

tPA Helpers in the Treatment of Acute Ischemic Stroke: Are They Ready for Clinical Use?

Jong S. Kim

Department of Neurology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea

Tissue plasminogen activator (tPA) is the only therapeutic agent approved to treat patients with acute ischemic stroke. The clinical benefits of tPA manifest when the agent is administered within 4.5 hours of stroke onset. However, tPA administration, especially delayed administration, is associated with increased intracranial hemorrhage (ICH), hemorrhagic transformation (HT), and mortality. In the ischemic brain, vascular remodeling factors are upregulated and microvascular structures are destabilized. These factors disrupt the blood brain barrier (BBB). Delayed recanalization of the vessels in the presence of relatively matured infarction appears to damage the BBB, resulting in HT or ICH, also known as reperfusion injury. Moreover, tPA itself activates matrix metalloproteases, further aggravating BBB disruption. Therefore, attenuation of edema, HT, or ICH after tPA treatment is an important therapeutic strategy that may enable clinicians to extend therapeutic time and increase the probability of excellent outcomes. Recently, numerous agents with various mechanisms have been developed to interfere with various steps of ischemia/reperfusion injuries or BBB destabilization. These agents successfully reduce infarct volume and decrease the incidence of ICH and HT after delayed tPA treatment in various animal stroke models. However, only some have entered into clinical trials; the results have been intriguing yet unsatisfactory. In this narrative review, I describe such drugs and discuss the problems and future directions. These "tPA helpers" may be clinically used in the future to increase the efficacy of tPA in patients with acute ischemic stroke.

Correspondence: Jong S. Kim
Department of Neurology, Asan Medical Center, University of Ulsan College of Medicine, 88 Olympic-ro 43-gil, Songpa-gu, Seoul 05505, Korea
Tel: +82-2-3010-3442
Fax: +82-2-474-4691
E-mail: jongskim@amc.seoul.kr

Received: March 11, 2019
Revised: April 4, 2019
Accepted: April 15, 2019

Keywords Tissue plasminogen activator; Stroke; Thrombolysis; Adjuvant therapy; Neuroprotection

Introduction

Currently, tissue plasminogen activator (tPA) is the only therapeutic agent approved to treat patients with acute ischemic stroke (AIS).¹ The clinical benefits of tPA treatment manifest when it is administered within 4.5 hours of stroke onset.²⁻⁴ However, intravenous (IV) tPA administration is associated with increased occurrence of intracranial hemorrhage (ICH) and hemorrhagic transformation (HT).^{3,5} Particularly, delayed tPA administration increases the risk of edema, HT, and ICH in both experimental animal models⁶ and patients with AIS.⁶

In the ischemic brain, vascular remodeling factors are upregulated and microvascular structures are destabilized, causing blood brain barrier (BBB) disruption. The breakdown of the BBB combined with delayed vessel reperfusion renders the ischemic brain tissue vulnerable to edema, HT, and ICH,⁷⁻¹⁰ a phenomenon often called reperfusion injury. In one clinical study, early BBB disruption, detected by gadolinium enhancement in magnetic resonance imaging (MRI), predicted a higher risk of symptomatic HT.¹¹ Moreover, tPA itself is neurotoxic and aggravates the brain damage caused by glutamic acid release after ischemia if it leaks into the brain parenchyma. tPA promotes

leukocyte infiltration, microglial activation, and free radical production in the ischemic brain.¹² More importantly, it activates matrix metalloproteases (MMPs) that further aggravate BBB disruption.^{9,13,14} Therefore, apart from recanalization, attenuation of the development of edema, HT, and ICH after tPA treatment is an important therapeutic consideration.

Although many neuroprotective agents interfere with the ischemic cascade,¹⁵ previous trials have failed to confirm their clinical efficacy in stroke patients, even when administered early.^{16,17} This therapeutic failure may occur because drug delivery to the ischemic brain area is poor in cases of decreased blood flow and occluded cerebral blood vessels.^{18,19} Neuroprotectants may work better when reperfusion therapy is concomitantly achieved.³ Particularly, if the neuroprotectants can also stabilize the BBB, they may be used as “tPA helpers” to protect dying neurons and decrease the incidence of ICH or HT,^{20–22} eventually resulting in more favorable functional outcomes in patients undergoing tPA therapy. They may enable clinicians extend the time window for tPA therapy. Agents inhibiting vascular thrombosis may also serve as tPA helpers.^{23,24} Drugs with various mechanisms have been developed for this purpose, and numerous preclinical studies have examined their efficacy. Unfortunately, few well-designed clinical trials have been published. In this narrative review, I describe the various potential tPA helpers that have been developed to increase the efficacy and decrease the side effects of tPA in patients with AIS.

Agents that have shown benefits in preclinical studies

Various drugs have been developed as tPA helpers, and mostly through mechanisms such as anti-inflammation, suppression of MMP activation, vascular protection, or a combination of these. Although the mechanisms are often multiple, I will describe them according to their main mechanisms (Table 1).

Anti-inflammation and suppression of MMP activation

Tumor necrosis factor- α inhibitor

In one study, investigators intracisternally administered either tumor necrosis factor TNF- α or a goat-anti-rabbit-TNF- α antibody to rabbits subjected to stroke.²⁵ TNF- α administration increased the incidence of stroke-induced hemorrhage from 18.5% to 53.3% (188% increase). Meanwhile, tPA administration (3.3 mg/kg) increased hemorrhage incidence from 18.5% to 76.5% (314% increase). This effect was reversed by an anti-TNF- α antibody; in the tPA+anti-TNF- α antibody group, the

hemorrhage rate was 38.8%.

GM6001

GM6001 binds to the active sites of MMPs and prevents conversion of pro-MMPs into active forms. It attenuates the degradation of tight junction proteins, such as zona occludens (ZO)-1 and occludin in rats, so it may ameliorate BBB damage.²⁶ In one study,²⁷ mice subjected middle cerebral artery (MCA) occlusion (MCAO) were treated with vehicle, delayed tPA (10 mg/kg; IV) alone, or tPA+GM6001 (100 mg/kg; intraperitoneal). GM6001 significantly reduced MMP-9 activation and inhibited the tPA-induced degradation of occludin and ZO-1. Treatment with GM6001 also significantly improved the survival rate and locomotor activity after 7 days.

Niaspan

Niaspan, an extended-release formulation of niacin, is widely used to increase the level of high-density lipoprotein cholesterol and prevent cardiovascular diseases. In one study, rats were subjected to embolic MCAO and treated with low-dose Niaspan alone (n=7; oral Niaspan; 20 mg/kg 4 hours after MCAO and then daily for 7 days), tPA alone (10 mg/kg; n=7), a combination (n=7), or saline control (n=9), 4 hours after stroke.²⁸ Low-dose Niaspan+tPA significantly improved functional outcomes and reduced infarct volume compared to the saline control group, while treatment with Niaspan or tPA alone did not. Combination treatment reduced apoptosis and decreased the expression of TNF- α and Toll-like receptors in the ischemic brain compared to the monotherapy groups. These results suggest that Niaspan's effect is mediated through the modulation of neuroinflammation.

Pyrrolidine dithiocarbamate

Pyrrolidine dithiocarbamate (PDTC) is a small molecule with anti-inflammatory and anti-oxidant properties. It inhibits nuclear factor (NF)- κ B activation and activates Akt protein, which is believed to be pro-survival.²⁹ In one study, rats were subjected to embolic right MCAO and administered IV tPA (10 mg/kg) 4 hours later. PDTC (50 mg/kg) was administered via gastric gavage either 30 minutes or 4 hours after stroke onset. Two days after the stroke, the neurological outcomes were evaluated, and the right frontal cortex area, an ischemic penumbral region, was harvested for analysis. PDTC administered 30 minutes but not 4 hours after the stroke reduced infarct volume and improved neurological functions. It also reduced the prevalence of tPA-induced hemorrhages and attenuated the expression of proinflammatory cytokines, oxidative stress, and MMP-2 activity in the right frontal area.³⁰ Given early, PDTC

Table 1. Drugs that showed benefits in animal models of stroke treated by tPA

Agent	Presumed mechanisms	Animal model	Comments
Anti-TNF- α antibody	Antiinflammation, MMP inactivation	Rabbit, embolic	
GM6001	MMP inactivation	Mice, transient MCAO	Inhibited tPA induced degradation of occludin
Niacin	Antiinflammation	Rat, embolic MCAO	Reduced expression of TNF- α and Toll-like receptors
Pyrrrolidine dithiocarbamate	Antiinflammation, Anti-oxidant	Rat, embolic MCAO	Inhibits nuclear factor (NF)- κ B activation
Granulocyte colony-stimulating factor	Improving angiogenesis	Rat, transient MCAO	Increased angiogenesis markers and VEGF expression
Bryostatin	Protein kinase C activator, MMP inactivation	Rat, embolic MCAO	
BAY 60-6583	Adenosine A2b receptor agonist, MMP inactivation	Rat, transient MCAO	
Progesterone	MMP inactivation	Rat, MCAO	
Baicalin	MMP inactivation	Rat, transient MCAO	
Neuroserpin	Blocking extravascular tPA effects	Rat, embolic MCAO	Reduced the blood brain barrier disruption detected by MRI
Transforming growth factor- β 1	Blocking extravascular tPA effects, MMP inactivation	Rat, MCAO	
Minocycline	Antiinflammation, MMP inactivation	Rat, embolic MCAO	
Uric acid	Anti-oxidant, Endothelial protection	Rat, embolic MCAO	
Statin	Antiinflammation, MMP inactivation, Anti-oxidant	Rabbit, embolic	
Edaravone	Antioxidant, Antiinflammation	Rat, transient MCAO	
GP IIb/IIIa receptor antagonist	Antiplatelet	Rat, embolic MCAO	Decreased microvascular platelet accumulation
3K3A-APC	Antiinflammation, antiapoptotic, antithrombotic	Mice, transient MCAO	
Fingolimod	Antiinflammation	Mice, transient MCAO	
Otaplimastat	Anti-inflammatory, MMP inactivation	Rat, embolic MCAO	
Epigallocatechin gallate	Antioxidant, MMP inactivation	Rat, transient MCAO	

tPA, tissue plasminogen activator; TNF, tumor necrosis factor; MMP, metalloproteases; MCAO, middle cerebral artery occlusion; VEGF, vascular endothelial growth factor; MRI, magnetic resonance imaging.

may have a neuroprotective effect in subjects using tPA.

