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BLOOD GOT TO RID THE BRAIN OF EXCESS GLUTAMATE: A NOVEL APPROACH TO THE TREATMENT OF HUMAN STROKE AND OF OTHER NEUROLOGICAL DISORDERS

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ГЛУТАМАТ-ОКСАЛОАЦЕТАТ ТРАНСАМИНАЗА КРОВИ ДЛЯ СНИЖЕНИЯ ИЗБЫТОЧНОГО ГЛУТАМАТА МОЗГА: НОВЫЙ ПОДХОД К ЛЕЧЕНИЮ ИНСУЛЬТА И ДРУГИХ НЕВРОЛОГИЧЕСКИХ РАССТРОЙСТВ

Ряд заболеваний мозга, таких как инсульт, характеризуется пагубной ролью избыточного уровня глутамата, присутствующего во внеклеточных жидкостях мозга. На основании отказа антагонистов глутаматного рецептора бороться с избыточным содержанием глутамата в головном мозге мы разработали совершенно новый нейропротективный подход, при котором избыточный глутамат в мозговых жидкостях устраняется с использованием поглотителей глутамата крови, которые увеличивают естественное перемещение глутамата из мозга в кровь. В двух статьях, опубликованных в журнале "Cerebral Blood Flow and Metabolism", полностью подтверждается эта нейропротективная концепция.

Ключевые слова: заболевания мозга, глутамат, инсульт.

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BLOOD GOT TO RID THE BRAIN OF EXCESS GLUTAMATE: A NOVEL APPROACH TO THE TREATMENT OF HUMAN STROKE AND OF OTHER NEUROLOGICAL DISORDERS

A number of brain diseases such as stroke are characterized by a deleterious role of the excess glutamate levels present in brain extracellular fluids. On the basis of the failure of glutamate receptor antagonists to deal with the excess glutamate in brain, we developed a completely novel neuroprotective approach in which the excess glutamate in brain fluids is eliminated using blood glutamate scavengers that increase the naturally-occuring brain-to blood glutamate efflux. Two papers of published in the *Journal of Cerebral Blood Flow* and *Metabolism* now fully prove this neuroprotective concept.

Key words: brain diseases, glutamate, stroke.

Two landmark papers from the laboratory of Jose Castillo at the University of Santiago de Compostela in Spain, which were published in the *Journal of Cerebral Blood Flow* and *Metabolism* (Campos et al. 2011b; Campos et al. 2011c) will undoubtedly cause a revolution in the future treatment of stroke. Ischemic stroke is a devastating disorder, often leading to death or long — lasting neurological disability. tPA (tissue plasminogen activator), the main treatment, is only effective for a small population of stroke victims, leaving millions of stroke victims worldwide facing a bleak situation.

In fact, the potential treatment they describe may become standard worldwide for a number of other brain pathologies that, like stroke, involve a transient or chronic excess of glutamate in brain fluids. These include epilepsy, dementias (eg., Alzheimer's disease), multiple sclerosis, Parkinson's disease and other hyperkinetic disorders, amyotrophic lateral sclerosis, pain syndromes and closed head injury and subarachnoid hemorrhage. These CNS pathologies are responsible for about one per cent of deaths and account for almost 11 per cent of the disease burden world-wide.

In the first paper (Campos et al. 2011b), the authors demonstrate the effectiveness of intravenous oxaloacetate — a blood glutamate scavenger, in treating rats with a transient occlusion of the middle cerebral artery (a rat model of stroke). Under the stringent STAIR guidelines (Philip et al. 2009), they observed that a bolus intravenous injection of oxaloacetate at 3.5mg/100 g but not at 1.5 mg/100g rat weight decreases both blood and brain glutamate levels by 70%, causes an 80% reduction in the brain infract volume already observed at 24 h reaching a maximum at 7 days and prevents the development of brain edema at 24 h and 3 days while the edema resolves itself spontaneously at 7 days.

The neuroprotective effect of oxaloacetate is due to the decrease it causes in blood glutamate levels as the result of the activation of the blood resident enzyme glutamate-oxaloacetate transminase (GOT (Gottlieb et al. 2003)). The latter enzyme causes a reversible reaction where blood glutamate reacts with oxaloacetate to transfer, via the cofactor pyridoxal phosphate, its amino group transforming glutamate into 2-ketoglutarate and oxaloacetate into aspartate.

