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MASTER THESIS PROJECT

Assessing the Interference of Drugs on Cellular Efflux Transporter Activity of Zebrafish Embryo by Rhodamine-B Dye Uptake Assay

Confidential

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ABSTRACT

ATP binding cassette (ABC) transporter proteins, including the zebrafish Abcb4 efflux pump, function as a cellular defense against a variety of various, potentially hazardous chemical substances, mediating so-called multixenobiotic resistance (MXR). As so-called chemosensitizers, some drugs target MXR proteins and impede their action, increasing the toxicity of other compounds that would otherwise be excreted. In this work the MXR system of zebrafish *(Danio rerio*) was tested for interference using 31 pharmaceutical drugs including 7 custom-made drugs. The dye accumulation assay with zebrafish embryos was used to record concentration dependent effects of test substances at 10 & 50 µM. In the dye accumulation assay, embryos at 72 hours after fertilization (hpf) were exposed to test chemicals and 1 µM rhodamine B for 2h. Rhodamine B tissue levels were used as a measure for an embryo's MXR transporter efflux activity (low levels corresponded to high activity; high levels to low activity). Positive controls included the well-known ABC protein substrate/inhibitor Cyclosporin A. Ten substances—MK-571, pranlukast, ivermectin, montelukast, sequinavir, Ko143, verapamil, JBM c21, JMC c14, and JMC c21—had a clear inhibitory effect on zebrafish embryos in a dose-dependent manner. Two substances, erlotinib and sulfinpyrazone, had no effect. When taken as a whole, our discovery that a variety of chemicals interfere with zebrafish's MXR efflux activity suggests that (1) efflux transporters may influence the bioaccumulation of various chemicals in fish and (2) some may operate as chemosensitizers. Further, it appears that efflux activity in zebrafish embryos was not only inhibited by Abcb4 subtype but also by other subtypes too, which will need to be addressed in further research.