Bryostatin (protein kinase C activator)

In one study, rats received either tPA (6 hours after MCAO) or tPA+bryostatin, a potent protein kinase C (PKC) activator (6 hours, followed by 3, 6, 9, 12, 15, and 18 days after MCAO). Bryostatin-treated rats showed improved survival, as well as reduced infarct volume, hemispheric swelling, and improved neurological function 21 days after MCAO.³¹ In another study,³² bryostatin (or vehicle) was administered 2 hours after MCAO, while IV tPA was administered 6 hours after MCAO. Rats treated with bryostatin prior to tPA administration showed decreased hemispheric swelling compared to rats treated with tPA alone. Administration of bryostatin also attenuated BBB disruption and HT, and down-regulated MMP-9 expression while up-regulating PKC expression 24 hours after MCAO.

BAY 60-6583 (adenosine A2b receptor agonist)

The adenosine A2b receptor (A2bR) regulates vascular protection. In one study,³³ investigators used a rat transient MCAO model to show that mRNA and protein expression of A2bR increased more after ischemia-reperfusion than did expression of the other three adenosine receptors. tPA administration reduced A2bR expression in ischemic brain microvessels. Post-treatment using BAY 60-6583 (1 mg/kg) at the start of reperfusion reduced lesion volume, brain swelling, BBB disruption, and tPA-exacerbated HT after 24 hours. BAY 60-6583 also inhibited tPA-enhanced MMP-9 activation, probably by elevating the expression of tissue inhibitor of MMP-1 and thereby reducing the degradation of tight junction proteins.

Progesterone

Progesterone has a neuroprotective effect in ischemia. In one

study, rats undergoing MCAO received either tPA treatment at 4.5 hours, or additional intraperitoneal progesterone treatment at 2 hours followed by subcutaneous progesterone injection 6 hours after MCAO. tPA treatment induced significant HT and brain swelling whereas progesterone+tPA ameliorated HT, edema, BBB permeability, and MMP-9 activation; it also prevented tPA-induced decreases in transendothelial electrical resistance and expression of occludin and claudin-5.³⁴

Baicalin

Baicalin (C₂₁H₁₈O₁₁; 5,6,7-trihydroxyflavone 7-O-β-d-glucuronide or baicalein 7-O-β-d-glucuronic acid or 7-d-glucuronic acid-5,6-dihydroxyflavone) is an important flavonoid component in the roots of *Scutellaria baicalensis* Georgi, which have been used as an oriental herbal medicine. Baicalin has neuroprotective properties mediated through various mechanisms, including anti-oxidative stress, anti-excitotoxicity, anti-apoptosis, anti-inflammation, neurogenesis stimulation, and upregulation of neuronal protective factors.³⁵ In a recent study,³⁶ rats were subjected to MCAO for 4.5 hours and then received IV tPA (10 mg/kg) for 0.5 hours, followed by a 19-hour reperfusion. Baicalin (50, 100, or 150 mg/kg) was administered via the femoral vein 4.5 hours after MCAO. Delayed tPA infusion increased HT, BBB damage, and apoptotic cell death in the ischemic brains, while co-treatment with baicalin significantly reduced HT, tPA-mediated BBB disruption, and MMP-9 activity.

Endothelial protection and improvements in angiogenesis

Granulocyte colony-stimulating factor

Rats undergoing MCAO were treated with vehicle, tPA (10 mg/kg), or tPA + IV granulocyte colony-stimulating factor (G-CSF) 300 μg/kg 6 hours after MCAO.³⁷ Twenty-four hours later, the rats given G-CSF+tPA displayed a 25% improvement in neurological functions and a 38.9% reduction in hemorrhage. Western blots showed that the rats given G-CSF+tPA had elevated levels of the angiogenesis markers angiopoietin-2 and von-Willebrand factor in the ischemic brain compared with rats treated using tPA only, as well as up-regulation of vascular endothelial growth factor. These findings suggest that G-CSF attenuated the delayed-onset, tPA-induced HT, probably by enhancing angiogenesis. In a subsequent study, G-CSF administration also increased the endothelial progenitor cell population and vasculogenesis in the ischemic brain.³⁸

Blocking extravascular tPA effects

Neuroserpin

Neuroserpin is a natural inhibitor of tPA and may reduce tPA-induced neurotoxicity. In one study, rats were subjected to embolic MCAO and treated using neuroserpin (16 μmol/L, at 3 hours)+tPA (10 mg/kg, at 4 hours; n=7), tPA alone (n=7), or saline (n=9).³⁹ MRI measurements were performed to assess BBB leakage. Neuroserpin+tPA significantly reduced BBB damage, brain edema, and ischemic lesion volume compared with tPA alone. Neuroserpin administration may block the extravascular effect of tPA.

Transforming growth factor-β1

Transforming growth factor-β1 (TGF-β1) promotes extracellular matrix accumulation. In one study,⁴⁰ rats were given saline, tPA (10 mg/kg) alone, or tPA+TGF-β1 (2.5 μg per animal; IV over 5 minutes) 3 hours after MCAO. Examination of the brain 24 hours after MCAO showed basement membrane damage, BBB disruption, and HT in rats treated with tPA. These effects were attenuated in rats co-treated with TGF-β1. Furthermore, TGF-β1 inhibited tPA-mediated activation of MMP-2 and MMP-9 and up-regulated plasminogen activator inhibitor-1 (PAI-1) expression in brain tissue. TGF-β1 may up-regulate PAI-1 expression in the ischemic brain, thereby reducing extravascular tPA activity by forming the PAI-1/tPA complex, resulting in stabilization of tPA-induced BBB disruption.

Drugs that have shown potential benefits in clinical studies

Drugs that have been examined in clinical studies are presented below and in Table 2. As they usually have diverse action mechanisms, they are not listed according their mechanisms.

Minocycline

Aside from anti-bacterial effects, minocycline has neuroprotective effects in various neurological disease processes.⁴¹ In a study using spontaneously hypertensive rats subjected to embolic MCAO, 1-hour tPA therapy restored perfusion and reduced infarction, whereas 6-hour tPA administration worsened HT. Minocycline+6-hour tPA therapy decreased plasma MMP-9 levels, reduced infarct volume, and decreased ICH.⁴² In another study involving rats with type 1 diabetes,⁴³ minocycline+tPA significantly reduced brain infarction, edema, and ICH 24 hours after stroke compared with saline or tPA monotherapy. The combination therapy also attenuated neutrophil infiltration, microglial activation, MMP-9 activation, and degradation of

Table 2. Drugs that showed benefits in clinical studies in patients treated by tPA

Agent	Trial name	Trial characteristics	No of patients	Enrollment from symptom onset (hr)	Administration	Result
Minocycline						
Lampl et al. (2007) ⁴⁵		R, open-label, evaluator-blinded	152	6–24	200 mg, once daily, oral, 5 days	Safe, better in NIHSS, mRS and BI
Padma Srivastava et al. (2012) ⁴⁶		R, single-blinded open-label study	50	6–24	200 mg, once daily, oral, 5 days	Safe, better in NIHSS, mRS and BI
Uric acid	URICO-ICTUS	R, D, P	411	<4.5	1,000 mg during tPA infusion	Safe, not proven to be effective
Simvastatin	STARS07	R, D, P	104 (half received tPA)	<12	40 mg once daily, oral, 90 days	Safe, not proven to be effective
Edaravone	YAMATO	R (for timing, no placebo), open label	165	<4.5	Oral, 30 mg twice/day, 7 days	Safe, timing of edaravone didn't affect recanalization rate
GP IIb/IIIa receptor antagonist						
Seitz et al. (2004) ²³		R, open-label	47	<4.5	0.4 µg/kg/min for 30 minutes followed by 0.1 µg/kg/min for 24 hours	Safe, decreased infarct volume
Li et al. (2016) ⁶⁸		R, open-label	41	<4.5	0.4 µg/kg/min for 30 minutes followed by 0.1 µg/kg/min for 24 hours	Safe, less re-occlusion at 24 hours, improved NIHSS score More favorable mRS at 3 months
Argatroban	ARTSS-2	R	90 (2 dosages)	<4.5	100 U/kg bolus+IV infusion for 48 hours	Safe, tendency for improved outcome
3K3A-APC	RHAPSODY	R, D, P	110 (4 dosages)	Patients undergoing tPA or EVT	IV infusion twice daily×5	Safe, decreased ICH rates and volume
Fingolimod		R, open label, evaluator blinded	47	4.5–72	0.5 mg every 12 hours×5	Safe, more favorable mRS
Otaplimastat	SAFE-TPA	R, D, P	69 (2 dosages)	<4.5	IV infusion twice daily×6	Safe, mRS distribution more favorable with low dosage
Epigallocatechin gallate		R, D, P	371	<4.5	500 mg daily×7 days	Safe, improved NIHSS score

