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Molecules in focus

Oxaloacetate: A novel neuroprotective for acute ischemic stroke

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ABSTRACT

It is well established that glutamate acts as an important mediator of neuronal degeneration during cerebral ischemia. Different kind of glutamate antagonists have been used to reduce the deleterious effects of glutamate. However, their preclinical success failed to translate into practical treatments.

Far from the classical use of glutamate antagonists employed so far, the systemic administration of *oxaloacetate* represents a novel neuroprotective strategy to minimize the deleterious effect of glutamate in the brain tissue after ischemic stroke. The neuroprotective effect of *oxaloacetate* is based on the capacity of this molecule to reduce the brain and blood glutamate levels as a result of the activation of the blood-resident enzyme glutamate-oxaloacetate transaminase.

Here we review the recent experimental and clinical results where it is demonstrated the potential applicability of *oxaloacetate* as a novel and powerful neuroprotective treatment against ischemic stroke.

1. Introduction

Ischemic stroke is one of major cause of death and incapacity in developed countries. Its incidence is increasing because of the progressive aging of the population in these countries. So far, the only treatment that has proved effective in clinical practice is thrombolysis. But it presents serious safety-related restrictions that limit its use to a small fraction of patients (<3%) (Kleindorfer et al., 2009). Therefore, it is important to develop new and effective therapeutic approaches that can improve the outcome after ischemic stroke.

It is well established that during ischemia, glutamate acts as an important mediator of neuronal degeneration, being released in large amounts from neurons and astrocytes, causing cellular overload of calcium, mainly through its action on calcium-permeable NMDA receptors. This overload of calcium leads to necrosis and breakdown of cellular structures including proteins, DNA, and membrane phospholipids (Lipton, 1999). The role of glutamate as one of the initial molecular mediators in the ischemic cascade resulted in the fact that most of the neuroprotective strategies used to date have focused on blocking this step.

With this aim, different experimental studies have observed good results when antagonists of glutamate receptors (e.g., *Dizocilpine*, *Aptigabel*, *Dextrorphan*, *Aptiganel*, or *Selfotel*) were used in animal models of cerebral ischemia, however when these treatments were tested in clinical trials, most of them did not show the efficacy as observed preclinically or they were associated with unacceptable adverse effects (Ginsberg, 2008).

Differing from classically used glutamate antagonists, the systemic administration of *oxaloacetate* may represent a novel neuroprotective strategy to minimize the deleterious effect of glutamate in the brain tissue after ischemic stroke.

The neuroprotective effect of *oxaloacetate* is based in its capacity to induce a reduction of blood glutamate as a result of the activation of the blood-resident enzyme glutamate-oxaloacetate transaminase (GOT). The enzyme GOT catalyzes the reversible transformation of *oxaloacetate* and glutamate to aspartate and α-ketoglutarate. Artificially increasing *oxaloacetate* concentration therefore shifts the equilibrium of the reaction to the right side, thereby decreasing glutamate levels in blood.

Based on this mechanism, when glutamate levels in brain fluids are elevated, *oxaloacetate* treatment causes a decrease of blood glutamate levels; this leads to a larger glutamate gradient between the brain and blood, which facilitates the decreases of extracellular levels of brain glutamate (Gottlieb et al., 2003; Teichberg et al., 2009) reflected in a reduction of ischemic damage.

2. Structure, turnover and biological function

Oxaloacetate (also called 2-Ketosuccinate, oxaloacetic acid, oxalacetic acid, oxalacetate) is a four-carbon molecule (Fig. 1) with a molecular weight of 130, involved in many metabolic pathways, including gluconeogenesis, citric acid cycle, glyoxylate cycle, urea cycle, and amino acid metabolism.

☆ In memoriam of Prof Vivian I. Teichberg.

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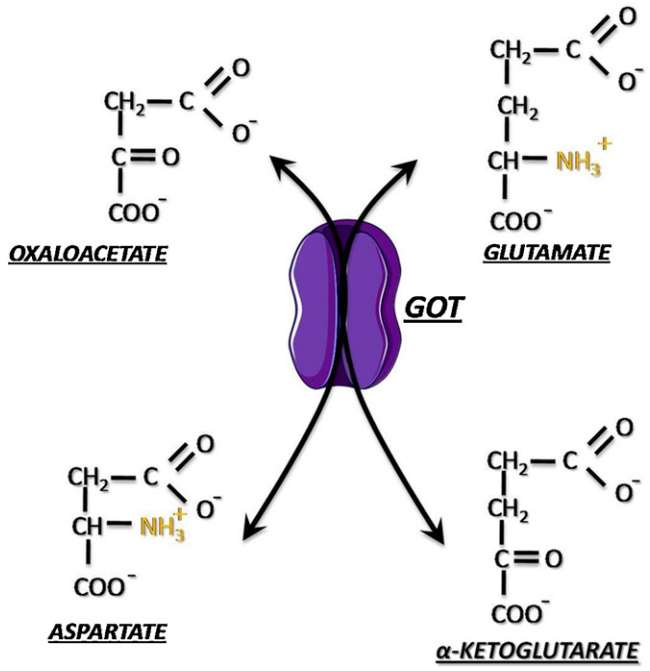

Fig. 1. Molecular structure of oxaloacetate. The neuroprotective effect of oxaloacetate is due to the decrease it causes in blood glutamate levels as a result of the activation of a blood-resident enzyme glutamate-oxaloacetate transaminase (GOT). The latter enzyme causes a reversible reaction in which glutamate reacts with oxaloacetate to transfer an amino group, transforming glutamate into α-ketoglutarate and oxaloacetate into aspartate. Artificially increasing oxaloacetate concentration shifts the reaction to the right, decreasing glutamate levels in blood.

