, CBF cerebral blood flow, EEG electroencephalogram, CCA Common Carotid Artery, ATP adenosine triphosphate

logical damage in rabbit embolic stroke models as late as 45 min after the cerebral embolic occlusion. rt-PA-related ICH did not occur when therapy was started 4 h after the onset of vascular occlusion. However, there was no benefit when treatment was delayed for 1 h. In both a small- and a large-clot rabbit embolic stroke model, there was no evidence that rt-PA changed the histological appearance of lesions compared with untreated controls. Zivin et al. [4], in a landmark 1985 Science study documented for the first time that rt-PA could, in fact, substantially improve neurological function after embolization with artificially made clots. It was primarily these data that paved the path for eventual FDA approval of IV rt-PA for stroke within 3 h in 1996 that was more recently extended to use within 4.5 h [4, 7–11].

Using awake baboons, Del Zoppo et al. [5] studied rt-PA-induced hemorrhagic transformation of ischemic brain within 3.5 h after MCA occlusion and 30 min of reperfusion. Three doses of rt-PA were infused over 1 h and compared with normal saline infusion. Peripheral (non-intracranial) hemorrhages were related to rt-PA dose, significant for the two highest doses. Peak plasma rt-PA levels were directly related to dose. No significant differences in the incidences or volumes of infarction-related hemorrhage occurred in any group compared with saline-treated animals that were sacrificed at 14 days. Their data suggest that rt-PA alone does not increase the risk (incidence or volume) of hemorrhagic infarction if administered within 3.5 h after MCA occlusion and reperfusion in baboons. Their data also suggest that rt-PA does not substantially decrease the infarct volume at any of the doses administered early after symptom onset.

rt-PA administered later [12] rather than earlier [6], albeit in different models, was more often associated with ICH. Lyden et al. [14] found no difference in the frequency of ischemic brain hemorrhagic transformation, however, when rt-PA was administered 10 min, 8 h, or 24 h after injection of autologous emboli. Del Zoppo et al. [2] infused rt-PA (0.3 mg/kg or 1.5 mg/kg) in baboons following 3 h of reversible acute MCA occlusion. Five of six animals at each dose (10 of 12 total) had petechial hemorrhagic infarction at 14 days compared with 7 of 12 control animals and infarct size did not differ between treated and untreated animals.

Vaugh et al. [14] demonstrated that rt-PA combined with IV aspirin synergistically and markedly prolonged the template bleeding time with a significant bleeding tendency. Administering reactivated PAI-1 can rapidly reverse this bleeding time prolongation.

Slivka and Pulsinella [12] investigated the hemorrhagic potential of both rt-PA (200,000 U; 10 % bolus remainder over 4 h) and SK; 10,000 U/kg bolus or 32,000 U/kg bolus, remainder over 4 h) initiated 24 h after experimental stroke in rabbits using a tandem common carotid and ipsilateral MCA occlusions with 2 h of halothane anesthesia. In addition, six rabbits were administered SK (10,000 U/kg bolus) 1 h after occlusion. Microscopic hemorrhage was frequently present in infarct tissue irrespective of treatment. Gross hemorrhagic infarction did not occur in rabbits either untreated or administered SK 1 h after occlusion but did occur in the other groups of treated animals. Two of 12 animals administered SK 24 h after injection had gross hemorrhages within the infarct. Only the rt-PA treated rabbits showed a significantly greater incidence of gross ICH than controls. Their data suggest that

the use of thrombolytic agents may increase the risk of microscopic hemorrhage unless the agents are administered early enough after onset of the insult.

In pioneering work that set the stage for well-conducted clinical trials, Zivin et al. [6] found that rt-PA-induced ICH did not occur more commonly than controls when therapy was started within 4 h after the onset of vascular occlusion in rabbit emboli model of ischemic stroke. rt-PA was administered at 1.0 mg/kg at 15, 30, or 60 min after small-clot embolization (24-h aged clot) and at 2 mg/kg at 45 and 60 min after small-clot embolization. rt-PA was also administered 30 min or 4 h after large-clot (1 mm<sup>3</sup>) embolization. Evaluations were performed blinded to the treatment group. The effective dose of clots required to produce a clinically apparent neurological disorder in 50 % of a group of animals was significantly greater in the rt-PA-treated rabbits when rt-PA was administered at 15, 30, or 45 min but not at 60 min post-embolization in either the 1 mg/kg or 2 mg/kg dose. In controls, grossly apparent ICH was present in 3 of 10 (30 %) animals.

When rt-PA was administered 30 min after embolization, 8 of 14 rabbits had gross hemorrhage. When rt-PA was delayed 4 h, 2 of 10 animals had such hemorrhages ( $p=NS$ ). In the small-clot model, ICH was uncommon, visible only microscopically, and only found in association with relatively large infarcts. The presence of microscopically visible intravascular clots was a function of the time between clot injection and animal death. In the large-clot model, the microscopy of the lesions in control- and rt-PA-treated rabbits was indistinguishable, and no difference was noted in neurological functions [13–15].

Lyden et al. [13] demonstrated in the rabbit emboli stroke model that ICH rates in the rt-PA (3 mg/kg or 5 mg/kg) or saline-treated group did not differ. rt-PA was infused 10 min, 8 h, and 24 h after emboli were instilled. Lyden et al. [15] also evaluated saline, rt-PA, or SK infusion in a similar rabbit embolic stroke model at various times of infusion to assess the rate of thrombolysis and ICH 24 h later. Only SK was associated with a significant increase in the rate of ICH compared with the saline controls. In animals administered 3 mg/kg, 5 mg/kg, or 10 mg/kg of rt-PA, there was no clear dose response for ICH, but there was for thrombolysis. However, only in rabbits who achieved thrombolysis was rt-PA associated with twice (24 %) the ICH rate as saline controls (12 %), whereas the hemorrhages were nearly identical in animals without thrombolysis.

Benes et al. [16] found that treatment with either rt-PA or UK significantly reduced the number of emboli present in a rabbit stroke model but only rt-PA significantly reduced the incidence of infarction. No animal suffered an ICH.

