Chlorpromazine disrupts structural integrity of hepatic cell membranes in human HepaRG cells and initiates a pro-inflammatory response

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INTRODUCTION

Chlorpromazine (CPZ) is a neuroleptic drug and prototype compound to study intrahepatic cholestasis (IHC). The exact mechanisms of CPZ induced IHC remain obscure, particularly the idiosyncratic aspect. Although murine models are commonly used for studying CPZ toxicity, better predict outcomes in pre-clinical trials, improved human in vitro models are desirable. We have developed a human HepaRG electrical cell-substrate impedance-sensing (ECIS) model capable of real-time, monitoring of CPZ toxicity. To assess dose-response effects on HepaRG cells.

AIM

To model IHC using CPZ in the HepaRG cell line and to assess molecular markers of membrane bound transporters and membrane integrity using qRTTPCR, immunocytostaining and a real time, non invasive impedance-based biosensor array.

MATERIALS AND METHODS

HepaRGs [Biopredic Int] were seeded on gold electrode ECIS arrays (8w10E+6 ibidi) at 250,000 cells/well HepaRG differentiation into stable hepatocyte-cholangiocyte co-culture was monitored using ECIS for 8 days [sampling time, 160 seconds; n=21]. Following establishment of polarized HepaRG co-culture (>200h) on ECIS arrays, chlorpromazine (25, 50, and 100 µM) was added to the culture medium and a 24 hour-based toxicity assay was conducted. Cells were seeded in parallel for immunocytostaining and mRNA extraction for qRTPCR analysis.

RESULTS SUMMARY

Cell viability showed no significant change between 25µM or 50µM indicating cells remained metabolically active. Expression of CYP3A4 (p=0.05) was upregulated at 25µM CPZ showing increased functionality while little change in expression was seen at 50µM (Fig. 1). Bile acid transporter ABCB11 (p=0.01) was down-regulated at 50µM while xenobiotic and phospholipid transporters ABCB11 (p<0.001), ABCB4 (p=0.01) (Fig. 2) as well as inflammatory markers TNFα (p=0.005) and IL6 (p=0.003) (Fig. 3) were up-regulated. This suggests an adaptive response with likely activation of inflammatory pathways for cell survival. ECIS modelling showed a dose-dependent loss of tight junctions (TJ) and some disruption of cellular adhesion (Fig. 4). Immunocytostaining verified a dose dependent loss of the TJ protein, ZO-1 and -actin cytoskeleton (Fig. 5).

CONCLUSIONS

Subtoxic doses of CPZ do not reduce viability or functionality of HepaRG cells, but induce inflammatory and adaptive responses shown by the up regulation of IL6, TNFα and NRF2. Dose dependent disruption of TJs can be seen through impedance and immunocytostaining. Bile acid transport is inhibited leading to likely increase of bile acids within the cell. Membrane bound transporters ABCB11 and ABCB4 are up-regulated in a dose dependent manner. While this is another indication of adaptive response, polymorphisms in these genes can induce cholestasis [1-3] and may provide an explanation for the idiosyncratic effect of CPZ.

REFERENCES


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