When glutamate levels in brain fluids are elevated, the activation of blood GOT with oxaloacetate, causes an acceleration of a naturally-occurring brain-to-blood glutamate efflux driven by the newly-established glutamate concentration gradient across the blood-brain barrier capillaries. Blood glutamate scavengers work only in the blood and this mechanism categorically differs from that of glutamate receptor antagonists that have to

cross the blood brain barrier from blood into brain in order to exert their neuroprotective effects.

In experimental stroke, several brain microdialyis studies have shown that glutamate levels increase substantially in brain extracellular fluids (Butcher et al. 1990; Globus et al. 1988; Guyot et al. 2001; Phillis et al. 1996) and exert a deleterious excicitotoxic effect on surrounding neurons.

In naïve rats, intravenous oxaloacetate was found to accelerate the transfer of radioactive glutamate into the bloodstream after its injection into the lateral ventricles and to decrease in parallel the blood glutamate levels (Gottlieb et al. 2003) The same phenomenon of brain-to blood glutamate efflux was observed in another supportive study using dual-probe brain microdialyis when glutamate was released from a delivery probe implanted in the striatum while a recovery probe collected it at a distance of 1 mm (Teichberg et al. 2009). Under such experimental conditions, intravenous oxaloacetate causes a very significant reduction (70%) of the released glutamate reaching the striatally-implanted recovery probe.

The impressive neuroprotective effect of oxaloacetate has been previously established in a rat model of head injury (Zlotnik et al. 2007), but that paper lacked the direct evidence — now provided for the first time by Campos and his colleagues (Campos et al. 2011b) by their use of magnetic resonance spectroscopy — that blood glutamate scavenging is the direct cause of a decrease of glutamate in the brain fluids within the infracted region. Thus, Campos et al. (Campos et al. 2011b) not only bring the final missing proof for the neuroprotective mechanism of blood glutamate scavenging, but also establish its effectiveness for the treatment of experimental stroke.

Of course, rats are not humans, and the corresponding dosage of oxaloacetate for a human patient would be huge as well as toxic.

Man indeed has about 5 liters of blood and the 1 ml solution that is likely to be injected to stroke patients should contain, as in rats, about 1 mmole of oxaloacetate. As this solution ought to be at a neutral pH, about 2 mmole NaOH are added to neutralize the acidity of oxaloacetate. Thus, the injected solution should be 10^{-3} mmole. 5000=5 M/l of Oxaloacetate and 10M/l NaCl which will obviously not be tolerated by patient.

One has here to take into consideration factors such as the volume of tissue distribution and the fact that one cannot find oxaloacetate in human blood (Haas et al. 1988). However, oxaloacetate must be present in human serum at a concentration not far from that found for citrate in human blood (citrate (87±36) μM) since the condensation of oxaloacetate with acetyl-CoA forms citrate. Oxaloacetate is also chemically unstable and decarboxylates spontaneously into pyruvate (pyruvate (43±10) μM in human blood) though the blood pyruvate may also come from the enzymatic activity of lactate dehydrogenase (lactate: (465±165) μM) (Haas et al. 1988). In addition to the above, oxaloacetate is a substrate for human GOT which has a turn over rate Kcat/Km value for oxaloacetate of 36900 s^{-1}/Mole, fast enough to eliminate oxaloacetate from human blood.

The second paper from the group of Jose Castillo (Campos et al. 2011c) makes the momentous jump from rat to human, in a fairly large cohort of several hundred stroke victims admitted to the emergency wards of two different hospitals. Using the same inclusion and exclusion criteria, they revealed two highly significant prognostic parameters for the future outcome of stroke patients in terms of the modified Rankin scale score at 3 months and the infarct size.

High blood serum glutamate levels (up to 1.5 times the normal values i. e 300 M) at the time of hospital admission is highly correlated with a poor outcome at three months,

confirming previously established results (Castillo et al. 1997). Normal serum glutamate but high blood GOT levels (twice the normal values) at admission is correlated with a good outcome. Interestingly, only age and the NIHSS at admission have a similar prognostic value at three months. In a further clinical paper (Campos et al. 2011a), blood glutamate pyruvate transaminase (GPT) is also shown to be a good prognostic value for the stroke outcome at three months though GOT is more robust than GPT. This fully confirms our own results with GPT in a rat model of closed head injury and where GPT and pyruvate are efficacious as neuroprotective agents but less than GOT and oxaloacetate (Zlotnik et al. 2008).

