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, to 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 qRTPCR, 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+ 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 (t>200h) on ECIS arrays, chlorpromazine (25, 50, and 100 µM) was added to the culture medium and a 24h impedance-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 ABCB1 ($p<0.001$), ABCB4 ($p<0.01$) (**Fig. 2**) as well as inflammatory markers TNFα ($p<0.005$) and IL6 ($p<0.005$)(**Fig. 3**) were up-regulated This suggests an adaptive response with likely activation of inflammatory pathways for cell survival. ECIS modelling showed a dose-dependant loss of tight junctions (TJ) and some disruption of cellular adhesion (**Fig. 4**). Immunocytostaining verified a dose-dependant loss of the TJ protein, ZO-1 and f-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 ABCB1 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.

Adaptive and inflammatory response

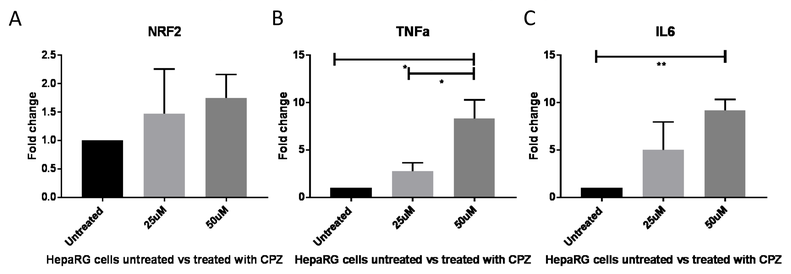
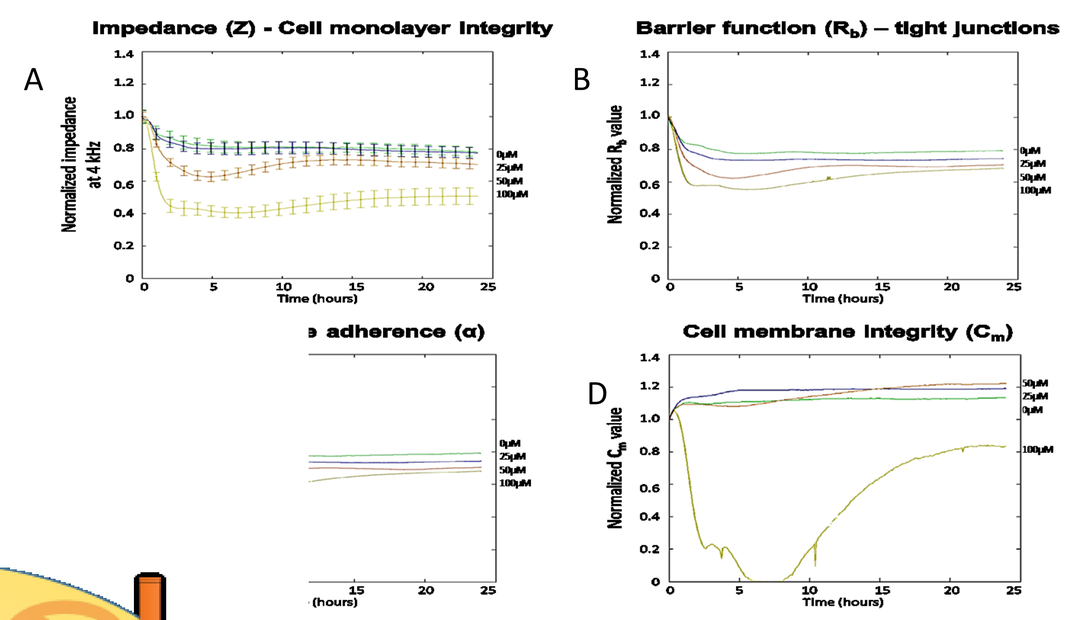


Figure 3: *mRNA expression of adaptive response **A)** NRF2 and cellular pro-inflammatory markers **B)** TNFa and **C)** IL6*

