



News & Views

Brain cytoprotection of ischemic stroke in the era of effective reperfusion

Di Wu^{a,b,1}, Ming Li^{a,b,1}, Marc Fisher^c, Xunming Ji^{a,b,*}^a Department of Neurology and China-America Institute of Neuroscience, Xuanwu Hospital, Capital Medical University, Beijing 100053, China^b Beijing Institute of Brain Disorders, Capital Medical University, Beijing 100053, China^c Department of Neurology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston 02215, USA

Acute ischemic stroke (AIS) treatment has been advanced by the development of recanalization therapies, especially endovascular thrombectomy. Thrombectomy has led to a better prognosis at 90 d not only in stroke patients because of large-vessel occlusion in the proximal anterior cerebral circulation, but also in those due to basilar-artery occlusion [1]. Thus, the number of eligible patients for revascularization will continue to increase, especially with the expanded therapeutic window (up to 24 h) for endovascular therapy in selected patients. However, the re-opened cerebral vessels and the restored perfusion to the ischemic brain do not necessarily result in better clinical outcomes. Functional independence was achieved only in approximately 50 % of patients receiving effective reperfusion treatments [2]. All cells in the neurovascular unit (NVU)—including neurons, astrocytes, microglia, pericytes, and the endothelia—are at risk of damage after stroke, thus cytoprotection might reduce ischemic brain injuries due to ischemic cascades [3]. Nerinetide has produced the most promising results of cytoprotective therapy after recanalization therapy in clinical trial of ESCAPE-NA1 (NCT02930018). The positive results highlighted the great potential of brain cytoprotection for ischemic stroke in the era of highly successful reperfusion. However, how to pre-test cytoprotection in preclinical studies and administer cytoprotective therapies for AIS patients in this era of effective reperfusion remains to be determined.

NVU protection, especially brain vessels. Over the past decades, neuroprotection of ischemic brain tissue has shifted from protecting neuron alone (or so-called neuroprotection) toward the protection of the neurovascular unit, appropriately termed cytoprotection [4]. The importance of protecting the neurovascular unit was derived from the original translational failures of protecting neuron alone strategy and was further proven by the findings that phenotype changes and cell communications occurred in NVU after focal ischemia. Modulation of microglia polarization to M1 or M2 phenotypes and adjustment of the phenotypes of astrocytes were closely associated with stroke outcomes in stroke models [5]. Moreover, the communication between different types of cells within NVU was also found to be related to stroke prognosis. The most important findings include ATP transfer between astro-

cytes and neurons and “help-me” signals mediating transition from injury to recovery within the NVU [4]. These findings extend the scope and targets of cytoprotection. However, vascular protection is still not well emphasized. Embolic clot or thrombosis in brain-supply arteries initiates ischemic damage in brain tissue in pathology. Damages to both the endothelium and the blood–brain barrier (BBB) occur during ischemia and reperfusion, especially when repeated clot retrieval with endovascular thrombectomy is needed. In patients, blood vessel damage enhances the risk of hemorrhage and can lead to a poor prognosis. Thus, vascular protection should be given more emphasis in the reperfusion era. Our previous study [6] found that focal ischemia induced subtle BBB leakage within 30 min after stroke in a mouse model by activating the Rho-associated protein kinase (ROCK)/myosin light chain (MLC) pathway and reducing early BBB disruption led to a better stroke outcome. Early normobaric hyperoxia (NBO) treatment mitigated ischemic BBB damage and improved neurological outcomes despite the delayed tissue plasminogen activator (tPA) treatment. Thus, we advocate that early BBB disruption may be a cause rather than a consequence of brain cell injury [6]. Pharmaceutical (such as cytoprotective drugs) and non-pharmaceutical (such as NBO, hypoxic preconditioning) therapies for stroke, directed at ameliorating the deleterious process affecting blood vessels or the BBB, should be assessed in future clinical trials when early BBB compromise has been identified. Vascular protection has gained more attention as recanalization therapy has become widely accepted. More pre-clinical experiments and clinical trials are still needed to provide definite evidence of this treatment approach.

