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Detoxification Mechanisms in Fish

Regulation and Function of Biotransformation and Efflux in Fish Exposed to Pharmaceuticals and Other Pollutants

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Dissertation Abstract

It is likely that fish are exposed to mixtures of several pharmaceuticals as well as other pollutants. This may result in increased adverse effects due to chemical interactions. Chemical interactions are challenging to predict and will be aided by increased knowledge on key detoxification mechanisms. In human, adverse drug-interactions can arise by interactions with the pregnane X receptor (PXR) and the target genes cytochrome P450 3A4 (CYP3A4) and P-glycoprotein (Pgp). These genes also exist in fish, but their functions are less understod in fish. The main focus in this thesis was to elucidate whether PXR regulates CYP3A and Pgp in fish and how pharmaceuticals interact with regulation of these genes and the functions of the proteins. We found weak induction of CYP3A and Pgp genes by two mammalian PXR ligands in rainbow trout hepatocytes. Besides, we found weak induction of hepatic PXR, CYP3A and Pgp expressions with PCBs in a killifish population that is non-responsive to CYP1A inducers. To further explore fish PXR activation, rainbow trout PXR was isolated, sequenced and expressed in a reporter assay. The reporter assay resulted in weak or no activation of rainbow trout PXR with a suite of prototypical PXR ligands. A CYP3B gene transcript was sequenced from the *Poeciliopsis lucida* hepatocellular carcinoma (PLHC-1) cell line. Basal expression of CYP3B was low in PLHC-1 cells and it was not responsive to exposure to PXR ligands. We have used both *in vitro* and *in vivo* fish models and we have analysed gene regulations and protein functions upon pharmaceutical exposures, both as single substance exposures and as a mixture exposure. Several pharmaceuticals were shown to inhibit the CYP1A catalytic functions and to interfere with efflux pumps activities in PLHC-1. Combined exposure of ethinylestradiol with the broad spectrum CYP inhibitor ketoconazole resulted in increased sensitivity to ethinylestradiol exposure in juvenile rainbow trout. This drug interaction was caused by inhibition of CYP1A and CYP3A enzyme activities in rainbow trout liver. In conclusion, pharmaceuticals affected both functions and regulations of key detoxification proteins in fish. Adverse toxicokinetic interactions via CYP1A and CYP3A inhibitions were demonstrated in rainbow trout.

Keywords: Fish, PXR, CYP1, CYP3, efflux, pharmaceutical, drug interaction