ECIS based toxicity assay



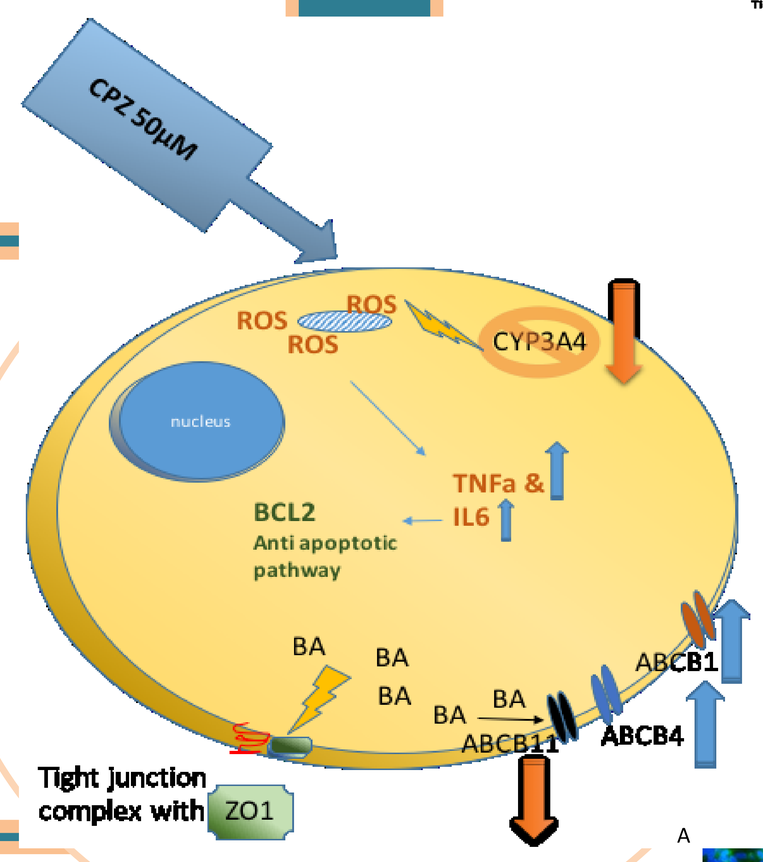


Figure 4 *Real-time |Z| monitoring showed highly-sensitive/ temporal dose-response to CPZ; with a decrease of impedance at all frequencies (A), indicating a global decline in cellular health; Subsequent |Z|-spectra modelling reflected significant early (1h) disruption of TJ (B), and the cell-substrate adhesion parameter, z-alpha (C); whilst only high dose CPZ disrupted cell membrane integrity (D);*

Functionality

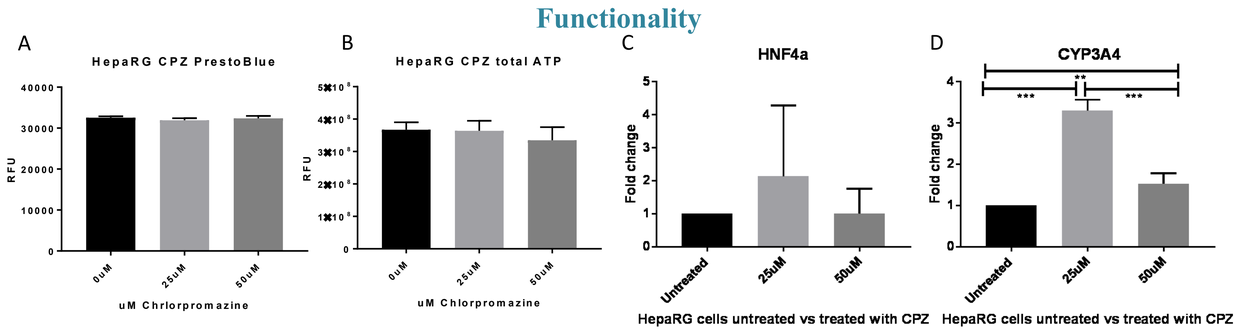


Figure 1 Viability and functionality of HepaRG cells exposed to CPZ: A) *Endpoint ATP-depletion assay and corresponding **B)** Prestoblue live-cell assay following 24h CPZ treatment. mRNA expression of hepatic functional marker **C)** HNF4α and **D)** CYP3A4*