The implication of these papers (Campos et al. 2011b; Campos et al. 2011c) is that stroke patients' chance of recovery will be significantly boosted by decreasing their blood glutamate levels to about 50% of normal values (from ~200 M to ~100 M)) by bolus intravenous administration of GOT i. e to a level of 60 units/liter around 3 times the normal range of GOT in clinical labs. This should bring about a neuroprotective decrease in glutamate in the extracellular fluids within and surrounding the infracted brain region.

A treatment with intravenous GOT is unlikely to have unwanted pathological consequences: Plasma glutamate fluctuates in any case by about 50% during the circadian cycle (Tsai and Huang 2000), most likely due to the accumulation of glutamate in brain fluids during intense neuronal activity or the REM phases of sleep. GOT, as well, is known to increase naturally, as it does in hepatitis, by several-hundred fold without laving any sort of pathology, either transient or permanent.

Thus, the stage is thus now set for the conducting of clinical trials not only for brain pathologies linked to the presence of excess glutamate in brain fluids but also for human cancers such as breast, colon, skin and lung cancer where a deleterious role for glutamate has been established (Brocke et al. ; Stepulak et al. 2009). All these cancer cells display on their surface both ionotropic and metabotropic glutamate receptors. Examining the expression of NMDA receptor subunits NR1-NR3B, AMPA receptor subunits GluR1-GluR4, kainate receptor subunits GluR5-GluR7, KA1, KA2 and metabotropic receptor subunits mGluR1-8, it was found that paraffin embedded samples from the above mentioned tumors were immunohistochemically stained for the selected subunits. The glutamate receptor subunits are differentially expressed in these human cancers at the mRNA and the protein level, and their expression is associated with the formation of functional channels. In all these cases, retrospective studies of the role of GOT will be relevant.

But the studies of Castillo and his colleagues hold larger implications. By adding a single test for glutamate/GOT in the routine clinical lab analysis, doctors apparently gain a new tool for diagnosing and regulating treatment, as well as following up the 50 different neurological disorders that display an excess glutamate in brain fluids.

Presently, blood, urine and CSF sample analysis is based on about 250 normal laboratory values, and in 95% of the cases bring the primary care physician to the initial diagnosis and therapy. When the treatment turns out to be ineffective, the 5% untreated patients are sent to the hospital emergency for further intensive testing — a factor that drives up the public health costs very significantly.

The question is: why are there only 250 normal lab values? An inspection of a two-dimensional gel electrophoresis of proteins and chromatograms for lipids in blood, urine and CSF sample reveal many more potential hits.

We suggest here to commit now more efforts for establishing the full human **"blufome"** (**bl**oodome, **u**rinome and cs**f**ome), an expanded and ultimate codex of the nor-

mal ranges of lab values that will be available to all primary care physicians in their daily diagnosis and decision about the appropriate therapeutic strategy to adopt. In this way, patient care will improve, costs will be reduced and, since only a fraction of the 5% of the patients will be referred to emergency wards, juggernaut hospitals will shrink accordingly.

Conclusions

With the papers of Campos et al. (2011) confirming our earlier results (Gottlieb et al. 2003; Teichberg et al. 2009; Zlotnik et al. 2009; Zlotnik et al. 2008; Zlotnik et al. 2007), blood glutamate scavenging with either oxaloacetate or GOT has become a fully demonstrated neuroprotective strategy in all cases where glutamate in present in excess in extracellular spaces. Its active components operate only via blood into which they cause an accelerated elimination. Their use appears to be the most attractive option in the treatment of human stroke.

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ЛІТЕРАТУРА

1. *Glutamate* receptors in pediatric tumors of the central nervous system / K. S. Brocke, C. Staufner, H. Luksch [et al.] // Cancer Biol Ther. – 2009. – № 9. – P. 455–468.

2. *Correlation* between amino acid release and neuropathologic outcome in rat brain following middle cerebral artery occlusion / S. P. Butcher, R. Bullock, D. I. Graham, J. McCulloch // Stroke. – 2000. – № 21. – P. 1727–1733.

3. *Blood* levels of glutamate oxaloacetate transaminase are stronger associated with good outcome in acute ishcemic stroke than glutamate pyruvate transaminase / F. Campos, M. Rodriguez-Yanez, M. Castellanos [et al.] // Clin Sci. – 2011. – № 121. – P. 11–18.

4. *Neuroprotection* by glutamate oxaloacetate transaminase in ischemic stroke: an experimental study / F. Campos, T. Sobrino, P. Ramos-Cabrer [et al.] // J Cereb Blood Flow Metab. – 2011. – № 31. – P. 1378–1386.

