Evaluation of the zebrafish embryo as an alternative model for hepatotoxicity testing

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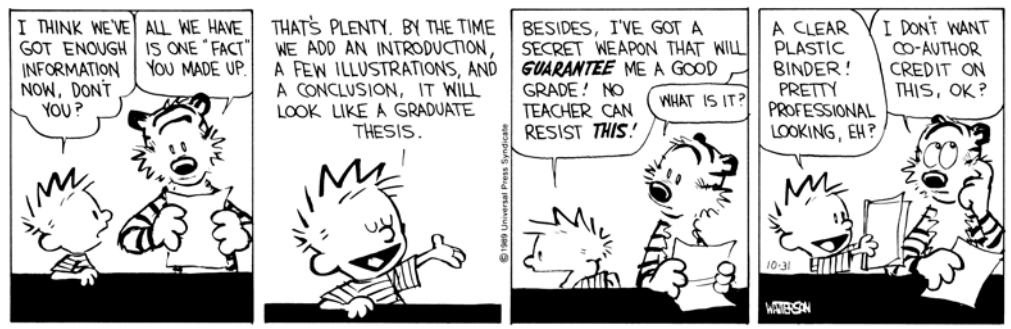
English Summary



English Summary

This thesis aims to establish whether the zebrafish embryo is a suitable alternative testing method for the prediction of hepatotoxic potential of compounds, thereby using toxicogenomic techniques. The goal was to establish improved alternative models to better predict human hepatotoxicity, thereby directly contributing to the reduction, refinement and replacement (3Rs) of animal experimentation.

In **chapter 1**, liver toxicity was introduced as an important target to study adverse drug effects. Especially, in mammals and particularly for xenobiotics that rely on oral uptake, many compounds provoke the first sign of toxicity in the liver. This is due to its function as a first pass organ in metabolism of toxic compounds, resulting in exposure of liver cells, mainly hepatocytes, to potentially toxic parent compounds and reactive metabolites. Hepatotoxicity is the result of various underlying molecular mechanisms arising from different xenobiotic-induced toxic phenotypes, of which cholestasis, steatosis and necrosis are most frequently observed. Cholestasis is a chronic condition and is phenotypically characterized by bile accumulation as a result of changes in intra- or extracellular bile flow or bile composition[34]. Steatosis may occur chronically as well and is characterized as an increase in cellular lipid content due to an increase in *de novo* synthesis of fatty acids or reduced lipid secretion or oxidation[19]. Necrosis is an acute condition and is characterized by cell death due to oxidative stress[4,33]. Traditionally, the hepatotoxic potential of compounds is tested using rodent studies. The aim of this thesis is to investigate whether the zebrafish embryo model combined with toxicogenomic techniques can be proposed as a promising alternative high-throughput method for hepatotoxicity testing.

In **chapter 2,** the applicability of the whole ZFE for hepatotoxicity testing was further underpinned by combining histopathology and next generation sequencing-based gene expression profiling. To this end, whole ZFE and adult zebrafish were exposed to a set of hepatotoxic reference compounds. Histopathology revealed compound and life-stage specific effects indicative of toxic injury in livers of whole ZFE and adult zebrafish. Next generation sequencing (NGS) was used to compare transcript profiles in pooled RNA samples of whole ZFE and livers of adult zebrafish. This revealed that hepatotoxicity-associated expression can be detected above the overall transcription noise in the whole embryo supporting the applicability of the whole ZFE model for compound-induced hepatotoxicity screening.

In **chapter 3,** we hypothesized that the detailed analysis of underlying mechanisms of hepatotoxicity in ZFE contributes to the improved identification of hepatotoxic properties of new compounds and to the reduction of the number of rodents used for chemical safety assessment. ZFEs were exposed to nine reference hepatotoxicants, targeted at

induction of cholestasis, steatosis and necrosis, and two non-hepatotoxic controls. Histopathology revealed various specific morphological changes in the ZFE hepatocytes indicative of cell injury. Gene expression profiles of the individual compounds were generated using microarrays. Regulation of single genes and of pathways could be linked to hepatotoxic responses in general, but phenotype-specific responses could not be distinguished. Hepatotoxicity-associated pathways included xenobiotic metabolism and oxidoreduction related pathways. Overall analysis of gene expression identified a small set of potential biomarkers specific for a common hepatotoxicity response.

To allow for verification of the histopathology, we applied another -omics technique, which was proteomics. Proteomics identified multiple markers for hepatotoxicity using the whole zebrafish embryo. Furthermore, we compared the gene expression results with the obtained proteomics results in **chapter 4**. Proteomics results showed that it is possible to identify markers after exposure to hepatotoxicants. These markers can be linked to enriched biological processes which are associated with hepatotoxicity. Furthermore, as observed in **chapter 3**, the zebrafish embryo is so far in our hands only able to distinguished general hepatotoxicity and not phenotype-specific hepatotoxicity.

In **chapter 5,** the zebrafish embryo was compared with the traditionally used models, including mice and rats. The ZFE model shares similarity at the pathway level after xenobiotic exposure with both *in vivo* and *in vitro* models. Concordance on the pathway level identified a single pathway to be altered across all hepatotoxic phenotypes. This analysis also suggest that pathways are better suited as hepatotoxicity markers than single genes as each model was found to effect different genes within the same pathway. Comparing at the single gene level showed that there were model specific changes and that outside of a model the overlap was fairly similar across models suggesting that on the gene level concordance cannot be readily identified. The advantage of the ZFE is that is can be used as a pre-screen to determine hepatotoxic potential of compounds, providing a quick and easy high-throughput testing model.

In **chapter 6**, we provided a general discussion of the obtained results including the assessment of the toxicokinetics parameters. Further, we addressed the limitations and strengths of this model. In addition, we provided the future perspectives of this model for the use in hepatotoxicity testing.