In summary, the studies by Phillips et al. [17, 18], Zivin et al. [4–6], Kissel et al. [19], Del Zoppo et al. [5] and Papadopolous et al. [20] taken together suggest that rt-PA reliably opens cerebral arteries occluded with either autologous or non-autologous embolic clots. Further, there is experimental data suggesting that rt-PA is more effective in inducing thrombolysis within precerebral vessels than systemic vessels [21]. At present, IV rt-PA at 0.9 mg/kg is licensed in many countries and appears to represent best practice and other drugs (see new thrombolytics below), doses or routes of administration should only be used in randomised controlled trials [22].

## rt-PA Analogs and Newer Thrombolytic Agents

### *Fb-Fb-CF rt-PA Analog (“Retaplast”)*

More recently, several new thrombolytic agents have been identified or synthesized and had gone into experimental and clinical studies, for both acute myocardial and cerebral ischemia. Analogs to rt-PA are available through recombinant DNA technologies and offer the possibility of an active portion of molecule that may have better fibrin specificity and penetration and a longer in vivo half-life than rt-PA [23].

Phillips et al. [24, 25] investigated the effects of a rt-PA analog, Fb-Fb-CF, in a rabbit embolic model. This analog consisted of the catalytic fragment of rt-PA and a dimer of the B fragment of staphylococcal protein A (Fb-Fb-CF) and has a longer serum half-life (90 min) than rt-PA (3 min). When Fb-Fb-CF was given as a bolus ( $n=10$ ) 15 min after embolization, cerebral reperfusion, documented angiographically, occurred in  $48 \pm 21$  min (range 30–90 min) while controls (saline treated,  $n=8$ ) did not reperfuse by 180 min ( $p<0.01$ ). Furthermore, reperfusion was demonstrated at  $66 \pm 32$  min post-treatment (range 30–90 min,  $n=11$ ) when the treatment was delayed 90 min after embolization (control =  $100 \pm 25$  min;  $n=12$ ); two spontaneous lyses occurred in controls ( $p<0.01$ ). One small macroscopic hemorrhage within an infarct was observed in the Fb-Fb-CF 15-min treated group (none in controls). In the 90-min group, microscopic hemorrhage was observed in four rt-PA-analog and three saline-treated animals. Plasma fibrinogen levels decreased 16 % immediately after and 19 % by 180 min following Fb-Fb-CF treatment ( $n=7$ ). No macroscopic or microscopic ICH was observed in noninfarcted brain regions although intraventricular hemorrhages occurred on one Fb-Fb-CF and two control animals only in the 90-min group.

This Fb-Fb-CF rt-PA analog (0.8 mg/kg, 1 mg = 500,000 IU) was given in a rabbit autologous clot cerebral embolization model [17], either 15 or 90 min after embolization, in conjunction with serial angiography [26]. Reperfusion was documented in both the 15- and 90-min groups treated with rt-PA analogs (different than controls) without a difference in median time to reperfusion in the two groups. In the 15-min group, 0 of 8 controls reperfused and in the 90-min group, 2 of 12 controls spontaneously reperfused. One small hemorrhage into a zone of infarction was observed in the 15-min rt-PA analog-treated group (0 in controls) and four hemorrhages into infarcts were observed in the 90-min group (three in controls), such that the risk of hemorrhage was essentially the same for rt-PA analog-treated rabbits and saline-treated rabbits. No additional studies have been conducted after these data were available.

### *Tenecteplase (TNK)*

Using the model developed by Lyden and Zivin, further studies of a mutant rt-PA, known as TNK were conducted. TNK, a genetically modified (“mutant”) form of wild-type rt-PA (3 induced substitutions in the rt-PA molecule) with a longer

biological half-life and greater fibrin specificity (14 times that of rt-PA), might have greater safety and/or efficacy [27, 28] although a phase IIb study in human was stopped prematurely without a definitive results [29] It offers the promise of more rapid [30] and complete thrombolysis. The longer half-life also offers the opportunity for bolus therapy over infusion therapy.

As critical lysis takes place at the surface of an arterial thrombus where PAI-1 levels can be very high, mutant rt-PA molecules with longer half-life and resistance to PAI-1 may be useful to reduce the contribution of PAI-1 to re-occlusion. TNK is 80-fold more resistance to PAI-1 than rt-PA [28]. In the small clot rabbit emboli model, TNK had a better pharmacologic profile than rt-PA – showing better lysis up to 3 h post stroke and no increase in ICH rate [31].

Intra-arterial TNK (1.5 mg/kg) has been shown to reduce infarct volume at 48 h post-stroke when given 2 h post MCA occlusion in a model of focal cerebral embolic ischemia (single fibrin-rich clot) in unanesthetized rats [32]. There was a strong trend ( $p=0.06$ ) for a reduction in lesion volume when TNK was given 4 h after occlusion. There was no increase in gross ICH in either the 2 h post- or 4 h post-occlusion treatment.

In the rabbit embolic stroke model, Chapman et al. [27] found hemorrhage in 26 % (6/23) of the control group, 80 % (16/20) of the wild-type rt-PA group, 57 % (12/21) in the 0.6 mg/kg TNK group, and 73 % (8/11) in the 1.5 mg/kg TNK group ( $p<0.01$ ). TNK shows comparable rates of recanalization, and may cause fewer hemorrhages, compared to wild-type rt-PA in a model of embolic stroke, although these results were not statistically significant. Unfortunately, there was also no difference in the size of the ICH with TNK compared to rt-PA [27]. No additional studies have been conducted after these data were available.