5. *High* blood glutamate oxaloacetate transaminase levels are associated with good functional outcome in acute ischemic stroke / F. Campos, M. Rodriguez-Yanez, M. Castellanos [et al.] // J Cereb Blood Flow Metab. – 2011. – № 31 (6). – P. 1387–1393.

6. *Castillo J.* Progression of ischaemic stroke and excitotoxic aminoacids / J. Castillo, A. Davalos, M. Noya // Lancet. – 1997. – № 349. – P. 79–83.

7. *Effect* of ischemia on the in vivo release of striatal dopamine, glutamate, and gamma-aminobutyric acid studied by intracerebral microdialysis / M. Y. Globus, R. Busto, W. D. Dietrich [et al.] // J Neurochem. – 1988. – № 51. – P. 1455–1464.

8. *Gottlieb M.* Blood-mediated scavenging of cerebrospinal fluid glutamate / M. Gottlieb, Y. Wang, V. I. Teichberg // J Neurochem. – 2003. – № 87. – P. 119–126.

9. *Real-time* measurement of glutamate release from the ischemic penumbra of the rat cerebral cortex using a focal middle cerebral artery occlusion model / L. L. Guyot, F. G. Diaz, M. H. O'Regan [et al.] // Neurosci Lett. – 2001. – № 299. – P. 37–40.

10. *Haas R. H.* High-performance liquid chromatographic measurement of selected blood citric acid cycle intermediates / R. H. Haas, J. Breuer, M. Hammen // J Chromatogr. – 1988. – № 425. – P. 47–57.

11. *Methodological* quality of animal studies of neuroprotective agents currently in phase II/III acute ischemic stroke trials / M. Philip, M. Benatar, M. Fisher, S. I. Savitz // Stroke. – 2009. – № 40. – P. 577–581.

12. *Phillis J. W.* Changes in extracellular amino acid neurotransmitters and purines during and fol-lowing ischemias of different durations in the rat cerebral cortex / J. W. Phillis, M. Smith-Barbour, M. H. O'Regan // Neurochem Int. – 1996. – № 29. – P. 115–120.

13. *Expression* of glutamate receptor subunits in human cancers / A. Stepulak, H. Luksch, C. Gebhardt [et al.] // Histochem Cell Biol. – 2009. – № 132. – P. 435–445.

14. *Homeostasis* of glutamate in brain fluids: an accelerated brain-to-blood efflux of excess glutamate is produced by blood glutamate scavenging and offers protection from neuropa-thologies / V. I. Teichberg, K. Cohen-Kashi-Malina, I. Cooper, A. Zlotnik // Neuroscience. – 2009. – № 158. – P. 301–308.

15. *Tsai P. J.* Circadian variations in plasma and erythrocyte glutamate concentrations in adult men consuming a diet with and without added monosodium glutamate / P. J. Tsai, P. C. Huang // J Nutr. – 2000. – № 130. – P. 1002–1004.

16. *The neuroprotective* effects of oxaloacetate in closed head injury in rats is mediated by its blood glutamate scavenging activity: evidence from the use of maleate / A. Zlotnik, S. E. Gruenbaum, A. A. Artru [et al.] // J Neurosurg Anesthesiol. – 2009. – № 21. – P. 235–241.

17. *The contribution* of the blood glutamate scavenging activity of pyruvate to its neuroprotective properties in a rat model of closed head injury / A. Zlotnik, B. Gurevich, E. Cherniavsky [et al.] // Neurochem Res. – 2008. – № 33. – P. 1044–1050.

18. *Brain* neuroprotection by scavenging blood glutamate / A. Zlotnik, B. Gurevich, S. Tkachov [et al.] // Exp Neurol. – 2007. – № 203. – P. 213–220.

