Published in final edited form as:

J Neural Transm. 2014 August ; 121(8): 971–979. doi:10.1007/s00702-014-1181-7.

Brain to blood glutamate scavenging as a novel therapeutic modality: a review

Matthew Boyko,

Department of Anesthesiology and Critical Care, Faculty of Health Sciences, Soroka Medical Center Ben Gurion University of the Negev, Beer Sheba, Israel

Shaun E. Gruenbaum,

Department of Anesthesiology, Yale University School of Medicine, New Haven, CT, USA

Benjamin F. Gruenbaum,

Department of Anesthesiology, Yale University School of Medicine, New Haven, CT, USA

Yoram Shapira, and

Department of Anesthesiology and Critical Care, Faculty of Health Sciences, Soroka Medical Center Ben Gurion University of the Negev, Beer Sheba, Israel

Alexander Zlotnik

Department of Anesthesiology and Critical Care, Faculty of Health Sciences, Soroka Medical Center Ben Gurion University of the Negev, Beer Sheba, Israel

Alexander Zlotnik: zlotnika@bgu.ac.il

Abstract

It is well known that abnormally elevated glutamate levels in the brain are associated with secondary brain injury following acute and chronic brain insults. As such, a tight regulation of brain glutamate concentrations is of utmost importance in preventing the neurodegenerative effects of excess glutamate. There has been much effort in recent years to better understand the mechanisms by which glutamate is reduced in the brain to non-toxic concentrations, and in how to safely accelerate these mechanisms. Blood glutamate scavengers such as oxaloacetate, pyruvate, glutamate–oxaloacetate transaminase, and glutamate–pyruvate transaminase have been shown to reduce blood glutamate concentrations, thereby increasing the driving force of the brain to blood glutamate efflux and subsequently reducing brain glutamate levels. In the past decade, blood glutamate scavengers have gained increasing international interest, and its uses have been applied to a wide range of experimental contexts in animal models of traumatic brain injury, ischemic stroke, subarachnoid hemorrhage, epilepsy, migraine, and malignant gliomas. Although glutamate scavengers have not yet been used in humans, there is increasing evidence that their use may provide effective and exciting new therapeutic modalities. Here, we review the laboratory evidence for the use of blood glutamate scavengers. Other experimental neuro-protective

treatments thought to scavenge blood glutamate, including estrogen and progesterone, beta-adrenergic activation, hypothermia, insulin and glucagon, and hemodialysis and peritoneal dialysis are also discussed. The evidence reviewed here will hopefully pave the way for future clinical trials.

Keywords

Blood glutamate scavenging; Neuroprotection

Introduction

Glutamate is a non-essential amino acid and the most abundant excitatory neurotransmitter in the brain, accounting for approximately 60 % of all neuromediators' activity. Glutamate is known to be neurotoxic at elevated concentrations (Zauner et al. 1996), and plays an important role in secondary brain injury following acute (Zauner et al. 1996; Castillo et al. 1996; Johnston et al. 2001) and chronic (Ferrarese et al. 2001; Shaw et al. 1995; Spranger et al. 1996) brain insults. Therefore, it is of vital importance that glutamate concentrations be tightly regulated in the brain to balance glutamate's important life-sustaining effects with its deleterious effects. In recent years, there has been great interest in understanding the mechanisms by which glutamate exerts its neurotoxic effects, as well the brain's innate mechanisms of protecting against the harmful effects of excess glutamate (Leibowitz et al. 2012; Lau and Tymianski 2010). Special attention has been paid to better understand the homeostatic mechanisms that maintain low concentrations of glutamate in the brain's extracellular fluids (1–10 μM) relative to the plasma (40–60 μM) (Rodriguez-Rodriguez et al. 2013; Mangia et al. 2012).

Moreover, there has been great interest in studying potential treatment modalities that may reduce the secondary brain damage associated with excess glutamate concentrations that follow an acute brain insult (Zlotnik et al. 2007). Historically, there has been much interest in studying the neuroprotective effects *N*-methyl-D-aspartate (NMDA) receptor antagonists (Ikonomidou and Turski 2002). Although administration of NMDA receptor antagonists has been neuroprotective in animal models of stroke and TBI (McCulloch 1992; Lee et al. 1999), clinical trials have either failed to demonstrate a therapeutic effect in humans or have worsened outcomes (Ikonomidou and Turski 2002; Muir 2006). It is thought that NMDA receptor blockade may interfere with processes in normal NMDA receptor signaling and maintenance of neuronal integrity, as well as with glutamate-facilitated neuronal repair after injury (Ikonomidou and Turski 2002; Zlotnik et al. 2009).

Another potential therapeutic modality that has shown great promise in reducing brain glutamate concentrations is the peripheral administration of pyruvate and oxaloacetate, termed "blood glutamate scavengers" (Gottlieb et al. 2003). Blood glutamate scavengers have received increased attention in recent years, and have now been extensively studied in animals across a wide range of contexts (Zlotnik et al. 2007, 2012; Boyko et al. 2011, 2012).

Mechanisms of glutamate homeostasis

Glutamate in the brain is primarily maintained intracellular. Despite its presence in high concentrations in the brain (5–15 mmol per kg), only a tiny fraction of glutamate exists in the extracellular space (Schousboe 1981). Whereas intracellular glutamate is not thought to be neurotoxic, extracellular glutamate (particularly at abnormally high concentrations) is known to exert neurotoxic effects (Teichberg 2011). Depending on the brain's energy needs and supply, glutamate is continuously released from cells and eliminated from the extracellular space (Danbolt 2001). Although the concentration of extracellular glutamate determines its capacity to stimulate glutamate receptors, extracellular glutamate concentrations are tightly maintained at low concentrations (Zlotnik et al. 2008).

