Development of a relay assay with plated human hepatocytes for the evaluation of slowly metabolized compounds

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Introduction

Routine application of metabolic stability screening assays in drug development has led to the accumulation of NCE with low hepatic metabolic clearance.

The metabolic fate (rate of hepatic metabolic clearance; metabolite profiles; metabolic pathways) of such NCEs cannot be readily evaluated using the routinely used in vitro systems such as HLM and human hepatocyte suspensions.

Di et al (2012) developed a relay assay with human hepatocyte suspension for the evaluation of compounds with low metabolic clearance. The assay involves incubation of a compound with human hepatocyte suspensions for 4 hrs, followed by removal of the hepatocytes, and re-incubation (relay) of the compound with a new hepatocyte suspension. With this relay methods, a compound can be incubated for 20 h with 5 relays (4 h per relay x 5 = 20 hr).

We report here a modified relay assay using primary cultured (plated) human hepatocytes. With our assay, each relay is 24 h. An accumulated incubation time of 72 h can be achieved with 3 relays (24 h x 3 = 72 hr).

This Plated Hepatocyte Relay Assay (PHRA) should be useful for the evaluation of slowly metabolized chemicals.

Materials & Methods

Materials:

Plateable Cryopreserved Human Hepatocytes (IVAL, Columbia, MD)
HH1023 (Donor 1), HH1031 (Donor 2), HH1036 (Donor 3), Pool of 3 donors
Collagen Coated 48 well plates (CellAffix™; APSciences Inc., Columbia, MD)
Universal Cryopreservation Recovery Medium (UCRM; IVAL).
Universal Primary Cell Plating Medium (UPCM; IVAL)
Hepatocyte Incubation Medium (HQM; IVAL)

Methods:

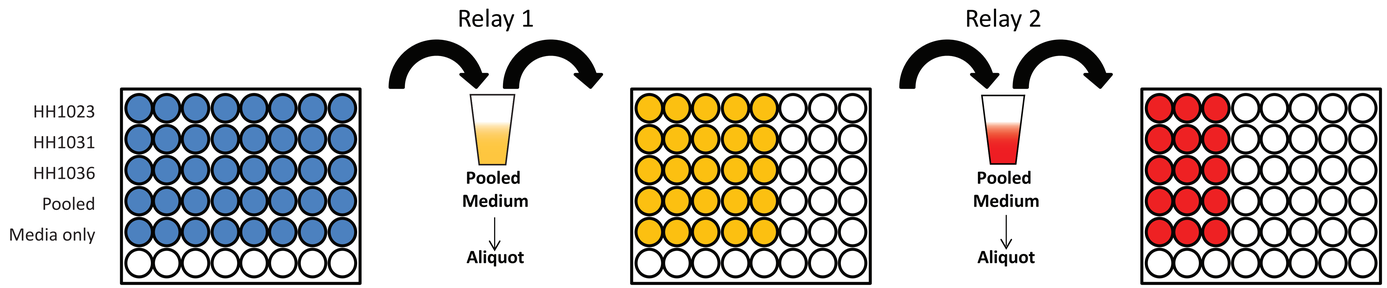
The samples were thawed, centrifuged at 3000 x g for 5 min and then analyzed by injecting into Agilent 1200 HPLC connected to API3000 Mass spectrophotometer.

The samples were separated on Eclipse C18 column, 4.6 x 75 mm and 3.5 µM particle size using 0.1 % formic acid in water and 0.1 % formic acid in acetonitrile as mobile phases.