Targets and routes for cytoprotection. The targets of cytoprotection have also evolved with the accumulation of data and evidence. The ischemic cascade is a multifaceted and complex process, including interconnected pathways that develop over time, such as glutamate release and Ca²⁺ overload in the acute phase, and inflammation and edema in the subacute phase. However, previous trials of cytoprotective therapy have only focused on pharmaceutical agents targeting one component of the ischemic cascade [3]. This may account for the translational failures in the past. Thus, agents that have multiple targets in the ischemic cascade and induce multiple cytoprotective effects have been recently tested in translational cytoprotection for ischemic stroke, such as uric acid (UA), nerinetide, and focal hypothermia [3]. However, multiple targets do not equal no differentiation. Recent data suggest

* Corresponding author.

E-mail address: jixm@ccmu.edu.cn (X. Ji).¹ These authors contributed equally to this work.

the importance of differentiating physiological and pathological effects for some potential therapies. Targeting the pathological changes after stroke, rather than those associated with physiological function, may have potential in clinical and translational studies [7]. For example, only pathophysiological glutamate overload leads to excitotoxicity and neuron death, while basal glutamatergic transmission is essential for neuronal survival. Thus, pre-clinical trials have not been successful when only directing glutamatergic inhibition, while inhibiting pathological glutamate overload has translational potential in stroke therapy [8]. Moreover, chronic phases of cytoprotection, such as stem cell therapy and physical rehabilitation, should also be considered in multi-pronged stroke treatments. Pre-clinical and clinical trials have shown evidence of enhanced recovery of motor function in the late stage of stroke after stem cell therapy [4]. Furthermore, the endovascular route will become an important approach to delivering adjunctive therapy directly to the ischemic region. This route has the advantage of targeting the damaged artery and brain tissue supplied by it in a precise way. Adjunctive therapies, such as focal hypothermia, especially when delivered locally through the intraarterial route, hold great translational potential in the reperfusion era. Our group determined the efficacy of focal hypothermia through an arterial micro-catheter in a rat model of ischemic stroke, and further proved its safety and efficacy in a rhesus monkey model of stroke [9]. This approach was tested in stroke patients undergoing endovascular therapy, demonstrating safety and potential efficacy. Thus, the cytoprotection strategy in the reperfusion era probably needs molecules or other therapies with multiple mechanisms of action and novel delivery routes through a micro-catheter can be considered.

Pre-hospital cytoprotection. Prehospital cytoprotection should be given more attention in the reperfusion era. The prehospital setting will be the ideal location to administrate cytoprotective agents. However, the unpredictable reperfusion status might minimize the beneficial effects of cytoprotective agents when used in pre-hospital setting. For example, the use of magnesium sulfate as a prehospital neuroprotectant was safe and allowed the start of therapy within 2 h after stroke onset, but it did not improve clinical outcomes within 90 d in the randomized clinical trial [10]. These drugs should be re-tested in the new era of reperfusion. Additionally, recent findings showed that mobile stroke units (MSUs), equipped with professional staff and a computed tomographic scanner, improved clinical outcomes compared with standard thrombolysis in the emergency department by initiating a faster prehospital treatment with tPA [11]. These recent technical advances highlight that cytoprotective treatments in the prehospital setting are feasible at present. There are many candidates for prehospital cytoprotection, including both pharmaceutical and non-pharmaceutical therapies, such as remote preconditioning and hypothermia. These non-pharmaceutical therapies can be well incorporated in the ambulance and prehospital setting. For example, the combination of selective surface cooling during prehospital transit and intra-arterial selective cooling infusion during and after thrombectomy will accelerate hypothermic induction and increase the accuracy of selectivity in the brain [9]. Thus, the initiation of treatments in patients as early as possible will be the most effective approach to achieve better clinical outcomes after stroke.

Novel endovascular materials for cytoprotection. The insights from the interdisciplinary community have led to breakthroughs in the revascularization of ischemic stroke by novel and refined approaches. Although tPA is approved by regulatory agencies for pharmacological thrombolysis, it has some limitations such as a relatively short therapeutic window (4.5 h) and half-life. The design of novel biomaterial-assisted vehicles can be used to increase the delivery efficiency for thrombolytic therapy [12]. As for mechanical thrombectomy, major complications due to

endovascular devices are vessel wall perforation and mechanical damage to vessel walls. Therefore, more attention should be paid to the flexibility of stent retrievers. Furthermore, cerebral venous thrombosis is another kind of ischemic stroke with an incidence slightly lower than ruptured intracranial aneurysms. However, the current thrombectomy devices are fabricated for arterial disease and are not developed for venous vasculature. The manufacture of dedicated endovascular tools for venous disease may be a future focus in neurosurgery. With the continued development of biomaterials, the use of customized biodegradable stents may induce cytoprotective effects by their degradation into components, such as Mg ions from Mg-based alloys, but this approach needs to be tested in appropriate experiments to confirm its efficacy [13]. The integration of sensors on a stent can also contribute to the operative success of endovascular neurosurgeons.