### ***Bat Saliva Plasminogen Activator (“Desmoteplase”)***

Vampire bats live on fresh blood. The saliva of the vampire bat has various factors that can maintain prolonged bleeding and preserve blood fluidity [33]. There are different molecular forms of DSPA (all single-chain molecules) [34, 35] that have about 85 % homology to human rt-PA, including DSPA $\alpha$ 1 (molecular weight 43 kDa) and DSPA $\alpha$ 2 (39 kDa). They possess a relative PAI-1 resistance, greatly enhanced fibrin-selectivity – without the systemic activation that leads to fibrinogen consumption, and a strict requirement of polymeric fibrin as a cofactor [33]. Terminal half-life for DSPA $\alpha$ 1 is 3 times longer than rt-PA. Also, DSPA does not promote kainite- or NMDA-mediated neurotoxicity [36]. Bleeding complications may also be reduced [37]. In a coronary thrombosis dog model of acute myocardial infarction, there was faster recanalization and less reocclusion with DSPA $\alpha$ 1 than rt-PA [38]. Currently, there is not enough evidence for clinical use in ischaemic stroke and further Phase III studies are underway. At this stage, desmoteplase remains an investigational compound [39].



## ***Newer Thrombolytics in Earlier Stages***

Although other thrombolytic agents (e.g. streptokinase; SK) have failed to show benefit over alteplase, there is still on-going research in search of alternative agents with higher target specificity and better safety profile. Beyond the three discussed above, where significant work was completed before interest decreased regarding their improvement over rt-PA, other more early stage potential thrombolytic agents include Staphylokinase, Saruplase, BB-1013, plasmin, and microplasmin. These are relatively early stage molecules that should be updated when more experimental work is completed.

## **rt-PA Hemorrhagic Conversion and Stroke Model Considerations**

Although in vitro clot systems have been used to understand the effect of adjuncts in combination with rt-PA on thrombolysis, to understand their roles in hemorrhagic conversion, the in vivo system of thromboembolic stroke with rt-PA is required. Further, reproducible models of hemorrhagic transformation associated with delayed administration of rt-PA similar to that observed in humans are required [40, 41]. Although there are unquestionable benefits from rt-PA thrombolytic activity, rt-PA also has deleterious effects on the ischemic brain including cytotoxicity and the increased permeability of the neurovascular unit with the development of cerebral edema and hemorrhagic transformation when it is delivered late after stroke [42]. Models that can study in detail the mechanisms of rt-PA-induced hemorrhagic conversion are also important in the evaluation of adjuncts to rt-PA [43].

Progress has been made in our understanding of the mechanisms of hemorrhagic conversion with rt-PA use. The significant involvement of oxidative stress/free radicals, inflammatory cytokines and proteins, and vascular/parenchymal neuroinflammation in the evolution of stroke-induced brain injury is now well-documented in animals and man [44, 45]. The extracellular matrix degradative protein matrix metalloproteinase (MMP)-9 is now recognized as a key player in both early stroke injury and the hemorrhage-producing effects of rt-PA in both animals and man [46, 47]. In addition to its role in clot lysis, rt-PA is also an extracellular protease and signaling molecule in the brain. rt-PA interacts with the NMDA-type glutamate receptor and facilitates excitotoxicity in the brain (i.e., can amplify excitotoxic calcium currents) and can also be vasoactive. Also, by augmenting matrix MMP further up-regulation after stroke, rt-PA induces further degradation of the extracellular matrix, increases neurovascular cell death, blood–brain barrier leakage, edema, and hemorrhagic conversion. Similar rt-PA toxic effects have been demonstrated in both human and animal stroke research [48–50]. Understanding the pathology of the evolution of brain injury in stroke and the pleiotropic toxic actions of rt-PA has provided the rationale for much of the adjuvants to thrombolysis molecules tested with rt-PA as discussed

below. New data from these studies are expected to provide novel therapeutic opportunities for combination stroke therapy.

Experimental models need to incorporate the additional comorbidities, other diseases, and of course aging in order to mimic the human stroke situation where these variables are important. Failure to include such variables important in the stroke patient population can result in poor translational value from preclinical models. For example, rt-PA-induced hemorrhage is dependent on blood pressure, and reduction of hypertension during fibrinolysis can reduce the risk of hemorrhagic transformation [51]. Diabetes and high glucose exhibit increased risk of hemorrhage with rt-PA use in stroke. rt-PA treatment in diabetic stroked rats significantly enlarged brain hemorrhage, augmented BBB leakage, and failed to decrease lesion volume and improve functional outcome [52]. However, early insulin glycemic control for ischemic stroke with diabetes mellitus can significantly improve outcome in rt-PA thrombolysis [53]. Similarly, work with aged animals needs to be carried out to understand the opportunity of adjuncts to thrombolytics as well. For example, in diabetic rats with embolic stroke, combination therapy with minocycline plus rt-PA may be beneficial in ameliorating inflammation and reducing infarction, brain swelling, and hemorrhage after ischemic stroke with diabetes mellitus/hyperglycemia [54]. These data emphasize the importance of making translational modification of experimental models that can better reflect and predict the clinical situation.

## ***Global Ischemia***

Moritomo et al. [55] studied heparin (100 U/kg) plus UK (3,000 U/kg IV) given 15 min of complete global ischemia for 6 h in dogs. The studied group experienced up to 70 % improvement in postischemic hypoperfusion CBF with significantly improved neurological outcome, suggesting that these two drugs together might improve impaired microcirculation. Such data also suggests a potential use in post-ischemic forebrain hypoperfusion.

## **Thrombolysis with New Thrombin Inhibitors**

Anticoagulants are highly effective for preventing cardioembolic stroke, but their effectiveness in non-cardioembolic stroke is unproven. Direct thrombin inhibitors (DTIs) are more effective than heparin in inhibiting platelet deposition and thrombus formation, and also show promise in preventing reocclusion after thrombolysis for both experimental thrombotic and embolic stroke. Of those being evaluated, argatroban will be discussed here, as it is the most advanced thrombin inhibitor and is being studied in stroke treatment trials.

Argatroban is used clinically because of its safe and effective antithrombotic action [56]. Thrombin is a blood-borne coagulation factor that contributes to

neurovascular injury during acute focal ischemia. Thrombin mediates severe vascular disruption and neuronal damage during ischemia. Thrombin activation is a key step in coagulation, and thrombin also has been shown to mediate endothelial permeability and cellular toxicity. Thrombin also can enter brain parenchyma due to increased ischemic vascular permeability, and thus augments neurotoxicity during ischemia. Thrombin's proteolytic activity is specifically associated with ischemia. Protease activated receptor-1, the presumptive thrombin receptor, appears to mediate ischemic neurovascular injury [57, 58]. Endogenous thrombin may play a role in this disturbance of microcirculation following cerebral ischemia [59].