tPA, tissue plasminogen activator; R, randomized; NIHSS, National Institutes of Health Stroke Scale; mRS, modified Rankin Scale; BI, Barthel index; URICO-ICTUS, Efficacy Study of Combined Treatment With Uric Acid and r-tPA in Acute Ischemic Stroke; D, double-blinded; P, placebo-controlled; STARS07, Stroke Treatment with Acute Reperfusion and Simvastatin 07; YAMATO, Tissue-Type Plasminogen Activator and Edaravone Combination Therapy; ARTSS-2, Argatroban with Recombinant Tissue Plasminogen Activator for Acute Stroke; IV, intravenous; RHAPSODY, The Safety Evaluation of 3K3A-activated protein C in Ischemic Stroke; EVT, endovascular thrombectomy; ICH, intracranial hemorrhage; SAFE-TPA, Safety and Efficacy of Otaplimastat in Patients with AIS Receiving recombinant tPA.

the tight junction protein claudin-5 in periinfarct brain tissues.

Minocycline is safe when administered to patients with AIS receiving tPA.⁴⁴ Two small randomized trials used the National Institutes of Health Stroke Scale (NIHSS), modified Rankin Scale (mRS), and Barthel index to show a beneficial effect of minocycline in patients with AIS receiving tPA after 7 days, 30 days,⁴⁵ and 3 months of follow-up.⁴⁶ In 2018, two systematic reviews were published.^{44,47} In patients with AIS, minocycline was associated with higher proportions of patients with 3-month mRS-scores of 0–2 (risk ratio, 1.59; 95% confidence interval [CI], 1.19 to 2.12; *P*=0.002; *I*²=58%), whereas reduced 3-month NIHSS scores (mean difference, 2.84; 95% CI, 5.55 to 0.13; *P*=0.04; *I*²=86%). Although data remain limited, minocycline is a promising neuroprotective agent in patients with AIS. The West Australian Intravenous Minocycline and tPA Stroke

Study (WAIMATSS)⁴⁸ aimed to determine the safety and efficacy of adding minocycline to tPA in patients with AIS. Although patients in the minocycline group had lower plasma MMP-9 levels, the main results are not yet available.⁴⁹

Uric acid

Uric acid is an endogenous antioxidant derived from purine metabolism. It also prevents the production of superoxide, nitric oxide, nitric oxide synthase (NOS), endothelial NOS, interleukin 18 in the ischemic arterial wall, and nitrosylated protein in the brain, suggesting that uric acid-mediated neuroprotection mainly drives from the protected cerebral vasculature.⁵⁰ In an experiment using thromboembolic MCAO rat models,⁵¹ IV uric acid (16 mg/kg) was injected 20 minutes after MCAO, whereas IV tPA (10 mg/kg) was administered after 3 hours. A third group

of rats received a combined treatment. Treatment using either uric acid or tPA reduced infarct volume compared with controls, but the protective effect was greater with uric acid+tPA.

Efficacy Study of Combined Treatment With Uric Acid and r-tPA in Acute Ischemic Stroke (URICO-ICTUS) trial⁵² was a randomized, double-blind, placebo-controlled, phase 2b/3 trial that recruited patients with AIS undergoing tPA treatment, who additionally received either IV uric acid (1,000 mg) or a placebo. The primary outcome was the proportion of patients with an mRS score of 0–1 after 90 days. Four hundred and eleven patients (211 receiving uric acid, 200 receiving a placebo) were enrolled. Thirty-nine percent of the patients who received uric acid and 33% in the placebo group had an excellent outcome (adjusted risk ratio, 1.23; 95% CI, 0.96 to 1.56; $P=0.099$). No significant differences were reported between groups with respect to death (13% vs. 16%), symptomatic ICH (4% vs. 3%), and serious adverse events (SAEs; 12% vs. 13%). The addition of uric acid to tPA therapy was safe, but did not improve clinical outcomes.

Secondary analysis showed that the primary outcome had been achieved in several subgroups, including those who had received endovascular thrombectomy (EVT) as part of their reperfusion therapy.⁵³ In addition, uric acid administration decreased the incidence of early neurological worsening.⁵² The Uric acid in Reperfusion Injury Control (URIC) trial was recently planned to validate the URICO-ICTUS results in a larger population of patients treated using EVT.⁵⁰

Statins

Although statins are widely-used lipid lowering agents, they have many other benefits; they have antioxidant properties, inhibit inflammation and MMP-9 activation, and increase nitric oxide bioavailability, thereby improving endothelial function.⁵⁴

One study⁵⁵ showed that IV tPA (3.3 mg/kg, IV) 1 hour following clot-embolization significantly increased hemorrhage volume (by 175%) and hemorrhage incidence (by 60%) compared to control in white rabbits. Simvastatin (20 mg/kg) administered subcutaneously 24 and 4 hours prior to clot embolization significantly decreased the incidence of tPA-induced hemorrhage ($P=0.022$) and infarct volume ($P=0.0001$). Another study⁵⁶ investigated the neuroprotective effects of atorvastatin in combination with delayed tPA therapy in rats subject to embolic MCAO. tPA infusion at 6 hours increased the incidence of HT and failed to reduce infarct volume compared with the control group. However, adjuvant treatment with atorvastatin at 4 hours reduced tPA-induced up-regulation of protease activated receptor 1 (PAR1), intercellular adhesion molecule-1 (ICAM-1), and MMP-9, and reduced cerebral microvascular

platelet levels, neutrophil count, and fibrin deposition compared with rats treated using tPA alone. Both studies suggested that statins can attenuate tPA-induced hemorrhage.

Stroke Treatment with Acute Reperfusion and Simvastatin (STARS) 07⁵⁷ was a multicenter, randomized, double-blind, placebo-controlled trial aiming to assess the efficacy and safety of simvastatin treatment in acute stroke. Patients with AIS were randomized into the oral simvastatin (40 mg) or placebo groups and treated once daily for 90 days. The primary outcome was the proportion of patients with mRS scores ≤ 2 after 90 days. One hundred-four patients were enrolled, with about half (55 patients) receiving IV tPA. No differences were found between the treatment arms regarding primary outcome (adjusted odds ratio, 0.99; 95% CI, 0.35 to 2.78; $P=0.98$) or rate of HT. In *post hoc* analyses of patients receiving tPA, a favorable effect of simvastatin treatment was noted, with a higher proportion of patients experiencing major neurological recovery (adjusted odds ratio, 4.14; 95% CI, 1.18 to 14.4; $P=0.02$).

The EUREKA study (effects of very early use of rosuvastatin in preventing recurrence of ischemic stroke),⁵⁸ attempted to examine the effect and safety of rosuvastatin in patients with AIS. This randomized, double-blind, multi-center trial compared rosuvastatin (20 mg) with a placebo in statin-naïve stroke patients who underwent diffusion-weighted imaging (DWI) within 48 hours of symptom onset. The primary outcome was occurrence of new ischemic lesions on follow-up DWI after 5 or 14 days. This trial was stopped early after randomization of 316 patients due to slow enrollment. Among 289 patients with at least one follow-up imaging, the frequency of new ischemic lesions on DWI (rosuvastatin 19.7% vs. placebo 23.6%) as well as infarct volume growth after 5 days, did not differ between the two groups. However, brain hemorrhages detected on gradient-recalled echo MRI occurred less frequently in the rosuvastatin group (6/137; 4.4%) than in the placebo group (22/152; 14.5%; $P=0.007$), suggesting that statin use in AIS may prevent HT, possibly associated with statin's effect in inhibiting MMP-9 activation.⁵⁴ Unfortunately, the number of patients who had received tPA was very low in this study (four in the rosuvastatin group, five in the placebo group), so it was impossible to assess the efficacy of rosuvastatin in preventing tPA-associated hemorrhages.

