Investigating Drug-Induced Mitochondrial Toxicity

Katalin Orban
Sygnature Discovery Ltd, BioCity, Pennyfoot Street, Nottingham NG1 1GR, United Kingdom
Contact: k.orban@sygnaturediscovery.com
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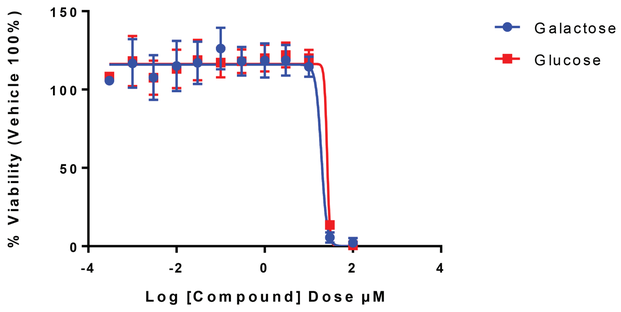
Introduction

Drug-induced mitochondrial dysfunction typically affects organs which heavily rely on oxidative phosphorylation (OXPHOS) such as heart, liver, kidney and nervous system. This dysfunction results in various negative side-effects including organ toxicities such as myopathies, hepatotoxicities, peripheral neuropathies as well as cardiovascular disorders.[1]
The mechanisms associated with mitochondrial toxicity are complex due to multiple modes of action. Therefore, a number of varied approaches are required to investigate potential mitochondrial liabilities of a new chemical entity. Herein, we present two approaches for high throughput screening to monitor hepatotoxicity, using three diagnostic control compounds. These approaches can discriminate primary mitochondrial toxicity from general cytotoxicity using the HepG2 cell line.

Pathomechanism vs toxicity shown in Glu-Gal assay

In normal glucose culture media, cancer cell lines
↓
use glycolysis
↓
not entirely rely on mitochondrial ATP production (Crabtree-effect)[2]
↓
replacing glucose with galactose in the cell media
↓
increases the reliance of the cells on mitochondrial OXPHOS to produce ATP
↓
cells become more susceptible to mitochondrial toxins

Tamoxifen



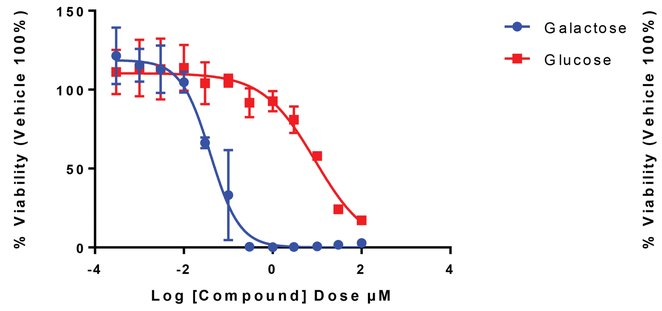
Gal EC50 (µM)	Glu EC50 (µM)	Glu/Gal Ratio
19	26	1.4

Tamoxifen:
- interact with ETC III
- catalyse mitochondrial H_2O_2
- cause lipid peroxidation to mitochondrial membranes
- deplete mtDNA

↓

- irreversible damage to the mitochondria
- multiple action for toxicity

Rotenone



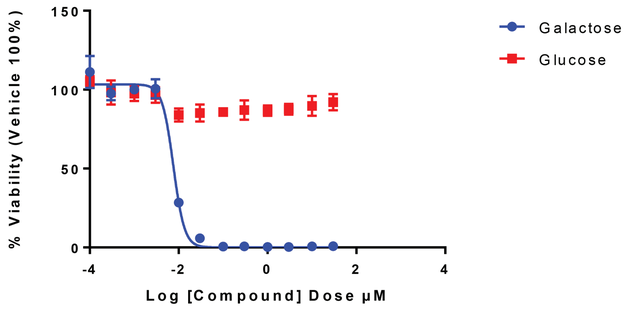
Gal EC50 (µM)	Glu EC50 (µM)	Glu/Gal Ratio
0.039	8.96	230

Rotenone:
- inhibition of ETC I therefore
- enhance the amount of mitochondrial ROS production
- increase caspase 3 activation

↓

- induce cell death
- partial mitochondria toxicant

Antimycin A



Gal EC50 (µM)	Glu EC50 (µM)	Glu/Gal Ratio
0.008	>30	>3750

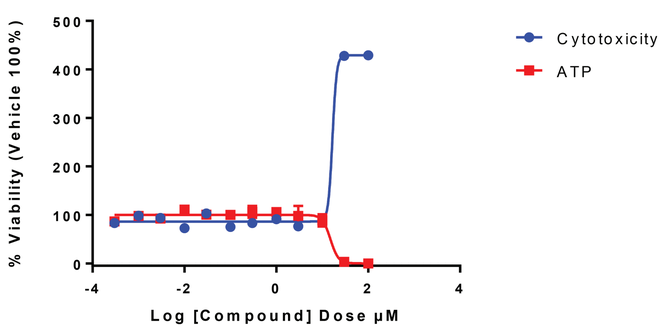
Antimycin A:
- inhibition of ETC III therefore
- increases cellular NADH
- contributes to ROS formation

