

Elucidating the role of mitochondrial dysfunction in drug-induced intrahepatic cholestasis

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Drug-induced intrahepatic cholestasis (DIC) represents the most frequent clinical manifestation of drug-induced liver (DILI), with bile acids (BAs) being recognised as the causative agents of toxicity. Whilst it is recognised that BA-induced toxicity is multi-mechanistic, research in isolated mitochondria and HepG2 cells has revealed that BA toxicity and mitochondrial dysfunction occur simultaneously in DIC. However, much of this prior research has been conducted using single BAs and thus overlooked the effects that a combination of BAs would have on the mitochondria.

HepaRG cells are a more suitable cell choice for DIC studies as they differentiate into hepatocytes and biliary-like cells and have a dynamic biliary system characterised by functional biliary transporters. Therefore, the aim of this research was to investigate whether BA mixture-induced mitochondrial toxicity could be detected concurrently in HepaRG cells and isolated mitochondria.

The mitochondrial toxicity of the BA mixtures was examined in HepaRG cells using Seahorse respirometry, alterations in mitochondrial membrane potential (MMP) and an acute metabolic modification assay. These results were then compared with the mitochondrial dysfunction detected in isolated mitochondria by changes in MMP and structural modifications.

It was demonstrated that 1000 x BA mix resulted in significant MMP depolarisation and structural alterations in isolated mitochondria. By contrast, BA-induced mitochondrial toxicity was not detected in HepaRG cells, as there were no significant changes in oxygen consumption rate, MMP or ATP levels between glucose and galactose media. BA mixtures were deemed cytotoxic as 1000 x BA caused a significant decrease in protein and retained LDH following 2 weeks treatment.

Overall, the results suggested that BA-induced mitochondrial toxicity does not precede cytotoxicity when studied in a whole cell system as opposed to when using isolated mitochondria. The toxicity of DIC is multi-mechanistic thus suggesting that a limitation of isolated mitochondria is their lack of cellular context and physiological relevance. This research has highlighted the importance of studying mitochondrial toxicity in different models simultaneously in order to gain mechanistic insight that is applicable to the *in vivo* pathophysiology.