There are several mechanisms by which glutamate concentrations are maintained at physiological concentrations in the brain. Na⁺-dependent glutamate transporters present on both nerve terminals and astrocytes are known to bind and uptake excess glutamate after synaptic or astrocytic release (Danbolt 2001). Na⁺-dependent glutamate transporters (EAAT3) on the antiluminal side of brain capillaries are also known to play an important role in maintaining normal concentrations of glutamate in the extracellular fluid of the brain (O'Kane et al. 1999), despite the highly unfavorable concentration gradient of the brain's extracellular fluid relative to blood plasma concentrations. In recent years, there has been much interest generated in better understanding this mechanism, and utilizing it for potential new therapeutic modalities in brain glutamate reduction (Zlotnik et al. 2007).

When the concentration of glutamate is elevated in the brain's extracellular fluid, glutamate is transported via the Na⁺-dependent transporters into the endothelial cell, where it accumulates. When the concentration of glutamate exceeds that of the blood, glutamate is transported across the luminal membrane by facilitated diffusion into the blood. In the blood, glutamate can then be redistributed from the plasma to the peripheral tissues (Marliss et al. 1971).

The brain to blood efflux of glutamate is a rapid and naturally occurring event. Hosoya (1999) observed the appearance of radiolabeled glutamate in the blood 20 min after injection in the CSF. Furthermore, after injection of radiolabeled glutamate in the lateral ventricles of naïve rats' brains, Gottlieb (2003) observed the immediate presence of un-metabolized glutamate in the blood that increased with time until reaching a plateau 40 min after injection.

Blood glutamate scavenging with oxaloacetate and pyruvate

It was demonstrated in rats that the rate of brain to blood efflux could be increased by creating a larger concentration gradient of glutamate between the brain and blood (Gottlieb et al. 2003). This was achieved with the use of blood resident glutamate scavenging enzymes glutamate-pyruvate transaminase (GPT) and glutamate-oxaloacetate transaminase (GOT). GPT and GOT, in the presence of their co-substrates pyruvate and oxaloacetate, respectively, convert glutamate to 2-ketoglutarate and its metabolites. After intravenous injection of pyruvate or oxaloacetate, the rate of brain to blood glutamate efflux was shown to increase. This supports the premise that brain extracellular glutamate levels can be

controlled in part by manipulating blood glutamate concentrations. The use of blood glutamate scavengers effectively resulted in the elimination of excess glutamate in the brain ventricles and CSF (Gottlieb et al. 2003), as well as from the brain parenchyma (Teichberg et al. 2009), as evidenced with the use of dual-probe microdialysis experiments. Likewise, peripheral injections of GOT and GPT in naïve rats, as a single treatment modality and in combination with its co-substrates oxaloacetate and pyruvate, respectively, have been shown to result in a reduction in blood glutamate (Zlotnik et al. 2007; Boyko et al. 2012a, b).

Evidence with blood glutamate scavengers shows potential for the development of novel, effective, and safe therapeutic agents that may be effective across a wide range of clinical contexts. Whereas NMDA receptor antagonists were ineffective or potentially harmful, blood glutamate scavengers do not act on glutamate receptors nor do they interfere with normal cellular signaling processes. Their action is only in the blood, and they accelerate a physiological mechanism of removing glutamate only from areas in which glutamate is pathologically elevated (Gottlieb et al. 2003). Furthermore, glutamate scavenging is a self-limiting process, and slows down and eventually stops as concentrations of glutamate in the brain are decreased (Zlotnik et al. 2009). In animal models, blood glutamate scavengers have been successfully utilized in many different experimental contexts, especially TBI and stroke, with promising results.

Traumatic brain injury

Following TBI, brain ECF and CSF concentrations of glutamate are known to increase in humans (Rose et al. 2002; Baker et al. 1993; Bullock et al. 1998) and rats (Palmer et al. 1993; Richards et al. 2003). In neurons, mitochondrial GOT has also been shown to decrease following blast exposure, which may result in mitochondrial dysfunction and decreased mitochondrial energy metabolism (Arun 2013).

Peripheral administration of oxaloacetate (Zlotnik et al. 2007, 2009, 2012) or pyruvate (Zlotnik et al. 2008, 2012) 60 min after TBI in rats results in a significant reduction in blood glutamate concentrations and concomitant improvement in neurological outcome. Zlotnik (2007) demonstrated that the blood glutamate-reducing effects of oxaloacetate and pyruvate are dose-dependent, and that the administration of very low doses (5 mM) of oxaloacetate or pyruvate did not result in a reduction of blood glutamate. However, when low doses of oxaloacetate or pyruvate were administered with GOT or GPT, respectively, the blood glutamate-lowering effects were restored. Seeing as GOT and GPT are too large to penetrate the blood brain barrier, this finding suggests that the therapeutic effects of oxaloacetate and pyruvate were mediated by blood glutamate scavenging and not by direct effects in the brain.

Oxaloacetate and pyruvate administration resulted in an increased number of surviving neurons in five different regions of the hippocampus, with preserved cellularity, 30 days after TBI (Zlotnik et al. 2012a). Co-administration of oxaloacetate with maleate, a GOT-blocker, was shown to prevent this glutamate reduction and improvement in neurological outcome. This provides strong evidence that the neuroprotective properties of oxaloacetate are primarily the result of blood glutamate scavenging, and not by some other mechanism (Zlotnik et al. 2009).

Stroke

It is well established that during brain ischemia, glutamate plays an important role in mediating neuronal damage (Lipton 1999), and higher glutamate concentrations in the blood and CSF are associated with an increased neurological deterioration after stroke in humans (Castillo et al. 1996, 1997) and rats (Castellanos et al. 2008). Furthermore, in patients with ischemic stroke, Campos (2011) reported an association between higher concentrations of GOT and GPT in the blood and good outcomes (GOT having a stronger association than GPT levels). In fact, patients presenting with lower GOT levels and higher blood glutamate levels were independently associated with a worsened neurological outcome at 3 months and a higher infarct volume (Campos et al. 2012a, b), which emphasizes the role that GOT plays in blood glutamate regulation.