↓

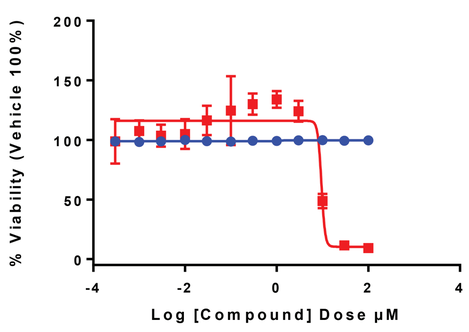
- genuinely mitochondria toxicant

Mitochondrial ToxGlo assay

Tamoxifen_Glu

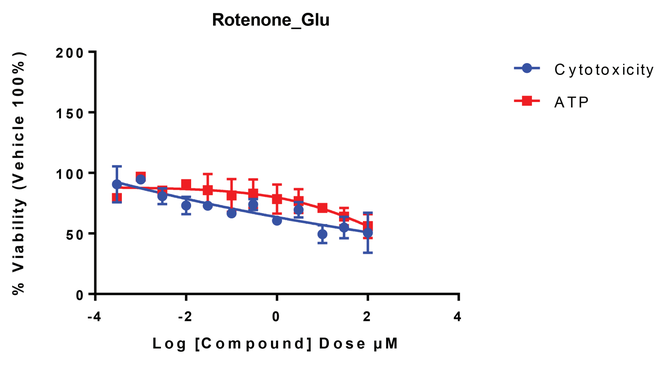


Tamoxifen_Gal

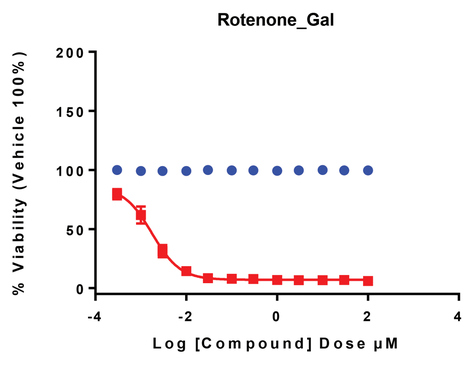


→ Cytotoxin

Rotenone_Glu

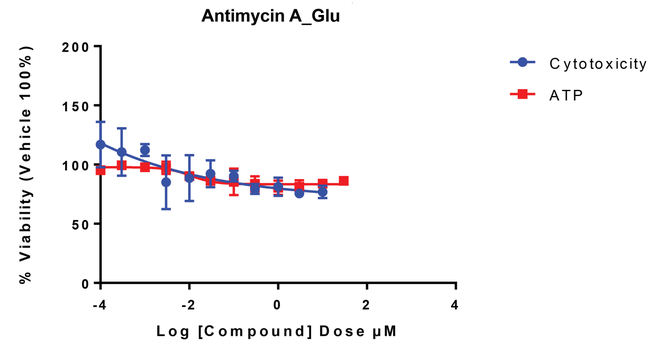


Rotenone_Gal

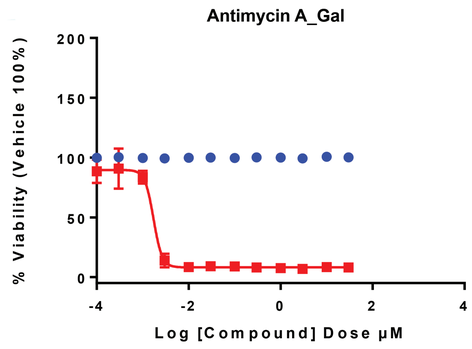


→ Mitotoxicant
Crabtree-effect was observed

Antimycin A_Glu



Antimycin A_Gal



→ Mitotoxicant
Crabtree-effect was observed

Figure 1: Assay chemistry provided by Promega

Summary

Data obtained using the three control compounds (Tamoxifen, Rotenone and Antimycin A) in various assays (Glu-Gal and ToxGlo) correlate and agree with their known pathomechanisms. This provides confidence that the discussed assays can indeed provide diagnostic tools for ranking lead compounds.
We have shown that by combining various assay chemistries and instrumentation it is possible to predict potential mitochondrial liabilities of new chemical entities at the early drug discovery stage. This can not only help reduce late-stage attrition, but can also help improve the safety profile of those drugs being brought to market.
Future work would involve setting up assays based on acidification and extracellular oxygen consumption as well as optimising the Mitochondrial ToxGlo assay with hepatocytes to gain more *in vivo*-like data.

References

1 Dykens et al. Expert Rev. Mol. Diagn., 2007, 7(2),161-175 **2** Marroquin et al. Toxicological Sciences, 2007, 97(2), 539–547