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Commentary

Excitotoxicity therapy for stroke patients still alive

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Brain ischemia is the fourth most-common cause of death and the leading cause of long-term disability in industrialized countries. Ischemia is defined as an insufficient blood supply resulting from thrombolytic or embolic stroke. Less frequently, stroke may be caused by haemorrhage or cardiac arrest. Relatively short interruptions of blood flow to the brain cause irreversible neuronal damage. The ischemic brain region may be separated into two regions; the core zone, where blood flow is reduced to less than 10–25% and the ischemic-penumbra, a tissue surrounding the infarcted core that can be rescued with rapid cerebral blood flow restoration. The only FDA-approved therapy for protecting the penumbra region consists of thrombolysis with recombinant tissue plasminogen activator (tPA). However, tPA is only applied in a limited number of cases (less than 10% of patients) and within a short therapeutic window (up to 4 [5]. hours after stroke onset). Research is therefore urgently required to seek alternative therapies that will prevent massive damage and transfer potential neuroprotective strategies from bench to bedside.

In a recent study published in *EBioMedicine*, María Perez-Mato and colleagues insert glutamate transporter EAAT2-encoding cDNA into mesenchymal stem cells (MSCs) and administer these engineered cells using systemic injection to treat animal models of stroke [1]. This approach is based on the principle of blood glutamate-grabbing therapies, a strategy on which the authors of this paper have been working for several years. In this study they go a step further, combining the intrinsic and pro-regenerative properties of MSCs with their neuroprotective features, as a result of the expression of the EAAT2 transporter.

Excitotoxicity is induced by an excess of extracellular glutamate ensued by overactivation of glutamate receptors, especially of the NMDA subtype. Attenuating glutamate excitotoxicity has proved beneficial in animal models of stroke, however clinical trials targeting NMDA receptors failed to improve symptoms in stroke patients. The design of those trials was probably hampered by a number of misconceptions and did not take into consideration the protection of other brain cells, oligodendrocytes, astrocytes, microglia and endothelial cells [2]. In addition, the complexity of the cascades mediating cell death was also underestimated. It is now known that subunit composition and NMDA location (synaptic or extra-synaptic) determines the signalling of cell survival or death [3]. More importantly, non-selective NMDA receptor antagonists may cause side effects as these receptors are key to numerous physiological actions in unaffected parts of the brain. Thus, strong inhibition of NMDA receptors blocks normal as well as pathological activity, leading to cognitive impairments, hallucinations and even coma, a risk which may have led to suboptimal doses being administered [4]. In this regard, the new therapeutic strategy proposed by the authors overcomes many of the drawbacks of previous NMDA receptor-based therapies.

Blood-glutamate-grabbing strategies are designed to reduce the excess of extracellular glutamate in the brain and hold great potential as a therapy in stroke. They are based on the principle that there is a flow of glutamate through the blood brain barrier (BBB). Endothelial cells contain glutamate transporters and can mobilize glutamate from the CNS parenchyma to the luminal zone of the blood stream through mechanisms which are not well understood. Since glutamate levels are higher in blood than in brain parenchyma, flow of glutamate from brain to blood can be promoted by reducing glutamate levels in the plasma. Different strategies have been designed to reduce plasma glutamate. The first approach tested was enzymatic and used glutamate-oxalacetate transaminase (GOT) that transforms glutamate into α-ketoglutatarate in the presence of oxalacetate (OxAc). Injection of GOT alone or in combination with OxAc,shifting the reaction towards glutamate degradation, successfully reduced plasma and brain glutamate, and diminished infarct size after transient middle cerebral occlusion (MCAO) in rats [5]. Interestingly, this approach has been validated in humans [6]. Pyruvate and riboflavin also have glutamate-grabbing properties [7] and the latter is being evaluated in a clinical trial (EudraCT number: 2014-003123-22). A second alternative was based on haemodialysis and peritoneal dialysis of glutamate. It was effective in animal models [8] and is being tested in human trials (EudraCt Number: 2012-000791-42). The new approach, described in this study in *EBioMedicine*, is a cell-based therapy using MSCs overexpressing the glutamate transporter EAAT2. The rationale of the idea is to use MSCs as a glutamate siphoning system that could, at the same time, release neurotrophic factors and anti-inflammatory cytokines, paving the way for a pro-regenerative environment. Indeed, it diminished glutamate levels in blood and significantly reduced the infarct size, similar to that for other glutamate-grabbing therapies. Importantly, at high dose these cells led to a further reduction in infarct size, surpassing the protection achieved by other glutamate-grabbing therapies. MSCs appear to provide protection by releasing neurotrophic and/or anti-inflammatory factors –albeit they do not access the CNS [9]– or by glutamate-grabbing; however, it is not as clear whether they are capable of combining the two effects. Further improvements to MSCs

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manipulations for EAAT2 overexpression would be needed to combine and maximize the benefits of the cells.

Several questions remain. One basic issue that still needs to be clarified is the mechanism of brain-to-blood glutamate flow. Endothelial cells can certainly take up glutamate from the brain through glutamate transporters (EAATs). However, the mechanism used by endothelial cells to release glutamate into the blood is not clearly understood. One candidate might be the cysteine-glutamate transporter, also known as system xc-, which exchanges cysteine for glutamate and is highly expressed at the BBB barrier. Extending our understanding of this mechanism could help to define new therapeutic targets. The other important question relates to the therapeutic window of these therapies and their potential for different types of patients. Here, any answer must in part await the results of ongoing clinical trials. Plasma glutamate can reach concentrations of up to 200 μM; higher levels act as a predictor of neurological deterioration in patients suffering from thrombolytic or embolic stroke. As with tPA treatment, the success of glutamate-grabbing therapies will be limited by the time that has elapsed since the onset of stroke. However, given the negligible side-effects of these therapies, it may be anticipated that it will be possible to administer these therapies to most patients, except those with haemorrhagic stroke [10].

In conclusion, the results published in this paper of *EBioMedicine* are good news for stroke patients as they build the road to potential therapeutic approaches complementing ongoing clinical trials based on blood-glutamate-grabbing. The results of proof-of-concept clinical trials are currently awaited and would shed light on the potential of these therapies for patients with acute ischemia stroke.

Disclosure

The authors declared no conflicts of interest.

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