Bioactivation Driven Toxicity Assay

Background information

Detect cytochrome P450 (CYP450) mediated chemically reactive metabolite (CRM) formation and CRM glutathione (GSH) interaction within metabolically competent cells *In Vitro* using Cyprotex's bioactivation driven toxicity assay.

- The removal of xenobiotics from the body is precipitated, in part, by the hepatic phase 1 CYP450 superfamily of metabolising enzymes.
- For certain compounds, such enzymatic interactions can result in the formation of chemically reactive metabolites (CRM) that in turn are capable of adverse cellular events through their interaction with cellular macromolecules and ultimately cytotoxicity[1].
- Within the liver, the glutathione (GSH)-conjugation system plays a central role in alleviating such cytotoxicity through the detoxification of CRM (GSH-adducts) and associated reactive oxygen species (ROS) formation[2].
- Sensitivity to CRM induced toxicity can be increased through the depletion of cellular GSH, which is achieved through the administration of a non-cytotoxic static concentration of the γ-glutamyl-cysteine synthetase inhibitor buthionine sulfoximine (BSO)[3].
- Through concurrent use of the non-specific CYP450 inhibitor 1-aminobenzotriazole (ABT) novel compounds can be identified as harbouring CRM within a cell-based system.
- Parallel compound treatment conditions are conducted, whereby cells receive compounds alongside either (+BSO) ±ABT or ±BSO to determine CYP450 mediate CRM formation potential and CRM:GSH binding potential, respectively (Figure 1.)
- CRM formation is determined through a sensitivity gap value (SGV) calculated at each tested compound concentration for each respective assay test condition. Test articles with SGV >12 are flagged as harbouring potential CRM potential.

Protocol

Cell Line
- Cryopreserved Primary Human Hepatocytes
- HepaRG

Analysis Platform
- Cellular ATP – Cytation 3 Cell Imaging Multi-Mode reader
- Cellular ROS - Cytation 3 Cell Imaging Multi-Mode reader

Test Compound Concentrations
7-point dose response curve with top concentration based on 100x C_{max} or solubility limit. 3 replicates per concentration. *

Compound Requirements
150 µL of a solution to achieve 100x C_{max} (200 x top concentration to maintain 0.5% DMSO) or equivalent amount in solid compound.

Time Points
24–48hr*

Quality Controls
Negative control:
0.5% DMSO (vehicle)
Positive controls:
Assay appropriate control

Data Delivery
Minimum effective concentration (MEC), AC50 value, sensitivity gap value (SGV) for cellular ATP and ROS content.

**Other options available upon request*

Figure 1

Representative bioactivation driven toxicity data in PHH and HepaRG cells models. PHH orange data points and curve fit, HepaRG green data points and curve fit. The +ABT test condition is represented with solid data points and curve, –ABT test condition is represented with empty data point and dashed line (A) Acetaminophen ±ABT cellular ATP, (B) Amiodarone ±ATP cellular ATP, (C) Diclofenac ±ABT cellular ATP, (D) Diclofenac ±ABT cellular ROS.

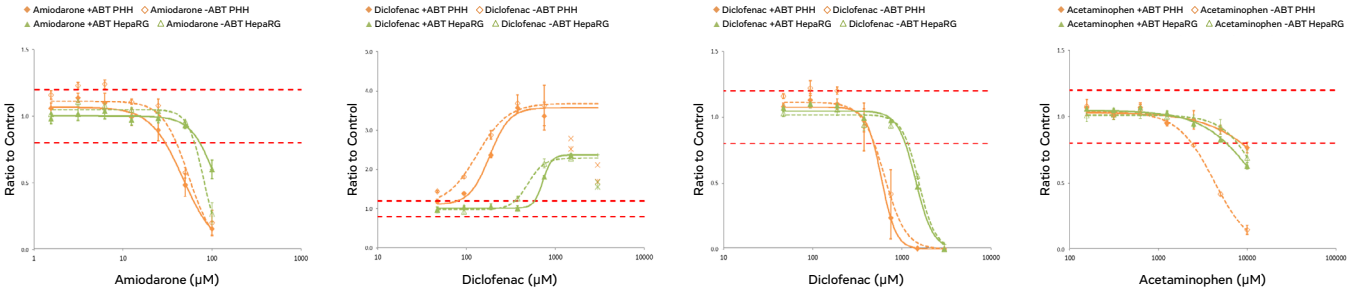

Table 1

Test set of compounds screened through PHH and HepaRG cell models with an SGV cut-off value >12 applied. SGV values have been calculated between each concentration of each compound for the respective endpoints. +VE = >12 SGV threshold. NR = no responder at the concentrations tested. If a positive SGV value was observed in either ROS or ATP (ABT/BSO) a positive was determined (Y). A combined assessment was assessed using both PHH and HepaRG cell models, same observation in both cell models is denoted by an asterisk (*).

