Investigating the effect of mitochondrial genome on antibiotic-induced liver injury

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Introduction

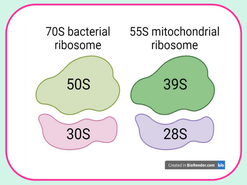
Mitochondrial dysfunction is a key mechanism of drug-induced liver injury (DILI)

Hepatocytes have many mitochondria

Synergy with other toxicity mechanisms[1]

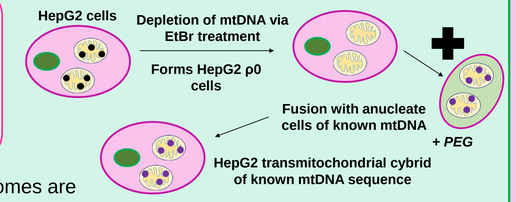
Mitochondrial genetics can influence susceptibility and severity of clinical adverse events [2,3]

Antibiotics are a main cause of DILI [1]



- Bacterial and mitochondrial ribosomes are structurally similar as a result of endosymbiosis[4]
- Drugs which inhibit bacterial ribosomes (e.g. linezolid and macrolides) may also inhibit mitoribosomes

Transmitochondrial cybrids allow us to assess the impact of mtDNA variation against a constant nDNA background



Haplogroup	12S rRNA SNPs	16S rRNA SNPs
B (HepG2 WT)	750G, 827G, 1438G	2706G
H (HepG2 cybrid)	750G, 751G, 951A	
J (HepG2 cybrid)	750G, 1438G	3010A

Methods

Growth of HepG2 WT (haplogroup B) and HepG2 transmitochondrial cybrids (haplogroups H & J)

↓

Treatment with linezolid or erythromycin for 24 or 72 hours

↓

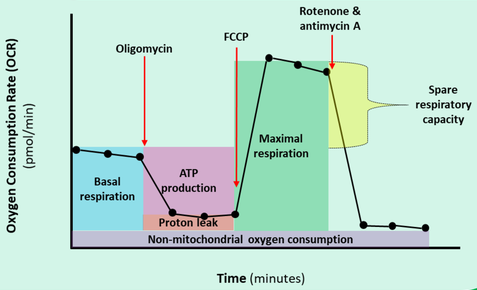
Assessment of mitochondrial function and composition

- **mtDNA copy number** – assessed using qPCR, probing for mtDNA- vs nDNA-encoded housekeeping genes
- **Mitoribosome function** – assessed using Western blot, probing for mitochondrial proteins translated by mitochondrial or cytoplasmic ribosomes (encoded by mtDNA and nDNA respectively)
- **Cellular bioenergetics** - assessed with high resolution respirometry using the Seahorse XFe96 analyser

→ *Oxygen consumption rate and proton flux are measured in real time, providing insight into mitochondrial respiration and glycolysis*

Aim

Utilise a panel of HepG2 WT and HepG2 transmitochondrial cybrids to investigate the effect of mitochondrial genome on susceptibility to toxicity caused by ribosome-targeting antibiotics *in vitro*



Results

Proposed progression of clinical toxicity

mtDNA not directly impacted

↕

Mito-encoded mRNA not directly impacted

↕

Linezolid / Erythromycin

↓

Inhibition of mitoribosomes

↓

↓ translation of mito-encoded mRNA

↓

↓ mito-encoded respiratory complexes

↓

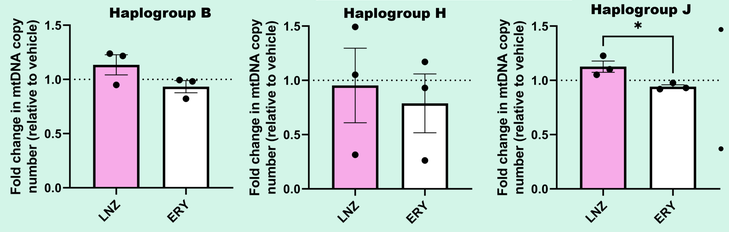
↓ oxidative phosphorylation

↓

- Lactic acidosis (mitochondrial)
- Microvesicular steatosis
- Disturbed hepatic function

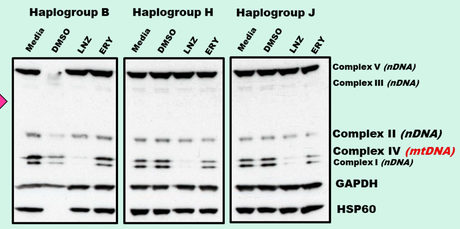
Mitochondrial DNA is not depleted by linezolid or erythromycin relative to vehicle control after 72 hours

