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Commentary

Rethinking a drug treatment failure on a traditional ALS target

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ABSTRACT

In a recent issue of Experimental Neurology, Boston-Hewes and colleagues used an assay of glutamate transport to screen 1040 FDA approved drugs in an attempt to identify compounds that would increase glutamate transport, a central function of astrocytes, and a potential biological target for neuroprotection for a variety of neurological disorders. They identified the compound nordihydroguaiaretic acid (NDGA) as a particularly good candidate for inducing glutamate transport. Pharmacological increases in glutamate transport could have a number of potential applications to diseases of the nervous system where glutamate excitotoxicity is thought to be a contributing factor to pathogenesis including Amyotrophic Lateral Sclerosis, Alzheimer's disease, Parkinson's disease, stroke, and epilepsy among others. They chose to test this compound in a model of Amyotrophic Lateral Sclerosis (ALS)—the SOD1G93A mouse. In both human ALS and rodent models of the disease, glutamate excitotoxicity and abnormalities in glutamate transporter biology more specifically, have been implicated in ALS disease propagation. Interestingly, while the authors nicely demonstrate that NDGA has a biological effect on glutamate transport in normal (wild type) central nervous system tissues both in vitro and in vivo, it was the somewhat unexpected (and often overlooked) findings in the ALS mouse model that makes this manuscript notable and suggests that rethinking translational approaches to drug therapies in ALS may be on the horizon.

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In both human and animal models of ALS, numerous studies suggest that glutamate is critical for normal synaptic neurotransmission but may be also be neurotoxic if not tightly regulated. What is the relationship of glutamate excitotoxicity to glutamate transporters? Glutamate levels in the extracellular space are regulated by glutamate transporters; responsible for maintaining a 1000 to 10,000-fold intracellular to extracellular gradient. Five plasma membrane glutamate transporter subtypes have been identified. In human tissues, they are referred to as excitatory amino acid transporters (EAAT) 1–5. EAAT1 (GLAST)(Storck et al., 1992; Shashidharan and Plaitakis, 1993) is primarily an astroglial transporter, and the principal transporter protein present during CNS development (Furuta et al., 1997b). Its concentrations in adult tissues are particularly high in the Bergmann glia of the cerebellum with less expression in the brain and spinal cord. EAAT2 (GLT1)(Shashidharan et al., 1994) is an astroglial transporter, and the pharmaceutical target in this current study. EAAT2 is responsible for up to 90% of all glutamate transport in adult central nervous system tissue(Tanaka, 1997; Danbolt et al., 1992). EAAC1 (EAAT3)(Kanai and Hediger, 1992) is a neuronal glutamate transporter with high densities on postsynaptic membranes. EAAT4 is a glutamate transporter largely limited to the Purkinje cells of the cerebellum(Fairman et al., 1995; Furuta et al., 1997a; Barpeled et al., 1997). EAAT5 is found primarily in the retina on photoreceptors and bipolar cells(Arriza et al., 1997; Pow and Barnett, 2000).

In humans, previous studies have demonstrated that cerebrospinal fluid glutamate levels may be elevated in subjects with sporadic ALS (Mei et al., 1996; Spreux-Varoquaux et al., in press). Several potential points along the glutamate transporter pathway have been implicated in the increase of extracellular glutamate. Abnormalities in glutamate transporter expression as a result of altered transcription, splicing, increased turnover of the transporter, altered trafficking of glutamate transporters, and reduced transport capacity are all potential sites where glutamate transporter dysfunction can occur.

The findings of abnormal glutamate homeostasis and glutamate transporter dysfunction in humans with ALS are, to some degree, mirrored in animal models of the disease. In all mutant SOD1 mouse transgenic models, including the G85R, G37R, and G93A mutants, a large reduction in the EAAT2 (GLT1) glutamate transporter was observed in end stage mutant SOD1 mice when compared with controls (Bruijn et al., 1998). Other lines of evidence implicating glutamate transporter loss or dysfunction in ALS biology include the development of a motor neuronopathy with antisense knockdown of EAAT2 (GLT1) (Rothstein et al., 1996), and an increase in the rate of motor decline accompanied by earlier motor neuron in the disease when mutant SOD1 mice are crossed with EAAT2 (GLT1) heterozygous mice (EAAT2 null mice often die of seizures) (Pardo et al., 2006).