Argatroban may be useful in cardioembolic stroke, increasing the improvement of recovery of stroke severity without increasing the risk of hemorrhage in one study [60]. The combination of Argatroban and IV rt-PA is potentially safe in patients with moderate neurological deficits due to proximal intracranial arterial occlusions and may produce more complete recanalization than rt-PA alone [61]. Importantly, argatroban may be an effective and safe for treatment of acute ischemic stroke presenting beyond 6 h of ischemic symptom onset [62]. In preclinical, rat studies, argatroban and rt-PA extends the window of opportunity for treatment of stroke to 4 h without increasing hemorrhagic transformation [63]. Currently, the combination of IV rt-PA with either low or high dose argatroban is being tested in a phase II randomized clinical trial (ARTSS-2).

## **“rt-PA Plus” Interventions: Adjuncts to Thrombolysis**

rt-PA is a serine protease found on endothelial cells, the cells that line the blood vessels. As an enzyme, it catalyzes the conversion of plasminogen to plasmin, the major enzyme responsible for clot (fibrinogen) breakdown. Thrombolytics in clinical use include alteplase (rt-PA; approved for ischemic stroke) and reteplase and TNK (approved for other peripheral clot conditions/myocardial ischemia). In stroke alteplase is substantially underused because of time to treatment limitations and concerns regarding adverse bleeding risk. This limitation has fuelled the search for other thrombolytic agents, which display greater fibrin dependence and selectivity, but lack detrimental effects within the central nervous system (which has been largely unsuccessful as discussed above).

The many failures of neuroprotective therapies in clinical trials can suggest that neuroprotection alone without restoration of tissue perfusion and vascular integrity may not be adequate for successful treatment of acute stroke. Many have suggested combination strategies are necessary to optimize the brain protection already provided by early thrombolysis/reperfusion [64–67].

Some studies evaluate compounds given concomitantly with alteplase to reduce the haemorrhage conversion rate and/or protect brain cells from injury associated with ischemic toxicity mechanisms at thrombolysis. For example, MMP inhibitors have been evaluated as potential combination therapy candidates because they prevent MMP-induced production (and *visa versa*) of the inflammatory cytokine

tumour necrosis factor- $\alpha$  (TNF $\alpha$ ). MMP-9 plays a very significant role in rt-PA toxicity because it is upregulated in ischemia and responsible for degradation of the extracellular matrix. This upregulation and degradation is further upregulated by rt-PA, thus resulting in leaky vessels/vasogenic edema in stroke and results in hemorrhagic conversion when rt-PA is used later than recommended to lyse the occlusive cerebrovascular clot.

Antioxidant reagents also have been put forward due to their free radical scavenging capabilities that can be protective and reduce expression of inflammation and toxic mediators that also upregulate MMPs and stimulate apoptosis. Earlier work in the rabbit large clot embolism model demonstrated the rt-PA effectively lysed blood clots and improved outcome but rt-PA also increased haemorrhage rate as in man. In rabbit or rodent studies, alteplase-induced haemorrhage rate was reduced significantly by administration of the MMP inhibitor batimastat (BB-94) or the spin trap (antioxidant) agent alpha-phenyl-N-t-butyl nitron (PBN) [65]. Other antioxidants have also demonstrated a potential use in conjunction with rt-PA ([67] and as listed below)

The focus here is “adjuncts to thrombolysis”. Adjuncts include therapeutic agents that have been used in pre-clinical, animal model studies in conjunction with rt-PA. Table 2.3 is a summary of research in animal models. All studies listed in this table used rats, except where indicated otherwise. Some of them have also been tested in clinical trials as a monotherapy and/or adjuncts to rt-PA, and this is also included. In addition, we have described the “adjuncts” mechanism(s) of action to help understand the actions and outcomes for consideration with thrombolysis. The data from preclinical/animal model studies with monotherapy and rt-PA adjunct therapy, and the results of clinical trials, if any, are also summarized in Table 2.3. Of interest here were treatment strategies that might reduce cerebral vascular and brain injury, decrease adverse rt-PA effects (e.g., hemorrhage), and extend the therapeutic time window for rt-PA use (i.e., thus are expected to improve access to rt-PA therapy for more patients). Thrombolysis may improve delivery of neuroprotectant molecules to the penumbral region increasing beneficial effect. Many neuroprotective agents exhibit synergistic effects with rt-PA preclinically, again including MMP inhibitors, free radical scavengers-antioxidant agents, NMDA and AMPA receptor antagonists, and anti-inflammatory and antiplatelet agents.

Among the data listed in Table 2.3, two leading GPIIb/IIIa antagonists have been significantly evaluated. Clinical evaluation of Abciximab is not expected to go further. For Eptifibatide (integrilin), a Phase II study has been recently completed and shows promise for a more definitive phase 3 trial [68]. The anti-coagulant Enoxaparin was not efficacious and the consensus for all unfractionated and low molecular heparins is that they do not have general utility for acute ischemic stroke. The results of the FAST MAG trial employing prehospital delivery of intravenous magnesium, typically within 60 min of stroke onset will be released within 6 months. The phase III trial of albumin therapy in acute ischemic stroke (ALIAS) was recently stopped due to futility. Hypothermia, claimed to be the “gold standard” for brain protection, will continue to be studied in the clinic. For Cerebrolysin, mixed efficacy results on monotherapy with no improvement in combination with rt-PA will likely halt future clinical work. There is no current indication that clinical studies will continue on Memantine.

