In vitro **Toxicology**

Mitochondrial Toxicity Assessment (Glu/Gal)

Background Information

'Clinical signs of drug induced mitochondrial impairment range from obvious and severe to more subtle reflections of modest loss of mitochondrial capacity such as exercise intolerance, malaise, and mild lactic acidosis.'

[1] Dykens JA and Will Y (2007) *Drug Discovery Today* **12**; 777-785

- Impairment of mitochondrial function is increasingly implicated in the etiology of drug-induced toxicity. For example, mitochondrial dysfunction was found to play a role in the toxicity of troglitazone and cerivastatin which were withdrawn from the US market in 2000 and 2001 respectively[1].
- Mitochondria produce >90% of the cellular energy requirements in the form of adenosine triphosphate (ATP) via oxidative phosphorylation.
- Many cell lines developed for use *in vitro* are metabolically adapted for growth under hypoxic and anaerobic conditions using high glucose media and derive most of their energy from glycosis rather than mitochondrial oxidative phosphorylation (a process termed the Crabtree effect). This reduces the cells susceptibility to mitochondrial toxicants[2].
- Circumventing the Crabtree effect by replacing glucose with galactose in the cell media increases the reliance of the cells on mitochondrial oxidative phosphorylation to obtain ATP. By comparing the toxic effects of different drugs in the glucose and galactose media, it is possible to detect mitochondrial impairment and identify if this is a primary effect or secondary to other cytotoxic mechanisms.[2]
- Cyprotex evaluates mitochondrial toxicity using HepG2 cells, U-87 MG cells or other cell lines (available on request).

Protocol

Media Assessed
Supplemented DMEM containing 25 mM glucose
Supplemented DMEM containing 10 mM galactose

Cell Types Available
HepG2, U-87 MG, other custom cell lines

Test Article Concentration
0.03, 0.1, 0.3, 1, 3, 10, 30 and 100 µM
(different concentrations available)

Final DMSO Concentration
0.5 %

Number of Replicates
3 replicates per concentration

Test Article Requirements
100 µL of 20 mM solution

Analysis Method
MTT [yellow; 3-(4, 5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide], determined by absorbance

Data Delivery
IC_{50} determination in the presence of glucose and galactose media
Minimum effective concentration (MEC) determination in the presence of glucose and galactose media
Fold change in Glu/Gal IC_{50}

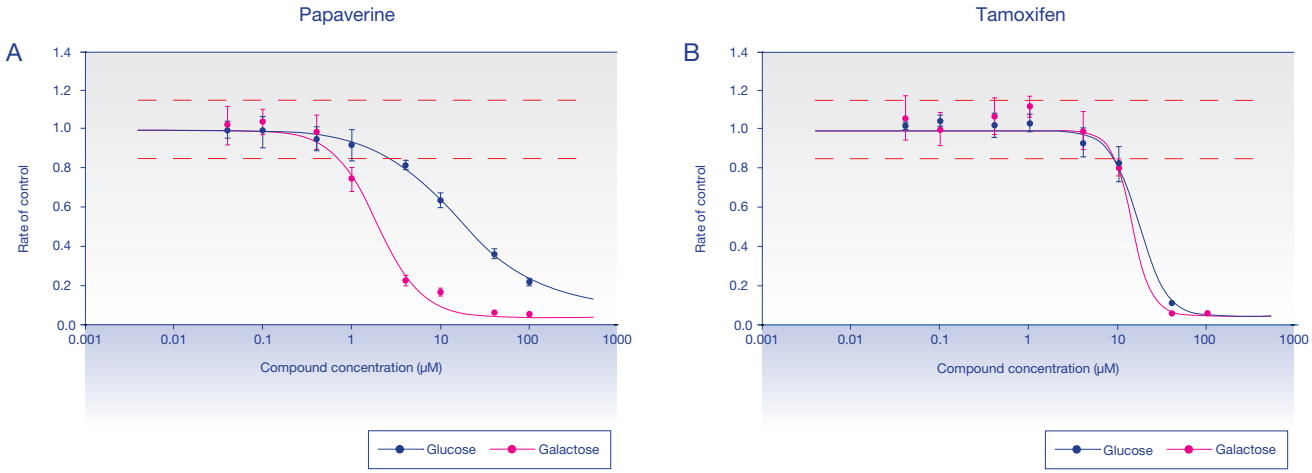
Drug induced mitochondrial toxicity is shown by members of important drug classes, including the thiazolidinediones, statins, fibrates, antivirals, antibiotics, and anticancer agents[2].

Mitochondrial Toxicity

The Cyprotex mitochondrial toxicity assay has been validated using a number of different mitochondrial toxicants and non-mitochondrial toxicant compounds.

Figure 1

Effect of papaverine (A) and tamoxifen (B) on HepG2 cell loss when cells are grown in glucose or galactose media.



A mitochondrial toxicant is indicated by a greater than three-fold change in IC_{50} value observed in the galactose media compared to the glucose media.

Figure 1 illustrates the data for the mitochondrial toxicant, papaverine (A), and the non mitochondrial toxicant, tamoxifen (B). A 7.91 fold increase in IC_{50} value is observed for papaverine in galactose media compared with glucose media (table 1). No fold change was observed with the non-mitochondrial toxicant (tamoxifen).

Table 1

IC_{50} fold change when HepG2 cells are exposed to papaverine or tamoxifen in galactose media compared with glucose media.

Compound	Media	Minimum Effective Concentration (µM)	IC_{50} (µM)	Fold Change in IC_{50}
Papaverine	Glucose	4	15.5	7.91
	Galactose	1	1.96	
Tamoxifen	Glucose	40	14.3	0.85
	Galactose	40	16.9	

References

[1] Dykens JA and Will Y (2007) The significance of mitochondrial toxicity testing in drug development. *Drug Discovery Today* **12**; 777-785

[2] Marroquin LD *et al*. (2007) Circumventing the Crabtree effect: Replacing media glucose with galactose increases susceptibility of HepG2 cells to mitochondrial toxicants *Toxicol Sci* **97(2)**; 539-547