Edaravone

Edaravone, a free radical scavenger, confers a neuroprotective effect by inhibiting vascular endothelial cell injury and ameliorating neuronal damage in ischemic brain models. In one study involving spontaneously hypertensive rats subjected to MCAO, the animals were administered vehicle alone, tPA alone, or

edaravone+tPA. Electron microscopic analyses showed that the basement membrane was disintegrated and detached from the astrocyte endfeet in tPA-treated animals, which was associated with HT. Edaravone prevented dissociation of the neurovascular unit, dramatically decreased HT, and improved the neurological score and survival rate of the rats.⁵⁹

Investigators in the Tissue-Type Plasminogen Activator and Edaravone Combination Therapy (YAMATO) study⁶⁰ attempted to assess whether edaravone administration before or during tPA therapy can expedite the recanalization of occluded arteries. This was a multicenter, prospective, randomized, and open-label study. One-hundred and sixty-five patients with AIS secondary to MCAO were randomly allocated to the early group (IV edaravone [30 mg] started before or during tPA) and the late group (edaravone started after tPA). Recanalization, ICH, and favorable outcome (mRS score of 0–2) after 3 months were similar between the groups, suggesting that the timing of edaravone infusion does not affect the clinical outcome. A recent, large observational study provided some evidence that edaravone is associated with better functional outcome when it is used in patients who have undergone acute EVT.⁶¹ Unfortunately, no qualified studies have yet compared edaravone+tPA with placebo+tPA.

GP IIb/IIIa receptor antagonist

The overall recanalization rate is approximately 46% after IV tPA.⁶² However, re-occlusion after initial recanalization occurs in 14% to 34% of patients and is associated with early neurological deterioration. Re-occlusion usually occurs due to activated platelet aggregation and endothelial damage.^{63,64} Although early administration of antiplatelet agents after IV tPA may prevent platelet aggregation and subsequent vascular re-occlusion, the Antiplatelet Therapy in Combination With Recombinant tPA Thrombolysis in Ischemic Stroke (ARTIS) trial concluded that early IV administration of aspirin (300 mg) shortly after recombinant tPA did not improve outcomes after 3 months, but that it significantly increased the rate of symptomatic ICH.⁶⁵ However this failure may have been due to the long-lasting and irreversible anti-platelet effects of aspirin.

A GP IIb/IIIa receptor antagonist has a short half-life and blocks the final pathway to platelet aggregation and thrombus formation. In one study, rats were subjected to embolic MCAO and treated with a GP IIb/IIIa antagonist, 7E3 F(ab')₂ (2 mg/kg)+tPA (10 and 5 mg/kg), tPA alone, 7E3 F(ab')₂ alone, or saline 4 hours after MCAO. The results showed that 7E3 F(ab')₂+tPA significantly reduced infarct volume and neurological deficits compared with the saline-treated rats, whereas monotherapy did not. Quantitative measurements of cerebral microvessels revealed that

7E3 F(ab')₂+tPA significantly increased the percentage of fluorescein isothiocyanate-dextran-perfused vessels, and that it decreased microvascular platelet accumulation and MMP-9 immunoreactivity compared with rats receiving monotherapy.⁶⁶

One open label clinical trial²³ studied 47 patients with AIS to investigate the clinical efficacy of IV tPA+tirofiban. Compared with tPA monotherapy (0.9 mg/kg), tPA (20 mg)+tirofiban (0.4 µg/kg/min for 30 minutes), followed by continuous infusion of 0.1 µg/kg/min for 24 hours, reduced the MRI-identified infarct volume on day 8 (50% vs. 30%, *P*<0.03). No symptomatic ICH was observed among the subjects. Another study⁶⁷ reported that MCA recanalization was achieved in 68% of 19 patients with acute MCAO using the combined treatment strategy, compared to 46% of patients treated using conventional IV tPA. In a recent prospective, open-label trial, Li et al.⁶⁸ re-investigated this issue in 41 patients with AIS. The incidence of ICH, mortality, and clinical outcomes were similar between the combined therapy group (tPA [0.9 mg/kg]+tirofiban [0.4 µg/kg/min for 30 minutes followed by continuous infusion of 0.1 µg/kg/min for at least 24 hours]) and the standard tPA therapy group. However, the combined therapy group showed less re-occlusion after 24 hours (2.4% vs. 22.0%, *P*=0.025), lower NIHSS score at day 7 or discharge (1 vs. 6, *P*=0.002), and more favorable functional outcomes after 3 months (70.7% vs. 46.2%, *P*=0.026). These preliminary trials suggest that combined therapy may be superior to tPA monotherapy.

Argatroban

Argatroban selectively inhibits free and clot-associated thrombin. In the rabbit arterial thrombosis model, it safely augments the benefits of tPA by improving the rate of recanalization and preventing re-occlusion.⁶⁹ In one study using the rat embolic MCAO model, either tPA or tPA+argatroban was administered 4 hours after MCAO. A significant reduction in lesion volume and fibrin deposition in the ipsilateral cortical microvasculature were observed in the argatroban+tPA group compared with controls. No increase in HT was observed.⁷⁰

The Argatroban Anticoagulation in Patients with Acute Ischemic Stroke (ARGIS-1) study showed that argatroban monotherapy, with mean doses of 1.2 and 2.7 µg/kg/min given within 12 hours, was safe in patients with AIS.¹¹ The Argatroban tPA Stroke Study⁷¹ was an open-label, pilot study of tPA+argatroban treatment in patients with AIS caused by proximal intracranial occlusion. During standard-dose IV tPA therapy, a 100-µg/kg bolus of argatroban, followed by infusion for 48 hours, was adjusted to correspond with a target partial thromboplastin time of 1.75×baseline. Significant ICH had occurred in four patients (6.2%) and recanalization had occurred in 29 patients (61%) at the 2-hour monitoring period.

Argatroban with Recombinant Tissue Plasminogen Activator for Acute Stroke (ARTSS-2) was a randomized, multicenter study that assessed the safety and functional outcome of adjunctive argatroban administered to tPA-treated patients who had not undergone EVT.²⁴ Patients were randomized to receive either no argatroban or argatroban (100 µg/kg bolus) followed by infusion of either 1 µg/kg/min (low dose) or 3 µg/kg/min (high dose) for 48 hours. Ninety patients were randomized: 29 to the tPA alone group, 30 to the tPA+low-dose argatroban group, and 31 to the tPA+high-dose argatroban group. After 90 days, 21%, 30%, and 32% of these patients had an mRS ≤1, respectively, while the rates of symptomatic ICH were similar. The result suggested that argatroban may provide a clinical benefit. Finally, the Safety and Feasibility of Argatroban, Recombinant Tissue Plasminogen Activator, and Intra-Arterial Therapy in Stroke (ARTSS-IA) study aimed to ascertain the feasibility and safety of tPA+argatroban in patients undergoing EVT.⁷² In this single-arm study, all 10 enrolled patients received argatroban before EVT, with no delays in time-metrics compared to non-study patients. Nine patients achieved good reperfusion (Treatment In Cerebral Ischemia grade ≥2b) and none had symptomatic ICH or EVT complications.

3K3A-activated protein C

Protein C is a plasma serine protease zymogen whose active form, activated protein C (APC), exerts a potent anticoagulant activity. In addition, APC has both anti-inflammatory and anti-apoptotic properties in cultured endothelial cells.⁷³ Preclinical studies have indicated that the G-protein-coupled receptor, PAR1, is necessary for APC's diverse pharmacologic effects, and biased signaling mediated by PAR1 is thought to be central to APC's clinical benefits.^{74,75}

In the mouse model of transient MCAO,⁷⁶ treatment using purified human plasma-derived APC (2 mg/kg), delivered IV either 15 minutes before or 10 minutes after stroke induction, reserved cerebral blood flow, reduced infarct volume and brain edema, eliminated brain neutrophil infiltration, reduced the number of fibrin-positive cerebral vessels, and inhibited ICAM-1 expression on ischemic cerebral blood vessels. These results suggest that APC's anti-inflammatory, antithrombotic, and neuroprotective effects improve BBB maintenance. Further studies showed that APC infused simultaneously with, or 3 hours after, IV tPA markedly reduced infarct volume and tPA-induced hemorrhage in mouse models of embolic MCAO. APC also inhibited the pro-inflammatory up-regulation of NF-κB by tPA, and the effects were not observed in PAR1 null mice.⁷⁷

Another study engineered APC by site-directed mutagenesis to produce a selective APC mutant that had three lysine resi-

dues replaced by three alanine residues (3K3A-APC). This mutant lacks >90% of the anticoagulant activity of APC, but retains normal cell signaling.⁷⁵ In a study using a rodent MCAO model,⁷⁸ when tPA was given 4 hours after MCAO, it provided no benefit; rather, it introduced bleeding. In contrast, additional administration of 3K3A-APC reduced infarct volume and eliminated tPA-associated bleeding.

3K3A-APC appears to be safe in human beings.⁷⁹ The Safety Evaluation of 3K3A-activated protein C in Ischemic Stroke (RHAPSODY) was a phase 2, randomized, controlled, blinded trial that aimed to determine the maximum tolerated dose of 3K3A-APC in AIS patients receiving IV tPA, EVT, or both.⁸⁰ One of four doses of 3K3A-APC (120, 240, 360, or 540 µg/kg) or placebo was randomly administered in 110 patients. The maximum tolerated dose was 540 µg/kg. Although there was no difference in prespecified ICH rates, 3K3A-APC reduced ICH rates compared to placebo from 86.5% to 67.4% ($P=0.046$) and total hemorrhage volume from an average of 2.1 ± 5.8 to 0.8 ± 2.1 mL ($P=0.066$).