Table 2.3 Preclinical to clinical update on adjuncts to thrombolysis

| Drug      | Description - mechanism of action   | Preclinical monotherapy   | Preclinical adjunct to thrombolysis  | Clinical data (if available)   |
|-----------|---|---|--|--|
| Abciximab | Antiplatelet drug; Glycoprotein IIb/IIIa antagonist; blocks final common pathway in platelet aggregation; favors endogenous thrombolysis (reduces thrombus growth and prevents thrombus reformation by competitive inhibition of fibrinogen). | Reduced brain infarction within a 3 h therapeutic window [88]. Most of the experimental data for glycoprotein IIb/IIIa inhibitors is for abciximab in animal models of ischemic stroke. | Adjutant treatment increases the therapeutic window for low-dose rt-PA. Reduces infarct volume and improves neurological outcome [89, 90]. In vitro and in vivo experimental studies suggest that combination therapy (fibrinolytic agent plus GPIIb/IIIa inhibitor) provide more complete lysis than fibrinolytics alone. | Both monotherapy and rt-PA adjutant: Safe alternative for achieving recanalization. Might improve clinical outcome and reduced risk of hemorrhage [91, 92].<br><br>Monotherapy: Abciximab (phase III AbESTT II trial) was tested in ischemic stroke patients who could be treated within 5 h of symptom onset. Due to an unfavorable benefit to risk profile during the study, the study was terminated early [93].<br>Abciximab plus rt-PA Clinical Study: In the ROSIE (ReoPro Retavase Reperfusion of Stroke Safety Study - Imaging Evaluation) study :<br><br>Reperfusion was relatively similar in the abciximab monotherapy and combination with rt-PA group [94]. |

(continued)

Table 2.3 (continued)

| Drug         | Description - mechanism of action   | Preclinical monotherapy                                       | Preclinical adjunct to thrombolysis  | Clinical data (if available)   |
|--------------|---|---|--|--|
| Eptifibatide | Antiplatelet drug: (glycoprotein IIb/IIIa inhibitor class). Blocks final common pathway in platelet aggregation. facilitates thrombolysis by reducing thrombus growth and prevent thrombus reformation by competitive inhibition with fibrinogen. | Reduces brain infarction and improves recanalization [95, 96] | Empirical evidence of efficacy from other glycoprotein IIb/IIIa inhibitors. In in-vitro human clot models, eptifibatide + rt-PA was found to be more effective at lysing clots [97, 98]. | The combination was evaluated in a multicenter phase II study, In the Combined Approach to Lysis Utilizing Eptifibatide and rt-PA in Acute Reperfusion (CLEAR trial) [4] study, the authors reported a paradoxical trend towards increased efficacy of the rt-PA-alone group compared with the combination arm. Despite this, since safety of the drug combination was deemed adequate, investigators have recently completed a second phase II trial (CLEAR-ER) [68, 99]. |

|  |   |  |  |   |
|--|---|--|--|---|
| Enoxaparin<br>(Low-molecular weight heparin) | Anti-coagulant. Enoxaparin binds to and accelerates the activity of antithrombin III. By activating antithrombin III, enoxaparin preferentially potentiates the inhibition of coagulation factors Xa and IIa. The anticoagulant effect is correlated to its ability to inhibit factor Xa. Factor Xa catalyzes the conversion of prothrombin to thrombin, so enoxaparin's inhibition of this process results in decreased thrombin and ultimately the prevention of fibrin clot formation. | Reduces brain edema, infarct volume, neuroprotective [87, 100].  | Improves clot lysis and blood flow restoration in response to rt-PA [101, 102].  | Monotherapy: In the Org 10172 in Acute Stroke (TOAST) trial, a large randomized, placebo-blinded study a low molecular weight heparin exhibited no long-term difference in functional outcome but did increase rate of ICH in the experimental (drug treatment) group compared to placebo [102, 103].<br>rt-PA adjunct: No apparent benefit; increases risk of ICH [104].<br>The general consensus is that neither unfractionated nor low molecular heparin, subcutaneous or i.v. heparinoids, or direct thrombin inhibitors show any significant benefit in clinical outcome in the setting of acute stroke. |
| Aspirin                                      | Non-steroidal antiinflammatory drug (NSAID). Antiplatelet   | Reduces perfusion deficit, anti-platelet aggregation, neuroprotective effect, improves outcome [105, 106]. | Aspirin therapy resulted in paradoxical antagonism of clot lysis and was associated with a more modest restoration of blood flow [105, 106]. | rt-PA adjunct therapy: Slightly favorable neurological outcome; Increased symptomatic intracranial hemorrhage events [107].   |

(continued)

Table 2.3 (continued)

| Drug        | Description - mechanism of action  | Preclinical monotherapy  | Preclinical adjunct to thrombolysis                                    | Clinical data (if available)  |
|-------------|--|--|--|---|
| Simvastatin | 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor (i.e., a statin). Exerts beneficial effects independent of cholesterol lowering; upregulates endothelial nitric oxide synthase (eNOS), resulting in more functional protein, augmentation of cerebral blood flow, and neuroprotection due to increased endothelial nitric oxide [108]. | Augments cerebral blood flow; neuroprotection; reduces extent of brain damage; reduces oxidative stress; enhances angiogenesis by increasing notch signaling pathway [109, 110]. | Adjuvant treatment reduces the odds of thrombolytic induced ICH [111]. | Monotherapy - ischemic stroke: From meta-analysis, insufficient data exists from randomized trials on statin safety and efficacy in ischemic stroke and TIA [112].<br>Monotherapy - ischemic stroke: Largest review and meta-analysis indicates that statin therapy at stroke onset improves outcome, a finding not observed in studies restricted to thrombolysis-treated patients. Suggests that randomized trials of statin therapy in acute ischemic stroke are needed. Adjunct to thrombolysis utility questioned [113].<br>Monotherapy for hemorrhagic stroke: Retrospective analysis indicate statin use is associated with improved long-term outcome at 12 months after ICH. Finding supports short-term benefits and possible long-term recovery benefits of statins [114]. |



