MURINE PRECISION-CUT LIVER SLICES AS A DISEASE MODEL TO PREDICT DRUG-INDUCED CHOLESTASIS

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TisuMR

Introduction

Stages of cholestasis

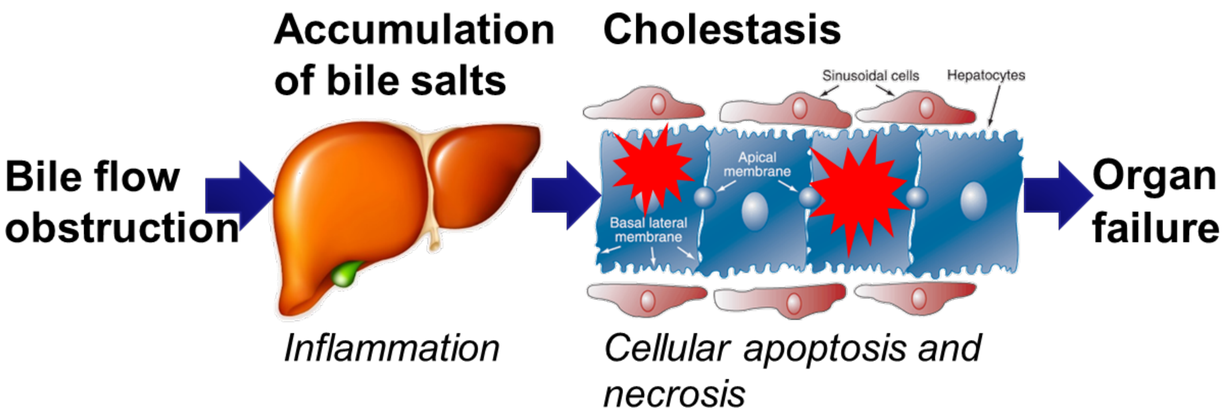

Figure 1: Four stages of cholestasis development in the liver.

Drug-induced cholestasis

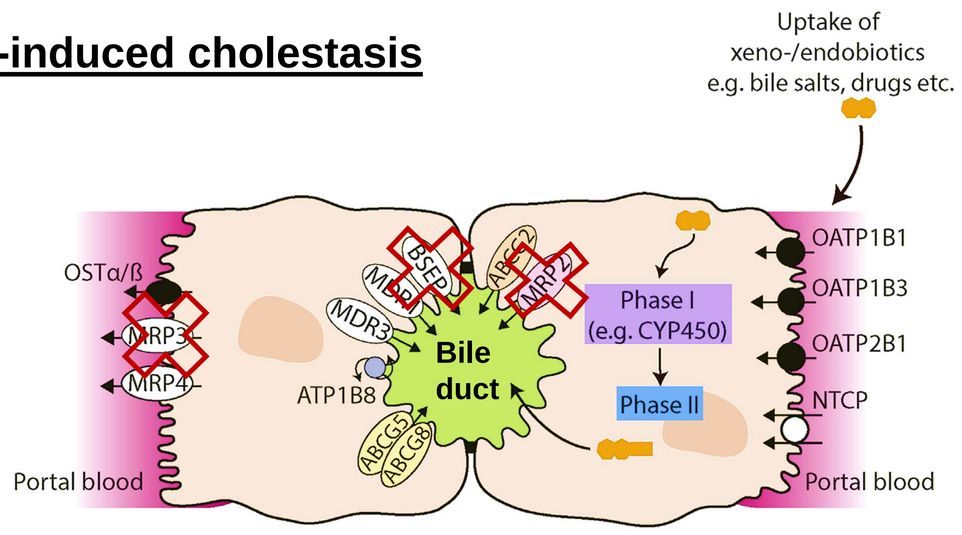

Figure 2: Graphic representation of drug-induced cholestasis with possible disease pathways (1). Drugs and bile acids are actively transported into hepatocytes and subsequently metabolized and secreted into the bile ducts by ATP-dependent transporters. However, in the presence of a cholestatic drug, biliary transport is obstructed(⊗) and hepatocytes accumulate bile acids in the cell interior. This accumulation is thought to cause hepatocellular apoptosis and necrosis.

Aim

Develop a disease model to study drug-induced cholestasis in precision-cut liver slices (PCLS) in real time.