In animal models of stroke, blood glutamate scavenging has provided promise as a potentially useful therapeutic intervention (Campos et al. 2012a, b). Immediately after subjecting rats to photothrombotic lesion and in incomplete forebrain ischemia model, peripheral oxaloacetate administration (1.2 mg/100 g over 30 min) resulted in decreased brain and blood glutamate, reduction in infarct size, and prevented the decreased amplitude of evoked potentials (Nagy et al. 2009). Oxaloacetate further prevented the long-term potentiation impairment in the rat CA1 hippocampal region induced by 2-vessel occlusion (Marosi et al. 2009). After middle cerebral artery occlusion (MCAO) in rats, Campos et al. (2011) further demonstrated that peripheral infusion of oxaloacetate 60 min following MCAO (250 mg/kg over 30 min) resulted in a reduction in blood and cerebral glutamate levels, with subsequent reduction in infarct size, smaller edema volume, and lower sensorimotor deficits compared with controls. Similarly, infusion of peripheral pyruvate (with or without GPT) 60 min after MCAO resulted in reduced blood glutamate concentrations, smaller volume of infarction, reduced brain edema, improved neurological outcome, and reduced mortality compared with controls (Boyko et al. 2011).

Subarachnoid hemorrhage (SAH)

Recent evidence suggests that glutamate scavengers also may be therapeutically useful in the treatment of SAH. In a rat model of SAH, oxaloacetate and pyruvate (250 and 125 mg/kg) were infused for 30, 60 min after induction of SAH (Boyko et al. 2012a, b). Infusion of the scavengers was associated with a significant reduction in blood and CSF glutamate. Furthermore, there was an improvement in neurological outcome 24 h after SAH demonstrated by blood brain barrier disruption, neurological severity score, and brain edema (Boyko et al. 2012a, b).

Epilepsy

There is much evidence that abnormally elevated glutamate is present in the brain of patients with medically refractory temporal lobe epilepsy (Lee et al. 2007; Eid et al. 2008a). As such, treatment with glutamate scavengers may provide a novel therapeutic modality in the treatment of such medically refractory seizures. Rats administered a single injection of peripheral oxaloacetate and pyruvate 30 min after being subjected to pilocarpine-induced status epilepticus (SE) resulted in complete prevention of SE-induced neuronal loss in the CA1 region of the hippocampus with a significant reduction in apoptosis (Carvalho et al.

2011). Despite evidence for neuroprotection, a reduction in seizure severity was not observed in that study. Glutamate scavenging may in part explain the anticonvulsant effects of the ketogenic diet (Yudkoff et al. 2004, 2007), but this has yet to be systematically studied.

Migraine headache

Recent evidence has demonstrated that glutamate may play an important role in the development of migraine symptoms (Ramadan 2003). Campos (2013) recently showed that migraine patients had lower peripheral GOT activity and higher levels of peripheral glutamate compared with controls. This has led some authors to suggest that new therapeutic modalities in migraine prophylaxis may involve blood glutamate reduction (Ferrari et al. 2009). Thus far, however, there are no experimental studies that demonstrate the use of blood glutamate scavengers in the treatment or prophylaxis of migraine headaches.

Glioma

Studies in the last few years have suggested that glutamate plays an important role in the growth and invasiveness of malignant gliomas, and may play an important role in the development of seizures that often accompany gliomas (de Groot and Sontheimer 2011; Sontheimer 2003, 2004; Ye and Sontheimer 1999). Interestingly, a recent study demonstrated that rats and mice subjected to oxaloacetate treatment (0.2 M solution in water 7 days after tumor cell implantation) displayed a smaller tumor volume, reduced tumor invasiveness, and prolonged survival (Ruban et al. 2012). This study, the first implicating the use of glutamate scavengers in invasive cancer, opens the doors for a new and exciting approach to adjuvants in the treatment of gliomas.

Organophosphate intoxication

Recent studies have suggested that glutamate may play an important role in the development of organophosphate-induced seizures and secondary brain damage (Ruban 2013). 10 min after paraoxon exposure, Ruban and colleagues (2013) infused oxaloacetate and GOT (3.95 mg/ 3 ml and 45 µg, respectively over 30 min) in rats. The infusion of blood glutamate scavengers significantly reduced neuronal damage and prevented the peripheral benzodiazepine receptor density elevation. This study showed promise that glutamate scavengers may be useful in the treatment of secondary brain damage in the setting of organophosphate toxicity.

Limitations of glutamate scavengers

In clinical practice, glutamate scavengers may have some important limitations. Studies with rat models of TBI, stroke, and SAH have demonstrated that the therapeutic window of glutamate scavengers is very short (Zlotnik et al. 2007, 2008; Boyko et al. 2011, 2012). For example, glutamate scavengers are effective in improving neurological outcomes after TBI when administered 30 and 60 min after TBI, but were ineffective when administered after 120 min (Zlotnik et al. 2007, 2008). The short therapeutic window of glutamate scavengers may be due to the fact that within a few minutes of an acute brain injury, glutamate levels sharply increase and slowly return to baseline by 120 min (Zlotnik et al. 2007). In contrast to

rats, however, the increased glutamate in humans following TBI and stroke is known to last for a much longer time period (Castillo et al. 1996; Baker et al. 1993). Therefore, it is conceivable (although not known for sure) that glutamate scavengers may be effective in humans if administered later in the time course following an acute brain insult (Boyko et al. 2012).

There are no safety studies that have been performed with oxaloacetate or pyruvate in humans, which has limited clinical investigations. The growing evidence for the efficacy of blood glutamate scavengers in animal studies emphasizes the need for such clinical studies. Because blood glutamate scavengers are thought to only work in the blood, and increase the brain to blood glutamate efflux only in areas where glutamate is pathologically elevated (Gottlieb et al. 2003), it is unlikely that they will produce unwanted side effects (Teichberg 2011). Plasma glutamate concentrations are known to vary by as much as 50 % during the circadian cycle (Tsai and Huang 2000), and GOT is known to increase by several 100-fold naturally without any transient or permanent pathological consequences. It should be noted, however that the effective dose of oxaloacetate may not be tolerated in humans, because large amounts of NaOH would be likely be needed to neutralize the acidity of oxaloacetate (Teichberg 2011).

