

● PERSPECTIVE

Towards reperfusion-centric preclinical stroke research: outside the box of “reperfusion injury”

Stroke is a major health issue of increasing significance for any society with an aging population. Globally, stroke is the second-leading cause of death with approximately 5.9 million fatal events in 2010, equivalent to 11.1% of all deaths. Yet, despite years of preclinical research on neuroprotection and a multitude of clinical trials, tissue plasminogen activator (tPA)-mediated recanalization remains the mainstay of acute ischemic stroke therapy, whereas tPA thrombolysis rarely provides benefits in the mechanical occlusion-based stroke models. This split between the bench and bedside raised the concern over the clinical applicability of neuroprotection in acute ischemic stroke. In this perspective commentary, we call for attention to the differences between mechanical-occlusion and thromboembolic stroke models in cerebral hemodynamics (**Figure 1A, B**), the implications of these differences in view of progressive pathobiology of ischemic stroke (**Figure 1C**), and the need and strategies towards reperfusion-centric preclinical stroke research.

Transient mechanical occlusion represents a minority of ischemic stroke: The difficulty in translating neuroprotection into stroke patients has many factors. The current trend emphasizes good laboratory practice, meta-analysis of multiple datasets, and international cooperation to improve preclinical stroke research. In addition, there is a minority view that the translational failure is due to over-reliance on transient mechanical occlusion model in preclinical stroke research (Hossmann, 2009). This is because mechanical occlusion models (*e.g.*, intraluminal suture middle cerebral artery occlusion, MCAO) rarely induces blood clotting, whereas cerebral blood flow recovers rapidly upon removal of vascular occlusion in this model, rendering it unresponsive to the real-world therapy of tPA thrombolysis (**Figure 1A**). In contrast, thromboembolic stroke models respond to intravenous tPA by gradual and often partial recovery of cerebral perfusion (**Figure 1B**).

So which of the two models captures the alterations of cerebral hemodynamics in stroke patients better? Clinical studies showed that the non-occlusion or spontaneous recanalization rate only approaches 28% in the first 6 hours after stroke onset, and merely rises above 50% after 3 to 4 days (Kassem-Moussa and Graffagnino, 2002). Even with intravenous tPA thrombolysis, 30–40% of occluded intracranial arteries reopens partially within the first 1 to 2 hours after initiation of the treatment as determined by cerebral angiography (Broderick and Hacke, 2002). In view of these findings, it is fair to conclude that transient mechanical occlusion-based models best mimic ischemic stroke with spontaneous recanalization, which constitutes

only a fraction of patients. In contrast, the MCAO model yields rapid reperfusion and a dubiously long therapeutic window, which led to the emphasis of “reperfusion injury” in preclinical research, while “improvement of reperfusion” is the priority in acute ischemic stroke therapy in real world (Hossmann, 2009). The recent success of endovascular thrombus-removal treatment in clinical trials further underscores the rationale of reperfusion-oriented ischemic stroke therapy (Berkhemer et al., 2014).

Moreover, the seventh Stroke Treatment Academic Industry Roundtable (STAIR) made a recommendation that “neuroprotective agents (shall) be tested in combination with reperfusion therapies, that is, on a background of intravenous tPA as a standard care”. Because the transient mechanical occlusion-based stroke models do not respond to intravenous tPA, these models have little opportunity to improve reperfusion therapies, although their utility in studying other aspects of stroke pathophysiology (*e.g.*, plasticity and neural regeneration) is well-justified.

Pathological events following ischemic stroke vary in time: Brain injury following stroke induces a series of complex pathophysiological events that evolve in time and space. In 1999, Drs. Dirnagl, Iadecola, and Moskowitz suggested a cascade of time-dependent key events, which remains the best framework to appreciate the progression of ischemic brain injury and predict the benefits of various treatments (Dirnagl et al., 1999). This cascade is reproduced in **Figure 1C** with minor modifications.

The human brain comprises about 2.5% of the body’s weight, but receives almost 15% of the cardiac output, attesting to its high vascular and oxygen demand for energy production. Thus, the first consequence of ischemic stroke in the absence of vascular reperfusion is energy failure, which leads to excitotoxicity and cellular self-cannibalism (autophagy) (Adhami et al., 2006). Whether therapies aimed at blocking glutamate excitotoxicity can prevent the thrombosis/energy failure-induced ischemic brain injury is yet to be determined. Meanwhile, repetitive peri-infarct depolarization (also called “spreading depression”) expands the unsalvageable ischemic tissue. Subsequently, inflammation and apoptosis modulates the throes between life and death near the border of ischemic core, and jointly determines the eventual infarct size. Less appreciated in the original 1999 scheme is that the brain possesses significant self-healing ability (plasticity), which could be augmented to reach greater recovery in neurological functions.