In addition to the current work discussed here, Trotti and colleagues have also contributed to the understanding of relationships between EAAT2 dysfunction in ALS in demonstrating that EAAT2 (GLT1) was a target of mutant SOD1 mediated oxidation (Trotti et al., 1999) and that its glutamate transporter capacity was reduced. They

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extended these observations in showing that in mutant SOD1 mice, EAAT2 was truncated by caspase-3 which resulted in normal trafficking to the cell membrane but that binding and transport of glutamate was reduced—possibly because caspase 3 cleavage affected the normal conformational arrangement of one of the transmembrane domains of the protein (Boston-Howes et al., 2006).

It is also worth noting that the only FDA approved drug for treating ALS acts on glutamatergic pathways. Riluzole appears to have several mechanisms of action on the glutamatergic system including the inhibition of glutamate release, blockade of amino acid receptors, and inhibition of voltage-dependent sodium channels on dendrites and cell bodies (Doble, 1996). Riluzole's efficacy was established in two important ALS clinical trials showing a modest but reproducible effect on survival (Lacomblez et al., 1996). The consistent results in these human clinical trials have lead to the use of riluzole as a standard-of-care in the pharmacological treatment of ALS.

Therefore, while glutamate excitotoxicity related to reduced glutamate transporter expression or dysfunction is likely not the sole cause of ALS or its progression, evidence does support it as a potential target for therapeutics.

With this body of data in mind, Boston-Howes and colleagues initially demonstrated in vitro, using a motor neuron-like immortalized cell line (MN1) which expresses glutamate transporters, that nordihydroguaiaretic acid (NDGA) was capable in increasing functional glutamate transport in a dose-dependent manner. Using pharmacologically specific inhibitors of EAAT2 (GLT1), the authors found that the increases in glutamate transport were related to specific NDGA-induced EAAT2 mediated transport. This was particularly relevant for its specificity for this subtype of transporter and also because EAAT2 is specifically lost in both human and animal models of ALS (among other neurological disorders). Thus, the increases in EAAT2-mediated uptake could have therapeutic relevance. NDGA is a compound that interferes with the arachidonic acid metabolic pathway; in particular NDGA blocks 5, 12, 15-Lipoxy-genases (LOX). Using a variety of specific LOX subtype inhibitors, the authors demonstrate that EAAT2 mediated increases in glutamate transport were related to the inhibition of the 5-LOX subtype Boston-Howes, et al. 2008.

The authors then demonstrated in synapotosomes from NDGA treated wild type mice, that EAAT2-mediated glutamate transport was again increased when administered subcutaneously for periods of up to 30 days. After this was established, they then tested the compound in mutant SOD1 mice. The mutant SOD1 mouse is one model of motor neuron disease and by far the most extensively studied, particularly with regard to pharmacotherapeutic testing of potential candidate drugs for ALS. The mutant SOD1 transgenic mouse develops a slowly progressive hindlimb paralysis with early death at approximately 130 days of age. This is accompanied by motor neuron loss, astrogliosis, and in most of the models, cytosolic SOD1 inclusions (Wong et al., 1995; Bruijn et al., 1998). Notably, the pathology is present in neural and non-neural cells (like astrocytes) in the CNS. The animals were treated with NDGA prior to symptom onset at 90 days of age. NDGA treated mutant SOD1 mice did not have a statistically significant improvement in lifespan or other behavioral measures of disease. After 10 days from the first implantation of an NDGA pellet, glutamate uptake activity was upregulated by approximately 50% compared to placebo-treated mice. However, after 20 or 30 days of treatment, the increase in glutamate uptake dropped to the same levels as in placebo-treated mice mutant SOD1 mice.