Other factors affecting blood glutamate concentrations

Healthy humans

Blood glutamate concentrations are thought to play an important role in brain glutamate homeostasis, and as discussed above, the rate of the brain to blood glutamate efflux is primarily determined by the relative ratio of brain to blood glutamate concentrations. In healthy humans, women have been shown to have lower blood glutamate, GOT, and GPT concentrations compared with men (Zlotnik et al. 2011a), which has been attributed to higher levels of estrogen and progesterone in women. Age and consumptions of liquids (including coffee, a known stimulator of the sympathetic nervous system) were not shown to have any significant impact on blood glutamate levels (Zlotnik et al. 2011b). Consumption of food without monosodium glutamate (MSG) does not result in an increased plasma glutamate concentration (Zlotnik et al. 2011a; Stegink et al. 1985), possibly due to the rapid metabolism of glutamate in humans (Tsai and Huang 2000). The consumption of food with MSG, however, has been shown to result in an increased plasma glutamate concentrations between 2 and 4 h after consumption (Stegink et al. 1983).

Estrogen and progesterone

Estrogen and progesterone are known to have neuroprotective effects in a wide range of neurodegenerative conditions (Slooter et al. 1999; Behl 2002; Tang et al. 1996) in humans. In healthy humans, there is evidence that blood glutamate concentrations inversely correlate with estrogen and progesterone. Zlotnik (2011) demonstrated that during the menstrual cycle, blood glutamate levels decrease as estrogen and progesterone levels increase. Furthermore, the same group (Tsesis 2013) demonstrated an inverse correlation between blood glutamate levels and levels of plasma estrogen and progesterone throughout normal pregnancy.

Experimental animal studies have demonstrated a significant improvement in neurological outcomes after stroke and TBI in rats pretreated or treated with either estrogen or progesterone (Yang 2000; Wang et al. 1999; Dubal et al. 1998, 1999). Furthermore, estrogen has been shown to delay the onset of kainic acid-induced seizures and reduce seizure-related mortality in rats (Veliskova et al. 2000). While several potential mechanisms for estrogen and progesterone's effects have been proposed, recent evidence suggests that an estrogen and progesterone-induced reduction in blood glutamate (and subsequent reduction in brain glutamate) may play an important role. Zlotnik (2012) demonstrated the blood glutamate scavenging properties of estrogen in the setting of TBI. Following the peripheral administration of estrogens, a decrease in blood glutamate levels was observed within 60 min of TBI. At 24 h after TBI, rats treated with estrogens had significantly more neurological recovery compared with the control group.

Stress and beta-adrenergic activation

Spontaneous reductions in blood glutamate levels after TBI were shown in part to be the result of a stress response and subsequent activation of the sympathetic nervous system (Zlotnik et al. 2010a, 2011, 2012). In particular, experimental activation of β 2-adrenergic receptors resulted in decreased blood glutamate levels, whereas selective β 2-adrenergic receptor blockade prevented isoproterenol-mediated reductions in blood glutamate levels in naïve rats (Zlotnik et al. 2011a). Similarly, after TBI, pretreatment with metoprolol or isoproterenol lead to a reduction of blood glutamate levels and improvement in neurological outcomes in rats (Zlotnik et al. 2012a). However, β 1-adrenergic receptor activation resulted in an increase in blood glutamate levels and prevented the spontaneous neurological improvement typically observed in rats after TBI. Similarly, β 2-adrenergic receptor blockade before such pretreatment prevented the reduction of blood glutamate levels and subsequent improvements in neurological outcome (Zlotnik et al. 2012a).

Hypothermia and hyperthermia

Recent evidence suggests that shift in blood glutamate levels that result from changes in body temperature may play an important role in hypothermia-induced neuroprotection. In naïve rats, induced mild to moderate hypothermia resulted in a reduction in blood glutamate levels, whereas severe hyperthermia resulted in increases in blood glutamate levels (Boyko et al. 2013). Experimental studies in rats have further demonstrated that mild hyperthermia (T 37–40 °C) is associated with a reduction of blood glutamate, likely due to an activation of the sympathetic nervous system (Zlotnik et al. 2010a). Severe hyperthermia (T 40–42 °C) was associated with increased blood glutamate concentrations, however, and could not be blunted with the administration of propranolol or oxaloacetate (Zlotnik et al. 2010a). Boyko (2013) demonstrated that mild and moderate hypothermia led to a reduction in blood glutamate in naïve rats with a corresponding increase in GOT and GPT levels.

In both animal (Berger et al. 2004) and human (Berger et al. 2002) studies of stroke, hypothermia was observed to result in decreased brain concentrations of glutamate. Using magnetic resonance imaging (MRI) data, Campos et al. (2012) further demonstrated that rats subjected to hyperthermia in the acute phase of stroke had larger lesion volumes at day 7 after stroke, whereas hypothermic animals exhibited the smallest lesion volumes. Although

systemic administration of glutamate reversed the neuro-protective effects of hypothermia, systemic administration of oxaloacetate prevented the deleterious effects of hyperthermia (Campos et al. 2012a, b), indicating that the impact of temperature on neurological outcome in the acute phase of stroke is related to changes in blood glutamate levels. These studies suggest that the neuroprotective properties of hypothermia may be at least in part due to blood glutamate scavenging.

Insulin and glucagon

Insulin and glucagon have also been shown to play an important role in regulating blood glutamate concentrations. In naïve rats, infusion of insulin and glucagon resulted in decreased glutamate levels (Zlotnik et al. 2011a). Whereas the effect of insulin was immediate and transient, the effect of glucagon was delayed but longer lasting, suggesting that adequate glucose concentrations are important promoting the activity of glutamate in pancreatic insulin and glucagon-secreting cells (Zlotnik et al. 2011a). Even more so, glucagon was shown to improve outcomes after TBI in mice (Fanne et al. 2011a), and both insulin and glucagon (regardless of glucose concentration) improved post-stroke outcomes in animal models by reducing glutamate concentrations in the blood and CSF (Fanne et al. 2011b). These data suggest that insulin and glucagon both may play an important role in scavenging blood glutamate, and this mechanism may be independent of their effect on modifying blood glucose levels.