This cascade of stroke pathobiology not only provides a framework of complex ischemic brain injury, but also a basis to predict the effects of various treatments. For example, according to this cascade, acute thrombolysis that reduces the size and duration of energy failure is likely to be more effective than anti-apoptosis therapies that target the penumbra area. Further, because transient mechanical occlusion-based stroke models yield rapid and near-complete recovery of blood flow, these models are poised to benefit from neuroprotective agents through circulation, whereas in real world, lack of vascular recanalization and

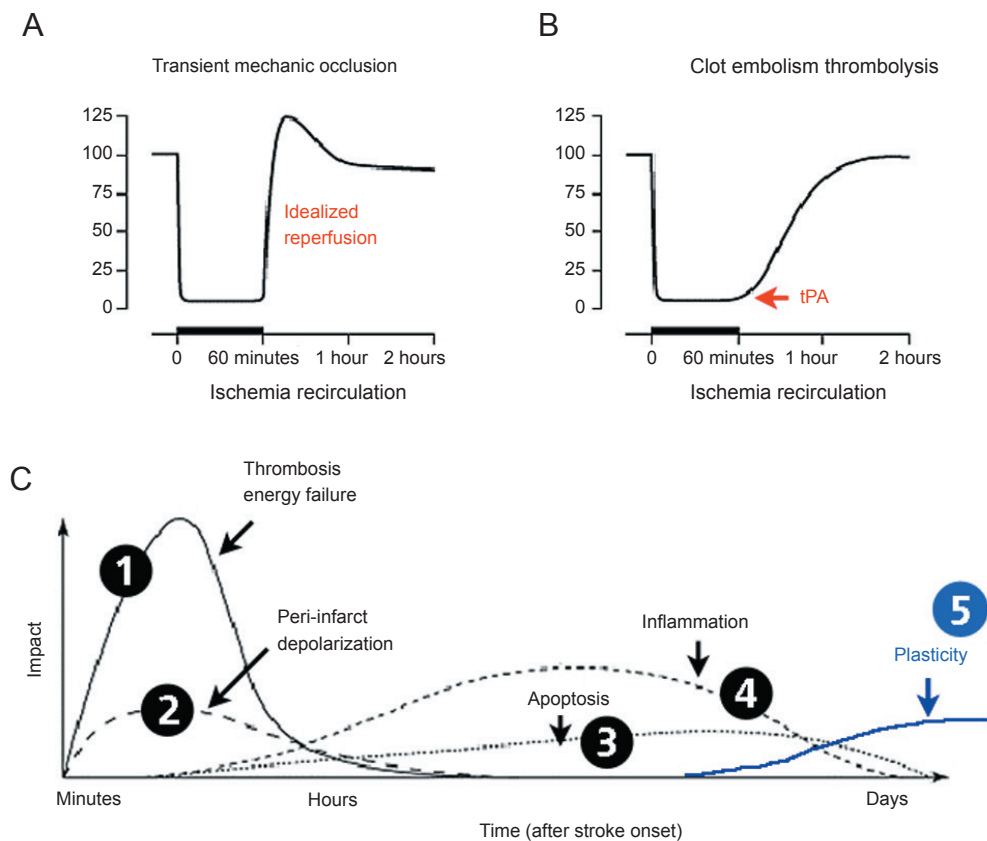


Figure 1 Distinct effects on hemodynamics by mechanic occlusion *versus* clot embolism in view of the progressive cascade of pathobiology events following cerebral ischemia.

(A, B) Transient mechanical occlusion-based stroke models produce rapid recovery of cerebral blood flow upon removal of vascular obstruction, while clot-embolism responds to intravenous tPA by gradual recovery of blood flow. This salient difference in cerebral hemodynamics bears great implications on the utility of these two preclinical stroke models. The y-axis indicates the relative cerebral blood flow value. (C) Ischemic brain injury induces a complex series of pathophysiological events including: (1) thrombosis and cessation of blood supply-induced energy failure that triggers excitotoxicity and autophagy, (2) peri-infarct depolarization that enlarges the unsalvageable tissue, (3) apoptosis in the ischemic penumbra, (4) sterile microglia activation secondary to neural injury and inflammation coupled to the influx of systemic immune cells, and finally (5) intrinsic self-healing and functional recovery (plasticity). This putative cascade provides a useful framework to comprehend the potential and limitation of various stroke therapies. (A, B are modified with permission from Hossmann, 2009; C from Dirnagl et al., 1999); tPA: tissue plasminogen activator.

tissue reperfusion remains the biggest challenge in acute stroke therapy (Broderick and Hacke, 2002; Khatri et al., 2005). This simple fact supports the importance of reperfusion-centric preclinical stroke research, which however is under-studied to date due to technical constraints and conceptual fixation of “reperfusion injury”.