In the glyoxylate and citric acid cycles, *oxaloacetate* is formed as the result of the catalysis by malate dehydrogenase (EC 1.1.1.37). In this reaction, the hydrogen atoms from malate are transferred to NAD+, forming NADH, H+ and *oxaloacetate*. *Oxaloacetate* can be converted to citrate with the addition of acetyl-CoA by the enzyme citrate synthase (EC 2.3.3.1). *Oxaloacetate* is a critical component in the production of ATP and must be constantly regenerated in order for the citric acid cycle and the electron transport chain to continue.

In the urea cycle and in the amino acid metabolism, aspartate is transaminated to form *oxaloacetate*, a reaction catalyzed by the enzyme GOT (EC 2.6.1.1). GOT, also called aspartate transaminase (AST), is a pyridoxal phosphate (PLP)-dependent enzyme which catalyzes the reversible transfer of an α-amino group between aspartate and glutamate, transforming glutamate into α-ketoglutarate and *oxaloacetate* into aspartate (Fig. 1).

Other enzymes that acts on *oxaloacetate* are pyruvate carboxylase (EC 6.4.1.1), phosphoenolpyruvate carboxykinase (PEPCK) (EC 4.1.1.49), both are involved in gluconeogenesis.

3. Possible medical applications

Because of its central role in energy metabolism, mainly in the citric acid cycle, *oxaloacetate* has been studied in the context of various conditions, with multiple beneficial properties.

Experimental studies have shown that *oxaloacetate* supplementation increases the lifespan of *Caenorhabditis elegans*, this effect being dependent on the transcription factor FOXO/DAF-16, and the energy sensor AMP-activated protein kinase (Williams et al., 2009). It has also been reported that *oxaloacetate* provides antioxidant protection when cells are submitted to stressful stimuli, such as hydrogen peroxide (Desagher et al., 1997; O'Donnell-Tormey et al., 1987), thiobarbituric acid reactive species (TBARS) (Puntel et al., 2005) or excitotoxic damage (Yamamoto and Mohanan, 2003).

The capacity of *oxaloacetate* to reduce glutamate levels in blood has opened the possibility of a new application in the treatment of diseases associated with high glutamate concentrations in brain, such as ischemic stroke.

Under normal physiological conditions, glutamate released into the synaptic cleft is quickly removed by excitatory amino acid transporters (EAATs) located on astrocytes and neurons (Hazell, 2007). In addition to astrocytes and neurons, the presence of EAATs has also been demonstrated in endothelial cells from the brain vasculature, which shows the relevant role of these cells in the maintenance of glutamate levels in the extracellular medium (O'Kane et al., 1999).

Under ischemic conditions, neurons and glial cells are permanently depolarized for a certain period of time. In this situation, their transporters are working in "backward" mode (Nicholls and Attwell, 1990; Szatkowski et al., 1990), further increasing the glutamate concentration in the extracellular fluid. Nevertheless, the transporters of the endothelial cells (whose membrane potential is much more stable during ischemia) are only in a position to remove the level of glutamate from the extracellular fluid during hypoperfusion.

The presence of EAATs in the abluminal membrane of and facilitative carriers for glutamate in the luminal membranes allow the diffusion of glutamate from the brain to the bloodstream following its concentration gradient. Therefore, a decrease of blood glutamate levels, by means of *oxaloacetate* administration, should lead to a larger glutamate gradient between the brain and blood, facilitating the lowering of extracellular glutamate in brain (Fig. 2).

To demonstrate this mechanism, the effect of *oxaloacetate* was studied in experimental animals in which radioactive glutamate was injected into the lateral ventricles. It was found that *oxaloacetate* induced a decrease in blood glutamate levels followed by an increase of the diffusion of radioactive brain glutamate into the blood (Gottlieb et al., 2003). Similar effects were observed in other study using two microdialysis probes, the first one infusing, the other one collecting glutamate; *oxaloacetate* treatment reduced the rate of glutamate collection by the second probe (Teichberg et al., 2009).

The first evidences of the neuroprotective effect with *oxaloacetate* in ischemia as the result of its capacity to reduce blood and brain glutamate levels were observed in rats submitted to photothrombotic lesion (Nagy et al., 2009) and in incomplete forebrain ischemia (2VO) model, where the administration of *oxaloacetate* was successful in restoring synaptic plasticity (LTP) in the CA1 region of the hippocampus in a dose-dependent manner (Marosi et al., 2009). However, although those studies showed a significant neuroprotective effect, a clear demonstration of the mechanism involved was not provided.

In order to demonstrate the effectiveness of *oxaloacetate* to reduce blood and brain glutamate levels and to induce neuroprotective effects after cerebral ischemia, we recently studied the effect of this molecule in a model of ischemia induced by transient occlusion of the middle cerebral artery (MCAO) (Campos et al., 2011c). Under the STAIR guidelines (Philip et al., 2009), we demonstrated that a bolus intravenous injection of *oxaloacetate* (3.5 mg/100 g), 90 min after occlusion, decreased blood glutamate levels, inducing an 80% decrease in infarct volume at 7 days after ischemia; edema was also reduced (Fig. 3). These effects were associated with reduced motor deficit. To confirm that the neuroprotective effect was due to decreased brain glutamate levels, we performed magnetic resonance spectroscopy (MRS) in the infarct region. Spectroscopic analysis revealed that the increase in brain glutamate seen in control animals after MCAO was clearly reduced in animals treated with *oxaloacetate*, demonstrating its mechanism of action.

In line with our findings, a recent study has also demonstrated that the capacity of the brain GOT to transform glutamate into