			Primary Human Hepatocytes				HepaRG				
Compound	LTKB Label	CRM	ROS SGV	ATP (ABT) SGV	ATP (BSO) SGV	Detected	ROS SGV	ATP (ABT) SGV	ATP (BSO) SGV	Detected	Response in either cell model
Glafenine	Withdrawn	Positive	+VE	-VE	NR	Y	-VE	NR	-VE	N	Y
Troglitazone	Withdrawn	Positive	-VE	-VE	-VE	N	+VE	-VE	-VE	Y	Y
Amiodarone	Box warning	Positive	-VE	-VE	-VE	N	NR	+VE	+VE	Y	Y
Flutamide	Box warning	Positive	+VE	-VE	-VE	Y	-VE	-VE	-VE	N	Y
Isoniazid	Box warning	Positive	NR	NR	NR	N	NR	NR	NR	N	Y
Nefazodone	Box warning	Positive	+VE	-VE	NR	Y	+VE	-VE	NR	Y	Y*
Acetaminophen	Warnings & precautions	Positive	+VE	+VE	+VE	Y	+VE	-VE	-VE	Y	Y*
Carbamazepine	Warnings & precautions	Positive	-VE	-VE	-VE	N	+VE	-VE	-VE	Y	Y
Diclofenac	Warnings & precautions	Positive	+VE	-VE	-VE	Y	+VE	-VE	-VE	Y	Y*
Disulfiram	Warnings & precautions	Positive	+VE	-VE	-VE	Y	NR	NR	NR	N	Y
Ibuprofen	Warnings & precautions	Positive	NR	NR	NR	N	NR	NR	NR	N	N*
Ticlopidine	Warnings & precautions	Positive	+VE	-VE	-VE	Y	-VE	-VE	-VE	N	Y
Dapsone	Warnings & precautions	Negative	-VE	NR	NR	N	-VE	NR	NR	N	N*
Niacin	Warnings & precautions	Negative	NR	-VE	NR	N	NR	NR	NR	N	N*
Acetylsalicylic Acid	No match	Negative	+VE	+VE	-VE	Y	-VE	-VE	-VE	N	Y
Caffeine	No match	Negative	-VE	NR	NR	N	NR	NR	-VE	N	N*
Mitomycin C	No match	Positive	-VE	-VE	+VE	Y	NR	-VE	-VE	N	Y

SGV values were calculated for each of the respective endpoints i.e. ROS, ATP (ABT) and ATP (BSO) and corresponding concentration to discriminate between any concentrations showing suitable shift between their respective test conditions (Figure 1).

Utilising an SGV cut-off of >12 for each respective endpoint a sensitivity of 62% for Primary Human Hepatocytes (PHH) cell model and a slightly lower sensitivity of 46% for the HepaRG cell model, interestingly 3 CRM positive compounds were detected in HepaRG alone. Therefore, when combining both cell models we detected 85% of the CRM positive compounds (Table 1.) Consequently, we would recommend using both cell models for test article screening.

References

[1] Thompson, R.A. et al. (2016) 'Reactive metabolites: Current and emerging risk and hazard assessments', Chemical Research in Toxicology, 29(4), pp. 505–533. doi:10.1021/acs.chemrestox.5b00410.

[2] Leung, L., Kalgutkar, A.S. and Obach, R.S. (2012) 'Metabolic activation in drug-induced liver injury', Drug Metabolism Reviews, 44(1), pp. 18–33. doi:10.3109/03602532.2011.605791.

[3] Xu, J., Oda, S. and Yokoi, T. (2018) 'Cell-based assay using glutathione-depleted HEPARG and HEPG2 human liver cells for predicting drug-induced liver injury', Toxicology in Vitro, 48, pp. 286–301. doi:10.1016/j.tiv.2018.01.019.

[4] Harada, K. et al. (2021) 'Cell-based high-throughput screening for the evaluation of reactive metabolite formation potential', Toxicology in Vitro, 74, p. 105159. doi:10.1016/j.tiv.2021.105159.