Reperfusion-centric preclinical stroke research is needed: Early artery reopening (recanalization) is a sign of favorable outcomes after tPA therapy. Yet, the percentage of recanalization within 1 hour after tPA therapy declines rapidly according to onset time-to-treatment from 45% (thrombolysis initiated < 90 min) to 16% (started > 270 min) (Muchada et al., 2014). Moreover, recanalization of large arterial occlusion does not guarantee reperfusion of the distal vascular bed, which is the best predictor of stroke outcomes (Khatri et al., 2005). The causes of reperfusion deficits despite recanalization of large arteries are complex, including breakup of the primary clot to occlude smaller arteries, compression of blood vessels by

edema, and secondary thrombosis in hypoxic-ischemic tissue. These observations suggest that reperfusion-centric preclinical stroke research is needed and may yield more fruitful results than neuroprotection research. Although this idea is covered in the concept of “neurovascular unit”, there has been limited progress in optimizing thromboembolic stroke models in the past, which we believe is essential for advancing the reperfusion-centric preclinical stroke research.

Current thromboembolic stroke models (delivery of *ex-vivo* pre-formed emboli *via* the common carotid artery, photochemically induced platelet-rich thrombi, or direct injection of thrombin to the MCA branch) all have technical limitations, including large variations in location and infarct size, poor response to tPA in platelet-rich thrombosis, and the need of craniectomy, respectively. To expand the repertoire of thromboembolic models for preclinical stroke research, we recently devised a simple and tPA-responding thrombotic model based on transient hypoxia-ischemia (tHI), which does not require craniectomy or

pre-formed emboli (Sun et al., 2014).

This new stroke model consists of reversible occlusion of the unilateral common carotid artery and delivery of 7.5% oxygen to adult mice through a face mask for 30 minutes, while keeping the animal rectal temperature at $37.5 \pm 0.5^\circ\text{C}$. Although hypoxia or unilateral reversible ligation of the carotid artery by itself each suppresses cerebral blood flow transiently, the combination of both insults leads to reperfusion deficits, fibrin and platelet deposition, and significant infarct in the MCA-supplied territory. Moreover, tail-vein injection of recombinant tPA (10 mg/kg) at 0.5, 1, or 4 hours post-tHI provided time-dependent reduction of mortality rate and infarct size (Sun et al., 2014). This new thrombotic model is simple and can be standardized across laboratories for comparison of experimental data, which will increase the rigor of preclinical stroke research. Furthermore, this model yields endogenous components into thrombosis under transient cerebral hypoxia-ischemia. Thus, it is highly suited for studying the vascular bed-specific hemostasis and hypercoagulable states in the brain.

The tHI-induced stroke model can be used to optimize the tPA thrombolytic therapy. We tested this application by comparing the effects of tPA thrombolytic treatment with and without Edaravone (Sun et al., 2014). Edaravone is a free radical scavenger that has high permeability across the blood-brain-barrier (Lapchak, 2010). A multitude of animal studies have shown that Edaravone has a strong ability to mitigate oxidative stress in various cells of the neurovascular unit, including neurons, platelets, the endothelium, and pericytes, to prevent thrombus formation. Clinically, Edaravone is approved for treating ischemic stroke within 24 hours of onset in Japan and has passed a Phase 1 trial in Europe as a safe stand-alone therapy of ischemic stroke (Kaste et al., 2013). Further, a recent open-label study showed that administration of Edaravone during tPA infusion enhances the early recanalization rate in stroke patients (Kimura et al., 2012). These findings suggest that Edaravone may be a potent adjuvant to improve tPA thrombolysis therapy.

Consistent with this possibility, we showed that post-tHI administration of Edaravone by itself reduces mortality rate and infarct size in the new thrombotic stroke model. Moreover, when Edaravone is combined with acute tPA-treatment, the infarct size is smaller than those in animals receiving tPA or Edaravone alone ($P < 0.05$) (Sun et al., 2014). These results implicate synergistic benefits of the combined tPA-Edaravone therapy in acute ischemic stroke, as well as, the utility of the tHI-induced thrombotic stroke model in preclinical stroke research.

In conclusion, we suggest that reperfusion-centric preclinical research is a new direction to bridge the bench and the bedside in acute stroke therapy. The newly introduced tHI thrombotic model can facilitate to this important task in stroke research.

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