Abstract 2255-439

Assessment of the genotoxicity of metabolites generated by S9 extracts or by hepatocytes using the GreenScreen HC GADD45a-GFP assay

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Introduction

ICH guidelines for the registration of pharmaceuticals require that *in vitro* genotoxicity assessments are carried out with and without a source of metabolic activation. Commonly this is provided by incubation with 'S9' liver extracts prepared from rats, exposed to Aroclor-1254 to induce some of the enzymes which drive biotransformation. S9 does not contain a full complement of active biotransformation enzymes, or co-factors, at sufficiently high levels to fully predict *in vivo* hazard, but the prevalence of pro-genotoxins justifies this early preview. However, even without S9, the current *in vitro* mammalian assays (MNT/MLA) frequently generate positive results for compounds that are negative *in vivo*. The predictivity of pro-genotoxicity needs improvement.

In the GreenScreen HC genotoxicity screening assay, p53-dependent regulatory elements of the GADD45a gene drive genotoxin-induced accumulation of GFP in the human TK6 cell line. The assay has a much higher specificity than the current assays. This poster provides data from flow cytometric analysis, demonstrating that it can be used successfully with S9 extracts.

Biotransformation using hepatocytes might provide better prediction of *in vivo* hazard although historically this has been confounded by the lack of reliability in supply, stability and their genetic diversity. Abcellute Ltd has developed a non-cryogenic method of preserving primary hepatocytes which maintains the cells for 4 days. Upon resuscitation, these cells behave identically to freshly isolated hepatocytes, containing all their phase I and II biotransformation enzymes. This poster also provides preliminary data from experiments in which pro-genotoxins have been exposed to intact hepatocytes co-cultured with GreenScreen HC reporter cells.

Methods

S9: Reporter (GADD45a-GFP) and control cells (reporter with GFP out of frame) were exposed to various chemicals in the presence of 1% S9 for 3 hours. Cells were washed then cultured for 45 hours in fresh medium.

Hepatocytes: Reporter and control cells were co-cultured with resuscitated rodent hepatocytes in the presence of the chemical for 21 hours. Reporter and control cells were then removed from the co-culture and incubated for a further 24 hours.

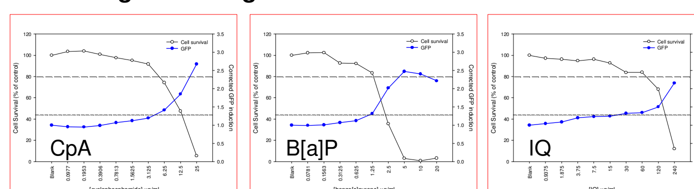
Both systems: Growth and GFP expression from reporter and control cells were assessed using flow cytometry. Background fluorescence was removed and mean GFP intensities normalised to give fold induction values over negative control. Propidium iodide exclusion was used to determine cell survival.

Results

1. Pro-genotoxins are effectively detected by GreenScreen HC after exposure to S9 or co-culture with preserved hepatocytes. Importantly, non genotoxins gave negative results after exposure to S9 or co-culture with hepatocytes.

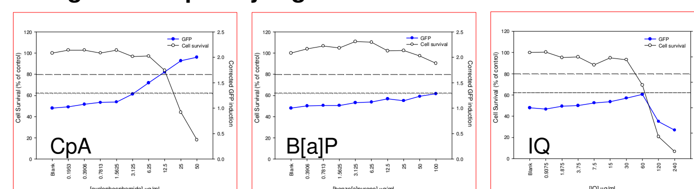
2. Unsurprisingly hepatocytes and S9 do not generate the same results.

Figure 1: S9-generated metabolite assessment



Representative data to demonstrate the genotoxicity of metabolites from three commonly cited pro-genotoxins exposed to S9 (—— Genetox —— Survival).

Figure 2: Hepatocyte-generated metabolite assessment



Representative data to demonstrate the genotoxicity of metabolites from pro-genotoxins using a co-culture methodology (—— Genetox —— Survival).

Conclusions

1. The GreenScreen HC assay can be used to assess the genotoxicity of metabolites generated by either S9 extracts or non-cryogenically preserved primary rat hepatocytes provided by Abcellute Ltd.

2. The use of flow cytometry increases the range of genotoxic assessments by allowing data to be collected from compounds which are, or could become, light absorbing or fluorescent following biotransformation

3. The differences in metabolism provided by S9 extracts from 'induced' livers and un-induced hepatocytes are readily apparent from the genotoxicity results. In this study, this appears to reflect increased CYP450 1A activity in S9 from Aroclor-induced rats.

Table 1: Compounds screened to date using exogenous metabolism in the GADD45a-GFP (GreenScreen HC) assay.

		Chemical tested		Biotransformation method			
			Main rat CYP450 family required	Hepatocytes	LEC µg/ml	S9	LEC µg/ml
Pro-genotoxins	1	1,2-benzanthracene	1A	NEG		POS	5.0
	2	2,4-diaminotoluene	UNK	NEG		NEG	
	3	2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP)	1A	NEG		POS	2.5
	4	2-amino-3,4-dimethylimidazo[4,5-f]quinoline (MEIQ)	1A	POS	25.0	POS	40.0
	5	2-amino-3-methylimidazo[4,5-f]quinoline (IQ)	1A	NEG		POS	120.0
	6	2-aminoanthracene	1A	NEG		POS	5.0
	7	3-amino-1-methyl-5H-pyrido[4,3-b]indole (Trp-P-2)	1A	POS	0.781	POS	1.25
	8	6-aminochrysene	1A	NEG		POS	2.5
	9	7,12-dimethylbenz[a]anthracene	1A	POS	20.0	POS	1.25
	10	aflatoxin B1	3A	POS	0.039	POS	0.039
	11	benzo[a]pyrene	1A	NEG		POS	2.5
	12	cyclophosphamide	2B/3A	POS	6.25	POS	6.25
	13	phenolphthalein	UNK	POS	79.4	POS	79.4
Genotoxins	1	4-nitroquinoline 1-oxide (4-NQO)		POS	0.0625	POS	1.0
	2	5-fluorouracil		NEG		POS	2.5
	3	busulfan*		POS	9.59	POS	19.9
	4	cis-diammineplatinum(II) dichloride (cisplatin)**		POS	2.0	POS	2.0
	5	etopside		POS	0.125	POS	8.0
	6	methyl methanesulphonate (MMS)*		POS	3.13	POS	6.25
	7	methylnitrosurea		POS	4.02	POS	4.02
	8	mitomycin C**		POS	0.3125	POS	0.5
	9	paclitaxel*		POS	0.3125	POS	0.02
Non-genotoxins	1	2,4-dichlorophenol		POS	40.5	NEG	
	2	benzoin		NEG		NEG	
	3	d-mannitol		NEG		NEG	
	4	epsilon-caprolactam		NEG		NEG	
	5	ethylene glycol		NEG		NEG	
	6	phenformin hydrochloride		NEG		NEG	
	7	pyridine		NEG		NEG	
	8	sodium chloride		NEG		NEG	
	9	sucrose		NEG		NEG	
	10	tetracycline hydrochloride		NEG		NEG	

LEC (**L**owest **E**ffective **C**oncentration) values are means from triplicate experiments; **UNK** = unknown; **POS** = positive genotoxicity call from the GADD45a-GFP reporter; ***** = positive genotoxicity call in experiments not using S9; ****** = positive genotoxicity call in experiments without S9 using a spectrophotometric reader; **NEG** = negative for genotoxicity.

Acknowledgments

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