Last, but potentially important in the reperfusion era, many old neuroprotectants might have translational potential, if restudied in the context of reperfusion. These drugs did not succeed in previous clinical trials due to low reperfusion rates before the widespread application of mechanical thrombectomy. Thus, it is recommended that old neuroprotectants be tested in animal models of ischemic stroke using thrombolysis and mechanical thrombectomy. Compared with development costs of approximately \$1.3 to \$1.7 billion for new drugs and a long testing period, finding novel applications of old drugs might be cost and time-saving [14].

The efficient system for translating cytoprotection. We also need an efficient system to enhance the translation from the bench to the bedside. Stroke models have played an important role in developing adjunctive neuroprotective drugs and interventional modalities, especially rodent models [15]. However, over 1000 experimental treatments based on stroke models did not lead to clinical benefits in stroke patients. As the first nearly successful neuroprotective agent, the translational process of nerinetide was a good example for future neuroprotectants. Nerinetide studies began with cell experiments and rodent models of stroke, then monkey model of stroke, and finally clinical trials. This new translational strategy, including multiple animal species and stages, helped to bridge the gap between experiments and clinical drug development. Thus, two independent models of stroke, especially one of which uses large animals, will decrease the cost and increase the possibility of translational success [16]. Moreover, much emphasis should be paid to bridging the gap between the bench and the bedside. At present, tPA thrombolysis and endovascular thrombectomy are widely used for stroke patients, however, these treatments are rarely used in stroke models. Thus, tPA thrombolysis and endovascular treatments should be recommended during studies using animal models. Furthermore, it is also important to improve study quality by using multiple centers, blinded treatment assignments, and uploading study data to a central repository as in clinical trials. These methods will significantly increase experimental data tractability and reliability, including physiological parameters, imaging scans, recordings of behavior changes, and type of intervention during experiments [16]. More attention should be given to ethical considerations in animal studies when designing experiments using stroke models. Thus, a comprehensive translational approach should be recommended for future cytoprotection studies.

To conclude, brain cytoprotection for ischemic stroke has exhibited novel characteristics in the reperfusion era. More attention should be paid to NVU protection and protective agents with multiple-targets, especially brain vessels. With recent advancements establishing effective ways to reopen occluded arteries, the spotlight is now shifting towards the study of initiating prehospital cytoprotection and developing new endovascular materials to reduce ischemic damage and improve prognosis. A novel translational system composed of a full-chain translational strategy from

