TOWARDS UNDERSTANDING THE MECHANISMS OF CHLORPROMAZINE- INDUCED HEPATIC TOXICITY USING A HUMAN HEPARG- BASED *in vitro* MODEL

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INTRODUCTION

Chlorpromazine (CPZ) is a neuroleptic drug and a prototype compound used to study intrahepatic cholestasis. The exact mechanisms of CPZ induced cholestasis remain unclear. We have previously shown, using impedance biosensor technology, that CPZ induces early, dose-dependent disruption of tight junctions even at subtoxic CPZ levels (25 μM) [1]. In this study we investigated the molecular mechanisms that may mediate the hepatic alterations induced by CPZ.

AIM

1. To investigate the molecular mechanisms that mediate intracellular alterations associated with cholestasis development induced by single dose of CPZ in the human HepaRG model.
2. To examine whether inflammation, oxidative stress, defense or apoptosis related markers are altered upon CPZ treatment.
3. To establish whether CPZ induces transcriptomic changes of cellular membrane transporters that may contribute to cholestasis..

METHODS

HepaRG cells were exposed to CPZ concentrations of 25 μM, 50 μM and 100 μM for 24h. Cell viability was assessed by ATP-depletion assay (CellTiter-Glo). Assessment of cytoskeleton intergrity was performed by TJ-associated /F-actin expression following CPZ challenge. A number of molecular markers were analysed using qPCR (n=3-6). The fold change in expression of target genes relative to the internal control genes (TOP1, UBC and GAPDH) was calculated. QRT-PCR data were presented as the fold change in gene expression normalised to the average value of 3 common endogenous reference genes and relative to control (untreated cells) [2] .

Results are presented as average ± SD. Differences between the different culture conditions (with or without CPZ) were detected applying a one way Anova test. Results were considered significant at p<0.05.

RESULTS

CPZ induced extensive cell death at 100 μM. At concentrations of 25 μM and 50 μM, cell viability by ATP-depletion assay was not significantly impaired. As previously demonstrated, cytoskeletal changes suggestive of tight junction disruption were seen.

CPZ provoked a dose dependent inflammatory response (9.6-fold, p<0.005 at 50 μM) for IL-6 as well as (8.3-fold , p<0.001 at 50 μM when compared with untreated cells and 5.6-fold , p<0.005 at 50 μM when compared with 25μM) for TNFα. This response was associated with 3.3-fold, p<0.05 and 1.5-fold , p<0.0001 cytochrome 3A4 up regulation at 25 and 50 μM respectively.

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).

Significant down regulation of Bax to Bcl2 ratio (p<0.0001) at both doses indicates lack of apoptosis induction in CPZ treated cells and is supported by insignificant changes in nuclear apoptotic (p53) marker and apoptosis inducing factor (AIF1).

Although, NRF2 transcription factor changes were not significant, its downstream genes were activated. For instance, GCLM was increased at 50μM (8.1- fold, p<0.01) but not at 25μM. p62 which belongs to the proteins turnover functional group regulated by NRF2, was also significantly upregulated at 50μM (16.6-fold, p<0.05).

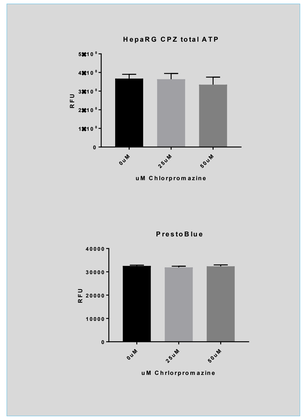

Figure 1. Measurement of HepaRG Viability at 25μM, 50μM and 100μM of CPZ (n=3). No significant difference was detected in total ATP levels and metabolic proficiency.