Fingolimod

Lymphocytes interact with endothelial cells and platelets (thrombo-inflammation), resulting in microvascular dysfunction and infarct growth in the ischemic brain. Fingolimod, a sphingosine-1-phosphate receptor modulator, blocks the egress of lymphocytes from lymphoid organs and reduces the vascular brain damage.⁸¹ In the mouse model of transient MCAO, fingolimod significantly reduced infarct size and improved functional outcome in wild-type mice. This protective effect was lost in lymphocyte-deficient Rag1^{-/-} mice. Fewer lymphocytes were present in the cerebral vasculature of fingolimod-treated wild-type mice, which showed reduced thrombosis and increased cerebral perfusion.⁸²

In an open-label, evaluator-blinded, multicenter trial,⁸³ investigators randomly administered tPA+oral fingolimod (0.5 mg) once daily for 3 days ($n=22$) or tPA only ($n=25$) in patients with AIS. Compared with patients receiving tPA alone, those who received the fingolimod+tPA exhibited fewer circulating lymphocytes, smaller lesion volumes (10.1 mL vs. 34.3 mL, $P=0.04$), less hemorrhage (1.2 mL vs. 4.4 mL, $P=0.01$), and attenuated neurological deficits (NIHSS scores: 4 vs. 2, $P=0.02$) on day 1. Furthermore, restrained lesion growth from day 1 to 7 (-2.3 mL vs. 12.1 mL, $P<0.01$), with better recovery on day 90 (mRS score ≤1: 73% vs. 32%, $P<0.01$) was evident in patients given the combined medication. There were no SAEs.

More recently, Tian et al.⁸⁴ conducted a prospective, randomized, open-label, blinded endpoint trial, enrolling patients with occlusion of the internal carotid artery or proximal middle ce-

rebral artery within 4.5 to 6 hours from symptom onset. Forty-six patients were randomly assigned to receive either tPA alone or tPA+fingolimod. Compared with the tPA monotherapy group, the fingolimod+tPA group exhibited greater early decrease in NIHSS score, as well as more favorable mRS distribution on day 90. They also exhibited a greater reduction in the non-perfusion lesion than those receiving tPA only, and this was accompanied by reduced infarct growth over 24 hours.

Otaplimastat

Otaplimastat, previously coded as SP-8203, is a small molecule with a quinazoline-2,4-dione scaffold. It has many potent neuroprotective effects mediated through anti-excitotoxic, anti-oxidant, and anti-inflammatory pathways.⁸⁵⁻⁸⁸ Preclinical evidence regarding otaplimastat has been evaluated in various animal models, including the rat focal and gerbil global ischemic models.⁸⁵⁻⁸⁸ When applied as a monotherapy, otaplimastat exhibits significant anti-ischemic and neuroprotective effects. Notably, in embolic stroke models, combined treatment with otaplimastat significantly reduced infarct volume, edema, ICH occurrence, and mortality after delayed tPA treatment (6 hours after ischemia onset).⁸⁸ Otaplimastat stabilizes the BBB in subjects receiving tPA by both inhibiting tPA-induced MMP activity and inducing tissue inhibitors of metalloproteinases (TIMPs) (submitted). Otaplimastat also decreased the expression of EMMPRIN/CD147, an inducer of MMP synthesis, whose expression is specifically elevated in cerebral vascular endothelial cells after delayed tPA treatment.

In a phase I study, up to 240 mg of otaplimastat was well tolerated, with no drug-related adverse events in 77 healthy volunteers (submitted). The Safety and Efficacy of Otaplimastat in Patients with AIS Receiving recombinant tPA (SAFE-TPA) study was a phase 2a trial that assessed the safety and efficacy of otaplimastat in patients receiving tPA.

This was a two-stage, multicenter trial in AIS patients receiving tPA. Stage 1 constituted a single-arm, open-label safety study in 11 patients. Otaplimastat (80 mg) was administered twice daily for 3 days. Stage 2 was a randomized, double-blind, placebo-controlled study involving 69 patients, assigned (1:1:1) to the otaplimastat (40 mg), otaplimastat (80 mg), or placebo group. IV otaplimastat or placebo was administered no later than 30 minutes after starting tPA infusion, and the study drugs were given twice daily six times, at intervals of 12 hours.

The primary endpoint was the occurrence of ICH on day 1. Secondary endpoints included SAEs, mortality, and mRS scores at 90 days (clinicaltrials.gov identifier: NCT02787278). No safety issues were encountered during stage 1. The incidence

of ICH during stage 2 was 0/22 with the placebo, 0/22 with otaplimastat (40 mg), and 1/21 with the 80 mg dose, indicating no treatment difference. No significant differences in SAE (13%, 17%, 14%) or deaths (8.3%, 4.2%, 4.8%) were observed among the three groups. Three adverse events (chills, muscle rigidity, hepatotoxicity) were judged to be related to otaplimastat. The mRS distribution was more favorable for otaplimastat 40 mg than for the placebo (submitted). Thus, IV otaplimastat adjunctive therapy in patients receiving tPA was feasible and generally safe, and the signal of improved functional outcome with otaplimastat 40 mg justifies further large trials (in submission). Currently, a phase IIb study is ongoing to obtain more evidence of otaplimastat's clinical efficacy.

Epigallocatechin gallate

Epigallocatechin gallate (EGCG) is an extract of green tea that has antioxidant and neuroprotective effects.⁸⁹ It improved limb paresis in a rat stroke model.⁹⁰ In one study, rats subject to transient MCAO received either IV tPA or tPA+EGCG (20 mg/kg) at 4 hours. Compared with monotherapy, tPA+EGCG significantly reduced infarct volume, edema, and BBB breakage; it also up-regulated PAI-1 and down-regulated MMP-2 and MMP-9 expression in the brain.⁹¹

In another study, 371 patients with AIS were randomly assigned according to their onset-to-treatment time. They were then treated with IV tPA+EGCG (500 mg/day for 7 days) or IV tPA+placebo. Treatment outcome was assessed using NIHSS scores and plasma levels of MMP-2 and MMP-9. The tPA+EGCG treatment significantly improved NIHSS scores compared with the placebo in patients with delayed onset-to-treatment time strata, whereas outcomes did not differ in patients treated <3 hours. This improved outcome may have been due to the reduction in plasma levels of MMP-2 and MMP-9, as both showed strong linear correlations with NIHSS score in all patients.⁹² These results suggest that EGCG could be used as an adjunctive drug during tPA treatment, especially when such treatment is delayed.

Can these drugs be used in reperfusion therapy without using tPA?

EVT is effective in patients with large artery occlusion.^{5,93,94} In those who visit >4.5 hours after symptom onset, EVT is performed without tPA therapy. The evidence regarding the drugs described above is typically based on favorable results in animal experiments, where the agents are applied in cases of delayed (4- or 6-hour) tPA therapy. Therefore, it remains unclear whether the drugs work in patients undergoing EVT

without tPA therapy.

Theoretically, the increased risk of ICH and HT after EVT does not differ significantly from that after IV tPA treatment,⁵ and failure to achieve good functional outcome occurs in up to 50% of patients, even those who have experienced successful EVT.⁹⁵ This lack of efficacy may be associated with persistent ischemic injury due to delayed treatment, poor cerebral collaterals, re-occlusion, and microcirculation impairment.^{50,95-99} In addition, oxidative damage, inflammation, edema formation, and HT associated with BBB breakdown appear to play a role, as described above. Thus, the mechanism of brain damage appears to be similar between tPA and EVT therapies.

In one study, investigators attempted to differentiate the pathophysiology of brain damage between mechanical reperfusion and IV tPA using spontaneously hypertensive rats.¹⁰⁰ Mechanical ischemia/reperfusion was achieved by using an intraluminal filament to occlude the MCA for 2 hours. Thrombolytic reperfusion was achieved by administering tPA 2 hours after embolic MCAO. Regional cortical blood flow was monitored using laser-Doppler flowmetry, and BBB permeability in the cortex was measured in terms of Evans blue dye leakage. Cortical MMP-9 levels were assessed using zymography and immunohistochemistry. Blood flow completely recovered during mechanical reperfusion in both the central and peripheral areas of the ischemic cortex whereas after IV tPA, reperfusion was incomplete, with moderate recovery in the periphery only. BBB permeability was mainly increased in the central regions of the ischemic cortex after mechanical reperfusion, but it was increased in both the central and peripheral areas after IV tPA. Overall, MMP-9 levels were higher after tPA therapy, even though ischemic injury was similar in both models after 24 hours. Thus, the profiles of blood flow recovery, BBB leakage, and MMP-9 up-regulation differ between mechanical and thrombolytic reperfusion after focal ischemia.

Thus, drugs that stabilize the BBB may be more useful in patients receiving IV tPA than in those treated with EVT. However, in our clinical practice, tPA treatment is performed earlier than EVT, and we only use EVT in patients that show persistent vascular occlusion after tPA therapy. Thus, this animal result cannot be directly applied to our clinical practice. Taken together, it is not yet clear whether these drugs are less effective in patients receiving EVT than in those treated with tPA, so further clinical trials using these drugs are warranted in patients receiving EVT as well.

Summary and future directions

As described above, many drugs have shown benefits in animal models, usually rat MCAO, treated with tPA. However, only a small number of drugs have entered into clinical study stage. For safety reasons, agents that are already in use in clinical practice (e.g., statins, minocycline, tirofiban, argatroban) can enter clinical studies more easily. Other drugs require more time and funding to be tested for safety (phase 1 study).