Maternal and fetal glutamate

The increased efflux of glutamate from the brain to the blood following reductions of blood glutamate concentrations may have other implications as well. Interestingly, Zlotnik (2012) demonstrated a correlation between glutamate concentrations and GOT levels in pregnant women's blood and fetal blood, with higher baseline concentrations present in fetal blood. As such, a reduction of maternal blood glutamate levels may in turn increase the rate of efflux from fetal blood to maternal blood, thereby reducing fetal brain glutamate levels that are associated with a worse neurological outcome after fetal asphyxia. Although speculative at this time, this may provide a novel therapeutic strategy for the in utero treatment of fetal asphyxia and may improve fetal neurological outcomes (Zlotnik et al. 2012a).

Hemodialysis and peritoneal dialysis

Another interesting and potentially useful method of reducing blood glutamate concentrations is the use of dialysis to filter the blood and remove excess of glutamate. It was recently demonstrated that compared with healthy controls, patients with end-stage renal failure on hemodialysis (HD) had higher concentrations of blood glutamate (Rogachev et al. 2012). During HD (especially in the first hour), glutamate concentrations decreased regardless of the size of filter pores, blood flow rate, or gender. Similarly, peritoneal dialysis (PD) resulted in decreases in blood glutamate (Rogachev et al. 2013; Godino Mdel 2013), with a corresponding increase of glutamate in the dialysis solution (Rogachev et al. 2013). In a rat model of stroke, the reduction of blood glutamate levels observed with PD was associated with a decrease in infarct area (Godino Mdel 2013).

These studies provide promising evidence that HD and PD may be an effective modality in reducing blood glutamate concentrations, thereby increasing the brain to blood glutamate efflux and reducing brain glutamate concentrations. This method is especially promising because its blood glutamate-reducing effects were long-lasting, compared with the transient effects observed after administration of blood glutamate scavengers. Furthermore, extracorporeal methods of blood glutamate reduction are advantageous in that they avoid the possible toxic effects that may accompany the administration of blood glutamate scavengers.

Conclusion

Experimental evidence with blood glutamate scavengers and other modalities thought to increase the brain to blood glutamate efflux have shown much promise in the treatment of acute brain insults. While some authors have postulated that the predicted effective dose of oxaloacetate in humans may be very large and not tolerated (Teichberg 2011), the administration of GOT or GPT, either as a single treatment or in combination with low doses of oxaloacetate or pyruvate, respectively, may provide a safe alternative. Clinical trials are the much-needed next step to studying the clinical effects of blood glutamate scavengers in humans. Furthermore, knowledge of the relationship between glutamate, glutamate scavengers, GOT, and GPT provides important insights they may 1 day be useful in guiding the clinical diagnosis, prognosis, and treatment regulation of acute brain insults in humans.

References

- Arun P, Abu-Taleb R, Oguntayo S, et al. Acute mitochondrial dysfunction after blast exposure: potential role of mitochondrial glutamate oxaloacetate transaminase. *J Neurotrauma*. 2013
- Baker AJ, Moulton RJ, MacMillan VH, Shedden PM. Excitatory amino acids in cerebrospinal fluid following traumatic brain injury in humans. *J Neurosurg*. 1993; 79:369–372. [PubMed: 8103092]
- Behl C. Oestrogen as a neuroprotective hormone. *Nat Rev Neurosci*. 2002; 3:433–442. [PubMed: 12042878]
- Berger C, Schabitz WR, Georgiadis D, Steiner T, Aschoff A, Schwab S. Effects of hypothermia on excitatory amino acids and metabolism in stroke patients: a microdialysis study. *Stroke*. 2002; 33:519–524. [PubMed: 11823663]
- Berger C, Schabitz WR, Wolf M, Mueller H, Sommer C, Schwab S. Hypothermia and brain-derived neurotrophic factor reduce glutamate synergistically in acute stroke. *Exp Neurol*. 2004; 185:305–312. [PubMed: 14736512]
- Boyko M, Zlotnik A, Gruenbaum BF, et al. Pyruvate's blood glutamate scavenging activity contributes to the spectrum of its neuroprotective mechanisms in a rat model of stroke. *Eur J Neurosci*. 2011; 34:1432–1441. [PubMed: 21936878]
- Boyko M, Melamed I, Gruenbaum BF, et al. The effect of blood glutamate scavengers oxaloacetate and pyruvate on neurological outcome in a rat model of subarachnoid hemorrhage. *Neurotherapeutics*. 2012a; 9:649–657. [PubMed: 22711471]
- Boyko M, Stepensky D, Gruenbaum BF, et al. Pharmacokinetics of glutamate-oxaloacetate transaminase and glutamate-pyruvate transaminase and their blood glutamate-lowering activity in naive rats. *Neurochem Res*. 2012b; 37:2198–2205. [PubMed: 22846966]
- Boyko M, Kuts R, Gruenbaum BF, et al. The role of hypothermia in the regulation of blood glutamate levels in naive rats. *J Neurosurg Anesthesiol*. 2013; 25:174–183. [PubMed: 23295267]
- Bullock R, Zauner A, Woodward JJ, et al. Factors affecting excitatory amino acid release following severe human head injury. *J Neurosurg*. 1998; 89:507–518. [PubMed: 9761042]

