Neuropharmacology xxx (2017) 1–9

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Invited review

Can adjunctive therapies augment the efficacy of endovascular thrombolysis? A potential role for activated protein C

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ARTICLE INFO

Article history:
Received 3 August 2017
Accepted 13 September 2017
Available online xxx

Keywords:
Activated protein C (APC)
Endovascular restorative neurosurgery
Mechanical neurothrombectomy
Neuroprotection
Neurorestoration
Stroke
Thrombolysis

ABSTRACT

In the management of acute ischemic stroke, vessel recanalization correlates with functional status, mortality, cost, and other outcome measures. Thrombolysis with intravenous tissue plasminogen activator has many limitations that restrict its applicability, but recent advances in the development of mechanical thrombectomy devices as well as improved systems of stroke care have resulted in greater likelihood of vessel revascularization. Nonetheless, there remains substantial discrepancy between rates of recanalization and rates of favorable outcome. The poor neurological recovery among some stroke patients despite successful recanalization confirms the need for adjuvant pharmacological therapy for neuroprotection and/or neurorestoration. Prior clinical trials of such drugs may have failed due to the inability of the agent to access the ischemic tissue beyond the occluded artery. A protocol that couples revascularization with concurrent delivery of a neuroprotectant drug offers the potential to enhance the benefit of thrombolysis. Analogs of activated protein C (APC) exert pleiotropic anti-inflammatory, anti-apoptotic, antithrombotic, cytoprotective, and neuroregenerative effects in ischemic stroke and thus appear to be promising candidates for this novel approach. A multicenter, prospective, double-blinded, dose-escalation Phase 2 randomized clinical trial has enrolled 110 patients to assess the safety, pharmacokinetics, and efficacy of human recombinant 3K3A-APC following endovascular thrombolysis.

Contents

Abbreviations: (AIS), acute ischemic stroke; (Akt), protein kinase B; (APC), activated protein C; (BBB), blood-brain barrier; (CNS), central nervous system; (EPCR), endothelial protein C receptor; (ERK1/2), extracellular signal-regulated kinase 1/2; (FDA), food and drug administration; (GA), general anesthesia; (IV tPA), intravenous tissue plasminogen activator; (LVO), large vessel occlusion; (MMP9), matrix metalloproteinase-9; (NFkB), nuclear factor kappa-light-chain-enhancer of activated B cells; (NMDA), *N*-methyl-D-aspartate; (NPCs), neural progenitor cells; (NSC), neural stem cell; (NVU), neurovascular unit; (PC), protein C; (PAR1), protease activated receptor 1; (PAR3), protease activated receptor 3; (Rac1), Ras-related C3 botulinum toxin substrate 1; (RhoA), Ras homolog gene family, member A; (Serpins), serine protease inhibitors; (STAIR), stroke therapy academic industry roundtable; (SVZ), subventricular zone; (IIa), thrombin; (TM), thrombomodulin; (tPA), tissue plasminogen activator; (TRAP), thrombin receptor-activating peptides; (wt), wild-type.

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https://doi.org/10.1016/j.neuropharm.2017.09.021
0028-3908/

1. Introduction

Stroke is the third leading cause of death worldwide and the number one cause of disability in the United States (Mortality and Causes of Death, 2015). Among the subset of stroke patients with persistent proximal vessel occlusion, up to 80% die within 90 days or fail to regain functional independence (Amar et al., 2015). It is estimated that for each minute during acute ischemic stroke (AIS), 1.9 million neurons, 14 billion synapses, and 12 km (7.5 miles) of myelinated fibers are destroyed (Saver, 2006).

Extensive research efforts have helped elucidate the pathophysiology underlying AIS and have characterized many processes of the ischemic cascade, including dysfunction of all elements of the neurovascular unit (neurons, astrocytes, microglia, and endothelial cells). Although these multiple injury mechanisms suggest myriad potential therapeutic approaches, the only medication approved by the United States Food and Drug Administration (FDA) for AIS remains intravenous tissue plasminogen activator (IV tPA), which targets the occlusive thrombus within a blood vessel. However, IV tPA has many limitations that restrict its widespread application, including a relatively short time window for delivery, low rates of recanalization in large vessel occlusion (LVO), and risks of intracranial bleeding (Amar et al., 2015). As a result of these and other reasons, only about 5% of AIS patients receive IV tPA (Jauch et al., 2013; Mozaffarian et al., 2015; Schwamm et al., 2013). Adjunctive drugs that counteract these limitations may expand the applicability of this therapy in the future.