Even when agents are proven to be safe, human trials are still limited by several factors. Firstly, it is difficult to obtain funds. Although drugs that have been in clinical use may have an advantage in terms of safety, pharmacological companies typically have insufficient enthusiasm for clinical studies aimed at different therapeutic targets and are unwilling to invest funds for old drugs.

Secondly, it would be difficult to recruit sufficient numbers of patients to carry out trials; tPA is administered to less than 10% of stroke patients. Moreover, because these agents are likely to work when administered quite early, the therapeutic time window for tPA helpers may be very narrow. One study showed that the median estimate of time from onset of ischemia to BBB disruption was 3.8 hours.¹⁰¹ Moreover, for severe stroke patients, it would be difficult to take the drugs orally. Future trials may have to consider administering the drugs during transportation (in the ambulance).

Thirdly, some drugs are difficult to use in a trial because of their original effects. For example, two statin trials described above suffered from early termination because of slow enrollment; investigators were uncomfortable withholding statins from AIS patients, given that much evidence indicates that statins prevent further stroke. Despite these difficulties, many ongoing trials are comparing the efficacy of tPA with that of tPA+helpers, and I hope that new therapeutic strategies will arise in the near future.

Finally, thrombolytic drugs with fewer side effects than tPA have been developed. One example is tenecteplase (TNK). It is not yet known whether this drug indeed has less marked tPA toxicity (personal communication with TNK investigators). Currently, ongoing trials are comparing the efficacy and hazards of tPA and TNK in patients with AIS, and tPA may be replaced by TNK in the future. It may then be necessary to carry out studies comparing TNK with tPA+tPA helpers. If TNK wins this race, would these tPA helpers become obsolete? I doubt it. As discussed above, although many of the drugs were tested in animal stroke models that use tPA, most are likely to be useful in ischemic/reperfusion damage unassociated with tPA as well. Thus, these drugs would also constitute TNK helpers, so future

studies may need to compare TNK with TNK+TNK helpers.

Disclosure

The author has no financial conflicts of interest.

Acknowledgments

This study was supported by a grant from the Korea Health Technology R&D project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health and Welfare, Republic of Korea (HI15C2796).

References

1. National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group. Tissue plasminogen activator for acute ischemic stroke. *N Engl J Med* 1995;333:1581-1587.
2. Hacke W, Kaste M, Bluhmki E, Brozman M, Dávalos A, Guidetti D, et al. Thrombolysis with alteplase 3 to 4.5 hours after acute ischemic stroke. *N Engl J Med* 2008;359:1317-1329.
3. Emberson J, Lees KR, Lyden P, Blackwell L, Albers G, Bluhmki E, et al. Effect of treatment delay, age, and stroke severity on the effects of intravenous thrombolysis with alteplase for acute ischaemic stroke: a meta-analysis of individual patient data from randomised trials. *Lancet* 2014;384:1929-1935.
4. Lees KR, Emberson J, Blackwell L, Bluhmki E, Davis SM, Donnan GA, et al. Effects of alteplase for acute stroke on the distribution of functional outcomes: a pooled analysis of 9 trials. *Stroke* 2016;47:2373-2379.
5. Goyal M, Menon BK, van Zwam WH, Dippel DW, Mitchell PJ, Demchuk AM, et al. Endovascular thrombectomy after large-vessel ischaemic stroke: a meta-analysis of individual patient data from five randomised trials. *Lancet* 2016;387:1723-1731.
6. Saver JL, Fonarow GC, Smith EE, Reeves MJ, Grau-Sepulveda MV, Pan W, et al. Time to treatment with intravenous tissue plasminogen activator and outcome from acute ischemic stroke. *JAMA* 2013;309:2480-2488.
7. Jickling GC, Liu D, Stamova B, Ander BP, Zhan X, Lu A, et al. Hemorrhagic transformation after ischemic stroke in animals and humans. *J Cereb Blood Flow Metab* 2014;34:185-199.
8. Shi ZS, Duckwiler GR, Jahan R, Tateshima S, Szeder V, Saver JL, et al. Early blood-brain barrier disruption after mechanical thrombectomy in acute ischemic stroke. *J Neuroimaging* 2018;28:283-288.
9. Wang X, Tsuji K, Lee SR, Ning M, Furie KL, Buchan AM, et al. Mechanisms of hemorrhagic transformation after tissue plasminogen activator reperfusion therapy for ischemic stroke. *Stroke* 2004;35(11 Suppl 1):2726-2730.
10. Simard JM, Kent TA, Chen M, Tarasov KV, Gerzanich V. Brain oedema in focal ischaemia: molecular pathophysiology and theoretical implications. *Lancet Neurol* 2007;6:258-268.
11. Kastrup A, Gröschel K, Ringer TM, Redecker C, Cordesmeyer R, Witte OW, et al. Early disruption of the blood-brain barrier after thrombolytic therapy predicts hemorrhage in patients with acute stroke. *Stroke* 2008;39:2385-2387.
12. Wang W, Li M, Chen Q, Wang J. Hemorrhagic transformation after tissue plasminogen activator reperfusion therapy for ischemic stroke: mechanisms, models, and biomarkers. *Mol Neurobiol* 2015;52:1572-1579.
13. Tsuji K, Aoki T, Tejima E, Arai K, Lee SR, Atochin DN, et al. Tissue plasminogen activator promotes matrix metalloproteinase-9 upregulation after focal cerebral ischemia. *Stroke* 2005;36:1954-1959.
14. Montaner J, Molina CA, Monasterio J, Abilleira S, Arenillas JF, Ribó M, et al. Matrix metalloproteinase-9 pretreatment level predicts intracranial hemorrhagic complications after thrombolysis in human stroke. *Circulation* 2003;107:598-603.
15. Dirnagl U, Iadecola C, Moskowitz MA. Pathobiology of ischaemic stroke: an integrated view. *Trends Neurosci* 1999;22:391-397.
16. Fisher M. New approaches to neuroprotective drug development. *Stroke* 2011;42(1 Suppl):S24-S27.
17. Savitz SI, Baron JC, Yenari MA, Sanossian N, Fisher M. Reconsidering neuroprotection in the reperfusion era. *Stroke* 2017;48:3413-3419.
18. Fisher M, Feuerstein G, Howells DW, Hurn PD, Kent TA, Savitz SI, et al. Update of the stroke therapy academic industry roundtable preclinical recommendations. *Stroke* 2009;40:2244-2250.
19. Albers GW, Goldstein LB, Hess DC, Wechsler LR, Furie KL, Gorelick PB, et al. Stroke Treatment Academic Industry Roundtable (STAIR) recommendations for maximizing the use of intravenous thrombolytics and expanding treatment options with intra-arterial and neuroprotective therapies. *Stroke* 2011;42:2645-2650.
20. Switzer JA, Hess DC, Ergul A, Waller JL, Machado LS, Portik-Dobos V, et al. Matrix metalloproteinase-9 in an exploratory trial of intravenous minocycline for acute ischemic stroke. *Stroke* 2011;42:2633-2635.
21. Powers WJ, Rabinstein AA, Ackerson T, Adeoye OM, Bambakidis NC, Becker K, et al. 2018 Guidelines for the early management of patients with acute ischemic stroke: a guideline for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke* 2018;49:e46-e110.
22. Amar AP, Sagare AP, Zhao Z, Wang Y, Nelson AR, Griffin JH,