- Campos F, Rodriguez-Yanez M, Castellanos M, et al. Blood levels of glutamate oxaloacetate transaminase are more strongly associated with good outcome in acute ischaemic stroke than glutamate pyruvate transaminase levels. *Clin Sci (Lond)*. 2011a; 121:11–17. [PubMed: 21265738]
- Campos F, Sobrino T, Ramos-Cabrer P, et al. High blood glutamate oxaloacetate transaminase levels are associated with good functional outcome in acute ischemic stroke. *J Cereb Blood Flow Metab*. 2011b; 31:1387–1393. [PubMed: 21266984]
- Campos F, Sobrino T, Ramos-Cabrer P, et al. Neuroprotection by glutamate oxaloacetate transaminase in ischemic stroke: an experimental study. *J Cereb Blood Flow Metab*. 2011c; 31:1378–1386. [PubMed: 21266983]
- Campos F, Sobrino T, Ramos-Cabrer P, Castillo J. Oxaloacetate: a novel neuroprotective for acute ischemic stroke. *Int J Biochem Cell Biol*. 2012a; 44:262–265. [PubMed: 22085530]
- Campos F, Perez-Mato M, Agulla J, et al. Glutamate excitotoxicity is the key molecular mechanism which is influenced by body temperature during the acute phase of brain stroke. *PLoS One*. 2012b; 7:e44191. [PubMed: 22952923]
- Campos F, Sobrino T, Perez-Mato M, et al. Glutamate oxaloacetate transaminase: a new key in the dysregulation of glutamate in migraine patients. *Cephalalgia*. 2013
- Carvalho AS, Torres LB, Persike DS, et al. Neuroprotective effect of pyruvate and oxaloacetate during pilocarpine induced status epilepticus in rats. *Neurochem Int*. 2011; 58:385–390. [PubMed: 21185899]
- Castellanos M, Sobrino T, Pedraza S, et al. High plasma glutamate concentrations are associated with infarct growth in acute ischemic stroke. *Neurology*. 2008; 71:1862–1868. [PubMed: 18971451]
- Castillo J, Davalos A, Naveiro J, Noya M. Neuroexcitatory amino acids and their relation to infarct size and neurological deficit in ischemic stroke. *Stroke*. 1996; 27:1060–1065. [PubMed: 8650715]
- Castillo J, Davalos A, Noya M. Progression of ischaemic stroke and excitotoxic aminoacids. *Lancet*. 1997; 349:79–83. [PubMed: 8996418]
- Danbolt NC. Glutamate uptake. *Prog Neurobiol*. 2001; 65:1–105. [PubMed: 11369436]
- de Groot J, Sontheimer H. Glutamate and the biology of gliomas. *Glia*. 2011; 59:1181–1189. [PubMed: 21192095]
- Dubal DB, Kashon ML, Pettigrew LC, et al. Estradiol protects against ischemic injury. *J Cereb Blood Flow Metab*. 1998; 18:1253–1258. [PubMed: 9809515]
- Dubal DB, Shughrue PJ, Wilson ME, Merchenthaler I, Wise PM. Estradiol modulates bcl-2 in cerebral ischemia: a potential role for estrogen receptors. *J Neurosci*. 1999; 19:6385–6393. [PubMed: 10414967]
- Eid T, Williamson A, Lee TS, Petroff OA, de Lanerolle NC. Glutamate and astrocytes—key players in human mesial temporal lobe epilepsy? *Epilepsia*. 2008a; 49(Suppl 2):42–52. [PubMed: 18226171]
- Eid T, Ghosh A, Wang Y, et al. Recurrent seizures and brain pathology after inhibition of glutamine synthetase in the hippocampus in rats. *Brain*. 2008b; 131:2061–2070. [PubMed: 18669513]
- Fanne RA, Nassar T, Mazuz A, et al. Neuroprotection by glucagon: role of gluconeogenesis. *J Neurosurg*. 2011a; 114:85–91. [PubMed: 20509730]
- Fanne RA, Nassar T, Heyman SN, Hijazi N, Higazi AA. Insulin and glucagon share the same mechanism of neuroprotection in diabetic rats: role of glutamate. *Am J Physiol Regul Integr Comp Physiol*. 2011b; 301:R668–R673. [PubMed: 21677268]
- Ferrarese C, Aliprandi A, Tremolizzo L, et al. Increased glutamate in CSF and plasma of patients with HIV dementia. *Neurology*. 2001; 57:671–675. [PubMed: 11524477]
- Ferrari A, Spaccapelo L, Pinetti D, Tacchi R, Bertolini A. Effective prophylactic treatments of migraine lower plasma glutamate levels. *Cephalalgia*. 2009; 29:423–429. [PubMed: 19170689]
- Godino Mdel C, Romera VG, Sanchez-Tomero JA, et al. Amelioration of ischemic brain damage by peritoneal dialysis. *J Clin Invest*. 2013; 123:4359–4363. [PubMed: 23999426]
- Gottlieb M, Wang Y, Teichberg VI. Blood-mediated scavenging of cerebrospinal fluid glutamate. *J Neurochem*. 2003; 87:119–126. [PubMed: 12969259]
- Hosoya K, Sugawara M, Asaba H, Terasaki T. Blood-brain barrier produces significant efflux of L-aspartic acid but not D-aspartic acid: in vivo evidence using the brain efflux index method. *J Neurochem*. 1999; 73:1206–1211. [PubMed: 10461913]