In the last few years, the role of mechanical neurothrombectomy in AIS therapy has expanded significantly. The current generation of aspiration and stent retrieval devices achieves recanalization in the majority of patients with LVO (Almekhlafi et al., 2014; Berkhemer et al., 2015; Campbell et al., 2015; Goyal et al., 2015; Nogueira et al., 2012; Saver et al., 2012, 2015) and detailed analysis of safety data confirms that neurothrombectomy procedures can be performed with minimal morbidity and mortality (Akins et al., 2014).

Nonetheless, the likelihood of functional independence following neurothrombectomy (14–58%) remains poor compared with rates of recanalization (60–90%) (Amar et al., 2015). This disparity underscores the need for adjunctive therapies that enhance the benefit of endovascular thrombolysis, such as pharmacological neuroprotection (Amar et al., 2015).

Thousands of preclinical studies and human trials with potential neuroprotective agents in AIS have been reported, but none has proven unequivocally efficacious and none has yet achieved FDA approval (Ginsberg, 2009; Tymianski, 2013). One plausible explanation for this failure is that the agent may not reach the ischemic tissue due to lack of perfusion. When administered systemically, neuroprotective agents might not traverse the occluded artery and must rely instead on collateral flow to ischemic tissue, but such collateral flow may be insufficient for adequate drug delivery. This provides impetus for a strategy coupling revascularization with the ancillary administration of a neuroprotective drug.

We have previously reviewed the foundation of clinical trials that administer an analogue of activated protein C (APC) after endovascular thrombolysis by IV tPA, mechanical neurothrombectomy, or both (Amar et al., 2015). APC confers pleiotropic benefits, such as stabilizing blood brain barrier (BBB) integrity, preventing propagation of thrombosis, enhancing fibrinolysis, promoting neuroprotection, attenuating inflammation, and facilitating neuroregeneration (Griffin et al., 2002, 2015; Zlokovic and Griffin, 2011). It represents a novel multiple-action multiple-target approach that addresses all components of the pathogenic triad (consisting of vascular damage, neuronal injury, and neuroinflammation) in AIS (Zlokovic and Griffin, 2011). Since the first report of its anti-inflammatory, cytoprotective, and antithrombotic properties in AIS (Shibata et al., 2001), APC has progressively fulfilled Stroke Therapy Academic Industry Roundtable (STAIR) criteria for drug development (Zlokovic and Griffin, 2011). The preclinical safety and pharmacokinetic profile of APC has been well characterized in mice and monkey models (Williams et al., 2012). A phase I safety study in normal human subjects has demonstrated that high dose bolus regimens of modified APC are well-tolerated in normal human subjects (Lyden et al., 2013), and a multicenter phase II dose-escalation clinical trial of intravenous administration for AIS (NCT02222714, NN104) is currently in progress (https://clinicaltrials.gov/ct2/show/record/NCT02222714) (Lyden et al., 2016).

2. Overview of activated protein C (APC) pathways

Protein C (PC) is a 62 kDa vitamin K-dependent secretary glycoprotein produced mainly by liver (Griffin et al., 1993). PC circulates at 70 nM in the blood as an inactive zymogen of the natural anticoagulant serine protease APC (Gruber and Griffin, 1992; Mosnier et al., 2007). PC binds to the endothelial cell protein C receptor (EPCR) at the endothelial cell surface (Fukudome and Esmon, 1994) and is activated by thrombomodulin (TM) receptor-bound thrombin (IIa) by cleavage at Arg169 and removal of a peptide fragment at the amino-terminal of PC heavy chain (Esmon, 2003; Essalmani et al., 2017) (Fig. 1). EPCR is also required for transport of APC across the BBB (Deane et al., 2009). APC associated with EPCR in caveolae microdomains (Russo et al., 2009) cleaves protease-activated receptor-1 (PAR-1) initiating cytoprotective signaling including altered gene expression, and anti-inflammatory, anti-apoptotic and barrier protective activities (Fig. 1). Normally plasma levels of APC are about 40 pM in healthy humans (Mosnier et al., 2007). APC is inactivated and cleared from plasma by serine protease inhibitors (Serpins) (Fig. 1). APC is a unique protease having potent anticoagulant and anti-inflammatory activities (Griffin et al., 2002). APC along with its cofactor protein S partially degrade and inactivate coagulation factors Va and VIIIa on the platelet membrane (Esmon, 2003; Marlar et al., 1982) (Fig. 1). APC is physiologically very important as heterozygous PC deficiency increases the risk for venous thrombosis in adults and the rare homozygous PC deficiency in neonates results in a fatal syndrome known as purpura fulminans if untreated (Griffin et al., 1981; Marlar et al., 1989). In mice, total

Please cite this article in press as: Amar, A.P., et al., Can adjunctive therapies augment the efficacy of endovascular thrombolysis? A potential role for activated protein C, Neuropharmacology (2017), https://doi.org/10.1016/j.neuropharm.2017.09.021