Materials and Methods

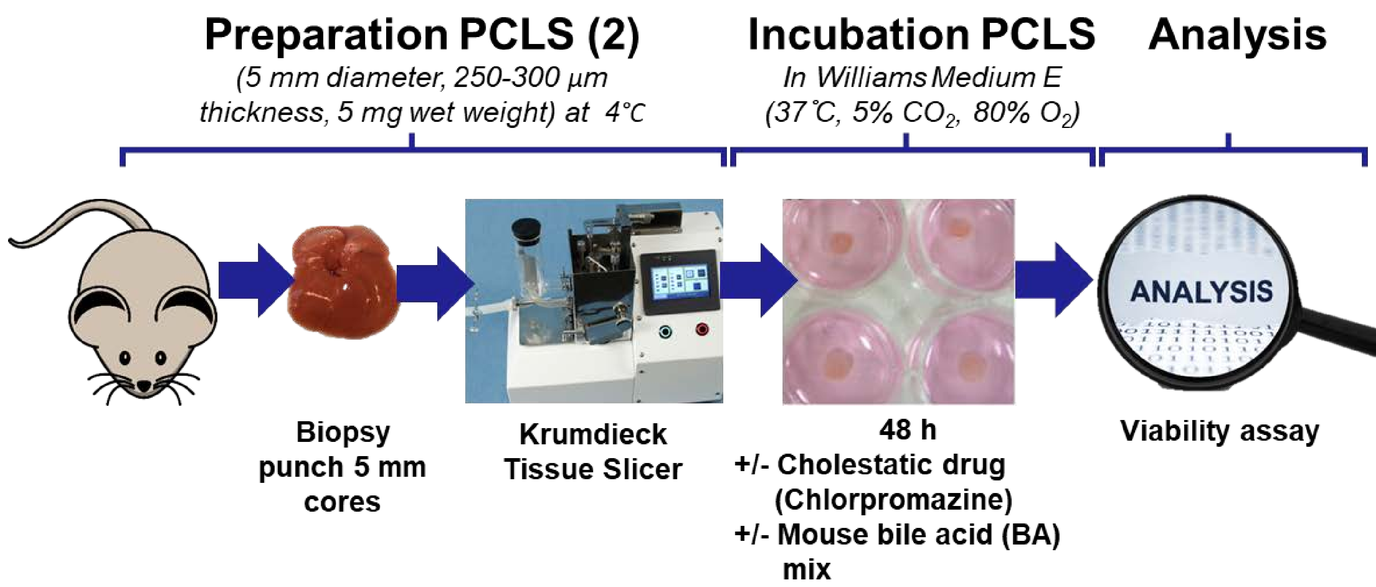

Figure 3: Overview of experimental procedures. Culture medium: Williams Medium E (WME) supplemented with glucose [25 mM] and gentamycin [50 µg/ml] (WME GG). Concentrations chlorpromazine (CPZ): 0, 5, 10, 15 and 20 µM. Concentration bile acid (BA) mix: 30 µM.

Results

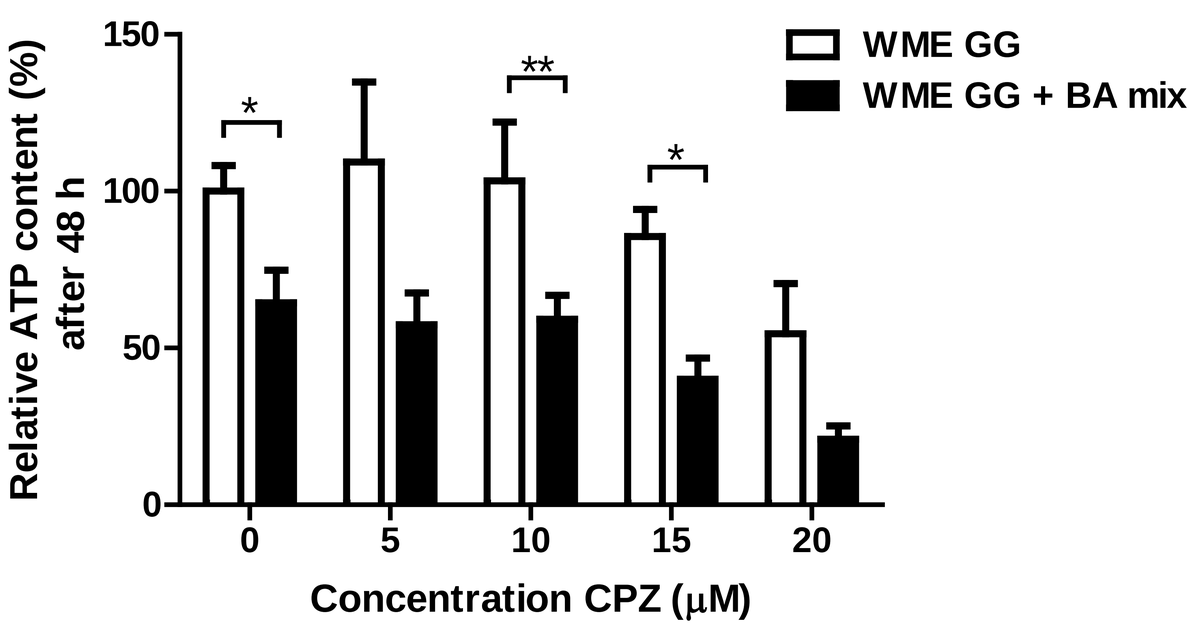

Figure 4: Bar chart of PCLS viability after 48 h incubation, presented with respect to percentage total ATP content of PCLS control slices after 48 h. PCLS were treated with different concentrations of CPZ [0, 5, 10, 15, 20 µM] with and without mouse BA mix [30 µM] (n=7 mice). * indicates $p<0.05$ and ** indicates $p<0.02$ significant decrease (two-tailed T-test).

Viability assay shows:

- Concentration-dependent effect of CPZ on PCLS:
  - No toxic effect for 5 and 10 µM CPZ;
  - Low toxicity for 15 µM CPZ;
  - Medium toxicity for 20 µM CPZ
  - Mouse BA mix causes toxicity.
- **Increased toxicity of CPZ + BA mix compared to drug treatment alone.**

Conclusions

CPZ + BA mix → leads to higher toxicity in PCLS than drug treatment alone.

These differences in toxicity are the first results of drug-induced cholestasis in mouse PCLS.

Outlook

Once optimized:

- **Incubate PCLS in continuously perfused microfluidic chip under controlled culture conditions**

  → **which will allow drug-induced cholestasis development to be monitored in real time.**

References

(1) Stieger B, Z.M. Mahdi. J Pharm Sci. 106: 2295-2301, 2017
(2) de Graaf IAM, et al. Nat Protoc. 5(9):1540–51, 2010

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