|             |   |   |   |   |
|-------------|---|---|---|---|
| Citicholine | Phosphatidylcholine (PC) biosynthesis intermediate. Neuroprotects by stimulation of PC synthesis, preserving cardiolipin/sphingomyelin levels, increases glutathione and glutathione reductase activity, restores Na <sup>+</sup> /K <sup>+</sup> + -ATPase activity; anti-glutamate effects, exerts neuroregenerative and restorative effects. | Neuroprotection, neuroregeneration [115, 116] with highlights of significant protection in ischemic and hemorrhagic animal stroke models [117].   | Effective protection with rt-PA [118, 119]  | Monotherapy: There is evidence from few clinical trials and drug surveillance studies that citocoline is safe and effective for ischemic stroke [120, 121] but efficacy in acute intervention has not been demonstrated [117, 122, 123].  |
| Magnesium   | Magnesium termed “nature’s physiologic calcium blocker”. It prevents cellular calcium influx and excitatory amino acid release in neurons by blockade of N-type and L-type calcium channels, prevents cellular calcium entry through NMDA-receptor channels   | Neuroprotective, but some studies fail to show reduced infarct volume [124, 125]. Preclinical data generally show 25 % level of Protection. Magnesium can produce post-ischemic hypothermia that can contribute to neuroprotective effects in studies that were not temperature-controlled [128]. | Magnesium sulfate neither potentiates nor inhibits tissue plasminogen activator-induced thrombolysis [126]. | Monotherapy: IMAGES trial: No overall reduction of death or disability (primary end-point), trend to benefit in lacunar stroke [127]. The Field Administration of Stroke Therapy–Magnesium (FASTMAG) Pilot Trial showed that magnesium intervention was feasible and safe with no serious adverse effects, and was associated with a beneficial functional outcome at 3-months [128]. |

(continued)

Table 2.3 (continued)

| Drug        | Description - mechanism of action   | Preclinical monotherapy   | Preclinical adjunct to thrombolysis   | Clinical data (if available)   |
|-------------|---|---|---|--|
| Albumin     | Plasma globular protein which provides oncotic pressure in vasculature. Plays a key role in restricting fluid leaking from the vasculature into the tissue. | Reduces infarcts when administered 4 h post-stroke [129]; Neuroprotection by ameliorating brain swelling, enhancing blood flow, maintaining vascular patency, preventing re-occlusion after thrombolysis [130]. | Albumin can ameliorate rt-PA-mediated blood-brain barrier permeability and ischemic brain injury in rats [131]. | Monotherapy: The pilot study Albumin in Acute Stroke (ALIAS) demonstrated that high-dose human albumin therapy is safe and may confer a neuroprotective effect within 5-h after acute ischemic stroke [132, 133]. These encouraging results led to a large placebo-controlled randomized multicentre phase III trial of albumin therapy in acute ischaemic stroke (ALIAS Part 2; [134]) that was stopped due to futility.<br><br>Under investigation in a large multi-center clinical trial [139]. |
| Hypothermia | Neuroprotective   | Hypothermia to 34°C has multiple neuroprotective effects and can reduce reperfusion associated injury [135, 136].   | Hypothermia to 34 °C rescues against rt-PA induced ICH and blood-brain barrier dysfunction [137, 138].          |  |

|              |  |  |  |  |
|--------------|--|--|--|--|
| Cerebrolysin | Neurotrophic peptidergic compound, mimics the action of endogenous neurotrophic factors, protecting stroke-injured neurons and promoting neuroplasticity and neurogenesis. | Enhances neurogenesis (probably via Sonic hedgehog signaling pathway), improves neuroplasticity deficits, reduces infarct volume, and improves functional outcome after MCAO in rats [140, 141]. | Enhances neurogenesis (probably via Sonic hedgehog signaling pathway), improves neuroplasticity deficits, reduces infarct volume, and improves functional outcome after MCAO in rats [140, 141]. | Monotherapy: Neutral results between treatment groups in double-blind, placebo-controlled randomized trial, however, favorable outcome in severely affected patients [142].<br>rt-PA adjuvant: Phase II trial: No improved outcome at day 90 in cerebrolysin+rt-PA group.<br>Cerebrolysin group had better neurological outcome compared to placebo group [143]. |
| Memantine    | Uncompetitive low affinity NMDA open-channel antagonist; Neuroprotective by blocking glutamate excitotoxicity [144].   | Improves neurological-behavioral outcomes in animal models [144–146].  | Improved safety of rt-PA in animal model studies; No effect on thrombolytic activity but prevented pro-neurotoxic effects of rt-PA [146, 147].   | No clinical trials.  |

(continued)

Table 2.3 (continued)

| Drug               | Description - mechanism of action  | Preclinical monotherapy  | Preclinical adjunct to thrombolysis  | Clinical data (if available)  |
|--------------------|--|--|--|---|
| NXY-059 (Cerovive) | Potent free radical trapping compound; antioxidant [148].  | Reduces cortical infarction. Improves neurological outcome. Reduces infarct volume [149, 150]. | Improves the safety of rt-PA by reducing rt-PA-induced hemorrhage [109, 151]. Meta-analysis of animal model data indicates that protection in experimental stroke might be overestimated and that efficacy in young, healthy, male animals is a poor predictor of clinical outcome in patients [76]. | <i>Monotherapy and rt-PA adjunct:</i><br>Ineffective [152].   |
| Uric Acid          | Uric acid is the major antioxidant in blood; scavenges hydroxyl radicals, hydrogen peroxide, peroxynitrite; Suppresses Fenton reaction; chelates transition metals, prevents lipid peroxidation [153]. | Protects from reperfusion or thromboembolic stroke [154, 155].                                 | Shows synergistic effects with rt-PA; reduces tyrosine nitration, neutrophil infiltration, infarct volume and neurological deficits [156].   | <i>Uric acid is rapidly consumed after stroke</i> [156]; Decreases lipid peroxidation and prevents an early fall of UA in patients treated with rt-PA [157]; Higher levels at stroke related to decreased infarct growth [158, 159]; Lower levels result in malignant infarctions and hemorrhagic transformation; higher levels related to decreased malondialdehyde and MMP-9 with rt-PA [159].<br>Current ongoing Phase IIb/3 trial on combination of UA and rt-PA given up to 4.5 h post-stroke [160]. |