Membrane bound transporters

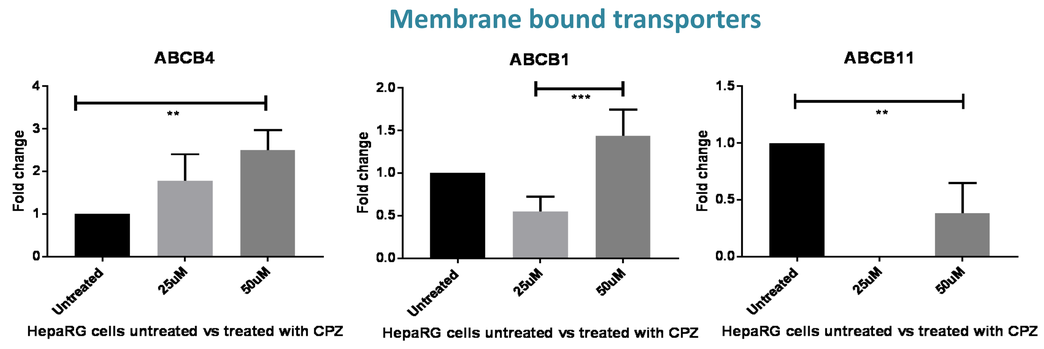


Figure 2 Membrane bound transporters: *mRNA expression of bile canalicular transporter ABCB11 (bile salt exporter pump), ABCB4 (phospholipids transporter) and ABCB1 (drug efflux transporter) was significantly changed. 2-fold higher expression of ABCB4 at 50µM CPZ ($p<0.01$) when compared with untreated cells and 1.5-fold, ($p<0.001$) higher expression of ABCB1at 50µM CPZ when compared with 25µM was detected. ABCB11 was inhibited at 25µM and significantly down regulated at 50µM ($p<0.01$).*

Immunocytostaining

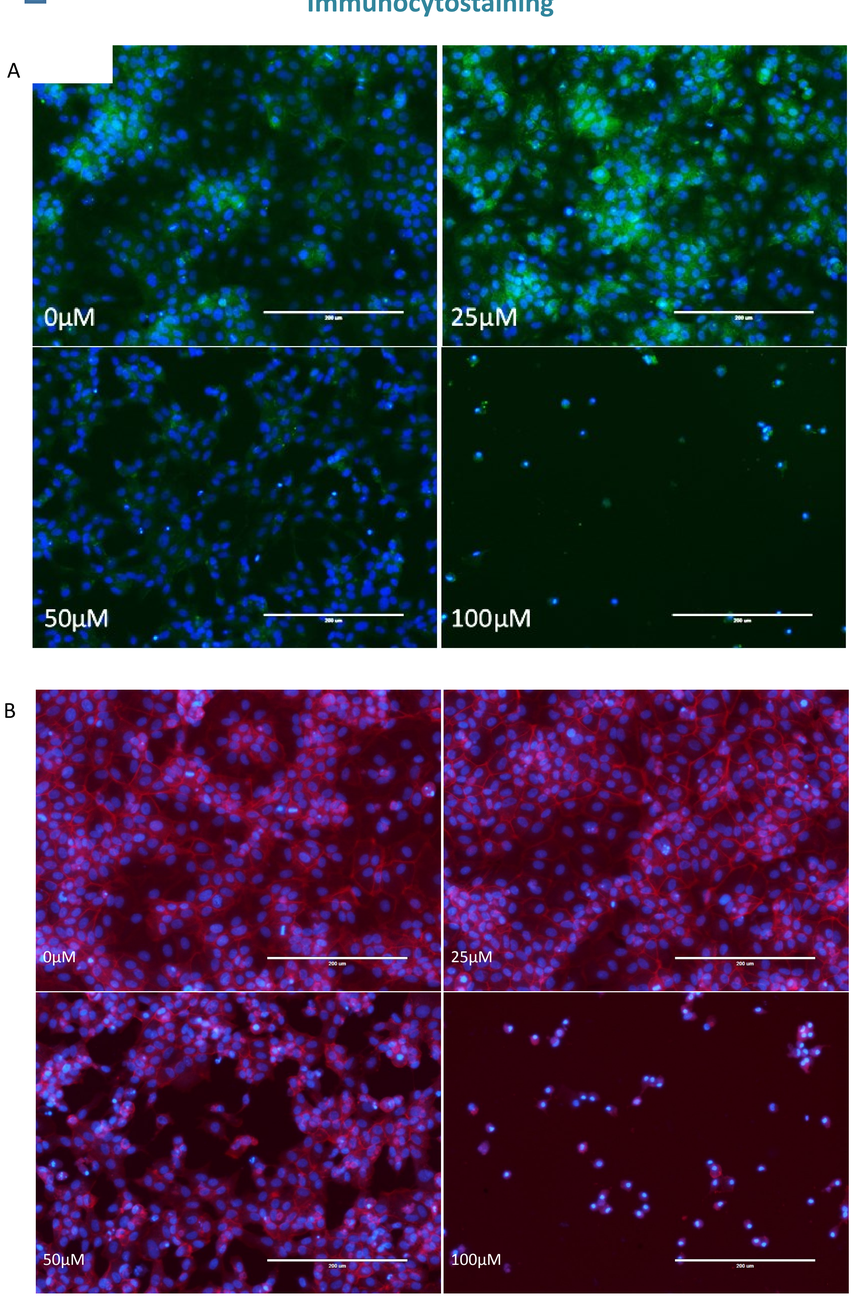


Figure 5 Immunocytostaining: *Dose dependent disruption of A) tight junction protein ZO1 and B) f-actin cytoskeleton at 0, 25, 50 and 100µM concentrations of CPZ*

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