Why was this? On examination of tissues from these mutant SOD1 mice, P-glycoprotein (P-gp) (also known as MDR[multi drug resistance 1]) was noted to increase as the disease progressed. In fact, at 120 days of age (near endstage in this model), densitometric analysis of immunoblots showed a nearly three-fold increase in P-gp expression. This protein is known as a drug-efflux transporter which is particularly abundant in neuroinflammatory disorders. While drug-efflux transporters have not been thoroughly studied in either human ALS neural tissues or animal models of the disease such as the mutant SOD1 mouse, poor drug responses in disorders such as cancer, infectious diseases, and CNS disorders such as epilepsy, depression, schizophrenia, and HIV-associated neurological dysfunction have been studied more extensively(Loscher and Potschka, 2005). These increases in P-gp appear to go beyond the constitutive expression of protein. Although the authors of the current study did not provide direct evidence that NDGA is transported by P-gp, the observation that this transporter increased during disease is suggestive of one possible mechanism for the differences seen between NDGA treated wild type and mutant SOD1 mice.

All cells express efflux transporters that protect them from endogenous or exogenous toxic substances (xenobiotics)(Schinkel et al., 1994). P-gp is among those efflux transporters and the first described for chemotherapy resistant cancer cells (Schinkel et al., 1994; Ling, 1997). P-gp is expressed on the luminal brain capillary endothelial cells which could implicate this efflux transporter as playing a role in drug resistance to disorders including ALS. Its potential role in central nervous system defense as well as in potentially complicated drug efficacy has been demonstrated in P-gp null mice or using P-gp inhibitors. In P-gp null animals, the brain to plasma levels of anticancer or antiepileptic compounds are increased when compared to wild type controls with intact P-gp function suggesting, at least in part, that reduced efficacy of CNS compounds may not be due to the absence of biological activity at the target but rather poor CNS penetration.

Complicating the potential for overcoming P-gp pathways in drug delivery itself is the observation that increases in P-gp expression may actually contribute to the etiology and pathogenesis of disorders such as Alzheimer's disease, Parkinson's disease, and brain HIV infection.

For example, increases in β-amyloid are believed to play a role in Alzheimer's disease pathogenesis through secretion from neural cells. Lam and colleagues demonstrated that P-gp serves as an Aβ-efflux pump which could contribute to the extracellular aggregation of this protein(Lam et al., 2001). In HIV encephalopathy, extensive P-gp immunolabeling of astroglial and microglial cells, but relatively less endothelial cell immunolabeling was observed. This contrasted with the HIV encephalopathy negative cases in which P-gp immunoreactivity was associated primarily with endothelial cells. Both the immunocytochemical and immunoblot analyses showed a significant positive correlation between P-gp expression and HIV RNA levels. The authors concluded that P-gp may be part of a central pathway mediating viral compartmentalization in the brains of HIV-infected individuals and may play a significant part in HIV disease progression in the brain (Langford et al., 2004). For Parkinson's disease, evidence has suggested a significant association with a MDR1 (P-gp) polymorphism, exposure to pesticides, and the development of Parkinson's disease. This association could lead to suggestions regarding the avoidance of pesticides in those with this specific MDR1 polymorphism (Drozdzik et al., 2003). As an extension of this hypothesis, the evaluation of the contribution of P-gp to the blood brain barrier was examined using [^{11}C]-verapamil, which is normally extruded from the brain by P-gp. In patients with Parkinson's disease, an 18% increase in [^{11}C]-verapamil was noted suggesting that abnormalities in the blood brain barrier, as related to P-gp, play a role in Parkinson's disease pathogenesis (Kortekaas et al., 2005).

What does the current study mean for ALS pathobiology and therapeutics? The observation that other proteins which reduce the bioavailability of drugs such as NDGA in this model should shed light on the possible explanation of differential observations of drugs which act on similar pathways and the divergent results seen in both mutant SOD1 mice and humans with ALS. This is a well-known interaction seen in the oncology community but likely underappreciated in the motor neuron biology and ALS clinical communities.

Indeed, several pharmacological compounds that have had effects in mutant SOD1 mice have not had efficacy in human ALS trials (Benatar, 2007). Several factors could account for this including the

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