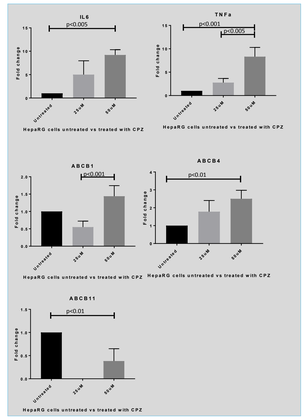

Figure 2. Expression of A) cellular proinflammatory markers and B) membrane transporters. Fold change in mRNA expression relative to untreated cells. (±SD) of IL-6 (n=3), TNFα (n=3), ABCB1(n=3), ABCB4(n=3) and ABCB11(n=3).

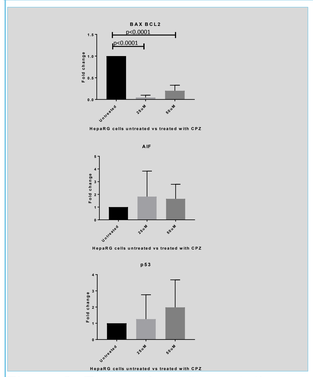

Figure 3. Expression of apoptotic and anti-apoptotic markers: Fold change in mRNA expression relative to untreated cells (±SD) of (a) ratio of apoptotic and anti-apoptotic factors Bax/Bcl2 (n=3) (b) pro-apoptotic genes p53 (n=3) and AIF1 (n=3).

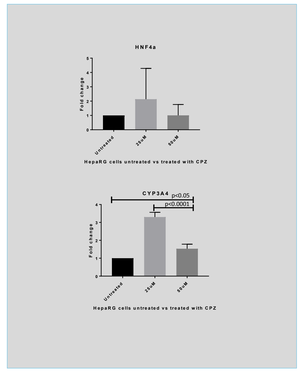

Figure 4. Expression of hepatic functional markers. Fold change (±SD) in mRNA expression relative to untreated cells (±SD) of (a) CYP3A4 (n=3) and (b) nuclear factor HNF4α (n=3).

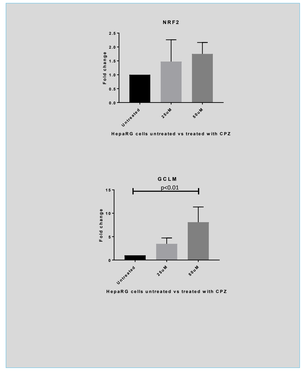

Figure 5. Expression of cellular defence system markers . Fold change in mRNA expression relative to untreated cells (±SD) of (a) NRF2 transcription factor (n=4) and its downstream gene (b) GCLM (n=3).

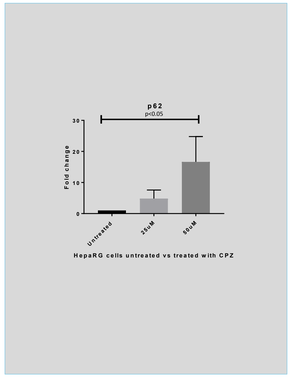

Figure 6. Expression of autophagy marker . Fold change in mRNA expression relative to untreated cells (±SD) of p62 (n=4)

CONCLUSIONS

Our work describes the molecular mechanism that mediates intracellular alterations associated with cholestasis development caused by single dose of CPZ in the human HepaRG model.

Complex transcriptomic assessment showed that:

1. CPZ negatively impacts the function of efflux transport proteins inducing inflammatory downstream effects, which activate adaptive responses for cell survival.
2. Accumulation of pro-inflammatory cytokines can trigger molecular events responsible for the disruption of membrane integrity and transporters function.
3. CPZ-induced biliary acid transporter (ABCB11) inhibition that may contribute to cholestasis. However expression of other membrane transporters (ABCB1 and ABCB4) was upregulated, which can be considered as an alternative response to escape cholestasis.
4. CPZ-induced adaptive processess (cytochrome 3A4, autophagy, defense system) that aim to protect cells against proteotoxicity and apoptosis.
5. Further understanding of the reparative pathways may be important for further understanding of drug induced cholestasis and target therapies.

REFERENCES

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