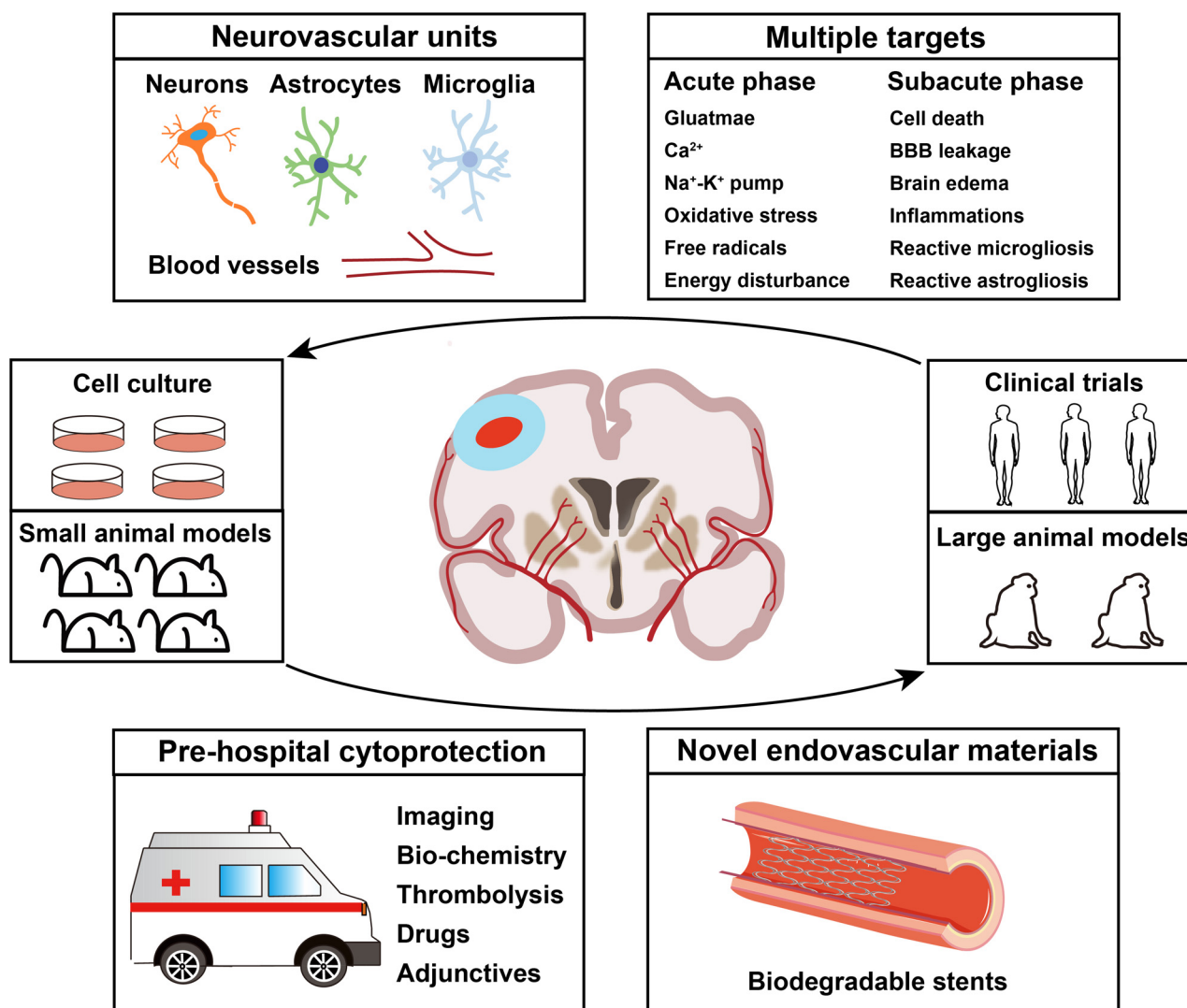


Fig. 1. Brain cytoprotection of ischemic stroke in the reperfusion era. Multiple cells in the neurovascular unit and various targets of the ischemic cascade should be considered for brain cytoprotection in the acute and subacute phases of ischemic stroke. Prehospital cytoprotection and novel endovascular materials should also be given more attention in the reperfusion era. It is also imperative to establish a full-chain translational strategy from cell culture to small animal models, large animal models, and then clinical trials to enhance the translation of interesting molecules and novel materials.

cell culture to small animal models, large animal models, and then clinical trials, will enhance the translation of interesting molecules and novel materials in an efficient way (Fig. 1).

Conflict of interest

The authors declare that they have no conflict of interest.

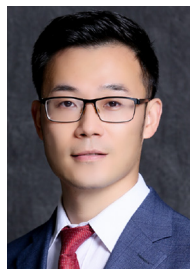
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Ming Li is an associate researcher at Xuanwu Hospital, Capital Medical University. His research interest lies in the design and fabrication of novel endovascular devices and functional materials for neuroprotection.



Di Wu is an associate professor at Xuanwu Hospital, Capital Medical University. His research focuses on establishing a full-chain translational strategy for stroke neuroprotection and enhancing the translation of novel neuroprotectants in pre-clinical and clinical trials.



Xunming Ji is the vice president of Capital Medical University, the president of Beijing Institute of Brain Disorders and the Stroke Center Director of Xuanwu Hospital. He is the pioneer in neuroprotective therapy for acute ischemic stroke patients, especially by non-pharmacological approaches, such as ischemic conditioning and selective hypothermia.