Acid Transporters; EAATs). Reuptake of glutamate from synaptic junctions after neuron excitation normally involves the participation of EAATs transporters located on astrocytes, which bind and remove the neurotransmitter for processing and recycling. Alternatively, when extracellular concentrations become elevated, sodium-dependent transport located on the antiluminal surface of brain capillary endothelial cells are able to transfer glutamate from the extracellular space. When glutamate accumulates in the endothelial cells to a concentration that exceeds plasma levels, it is moved via facilitated diffusion through the luminal side into the blood stream. In this regard, the endothelial regulation of glutamate concentration can occur despite unfavorable concentration gradients from brain to plasma[4, 5].

The failure of nutrient supply after ischemia causes a neuronal depolarization leading to a massive release of glutamate to the extracellular space. In addition, due to glutamate uptake is a high energy-dependent process, this restriction of energy causes a drastic disruption of the glutamate transporters enhancing the excitotoxic effect to trigger the death of neurons[6, 7].

More than 15 years ago, we reported, in clinical studies, that glutamate is critical for neuronal damage after ischemic stroke[8-10]. Ischemic patients presented higher blood and Cerebrospinal fluid (CSF) glutamate levels than control subjects at admission[8, 9], suggesting that glutamate concentrations above 200 μM in plasma acted as an important predictor of neuronal damage at 48 hours, with a sensitivity of 85% and a specificity of 97%[11]. Moreover, high levels of glutamate in the plasma for at least 24 hours was associated with early neurological deterioration, whereas in patients with stable ischemic stroke, glutamate levels dropped to normal values less than 6 hours from onset[11]. All of these clinical data demonstrated for the first time the critical role of glutamate in stroke pathology and suggested a source for new strategies for the research of new protective therapies based on the inhibition of glutamate toxicity. Therefore, controlling the increase of extracellular glutamate may confer neuroprotection by terminating multiple downstream death signaling cascades at their converging upstream initiation point.

Knowledge of the molecular mechanisms involved in glutamate excitotoxicity after cerebral ischemia allowed researchers to develop promising pharmacological strategies against this neurotoxic process. In the beginning, the major focus of research centered on NMDA receptors (NMDAr) antagonism. NMDAr provided a logical target for drug design because it represented a major gateway for the myriad of other downstream effects of glutamate excitotoxicity[7].

Several classes of NMDAr antagonists with different sites of action were developed. Though showing promise in animal studies, antagonist drugs have largely failed in randomized, controlled clinical trials in humans. A variety of reasons have been postulated to explain the lack of success for these NMDAr-targeting therapies. Many of these compounds lack sufficient brain penetrance while exhibiting significant dose-limiting side effects. And others caused adverse events as hallucinations, agitations, catatonia, peripheral sensory loss, nausea, and elevation in blood pressure[7].

Blood to Brain Glutamate Grabbing

High glutamate concentrations at the synaptic cleft are rapidly reduced by the action of glutamate transporters present primarily on astrocytes surrounding the nerve terminal to prevent glutamate excitotoxicity. In addition to astrocytes, EAATs, on the antiluminal membrane act to accumulate the excess extracellular glutamate into the endothelial cells. When the endothelial glutamate concentration becomes higher than the blood glutamate concentration, glutamate is transported into the blood by means of facilitated diffusion, a mechanism that facilitates blood excretion of glutamate from the brain[4,5,7]. Based on this mechanism, a decrease of blood glutamate levels by means of glutamate scavengers or grabbers leads to a larger glutamate gradient between the brain and blood, facilitating the lowering of extracellular glutamate in the brain, and reducing the toxic effects of this neurotransmitter. Therefore, manipulating this mechanism may have potential neuroprotective effects after stroke[5, 12].

To demonstrate this glutamate grabbing hypothesis, the blood resident enzyme glutamate-oxaloacetate transaminase (GOT), which transforms glutamate into α-ketoglutarate and aspartate in the presence of oxaloacetate (OxAc), was used. This enzyme, when OxAc is artificially increased shifts the equilibrium of the reaction to the right side, thereby decreasing glutamate levels in blood[5,12].

The first evidence of the neuroprotective effect with OxAc in cerebral ischemia was observed in rats submitted to photothrombotic lesions[13.] This effect was subsequently probed in a model of ischemia induced by the transient occlusion of the middle cerebral artery (MCAO)[14]. Under the STAIR guidelines OxAc was provided 90 min after occlusion, leading to decreased blood glutamate levels, following by a decrease in infarct volume and edema after ischemia. These effects were associated with a reduction in motor deficit. To confirm that the neuroprotective effect was due to a decrease in brain glutamate levels, spectroscopic analysis revealed that the increase in brain glutamate observed in control animals after MCAO was clearly reduced in animals treated with OxAc. These results were also validated by other independent laboratories[15].

To further demonstrate the clinical relevance of these pre-clinical results as potential therapeutic strategies, high blood levels of GOT was later hypothesized to be correlated with lower blood glutamate levels and subsequently with a better functional outcome. To test this hypothesis, two independent clinical and observational studies were performed, in which the primary end point was functional outcome at 3 months[16,17]. In these studies, patients with good outcomes showed lower glutamate levels and higher GOT levels in blood samples collected at admission. A significant inverse correlation was observed between GOT and glutamate levels. The favorable effect on functional outcome was also supported by reduced lesion volumes. These clinical findings represented the first clinical evidence of the neuroprotective effect of blood glutamate grabbing mechanisms in ischemic stroke patients and supported the potential applicability of OxAc or GOT as future treatments for acute ischemic stroke.

Indeed, human recombinant GOT1 (rGOT1) was later tested in the rat model of ischemia induced by transient MCAO and it was observed a reduction in serum and brain glutamate levels, resulting in a reduction in infarct volume and sensorimotor deficit[18].

Repositioning of Drugs against Glutamate Toxicity

All preclinical studies on different models and the clinical observational analysis reported guarantee the therapeutic efficacy of the reduction of blood glutamate as well as the glutamate grabber drugs; however, translation to clinical practice has critical steps before their use in humans. These critical steps are those necessary to develop a clinical trial, which will involve high financial support and risk of investment for the sponsors interested in the study. In addition, the repeated failure of protective drugs against glutamate excitotoxicity in clinical trials has reduced the trust of pharmaceutical companies and other sponsors in stroke studies.

Aiming to demonstrate the clinical efficacy glutamate grabbers in humans and reduce the risk of investment in the study, the pharmacological strategy known as drug repositioning represents an interesting alternative to find new grabbing drugs[19,20].This allowed researchers to search drugs already known and used for other pathologies and in which the clinical phase I and phase II were already completed, reducing the risk of investment for sponsors in case the clinical study result failed.

Following this pharmacological strategy, a new clinical study (proof of concept) with a new grabbing drug is currently ongoing (EudraCT Number: 2014-003123-22).

In conclusion, the critical role of glutamate excitotoxicity has been long described as a key molecular cause of neuronal injury from stroke. At present, we have a better understanding of the mechanisms by which elevated glutamate ultimately leads to cell death; unfortunately all clinical trials that have been primarily based on the inhibition of glutamate excitotoxicity through glutamate antagonist to date have failed. Currently, many experimental evidences in different models of diseases use blood glutamate grabbers as effective treatments against neuronal damage induced by glutamate excitotoxicity. Future clinical trials (some of them in progress) will allow to know the application of this novel therapeutic strategy.

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