- et al. Can adjunctive therapies augment the efficacy of endovascular thrombolysis? A potential role for activated protein C. *Neuropharmacology* 2018;134(Pt B):293-301.
23. Seitz RJ, Meisel S, Moll M, Wittsack HJ, Junghans U, Siebler M. The effect of combined thrombolysis with rtPA and tirofiban on ischemic brain lesions. *Neurology* 2004;62:2110-2112.
 24. Barreto AD, Ford GA, Shen L, Pedroza C, Tyson J, Cai C, et al. Randomized, multicenter trial of ARTSS-2 (Argatroban With Recombinant Tissue Plasminogen Activator for Acute Stroke). *Stroke* 2017;48:1608-1616.
 25. Lapchak PA. Tumor necrosis factor-alpha is involved in thrombolytic-induced hemorrhage following embolic strokes in rabbits. *Brain Res* 2007;1167:123-128.
 26. Chen W, Hartman R, Ayer R, Marcantonio S, Kamper J, Tang J, et al. Matrix metalloproteinases inhibition provides neuroprotection against hypoxia-ischemia in the developing brain. *J Neurochem* 2009;111:726-736.
 27. Mishiro K, Ishiguro M, Suzuki Y, Tsuruma K, Shimazawa M, Hara H. A broad-spectrum matrix metalloproteinase inhibitor prevents hemorrhagic complications induced by tissue plasminogen activator in mice. *Neuroscience* 2012;205:39-48.
 28. Shehadah A, Chen J, Cui Y, Zhang L, Roberts C, Lu M, et al. Combination treatment with low-dose Niaspan and tissue plasminogen activator provides neuroprotection after embolic stroke in rats. *J Neurol Sci* 2011;309:96-101.
 29. Liu SF, Ye X, Malik AB. Inhibition of NF-kappaB activation by pyrrolidine dithiocarbamate prevents in vivo expression of proinflammatory genes. *Circulation* 1999;100:1330-1337.
 30. Wang Z, Shan W, Cao J, Wintermark M, Huang W, Zuo Z. Early administration of pyrrolidine dithiocarbamate extends the therapeutic time window of tissue plasminogen activator in a male rat model of embolic stroke. *J Neurosci Res* 2018;96:449-458.
 31. Tan Z, Turner RC, Leon RL, Li X, Hongpaisan J, Zheng W, et al. Bryostatins improves survival and reduces ischemic brain injury in aged rats after acute ischemic stroke. *Stroke* 2013;44:3490-3497.
 32. Tan Z, Lucke-Wold BP, Logsdon AF, Turner RC, Tan C, Li X, et al. Bryostatins extends tPA time window to 6 h following middle cerebral artery occlusion in aged female rats. *Eur J Pharmacol* 2015;764:404-412.
 33. Li Q, Han X, Lan X, Hong X, Li Q, Gao Y, et al. Inhibition of tPA-induced hemorrhagic transformation involves adenosine A2b receptor activation after cerebral ischemia. *Neurobiol Dis* 2017;108:173-182.
 34. Won S, Lee JH, Wali B, Stein DG, Sayeed I. Progesterone attenuates hemorrhagic transformation after delayed tPA treatment in an experimental model of stroke in rats: involvement of the VEGF-MMP pathway. *J Cereb Blood Flow Metab* 2014;34:72-80.
 35. Sowndhararajan K, Deepa P, Kim M, Park SJ, Kim S. Neuroprotective and cognitive enhancement potentials of baicalin: a review. *Brain Sci* 2018;8:E104.
 36. Chen H, Guan B, Chen X, Chen X, Li C, Qiu J, et al. Baicalin attenuates blood-brain barrier disruption and hemorrhagic transformation and improves neurological outcome in ischemic stroke rats with delayed t-PA treatment: involvement of ONOO(-)-MMP-9 pathway. *Transl Stroke Res* 2018;9:515-529.
 37. dela Peña IC, Yoo A, Tajiri N, Acosta SA, Ji X, Kaneko Y, et al. Granulocyte colony-stimulating factor attenuates delayed tPA-induced hemorrhagic transformation in ischemic stroke rats by enhancing angiogenesis and vasculogenesis. *J Cereb Blood Flow Metab* 2015;35:338-346.
 38. Dela Peña IC, Yang S, Shen G, Fang Liang H, Solak S, Borlongan CV. Extension of tissue plasminogen activator treatment window by granulocyte-colony stimulating factor in a thromboembolic rat model of stroke. *Int J Mol Sci* 2018;19:E1635.
 39. Zhang Z, Zhang L, Yepes M, Jiang Q, Li Q, Arniego P, et al. Adjuvant treatment with neuroserpin increases the therapeutic window for tissue-type plasminogen activator administration in a rat model of embolic stroke. *Circulation* 2002;106:740-745.
 40. Cai Y, Liu X, Chen W, Wang Z, Xu G, Zeng Y, et al. TGF-beta1 prevents blood-brain barrier damage and hemorrhagic transformation after thrombolysis in rats. *Exp Neurol* 2015;266:120-126.
 41. Brundula V, Rewcastle NB, Metz LM, Bernard CC, Yong VW. Targeting leukocyte MMPs and transmigration: minocycline as a potential therapy for multiple sclerosis. *Brain* 2002;125(Pt 6):1297-1308.
 42. Murata Y, Rosell A, Scannevin RH, Rhodes KJ, Wang X, Lo EH. Extension of the thrombolytic time window with minocycline in experimental stroke. *Stroke* 2008;39:3372-3377.
 43. Fan X, Lo EH, Wang X. Effects of minocycline plus tissue plasminogen activator combination therapy after focal embolic stroke in type 1 diabetic rats. *Stroke* 2013;44:745-752.
 44. Malhotra K, Chang JJ, Khunger A, Blacker D, Switzer JA, Goyal N, et al. Minocycline for acute stroke treatment: a systematic review and meta-analysis of randomized clinical trials. *J Neurol* 2018;265:1871-1879.
 45. Lampl Y, Boaz M, Gilad R, Lorberboym M, Dabby R, Rapoport A, et al. Minocycline treatment in acute stroke: an open-label, evaluator-blinded study. *Neurology* 2007;69:1404-1410.
 46. Padma Srivastava MV, Bhasin A, Bhatia R, Garg A, Gaikwad

- S, Prasad K, et al. Efficacy of minocycline in acute ischemic stroke: a single-blinded, placebo-controlled trial. *Neurol India* 2012;60:23–28.
47. Sheng Z, Liu Y, Li H, Zheng W, Xia B, Zhang X, et al. Efficacy of minocycline in acute ischemic stroke: a systematic review and meta-analysis of rodent and clinical studies. *Front Neurol* 2018;9:1103.
 48. Blacker DJ, Prentice D, Alvaro A, Bates TR, Bynevelt M, Kelly A, et al. Reducing haemorrhagic transformation after thrombolysis for stroke: a strategy utilising minocycline. *Stroke Res Treat* 2013;2013:362961.
 49. Peña ID, Borlongan C, Shen G, Davis W. Strategies to extend thrombolytic time window for ischemic stroke treatment: an unmet clinical need. *J Stroke* 2017;19:50–60.
 50. Chamorro Á. Neuroprotectants in the era of reperfusion therapy. *J Stroke* 2018;20:197–207.
 51. Romanos E, Planas AM, Amaro S, Chamorro A. Uric acid reduces brain damage and improves the benefits of rt-PA in a rat model of thromboembolic stroke. *J Cereb Blood Flow Metab* 2007;27:14–20.
 52. Amaro S, Laredo C, Renú A, Llull L, Rudilosso S, Obach V, et al. Uric acid therapy prevents early ischemic stroke progression: a tertiary analysis of the URICO-ICTUS trial (efficacy study of combined treatment with uric acid and r-tPA in acute ischemic stroke). *Stroke* 2016;47:2874–2876.
 53. Chamorro Á, Amaro S, Castellanos M, Gomis M, Urrea X, Blasco J, et al. Uric acid therapy improves the outcomes of stroke patients treated with intravenous tissue plasminogen activator and mechanical thrombectomy. *Int J Stroke* 2017;12:377–382.
 54. Cimino M, Gelosa P, Gianella A, Nobili E, Tremoli E, Sironi L. Statins: multiple mechanisms of action in the ischemic brain. *Neuroscientist* 2007;13:208–213.
 55. Lapchak PA, Han MK. The 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor simvastatin reduces thrombolytic-induced intracerebral hemorrhage in embolized rabbits. *Brain Res* 2009;1303:144–150.
 56. Zhang L, Chopp M, Jia L, Cui Y, Lu M, Zhang ZG. Atorvastatin extends the therapeutic window for tPA to 6 h after the onset of embolic stroke in rats. *J Cereb Blood Flow Metab* 2009;29:1816–1824.
 57. Montaner J, Bustamante A, García-Matas S, Martínez-Zabaleta M, Jiménez C, de la Torre J, et al. Combination of thrombolysis and statins in acute stroke is safe: results of the STARS randomized trial (stroke treatment with acute reperfusion and simvastatin). *Stroke* 2016;47:2870–2873.
 58. Heo JH, Song D, Nam HS, Kim EY, Kim YD, Lee KY, et al. Effect and safety of rosuvastatin in acute ischemic stroke. *J Stroke* 2016;18:87–95.
 59. Yamashita T, Kamiya T, Deguchi K, Inaba T, Zhang H, Shang J, et al. Dissociation and protection of the neurovascular unit after thrombolysis and reperfusion in ischemic rat brain. *J Cereb Blood Flow Metab* 2009;29:715–725.
 60. Aoki J, Kimura K, Morita N, Harada M, Metoki N, Tateishi Y, et al. YAMATO study (tissue-type plasminogen activator and edaravone combination therapy). *Stroke* 2017;48:712–719.
 61. Enomoto M, Endo A, Yatsushige H, Fushimi K, Otomo Y. Clinical effects of early edaravone use in acute ischemic stroke patients treated by endovascular reperfusion therapy. *Stroke* 2019;50:652–658.
 62. Rha JH, Saver JL. The impact of recanalization on ischemic stroke outcome: a meta-analysis. *Stroke* 2007;38:967–973.
 63. Rubiera M, Alvarez-Sabin J, Ribo M, Montaner J, Santamarina E, Arenillas JF, et al. Predictors of early arterial reocclusion after tissue plasminogen activator-induced recanalization in acute ischemic stroke. *Stroke* 2005;36:1452–1456.
 64. Saqqur M, Molina CA, Salam A, Siddiqui M, Ribo M, Uchino K, et al. Clinical deterioration after intravenous recombinant tissue plasminogen activator treatment: a multicenter transcranial Doppler study. *Stroke* 2007;38:69–74.
 65. Zinkstok SM, Roos YB; ARTIS investigators. Early administration of aspirin in patients treated with alteplase for acute ischaemic stroke: a randomized controlled trial. *Lancet* 2012;380:731–737.
 66. Zhang L, Zhang ZG, Zhang R, Morris D, Lu M, Coller BS, et al. Adjuvant treatment with a glycoprotein IIb/IIIa receptor inhibitor increases the therapeutic window for low-dose tissue plasminogen activator administration in a rat model of embolic stroke. *Circulation* 2003;107:2837–2843.
 67. Straub S, Junghans U, Jovanovic V, Wittsack HJ, Seitz RJ, Siebler M. Systemic thrombolysis with recombinant tissue plasminogen activator and tirofiban in acute middle cerebral artery occlusion. *Stroke* 2004;35:705–709.
 68. Li W, Lin L, Zhang M, Wu Y, Liu C, Li X, et al. Safety and preliminary efficacy of early tirofiban treatment after alteplase in acute ischemic stroke patients. *Stroke* 2016;47:2649–2651.
 69. Jang IK, Gold HK, Leinbach RC, Fallon JT, Collen D. In vivo thrombin inhibition enhances and sustains arterial recanalization with recombinant tissue-type plasminogen activator. *Circ Res* 1990;67:1552–1561.
 70. Morris DC, Zhang L, Zhang ZG, Lu M, Berens KL, Brown PM, et al. Extension of the therapeutic window for recombinant tissue plasminogen activator with argatroban in a rat model of embolic stroke. *Stroke* 2001;32:2635–2640.
 71. Barreto AD, Alexandrov AV, Lyden P, Lee J, Martin-Schild S,

