**ABSTRACT**

Membrane transporters play crucial roles on brain physiology not only as the gatekeepers controlling CNS drug entry and drugs across the blood-brain-barrier (BBB), more importantly, they directly modulate key biological processes such as neurotransmission, energy metabolism and antioxidant defense. Pharmacological modulations of transporters including SERT, NET and DAT have been successfully in clinical trials for CNS diseases such as depression and epilepsy; on the other hand, undesirable intervention of certain CNS transporters such as EAATs and xCT are believed to be associated with both acute and chronic CNS adverse effects such as excitotoxicity and Parkinsonism. Despite its critical implication in understanding and predicting CNS toxicity, information about inhibition of drugs against the 10+ transporters expressed in the CNS (in contrast to on the BBB) is sparse. Against this backdrop, we have developed cell-based assays for more than 15 key CNS transporters and tested nearly 40 pharmaceuticals (CNS- and peripherally acting) and neurotoxins for their effects on these transporters. It is not surprising that the prevalence of transporter inhibition by neurotoxins was found to be higher than marketed CNS drugs. For example, L-Quiqualic acid, a naturally occurring excitotoxic agent, was found to be a potent inhibitor of xCT, a transporter with major role on glutathione (GSH) synthesis and homeostasis in the brain, suggesting that GSH depletion could be one mechanism of L-Quiqualic acid to neurotoxicity. Surprisingly, adeno-synthetic inhibitor L-Alanose was found to inhibit EAATs and xCT, raising the potential for CNS adverse effects by this experimental drug.

**MATERIALS AND METHODS**

xCT (SLCTA11) and EAATs (SLCA1-3) are currently subjects of high interest within the pharmaceutical industry as both a therapeutic and an imaging standard. L-Glutamate (L-Glu) is the primary excitatory neurotransmitter in the mammalian CNS. Through its activation of a wide variety of excitatory amino acid receptors, L-Glu mediated signaling contributes to synaptic neurotransmission. Concentrations of L-Glu in the CNS are regulated by a family of excitatory amino acid transporters (EAATs) that rapidly concentrate it in glia cells and neurons, and thereby limit its extracellular accumulation. In addition to glutamate transporters, levels of extracellular glutamate are controlled by the cystine/glutamate antiporter xCT, which releases intracellular glutamate in exchange for cystine, the production of glutathione, the major cellular antioxidant. Minor alterations of extracellular glutamate levels by EAATs and xCT in the brain therefore have the potential to drastically alter glutamate neurotransmission. In addition, the glutamate signaling is also regulated by other transporters such as as-1 (SLCTA10) and ASC2 (SLCT12A5).

The neurotransmitter L-Methyl-L-alanine (BMA) is considered to be an agent responsible for the rate of ALS/Parkinson dementia observed in the island of Guam. Recent work has demonstrated that BMAA interacts with the xCT transport system and modifies glutamate neurotransmission, leading to neurodegeneration. Similar observations have been made for Oxalidamino propanoic acid (ODAP), which is a structural analogue of the neurotransmitter glutamate and is the neurotransmitter responsible for lethality.

Investigating the possible interactions of drugs/compounds with these transporters would have a large impact on CNS therapeutic development, through explaining causes of certain CNS side effects of drugs, revealing potential new therapeutic indications of existing drugs, and discovering potential therapeutic benefits of inhibiting key neurotransmitter transporters in treating various CNS diseases.

**RESULTS (cont’d)**

Table 1. Probing substrates and inhibitors used in the transport assay

<table>
<thead>
<tr>
<th>Probe substrate</th>
<th>Standard Inhibitor</th>
<th>EAAT1</th>
<th>EAAT2</th>
<th>EAAT3</th>
<th>xCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamic acid</td>
<td>Glutamic acid</td>
<td>1</td>
<td>1</td>
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</tr>
<tr>
<td>L-Glutamate</td>
<td>L-Glutamate</td>
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</tr>
<tr>
<td>L-Glu-Cystine</td>
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<td>1</td>
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<tr>
<td>L-Glu-Acetic</td>
<td>L-Glu-Acetic</td>
<td>1</td>
<td>1</td>
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</tr>
<tr>
<td>L-Glu-Asparinate</td>
<td>L-Glu-Asparinate</td>
<td>1</td>
<td>1</td>
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<td>1</td>
</tr>
</tbody>
</table>

**CONCLUSIONS**

None of the 30 marketed CNS drugs were observed to dramatically inhibit the activity of EAAT2—a major EAAT responsible for glutamate recycling.

In a screening study involving six amino acid analog neurotransyn loops, L-Alanose was found to significantly inhibit all three EAATs. It has attenuated the activity of xCT at higher concentrations. Although L-Alanose is being developed as an anti-tumor drug acting peripherally, BBB leakage is present in late stage cancer patients, raising the concern for potential CNS toxicities.

The remaining five compounds at 100 µM significantly suppressed xCT uptake. Quisqualic acid demonstrated a robust reduction of xCT activity with an IC₅₀ of 69 µM. This inhibition may result in disruption of GSH production and play a role in its well-known neurotoxicity. as-1 and ASC2 were either not affected or only moderately suppressed by the six amino acid analogs.

**ACKNOWLEDGEMENTS**

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