|                     |  |  |   |   |
|---------------------|--|--|---|---|
| Edaravone (Radicut) | Potent lipophillic free radical scavenger-antioxidant (hydroxyl, peroxy), and superoxide radicals [67, 123, 161, 162]. | Inhibits neuronal death, microglia neurotoxicity, inflammation, lipooxygenase, oxidation of LDL; increases vascular endothelial growth factor, aquaporin-4, and MMP-9 [67, 69]; Reduced post-stroke increased AQP4, infarcts and neurological deficits [163] | In rabbit embolic stroke decreased behavioral deficits [69]; Expression of neurocan, Sema3A, Nogo-R, GAP43, and DCC decreased by rt-PA, but edaravone adjunct prevented reductions of these restorative protein changed by rt-PA [164]; Inhibits MMP-9 and hemorrhage when used with rt-PA [165]. | <i>Japan approval ischemic stroke (2001)</i> [162]; Lower serum levels of MMP-9 when it is given within 12–36 h of stroke onset [166]; Edaravone dose-dependently increases post-stroke functional recovery [167].<br><i>In combination with rt-PA</i> , lowers incidence of brain edema and white matter tissue injury more than placebo or rt-PA treatments alone [168].<br>More clinical data on efficacy and rt-PA adjuvant utility required [161]. |
|---------------------|--|--|---|---|

(continued)

Table 2.3 (continued)

| Drug   | Description - mechanism of action  | Preclinical monotherapy  | Preclinical adjunct to thrombolysis  | Clinical data (if available)  |
|--|--|--|--|---|
| Hyperbaric Oxygenation (HBO)<br>(Normbaric Oxygenation Often used as well) | Rationale: Reopens occluded vessels and increases additional O2 essential for survival of malfunctioning neural tissue; Minimizes secondary injury to brain tissue; Restores neuronal function in "penumbra; HBO increases the O2 concentration in tissue with impaired blood supply; Preclinical and clinical studies demonstrated positive effects of HBO therapy [169]. | Reduces glutamate release and the formation of hydroxyl free radicals [170]; improves energy metabolism in protected infarct border zones [171]; | With rt-PA it decreased infarction BBB injury, hemorrhage and matrix metalloproteinases [172]; Decreased longer term macrophage accumulation with reduction of neurological deficits [173]; Stabilized BBB and reduced MMP-2 [174]; reduces infarct volume and improves functional outcome [175]; Best efficacy and improvement of rt-PA therapy within first 3 h post-stroke [176]; | <i>Monotherapy pilot study:</i> significantly reduced NIHSS scores at 1 month [144]; Clinical studies on efficacy of hyperoxia on brain injury indicate safety, efficacy and practicality. Safety concerns and side effects need to be considered (e.g., especially pulmonary pathology, respiratory failure and theoretical risks) but a neuroprotective role of hyperoxia is supported and further studies are warranted [177]. |

|                                |  |   |  |   |
|--------------------------------|--|---|--|---|
| Fasudil (Rho kinase inhibitor) | Potent Rho-kinase (ROCK) inhibitor; central roles in actin cytoskeleton involved in a contraction, adhesion, migration, proliferation, and apoptosis; vasodilator with efficacy shown in human pulmonary hypertension [178, 179]; has been used in cerebral vasospasm (e.g., as occurs in subarachnoid hemorrhage); Also shown to improve cognition in models of ischemia. | Reduced infarct and neurological [138, 180–184]; Increased eNOS and decreased iNOS expression [67, 180]; Increases cerebral blood flow [181, 184]; vascular protective [138, 183–185]; Decreased MMP-9 and BBB injury [183, 184].   | Facudil with delayed rt-PA decreased hemorrhagic transformation but did not reduce infarct volume [184]  | No clinical trials.   |
| Minocycline                    | Broad-spectrum tetracycline antibiotic; Highly lipid soluble and brain penetrant; Exhibits significant neuroprotective and anti-inflammatory in neurodegenerative diseases and stroke; Decreases cytokine inflammatory signaling apoptosis and microglia activation [186–188].   | Decreased infarcts, brain swelling and neurological deficits; decreased MMP-9/BBB injury [189–191] and ICE, COX-2, PGE2 [192]; Protects aged male and female mice [193] and diabetic and hypertensive rats [194]; Does not protect in MMP-9 KO mice [195]; Decreases apoptosis [196], microglia expressing HMGB1 [197] and leukocytes, 5-LOX [198]; No effect in female rats [199]. | Minocycline plus Delayed rt-PA therapy with minocycline reduced brain infarction, intracerebral hemorrhage, and extended rt-PA to treat [200]; decreased MMP-9 levels, reduced deficits, and hemorrhage [201]; MMP-9 was correlated with infarcts and hemorrhage [200]; Also decreased IL-1 $\beta$ , neutrophil infiltration and microglia activation in diabetic rats [54, 202]. | Minocycline improved ischemic stroke functional outcome [189, 203] and reduced MMP-9 in controls and rt-PA treatment [204].<br><i>Initiated Trial:</i> The WAIMATSS is a multicentre, prospective, and randomised pilot study of intravenous minocycline, 200 mg 12 hourly for 5 doses, compared with standard care, in patients with ischaemic stroke treated with intravenous rt-PA. The primary endpoint is hemorrhagic conversion with rt-PA [166]. |

(continued)