- Ikonomidou C, Turski L. Why did NMDA receptor antagonists fail clinical trials for stroke and traumatic brain injury? *Lancet Neurol.* 2002; 1:383–386. [PubMed: 12849400]
- Johnston MV, Trescher WH, Ishida A, Nakajima W. Neuro-biology of hypoxic-ischemic injury in the developing brain. *Pediatr Res.* 2001; 49:735–741. [PubMed: 11385130]
- Lau A, Tymianski M. Glutamate receptors, neurotoxicity and neurodegeneration. *Pflugers Arch.* 2010; 460:525–542. [PubMed: 20229265]
- Lee JM, Zipfel GJ, Choi DW. The changing landscape of ischaemic brain injury mechanisms. *Nature.* 1999; 399:A7–A14. [PubMed: 10392575]
- Lee TS, Mane S, Eid T, et al. Gene expression in temporal lobe epilepsy is consistent with increased release of glutamate by astrocytes. *Mol Med.* 2007; 13:1–13. [PubMed: 17515952]
- Leibowitz A, Boyko M, Shapira Y, Zlotnik A. Blood glutamate scavenging: insight into neuroprotection. *Int J Mol Sci.* 2012; 13:10041–10066. [PubMed: 22949847]
- Lipton P. Ischemic cell death in brain neurons. *Physiol Rev.* 1999; 79:1431–1568. [PubMed: 10508238]
- Mangia S, Giove F, Dinuzzo M. Metabolic pathways and activity-dependent modulation of glutamate concentration in the human brain. *Neurochem Res.* 2012; 37:2554–2561. [PubMed: 22846967]
- Marliss EB, Aoki TT, Pozefsky T, Most AS, Cahill GF Jr. Muscle and splanchnic glutamine and glutamate metabolism in postabsorptive and starved man. *J Clin Investig.* 1971; 50:814–817. [PubMed: 5547277]
- Marosi M, Fuzik J, Nagy D, et al. Oxaloacetate restores the long-term potentiation impaired in rat hippocampus CA1 region by 2-vessel occlusion. *Eur J Pharmacol.* 2009; 604:51–57. [PubMed: 19135048]
- McCulloch J. Excitatory amino acid antagonists and their potential for the treatment of ischaemic brain damage in man. *Br J Clin Pharmacol.* 1992; 34:106–114. [PubMed: 1419472]
- Muir KW. Glutamate-based therapeutic approaches: clinical trials with NMDA antagonists. *Curr Opin Pharmacol.* 2006; 6:53–60. [PubMed: 16359918]
- Nagy D, Marosi M, Kis Z, et al. Oxaloacetate decreases the infarct size and attenuates the reduction in evoked responses after photothrombotic focal ischemia in the rat cortex. *Cell Mol Neurobiol.* 2009; 29:827–835. [PubMed: 19259807]
- O’Kane RL, Martinez-Lopez I, DeJoseph MR, Vina JR, Hawkins RA. Na(+)-dependent glutamate transporters (EAAT1, EAAT2, and EAAT3) of the blood-brain barrier. A mechanism for glutamate removal. *J Biol Chem.* 1999; 274:31891–31895. [PubMed: 10542215]
- Palmer AM, Marion DW, Botscheller ML, Swedlow PE, Styren SD, DeKosky ST. Traumatic brain injury-induced excitotoxicity assessed in a controlled cortical impact model. *J Neurochem.* 1993; 61:2015–2024. [PubMed: 7504079]
- Ramadan NM. The link between glutamate and migraine. *CNS Spectr.* 2003; 8:446–449. [PubMed: 12858134]
- Richards DA, Tolia CM, Sgouros S, Bowery NG. Extracellular glutamine to glutamate ratio may predict outcome in the injured brain: a clinical microdialysis study in children. *Pharmacol Res.* 2003; 48:101–109. [PubMed: 12770522]
- Rodriguez-Rodriguez P, Almeida A, Bolanos JP. Brain energy metabolism in glutamate-receptor activation and excitotoxicity: role for APC/C-Cdh1 in the balance glycolysis/pentose phosphate pathway. *Neurochem Int.* 2013; 62:750–756. [PubMed: 23416042]
- Rogachev B, Ohayon S, Saad A, et al. The effects of hemodialysis on blood glutamate levels in chronic renal failure: implementation for neuroprotection. *J Crit Care.* 2012; 27(743):e1–e7. [PubMed: 23084134]
- Rogachev B, Tsesis S, Gruenbaum BF, et al. The effects of peritoneal dialysis on blood glutamate levels: implementation for neuroprotection. *J Neurosurg Anesthesiol.* 2013; 25:262–266. [PubMed: 23752045]
- Rose ME, Huerbin MB, Melick J, et al. Regulation of interstitial excitatory amino acid concentrations after cortical contusion injury. *Brain Res.* 2002; 943:15–22. [PubMed: 12088834]
- Ruban A, Berkutski T, Cooper I, Mohar B, Teichberg VI. Blood glutamate scavengers prolong the survival of rats and mice with brain-implanted gliomas. *Invest New Drugs.* 2012; 30:2226–2235. [PubMed: 22392507]

