ZEBRAFISH: AN *IN VIVO* SCREENING TOOL TO EVALUATE ORGANIC CATION TRANSPORTER FUNCTION

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BACKGROUND

The organic cation transporter OCT2 is expressed in renal tubular cells, dorsal root ganglions, and outer hair cells of the cochlea, where it regulates the uptake of endogenous and xenobiotic substrates, such as cisplatin and oxaliplatin. We previously reported that certain tyrosine kinase inhibitors (TKIs) with YES1-kinase inhibitory ability significantly inhibit human, rat, and mouse OCT2 and inhibit the uptake of chemotherapeutic agents such as cisplatin and oxaliplatin into healthy cells, including hair cells of cochlea (Sprowl et al., Nat Commun, 2016; Huang et al., J Clin Invest 2020). The functional equivalent of hair cells present in the mammalian cochlea in zebrafish (*Danio rerio*) consists of a lateral line with hair cell clusters commonly known as neuromasts. We hypothesized that a similar regulatory mechanism exists in zebrafish by which cisplatin accumulates into neuromasts to initiate its toxicity, and that this process is sensitive to inhibition by TKIs.

METHODS

Sequence alignment was performed with MAFFT v7. Inhibition of cellular uptake of OCT2 specific substrates, including cisplatin and the fluorescent dye ASP, by various TKIs was performed in HEK293 and Hela cells transfected with human OCT2 (hOCT2) or its zebrafish normal or mutated orthologue, drOCT1. *In vivo* studies were performed in zebrafish larvae at 5 days post fertilization, as well as in adult wild-type and OCT2-deficient mice.

RESULTS

The protein sequences of OCT2 and drOCT1 were 71% identical, and YES1-tyrosine phosphorylation sites were conserved. Uptake studies indicated that hOCT2 and drOCT1 could be inhibited by the same TKIs, including dasatinib (63.6% vs 66.5%) and nilotinib (53.6% vs 68.8%), with comparable IC50 values. Hela cells expressing hOCT2 or drOCT1 accumulated cisplatin by >2-fold compared to control cells, and these processes were sensitive to inhibition by the same TKIs. Next, we found that Tyr to Phe mutagenesis in drOCT1 at corresponding functional phosphorylation sites in hOCT2 was associated with impaired transport function, suggesting that a similar regulatory mechanism exists for both the mammalian and zebrafish transporters. *In vivo*, we found that neuromasts accumulate ASP, and that cisplatin treatment caused a disruption of neuromasts and control of hearing and balance, as determined from a seeker response assay. Validation of these concepts in mice indicated that a pretreatment with dasatinib (10 mg/kg, p.o.) protected against cisplatin-induced high frequency hearing loss in a manner similar to that observed in OCT2-deficient mice.

CONCLUSION

These studies provide new insights into the regulatory mechanism and role of drOCT1 in cisplatin-induced toxicities in zebrafish, and support the further translational exploration of OCT2 inhibitors as a preventative strategy to ameliorate a debilitating toxicity associated with a commonly used chemotherapeutic agent.

Reference

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2. Huang KM, Leblanc AF, Uddin ME, Kim JY, Chen M, Eisenmann ED, Gibson AA, Li Y, et al. Neuronal uptake transporters contribute to oxaliplatin neurotoxicity in mice. J Clin Invest. 2020 1;130(9):4601-4606. doi: 10.1172/JCI136796.