Analysis:

Ln of % parent drug remaining vs time of incubation (hrs) was plotted and following equations were used to calculate T1/2 and Clint :
T1/ 2= ln 2/-(slope of the ln % remaining of drug vs. time plot)
CLint = ln 2 X (1/T1/ 2(min)) X (ml incubation/0.6 M cells) X (120 M cells/g liver) X (21 g liver/kg)

Procedures



Day 1 (Plate 1)

1) Thaw and plate human hepatocytes (Plate 1) and allow 4 h for attachment
2) T= 0 hr: Replace medium to that containing chemicals to be evaluated (remove an aliquot as no hepatocyte control)
3) Incubate for 24 h

Day 2 (1st Relay) (Plate 2)

1) Thaw fresh vials and plate hepatocytes to prepare the first relay plate (Plate 2).
2) T= 24 h: Pool the media containing the same chemical in all the wells in Plate 1. Remove aliquots for LCMS analysis.
3) Replace medium in Plate 2 with the pooled medium for the 1st Relay (T= 24 to 48 h). Incubate for 24 h.

Day 3 (2nd Relay) (Plate 3)

1) Thaw fresh vials and plate hepatocytes (Plate 3).
2) T= 48 h: Pool the media containing the same chemical in all the wells in Plate 2. Remove aliquots for LCMS analysis.
3) Replace medium in Plate 3 with the pooled medium for the 2nd Relay (T= 48 to 72 h). Incubate for 24 h.
4) T=72 h: Collect samples for analysis.

Results

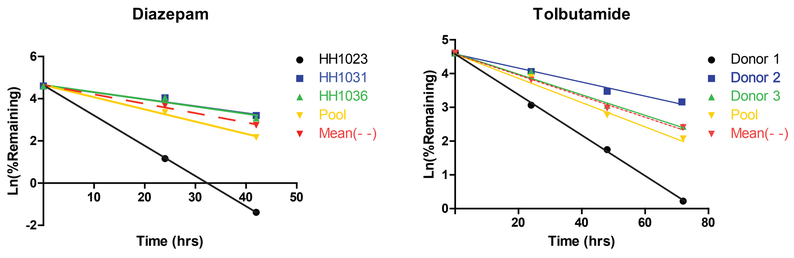


Table 1. Comparison of in vivo human intrinsic clearance with in vitro intrinsic clearance using the PHRA method

Drug	Donor	Slope (-K)	r2	$T_{1/2}$ (hrs)	$CL_{int\ (in\ vitro)}$ $ml.min^{-1}.kg^{-1}$	$CL_{int\ (in\ vivo\ human)}$ $ml.min^{-1}.kg^{-1}$	Fold Difference between In Vitro and In Vivo
Tolbutamide	Donor 1 (Lot # HH1023)	0.06	0.99	11.5	**4.2±0.09**	**4.9** Brown et. al. 2007, Li, D., et al., 2012	1.16
	Donor 2 (Lot # HH1031)	0.02	0.98	33.4	1.45± 0.13		3.38
	Donor 3 (Lot # HH1036)	0.03	0.99	22.13	2.19± 0.18		2.24
	Pool	0.04	0.99	19.07	2.54± 0.17		1.93
Diazepam	Donor 1 (Lot # HH1023)	0.14	1	4.9	**10 ± 0.03**	**15** Hallifax et. al. 2010, Li, D., et al, 2012	1.50
	Donor 2 (Lot # HH1031)	0.03	0.96	21.1	2.3 ± 0.46		6.52
	Donor 3 (Lot # HH1036)	0.04	0.94	19.5	2.48± 0.6		6.05
	Pool	0.06	0.99	13.9	3.5 ± 0.27		4.29

Summary

1) Linear time dependent disappearance of both diazepam and tolbutamide were observed, with tolbutamide < diazepam in metabolic clearance as expected.
2) Individual differences in clearance was observed for the three lots of hepatocytes. Pooled hepatocytes provide data similar to the mathematical average of the individual donors.
3) The calculated in vivo hepatic clearance is similar to that reported for diazepam and tolbutamide

Conclusions

The Plated Hepatocyte Relay Assay (PHRA) should be applicable for the evaluation of the metabolic fate of slowly metabolized chemicals that otherwise cannot be readily evaluated using current HLM and hepatocyte assays

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