Investigating the role of glutamine transporters in uptake of biologically inert platinum-compounds for targeting cancer cells

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Current platinum (Pt) chemotherapy drugs such as cisplatin rely mainly on passive diffusion for uptake into cells, leading to severe off target side effects including peripheral neuropathy, which is often the dose limiting side effect [1]. The amino acid glutamine plays important roles in cellular metabolism and has been extensively studied for its increased demand in cancer cells [2]. The Alanine Serine Cysteine Transporter (ASCT) 2, a major glutamine transporter, has shown to be upregulated in a variety of cancers. ASCT2 belongs to the SLC1A family of membrane transporters and is an obligatory exchanger of neutral amino acids. This means that each influx event of one neutral amino acid and 3 sodium ions corresponds to an efflux event of the same, leading to an overall electroneutral transport cycle (Fig. 2). However, during this transport cycle, an uncoupled chloride conducting state is activated, allowing transporter function to be measured via electrophysiology. In an attempt to improve selectivity of these drugs, biologically inactive amino acid targeted drug delivery systems, Pt-aspartate and Pt-succinate, were developed (Fig. 1). This project aimed to determine if these Pt-aspartate and Pt-succinate compounds enter cells via ASCT2 .

Membrane transporters were expressed in a *Xenopus laevis* oocyte epression model and their transport activity was assessed via two-electrode voltage clamp electrophysiology. When the Pt compounds were applied alone, they did not generate transporter currents, suggesting that they are unable to be transported by ASCT2. Instead, inhibition of the "leak current" was observed, suggesting they bind to and block substrate binding. When applied in the presense of substrate, both Pt compounds reduced transport currents and inhibit radiolabelled substrate uptake, with Pt-aspartate more potent than Pt-succinate. Future research will expand the selectivity testing outside the SLC1A family to assess alternative protein-mediated uptake routes, such as the large neutral amino acid transporters (LAT) and the sodium-coupled neutral amino acid transporters (SNAT). Previous work in cell lines showed these Pt compounds were able to cross the cell membrane, however this may be via an alternative transport mechanism, such as endocytosis or diffusion. If these compounds are found to selectively enter cancer cells via one of the suggested mechanisms, this research could lead to the start of a new and safer class of Pt-chemotherapy drugs.

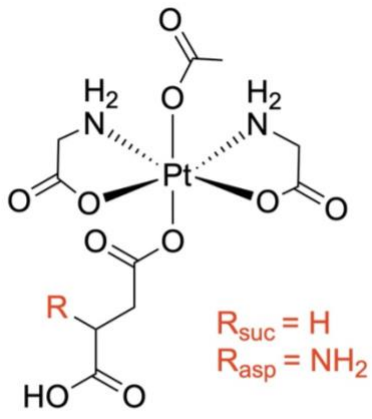

Figure 1: Chemical structure of the compounds Pt-succinate (R_{suc}) and Pt-aspartate (R_{asp}).

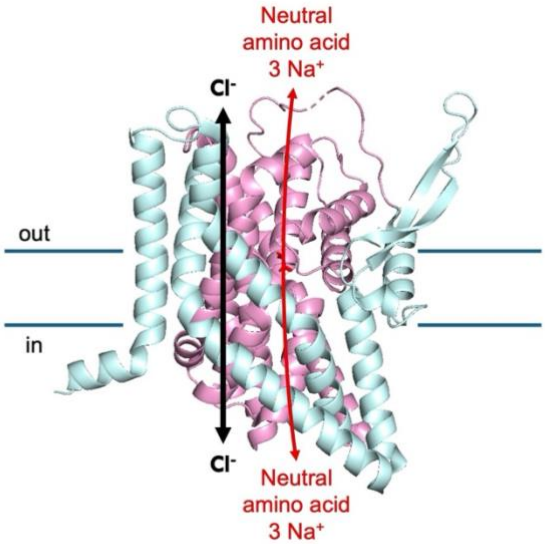

Figure 2: Ribbon Structure of ASCT2 showing the scaffold domain (blue) and transport domain (pink). Alanine (red) is shown bound. Transport stoichiometry is shown.

1. Amptoulach, S. and Tsavaris, N., *Neurotoxicity caused by the treatment with platinum analogues.* Chemother Res Pract, 2011. **2011**: p. 843019.
2. Freidman, N., et al., *Amino acid transporters and exchangers from the SLC1A family: structure, mechanism and roles in physiology and cancer.* Neurochem Res, 2020. **45**(6): p. 1268-1286.

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