Table 2.3 (continued)

| Drug | Description - mechanism of action   | Preclinical monotherapy   | Preclinical adjunct to thrombolysis  | Clinical data (if available)  |
|------|---|---|--|---|
| HDL  | HDLs display pleiotropic effects including antioxidant, anti-apoptotic, anti-inflammatory, anti-thrombotic or anti-proteolytic properties that account for their protective action on endothelial cells. Vasodilatation via production of nitric oxide [205]. | rt-PA alone increased mortality and rate of hemorrhagic transformation at 24 h in two models of prolonged focal ischemia [206]. | Cotreatment of rt-PA with HDL significantly reduced stroke-induced mortality and rt-PA-induced intracerebral parenchymal hematoma. In vitro endothelial cell assay exhibited decreased BBB-permeability and increased endothelial cell organization [206]. | <i>Risk for stroke evaluated:</i> Increased HDL-C levels are associated with reduced risk of ischemic stroke in the elderly and among different racial or ethnic groups [207]; Trend identified for higher risk of stroke with lower HDL; In patients with recent stroke or transient ischemic attack and no coronary heart disease, lower baseline HDL predicted the risk of recurrent stroke [208]. |



|                              |   |   |   |   |
|------------------------------|---|---|---|---|
| Fingolimod (FTY720, Gilenya) | Sphingosine analog; when phosphorylated acts on sphingosine 1-phosphate receptors; Regulates cell proliferation, apoptosis, adhesion, migration, differentiation/morphogenesis, inflammation, and/or blood–brain barrier (BBB) integrity [209, 210] | Reduces deficits and infarcts [211]; Reduces edema, lymphocytes, improves deficits in hemorrhagic [185]; Reduces neutrophils, microglia and ICAM-1 positive blood vessels [212] and apoptosis [213, 214]. | Reduces the risk of hemorrhagic transformation associated with delayed rt-PA [216]; significantly reduced brain atrophy, neuronal cell loss and inflammation in hemorrhagic stroke [217]. | No clinical trials currently planned for acute ischemic stroke. Clinical trials did lead to the approval of fingolimod for treatment of multiple sclerosis [218]; data here demonstrated benefit on relapses, disability progression, magnetic resonance imaging (MRI) activity, and brain volume loss. |
|                              | Metaanalysis of preclinical data indicates protection but suggests aged animals and comorbidities like diabetes and hypertension be evaluated [215].  |   |   |   |
| Erythropoietin               | Glycoprotein hematopoietic factor regulates red blood cells; Neuroprotective effects. Erythropoietic effects. Enhances angiogenesis and neurogenesis.   | Reduces atrophy. enhances angiogenesis, neurogenesis, and axonal remodeling. Antiapoptotic, antioxidant, anti-inflammatory. Reduces infarct, and neurological deficits [219, 220].                        | EPO exacerbates rt-PA-induced brain hemorrhage without reduction of ischemic injury when administered 6 h after stroke in a rat model of embolic stroke [70].                             | <i>Monotherapy:</i> Improves neurological outcome and decreases infarct volume [72, 221].<br><i>rt-PA adjuvant:</i> Increased hemorrhage and mortality with rt-PA treatment [71].   |

The free radical scavengers, NXY-059 was a failure and was very disappointing for a variety of “translational” and “strategy in stroke drug discovery” reasons [67, 69] However, uric acid and Edaravone are promising and still moving forward clinically. For hyperbaric and normobaric oxygenation, the preclinical data looks very interesting and supportive, but proceeding into clinical trials for monotherapy and use with rt-PA will be done carefully in light of potential risks. The Rho Kinase inhibitor, Fasudil, has an interesting preclinical profile of activity (i.e., very encouraging mixed actions that can be efficacious). However, much more work with this and perhaps other inhibitors are required before clinical entry. Minocycline is a case where an old drug has been repositioned for new use in stroke. The data are very exciting and the WAIMATSS pilot study may provide more direction to what might be an effective anti-inflammatory-protectant stroke therapy that might also provide an adjunct to and extent the use of rt-PA.

The use of HDL in stroke is in a very early stage but certainly is interesting based upon its known cardiovascular protective effects. Fingolimod results also are very interesting. Although fingolimod is currently approved for use in multiple sclerosis, its use has been limited by concerns regarding cardiac effects, infection, and macular edema as well as the relative lack of long-term safety data for this drug with a novel mechanism of action. As is now preferred in preclinical research, a meta-analysis of all animal data on fingolimod has been published, and it has been suggested that the drug be evaluated in aged animals, and that comorbidities like diabetes and hypertension be studied with its use to increase confidence in the preclinical to clinical translation prior to initiating clinical trials in stroke.

Erythropoietin is probably one of the most studied molecules in stroke. It clearly has demonstrated exceptional efficacy in both brain protection and brain restoration of function after injury has occurred. However, the effects that stopped the clinical trial was indeed combined use with rt-PA. The hematopoietic factor increased rather than decreased MMP-9 and thus exacerbates and increases the occurrence of hemorrhagic conversion preclinically [70] and clinically [71]. The preclinical work can predict this clearly and will be carefully checked for any “rt-PA plus” stroke molecules in the future. The use of erythropoietin for monotherapy and certainly for functional restoration following stroke will continue forward [72].

## Summary and Conclusions

Data from experimental cerebral ischemia studies have consistently demonstrated the need to treat acute clinical stroke within a few hours or less to effectively reduce stroke morbidity and mortality [73, 74]. Specifically, with reversible MCA occlusion models of focal cerebral ischemia animals uniformly survive without debilitating but with some neurologic deficit if the occlusion is for less than 2–3 h [75]. In primates, MCA occlusion for 3 h or less will lead to clinical improvement and a decrease in infarct size, with significant recovery generally associated with less than 2 h of MCA occlusion [76, 77]. Therefore, it appears unlikely that on the average

ischemic brain can be salvaged if vascular occlusion persists longer than 4–6 h (similar to the pathophysiology of myocardial ischemia). If rt-PA is administered immediately after experimental embolic occlusion, significant reduction in neurologic damage occurs [4]. Other “more recently studied” thrombolytics have not improved upon rt-PA’s efficacy to date. However, thrombolytic therapy in stroke has come a long way [222]. Perhaps new thrombolytics will offer the promise of more effective and safer acute pharmacological recanalization strategies, although it is too early to tell. However, basic and clinical research continue to identify, evaluate and discover potential newer approaches to thrombolysis and cellular-brain protection. Adjuncts to thrombolysis research are currently addressing our need to fill the therapeutic void in stroke. Our positive vision for the future is a transformation from the current and unfortunately unacceptable delivery of rt-PA to only a small number of patients to the optimized use of well-defined combination therapies that are designed to reach many more patients in the future.

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