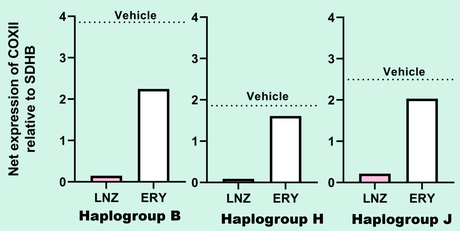
Mean ± SEM, n=3



- Haplogroup J cybrids treated with 100 μM erythromycin for 72 hours had significantly less copies of mtDNA than cells treated with 100 μM linezolid ($P < 0.05$)
- Greater variation in mtDNA copy number was observed in haplogroup H cybrids

Treatment with 100 μM linezolid or erythromycin decreases expression of mitoribosome-dependent protein COXII

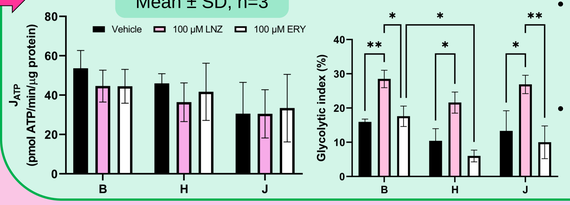




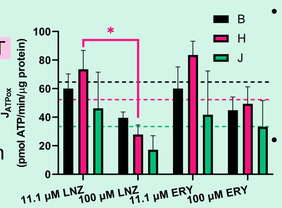
- Reduced expression of mtDNA-encoded proteins in the absence of decreased mtDNA copy number indicates inhibition of mitoribosomes
- SDHB chosen for comparison as Complex II is entirely nDNA-encoded
- Decreased expression of nDNA-encoded ETC subunits may be caused by instabilities induced by the depletion of mtDNA-encoded subunits[5,6]

- **HepG2 WT and HepG2 transmitochondrial cybrids retain bioenergetic capacity despite loss of mitoribosome-dependent proteins**
- **Treatment with 100 μM linezolid significantly induces glycolysis in all cell types, indicating mitochondrial dysfunction**

Mean ± SD, n=3



- Linezolid causes most significant increase in glycolysis in HepG2 WT which contain SNP m.2706A→G within 16S rRNA encoding region
- Treatment with erythromycin does not significantly induce glycolysis in any cell type – consistent with less clinical severity vs linezolid



- A biphasic dose response is observed in both HepG2 transmitochondrial cybrids following treatment with either drug for 72 hrs
- There is no significant change in the basal rates of oxidative respiration between haplogroups

Conclusions

- The most **significant drug-induced increases in glycolytic index** were observed in **HepG2 WT (haplogroup B)** which contain the **SNP m.2706A→G**, a SNP clinically **associated with drug-induced hyperlactatemia** [2]
- The **basal rate of ATP production via oxidative phosphorylation did not vary significantly between haplogroups**, but haplogroup J had lower respiration compared to haplogroups H and B, reflecting the literature [7]
- **HepG2** WT and transmitochondrial cybrids can **recapitulate drug-induced mitochondrial dysfunction** following dosing with ribosome-targeting antibiotics
- **Reduced expression of mtDNA-encoded proteins** in the absence of decreased mtDNA copy number **indicates inhibition of mitoribosomes**
- **Linezolid caused greatest reduction of JATPox**, consistent with the greater clinical incidence and severity of linezolid-induced hepatotoxicity relative to erythromycin

Next steps

Nuclear magnetic resonance (NMR) metabolomics is being utilised in order to explore further the metabolic differences between HepG2 WT (haplogroup B) and HepG2 transmitochondrial cybrids (haplogroups H and J).

Pilot study has shown significant metabolomic differences between cell type, treatment, and time points.

References: 1. Chalasani N et al.; United States Drug Induced Liver Injury Network. Features and Outcomes of 899 Patients With Drug-Induced Liver Injury: The DILIN Prospective Study. Gastroenterology. 2015 Jun;148(7):1340-52.e7; 2. Garrabou G et al. Influence of Mitochondrial Genetics on the Mitochondrial Toxicity of Linezolid in Blood Cells and Skin Nerve Fibers. Antimicrob Agents Chemother. 2017 Aug 24;61(9):e00542-17; 3. Pham, V.H. et al. The frequency of the known mitochondrial variants associated with drug-induced toxicity in a Korean population. BMC Med Genomics 15, 3 (2022); 4. Soriano, A., O. Miró and J. Mensa (2005). "Mitochondrial toxicity associated with linezolid." NEJM 353(21): 2305-2306;). 5. Mineri R et al., (2009) How Do Human Cells React to the Absence of Mitochondrial DNA?. PLOS ONE 4(5): e5713.; 6. Vartak R, Deng J, Fang H, Bai Y. Redefining the roles of mitochondrial DNA-encoded subunits in respiratory Complex I assembly. Biochim Biophys Acta. 2015;1852(7):1531-1539; 7. Ball AL et al. Assessment of the impact of mitochondrial genotype upon drug-induced mitochondrial dysfunction in platelets derived from healthy volunteers. Arch Toxicol. 2021 Apr;95(4):1335-1347. doi: 10.1007/s00204-021-02988-3. Epub 2021 Feb 13.

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