Reviewer’s report

Title: Treatment of mouse liver slices with cholestatic hepatotoxicants results in down-regulation of Fxr and its target genes

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Reviewer: Peter Olinga

Reviewer’s report:

This manuscript describes development of new screening model that allow identifying drugs with cholestatic properties. The results described in the manuscript are interesting, but it is not always clear how these results were derived:

Major Compulsory Revisions

Questions to the authors:

Introduction

The authors describe in their introduction that CSA and CZ are cholestatic compounds in rat and human. Are these compounds also cholestatic in the mouse?

They state “For steatosis valproic acid and amiodarone were selected. As pro-necrotic drugs paraquat and isoniazid were used” Please add references.

Method:

In there preparation of liver slices they state that the weight is 6 mg, how did they determine the weight of the slice?

Results:

In figure 2 it is not explained what a black box means.

Please combine table 1 for Csa and CPZ

How did the authors perform the Metacore analysis, did they choose the human or the mouse analysis within metacore?

“In a further analysis, we focused on those pathways that, using MetaCore, were found to be most significantly altered either by both drugs or specifically by one drug.” How was the significance calculated?

How was determined that FXR-regulated cholesterol and bile acids cellular transport pathway is the most significantly altered if cholestasis is not one of the terms in table 1. This is also the case for the other pathway mentioned, how did the authors determine that these are significant different? And on what basis did they choose these different pathways?
When the enrichment analysis was performed no FXR gene came-up even if it is important gene in the other pathways mentioned in the transcriptome analysis section. They only mention in the legend of figure 5 that FXR is involved. In figure 5 no 73 genes are depicted. The authors claim that these genes can be categorized in five functional clusters, however in the figure only about 50 genes are shown.

Microarray data verification: Why did the author choose these 4 genes. What were the criteria for taking these 4 genes? Top 4 genes of all the 73 genes that were highly regulated?

Biochemical analysis: The Fouchet staining figure 8 do E and F have the same magnification?

Discussion:
The authors refer to an in vitro study where CsA led to lipid droplets, do authors have indications that this also happens in slices.

They also discuss that gene sets related to T-cells are affected by CsA, are T-cells present in the slices? Is this not an artifact, because some of the genes (which genes?) that are affected may play a role within T cells, but these genes may also important in other gene sets, and as T-cells are probably absent it is unlikely that T-cells are affected in slices by CsA.

Is it known from other studies that the vacuolization by CsA may be due to disturbance in protein secretion?

The authors conclude a difference in immunosuppressive effect between CsA and CPZ, however T-cells are not present in the slice (or have the authors evidence that these are present in the slice), but both compounds are down-regulating the Kupffer gene sets, therefore the immunosuppressive effect of CsA and CPZ is not different?

Also in the discussion it is not clear why these 4 genes have been chosen as potential biomarkers. Could the authors elaborate on this?

Relevance of mouse PCLS: the genes that were identified as possible biomarkers were they regulated in the human samples?

Conclusion:
The authors conclude that ex vivo is comparable with in vivo, but there was no direct comparison with ex vivo mouse with in vivo mouse.

Minor Essential Revisions:
Nano Drop measurements: Typo it is 230/260 nm

Level of interest: An article of importance in its field

Declaration of competing interests:
I declare that I have no competing interests