REFERENCES

1. Brocke K.S., Staufner C., Luksch H., Geiger K.D., Stepulak A., Marzahn J., Schackert G., Temme A., Ikonomidou C. Glutamate receptors in pediatric tumors of the central nervous system. *Cancer Biol Ther* 2009; 9: 455-468

2. Butcher SP., Bullock R., Graham DI., McCulloch J. Correlation between amino acid release and neuropathologic outcome in rat brain following middle cerebral artery occlusion. *Stroke* 2000; 21: 1727-1733

3. Campos F., Rodriguez-Yanez M., Castellanos M., Arias S., Perez-Mato M., Sobrino T., Blanco M., Serena J., Castillo J. Blood levels of glutamate oxaloacetate transaminase are stronger associated with good outcome in acute ishcemic stroke than glutamate pyruvate transaminase. *Clin Sci (Lond*) 2011 Jul; 121(1): 11-7

4. Campos F., Sobrino T., Ramos-Cabrer P., Argibay B., Agulla J., Perez-Mato M., Rodriguez-Gonzalez R., Brea D., Castillo J. Neuroprotection by glutamate oxaloacetate transaminase in ischemic stroke: an experimental study. *J Cereb Blood Flow Metab* 2011 Jul; 31(1): 1378-1389

5. Campos F., Sobrino T., Ramos-Cabrer P., Castellanos M., Blanco M., Rodriguez-Yanez M., Serena J., Leira R., Castillo J. High blood glutamate oxaloacetate transaminase levels are associated with good functional outcome in acute ischemic stroke. *J Cereb Blood Flow Metab.* 2011 Jun; 31(6): 1387-1393.

6. Castillo J., Davalos A., Noya M. Progression of ischaemic stroke and excitotoxic aminoacids. *Lancet* 1997; 349: 79-83.

7. Globus M.Y., Busto R., Dietrich W.D., Martinez E., Valdes I., Ginsberg M.D. Effect of ischemia on the in vivo release of striatal dopamine., glutamate., and gamma-aminobutyric acid studied by intracerebral microdialysis. *J Neurochem* 1988; 51: 1455-1464.

8. Gottlieb M., Wang Y., Teichberg VI. Blood-mediated scavenging of cerebrospinal fluid glutamate. *J Neurochem* 2003; 87: 119-126.

9. Guyot L.L., Diaz F.G., O'Regan M.H., McLeod S., Park H., Phillis J.W. Real-time measurement of glutamate release from the ischemic penumbra of the rat cerebral cortex using a focal middle cerebral artery occlusion model. *Neurosci Lett* 2001; 299: 37-40.

10. Haas RH., Breuer J., Hammen M. High-performance liquid chromatographic measurement of selected blood citric acid cycle intermediates. *J Chromatogr* 1988; 425: 47-57.

11. Philip M., Benatar M., Fisher M., Savitz SI. Methodological quality of animal studies of neuro-protective agents currently in phase II/III acute ischemic stroke trials. *Stroke* 2009; 40: 577-581.

12. Phillis J.W., Smith-Barbour M., O'Regan M.H. Changes in extracellular amino acid neurotrans-mitters and purines during and following ischemias of different durations in the rat cerebral cortex. *Neurochem Int* 1996; 29: 115-120.

13. Stepulak A., Luksch H., Gebhardt C., Uckermann O., Marzahn J., Sifringer M., Rzeski W., Stauf-ner C., Brocke K.S., Turski L., Ikonomidou C. Expression of glutamate receptor subunits in human cancers. *Histochem Cell Biol* 2009; 132: 435-445.

14. Teichberg V.I., Cohen-Kashi-Malina K., Cooper I., Zlotnik A. Homeostasis of glutamate in brain fluids: an accelerated brain-to-blood efflux of excess glutamate is produced by blood glutamate scavenging and offers protection from neuropathologies. *Neuroscience* 2009; 158: 301-308

15. Tsai P.J., Huang P.C. Circadian variations in plasma and erythrocyte glutamate concentrations in adult men consuming a diet with and without added monosodium glutamate. *J Nutr* 2000; 130: 1002S-1004S.

16. Zlotnik A., Gruenbaum S.E., Artru A.A., Rozet I., Dubilet M., Tkachov S., Brotfain E., Klin Y., Shapira Y., Teichberg V.I. The neuroprotective effects of oxaloacetate in closed head injury in rats is mediated by its blood glutamate scavenging activity: evidence from the use of maleate. *J Neurosurg Anesthesiol* 2009; 21: 235-241.

17. Zlotnik A., Gurevich B., Cherniavsky E., Tkachov S., Matuzani-Ruban A., Leon A., Shapira Y., Teichberg V.I. The contribution of the blood glutamate scavenging activity of pyruvate to its neuroprotective properties in a rat model of closed head injury. *Neurochem Res* 2008; 33: 1044-1050.

18. Zlotnik A., Gurevich B., Tkachov S., Maoz I., Shapira Y., Teichberg V.I. Brain neuroprotection by scavenging blood glutamate. *Exp Neurol* 2007; 203: 213-220.

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