**Introduction:**

Drug-induced cholestasis (DIC) represents the most frequent clinical manifestation of drug-induced liver injury (DILI), with bile acids (BAs) being recognised as the causative agent of toxicity. Whilst it is understood that BA-induced toxicity is multi-mechanistic, research in isolated mitochondria has revealed that BA toxicity and mitochondrial dysfunction occur concurrently in DIC. The aim of this study was to investigate whether BA-induced mitochondrial toxicity could be detected simultaneously in isolated mitochondria and a more relevant hepatic model, HepaRG cells. HepaRG cells are a suitable cell choice for DIC studies as they express functioning bile canaliculi and differentiate into a mixed population of hepatocytes and biliary-like cells, both of which are implicated during DIC.

**Method:**

A physiological mixture composed of the 6 most abundant BAs in human plasma was prepared (1 x BA). Mitochondria were isolated from HepG2 cells using a semi-automated method utilising Pump Controlled Cell Rupture and were acutely exposed to the mixtures before mitochondrial membrane potential (MMP) and structural alterations were measured. Microscopy was undertaken to confirm the correct phenotype of HepaRG cells. HepaRG cells were treated with mixtures (DMSO ≤ 0.5% for 24 and 72 hours or 1 and 2 weeks). Mitochondrial toxicity was examined using several techniques; Seahorse respirometry, MMP and an acute metabolic modification assay. Data are given as mean ± SEM (n = 3) and analysis was performed using a one-way ANOVA with a Dunnett’s test or a Kruskal-Wallis test.

**Results:**

HepaRG cells were shown to be correctly polarised and express functional biliary transporters. In isolated mitochondria, 1000 x BA resulted in significant MMP depolarisation (20.66 ± 1.94 %) and an optical density decrease (10.48 ± 4.24 %). Contrarily, BA-induced mitochondrial toxicity was not detected in HepaRG cells as there were no significant changes in MMP, oxygen consumption rate or ATP levels between glucose and galactose media. BA mixtures were deemed cytotoxic as 1000 x BA resulted in a significant decrease in protein (60.50 ± 8.88 %) and retained LDH (69.38 ± 1.57 %) following 2 weeks treatment.

**Conclusion:**

Acute treatment of isolated mitochondria with BA mixtures resulted in the detection of toxicity. However, the data from HepaRG cells indicates that BAs are not mitotoxic but cytotoxic. The toxicity of DIC is multi-mechanistic meaning the use of isolated mitochondria lacks physiological relevance and neglects physiological cellular protective mechanisms, thus implying that mitochondrial toxicity detected in isolated mitochondria is artificial.