- Ruban A, Mohar B, Jona G, Teichberg VI. Blood glutamate scavenging as a novel neuroprotective treatment for paraoxon intoxication. *J Cereb Blood Flow Metab.* 2013
- Schousboe A. Transport and metabolism of glutamate and GABA in neurons and glial cells. *Int Rev Neurobiol.* 1981; 22:1–45. [PubMed: 6115823]
- Shaw PJ, Forrest V, Ince PG, Richardson JP, Wastell HJ. CSF and plasma amino acid levels in motor neuron disease: elevation of CSF glutamate in a subset of patients. *Neurodegeneration.* 1995; 4:209–216. [PubMed: 7583686]
- Slooter AJ, Bronzova J, Witterman JC, Van Broeckhoven C, Hofman A, van Duijn CM. Estrogen use and early onset Alzheimer's disease: a population-based study. *J Neurol Neurosurg Psychiatry.* 1999; 67:779–781. [PubMed: 10567497]
- Sontheimer H. Malignant gliomas: perverting glutamate and ion homeostasis for selective advantage. *Trends Neurosci.* 2003; 26:543–549. [PubMed: 14522147]
- Sontheimer H. Ion channels and amino acid transporters support the growth and invasion of primary brain tumors. *Mol Neurobiol.* 2004; 29:61–71. [PubMed: 15034223]
- Spranger M, Krempien S, Schwab S, Maiwald M, Bruno K, Hacke W. Excess glutamate in the cerebrospinal fluid in bacterial meningitis. *J Neurol Sci.* 1996; 143:126–131. [PubMed: 8981310]
- Stegink LD, Filer LJ Jr, Baker GL. Plasma amino acid concentrations in normal adults fed meals with added monosodium L-glutamate and aspartame. *J Nutr.* 1983; 113:1851–1860. [PubMed: 6886827]
- Stegink LD, Filer LJ Jr, Baker GL. Plasma glutamate concentrations in adult subjects ingesting monosodium L-glutamate in consomme. *Am J Clin Nutr.* 1985; 42:220–225. [PubMed: 2862786]
- Tang MX, Jacobs D, Stern Y, et al. Effect of oestrogen during menopause on risk and age at onset of Alzheimer's disease. *Lancet.* 1996; 348:429–432. [PubMed: 8709781]
- Teichberg VI. GOT to rid the body of excess glutamate. *J Cereb Blood Flow Metab.* 2011; 31:1376–1377. [PubMed: 21448218]
- Teichberg VI, 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. [PubMed: 18423998]
- Tsai PJ, Huang PC. 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. [PubMed: 10736370]
- Tsesis S, Gruenbaum BF, Ohayon S, et al. The effects of estrogen and progesterone on blood glutamate levels during normal pregnancy in women. *Gynecol Endocrinol.* 2013
- Veliskova J, Velisek L, Galanopoulou AS, Sperber EF. Neuroprotective effects of estrogens on hippocampal cells in adult female rats after status epilepticus. *Epilepsia.* 2000; 41 (Suppl 6):S30–S35. [PubMed: 10999516]
- Wang Q, Santizo R, Baughman VL, Pelligrino DA, Iadecola C. Estrogen provides neuroprotection in transient forebrain ischemia through perfusion-independent mechanisms in rats. *Stroke.* 1999; 30:630–637. [PubMed: 10066863]
- Yang SH, Shi J, Day AL, Simpkins JW. Estradiol exerts neuroprotective effects when administered after ischemic insult. *Stroke.* 2000; 31:745–749. discussion 9–50. [PubMed: 10700514]
- Ye ZC, Sontheimer H. Glioma cells release excitotoxic concentrations of glutamate. *Cancer Res.* 1999; 59:4383–4391. [PubMed: 10485487]
- Yudkoff M, Daikhin Y, Nissim I, Lazarow A, Nissim I. Ketogenic diet, brain glutamate metabolism and seizure control. *Prostaglandins Leukot Essent Fatty Acids.* 2004; 70:277–285. [PubMed: 14769486]
- Yudkoff M, Daikhin Y, Melo TM, Nissim I, Sonnewald U, Nissim I. The ketogenic diet and brain metabolism of amino acids: relationship to the anticonvulsant effect. *Annu Rev Nutr.* 2007; 27:415–430. [PubMed: 17444813]
- Zauner A, Bullock R, Kuta AJ, Woodward J, Young HF. Glutamate release and cerebral blood flow after severe human head injury. *Acta Neurochir Suppl.* 1996; 67:40–44. [PubMed: 8870800]
- Zlotnik A, Gurevich B, Tkachov S, Maoz I, Shapira Y, Teichberg VI. Brain neuroprotection by scavenging blood glutamate. *Exp Neurol.* 2007; 203:213–220. [PubMed: 17014847]

- Zlotnik A, Gurevich B, Cherniavsky E, et al. 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. [PubMed: 18080187]
- Zlotnik A, Gruenbaum SE, Artru AA, et al. 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. [PubMed: 19543002]
- Zlotnik A, Klin Y, Kotz R, et al. Regulation of blood L-glutamate levels by stress as a possible brain defense mechanism. *Exp Neurol.* 2010a; 224:465–471. [PubMed: 20493841]
- Zlotnik A, Gurevich B, Artru AA, et al. The effect of hyperthermia on blood glutamate levels. *Anesth Analg.* 2010b; 111:1497–1504. [PubMed: 21048094]
- Zlotnik A, Ohayon S, Gruenbaum BF, et al. Determination of factors affecting glutamate concentrations in the whole blood of healthy human volunteers. *J Neurosurg Anesthesiol.* 2011a; 23:45–49. [PubMed: 21248494]
- Zlotnik A, Gruenbaum BF, Mohar B, et al. The effects of estrogen and progesterone on blood glutamate levels: evidence from changes of blood glutamate levels during the menstrual cycle in women. *Biol Reprod.* 2011b; 84:581–586. [PubMed: 20980684]
- Zlotnik A, Klin Y, Gruenbaum BF, et al. The activation of beta2-adrenergic receptors in naive rats causes a reduction of blood glutamate levels: relevance to stress and neuroprotection. *Neurochem Res.* 2011c; 36:732–738. [PubMed: 21207137]
- Zlotnik A, Gruenbaum BF, Klin Y, et al. The effects of insulin, glucagon, glutamate, and glucose infusion on blood glutamate and plasma glucose levels in naive rats. *J Neurosurg Anesthesiol.* 2011d; 23:323–328. [PubMed: 21836527]
- Zlotnik A, Tsisis S, Gruenbaum BF, et al. Relationship between glutamate, GOT and GPT levels in maternal and fetal blood: a potential mechanism for fetal neuroprotection. *Early Hum Dev.* 2012a; 88:773–778. [PubMed: 22633534]
- Zlotnik A, Sinelnikov I, Gruenbaum BF, et al. Effect of glutamate and blood glutamate scavengers oxaloacetate and pyruvate on neurological outcome and pathohistology of the hippocampus after traumatic brain injury in rats. *Anesthesiology.* 2012b; 116:73–83. [PubMed: 22129535]
- Zlotnik A, Leibowitz A, Gurevich B, et al. Effect of estrogens on blood glutamate levels in relation to neurological outcome after TBI in male rats. *Intensive Care Med.* 2012c; 38:137–144. [PubMed: 22124768]
- Zlotnik A, Klin Y, Gruenbaum BF, et al. Beta2 adrenergic-mediated reduction of blood glutamate levels and improved neurological outcome after traumatic brain injury in rats. *J Neurosurg Anesthesiol.* 2012d; 24:30–38. [PubMed: 21979171]