- Shen L, et al. The argatroban and tissue-type plasminogen activator stroke study: final results of a pilot safety study. *Stroke* 2012;43:770-775.
72. Berekashvili K, Soomro J, Shen L, Misra V, Chen PR, Blackburn S, et al. Safety and feasibility of argatroban, recombinant tissue plasminogen activator, and intra-arterial therapy in stroke (ARTSS-IA Study). *J Stroke Cerebrovasc Dis* 2018; 27:3647-3651.
 73. Joyce DE, Gelbert L, Ciaccia A, DeHoff B, Grinnell BW. Gene expression profile of antithrombotic protein c defines new mechanisms modulating inflammation and apoptosis. *J Biol Chem* 2001;276:11199-11203.
 74. Griffin JH, Zlokovic BV, Mosnier LO. Activated protein C: biased for translation. *Blood* 2015;125:2898-2907.
 75. Griffin JH, Zlokovic BV, Mosnier LO. Activated protein C, protease activated receptor 1, and neuroprotection. *Blood* 2018;132:159-169.
 76. Shibata M, Kumar SR, Amar A, Fernandez JA, Hofman F, Griffin JH, et al. Anti-inflammatory, antithrombotic, and neuroprotective effects of activated protein C in a murine model of focal ischemic stroke. *Circulation* 2001;103:1799-1805.
 77. Cheng T, Petraglia AL, Li Z, Thiyagarajan M, Zhong Z, Wu Z, et al. Activated protein C inhibits tissue plasminogen activator-induced brain hemorrhage. *Nat Med* 2006;12:1278-1285.
 78. Wang Y, Zhang Z, Chow N, Davis TP, Griffin JH, Chopp M, et al. An activated protein C analog with reduced anticoagulant activity extends the therapeutic window of tissue plasminogen activator for ischemic stroke in rodents. *Stroke* 2012;43: 2444-2449.
 79. Lyden P, Levy H, Weymer S, Pryor K, Kramer W, Griffin JH, et al. Phase 1 safety, tolerability and pharmacokinetics of 3K3A-APC in healthy adult volunteers. *Curr Pharm Des* 2013; 19:7479-7485.
 80. Lyden P, Pryor KE, Coffey CS, Cudkowicz M, Conwit R, Jadhav A, et al. Final results of the RHAPSODY trial: a multi-center, phase 2 trial using a continual reassessment method to determine the safety and tolerability of 3K3A-APC, a recombinant variant of human activated protein c, in combination with tissue plasminogen activator, mechanical thrombectomy or both in moderate to severe acute ischemic stroke. *Ann Neurol* 2019;85:125-136.
 81. Fu Y, Hao J, Zhang N, Ren L, Sun N, Li YJ, et al. Fingolimod for the treatment of intracerebral hemorrhage: a 2-arm proof-of-concept study. *JAMA Neurol* 2014;71:1092-1101.
 82. Kraft P, Göb E, Schuhmann MK, Göbel K, Deppermann C, Thielmann I, et al. FTY720 ameliorates acute ischemic stroke in mice by reducing thrombo-inflammation but not by direct neuroprotection. *Stroke* 2013;44:3202-3210.
 83. Zhu Z, Fu Y, Tian D, Sun N, Han W, Chang G, et al. Combination of the immune modulator fingolimod with alteplase in acute ischemic stroke: a pilot trial. *Circulation* 2015;132:1104-1112.
 84. Tian DC, Shi K, Zhu Z, Yao J, Yang X, Su L, et al. Fingolimod enhances the efficacy of delayed alteplase administration in acute ischemic stroke by promoting anterograde reperfusion and retrograde collateral flow. *Ann Neurol* 2018;84:717-728.
 85. Noh SJ, Lee JM, Lee KS, Hong HS, Lee CK, Cho IH, et al. SP-8203 shows neuroprotective effects and improves cognitive impairment in ischemic brain injury through NMDA receptor. *Pharmacol Biochem Behav* 2011;100:73-80.
 86. Noh SJ, Lee SH, Shin KY, Lee CK, Cho IH, Kim HS, et al. SP-8203 reduces oxidative stress via SOD activity and behavioral deficit in cerebral ischemia. *Pharmacol Biochem Behav* 2011; 98:150-154.
 87. Kim WK, Ju C, Jalin AMAA, Ryu JM, Cho IH, Kim WS, et al. Therapeutic efficacy and pharmacological mechanisms of sp-8203 for treatment of cerebral ischemia. *Stroke* 2015;46(Suppl 1):ATP239.
 88. Ju C, Anthony Jalin A, Song HY, Kim WK, Ryu JM, Cho GS, et al. Extension of therapeutic time window of tissue plasminogen activator with sp-8203 combination therapy in rat embolic stroke models. *European Stroke Journal* 2016;1(1 Suppl):581.
 89. Han J, Wang M, Jing X, Shi H, Ren M, Lou H. (-)-Epigallocatechin gallate protects against cerebral ischemia-induced oxidative stress via Nrf2/ARE signaling. *Neurochem Res* 2014; 39:1292-1299.
 90. Lim SH, Kim HS, Kim YK, Kim TM, Im S, Chung ME, et al. The functional effect of epigallocatechin gallate on ischemic stroke in rats. *Acta Neurobiol Exp (Wars)* 2010;70:40-46.
 91. You YP. Epigallocatechin gallate extends the therapeutic window of recombinant tissue plasminogen activator treatment in ischemic rats. *J Stroke Cerebrovasc Dis* 2016;25:990-997.
 92. Wang XH, You YP. Epigallocatechin gallate extends therapeutic window of recombinant tissue plasminogen activator treatment for brain ischemic stroke: a randomized double-blind and placebo-controlled trial. *Clin Neuropharmacol* 2017;40:24-28.
 93. Albers GW, Marks MP, Kemp S, Christensen S, Tsai JP, Ortega-Gutierrez S, et al. Thrombectomy for stroke at 6 to 16 hours with selection by perfusion imaging. *N Engl J Med* 2018;378: 708-718.
 94. Nogueira RG, Jadhav AP, Haussen DC, Bonafe A, Budzik RF, Bhuva P, et al. Thrombectomy 6 to 24 hours after stroke with a mismatch between deficit and infarct. *N Engl J Med*

- 2018;378:11-21.
95. Shi ZS, Liebeskind DS, Xiang B, Ge SG, Feng L, Albers GW, et al. Predictors of functional dependence despite successful revascularization in large-vessel occlusion strokes. *Stroke* 2014;45:1977-1984.
96. Kawiorski MM, Martínez-Sánchez P, García-Pastor A, Calleja P, Fuentes B, Sanz-Cuesta BE, et al. Alberta Stroke Program Early CT Score applied to CT angiography source images is a strong predictor of futile recanalization in acute ischemic stroke. *Neuroradiology* 2016;58:487-493.
97. Gilberti N, Gamba M, Premi E, Costa A, Vergani V, Delrio I, et al. Leukoaraiosis is a predictor of futile recanalization in acute ischemic stroke. *J Neurol* 2017;264:448-452.
98. Hussein HM, Georgiadis AL, Vazquez G, Miley JT, Memon MZ, Mohammad YM, et al. Occurrence and predictors of futile recanalization following endovascular treatment among patients with acute ischemic stroke: a multicenter study. *AJNR Am J Neuroradiol* 2010;31:454-458.
99. Kim BM. Causes and solutions of endovascular treatment failure. *J Stroke* 2017;19:131-142.
100. Aoki T, Sumii T, Mori T, Wang X, Lo EH. Blood-brain barrier disruption and matrix metalloproteinase-9 expression during reperfusion injury: mechanical versus embolic focal ischemia in spontaneously hypertensive rats. *Stroke* 2002;33:2711-2717.
101. Warach S, Latour LL. Evidence of reperfusion injury, exacerbated by thrombolytic therapy, in human focal brain ischemia using a novel imaging marker of early blood-brain barrier disruption. *Stroke* 2004;35(11 Suppl 1):2659-2661.