ACCURATE PREDICTION OF HEPATIC CLEARANCE FOR LOW-CLEARANCE COMPOUNDS USING MICROPATTERNED HEPATOCYTE-STROMAL CELL CO-CULTURES (HEPATOPAC®)

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ABSTRACT

Generating accurate *in vitro* intrinsic metabolic stability and clearance data is an important aspect of predicting *in vivo* human clearance and establishing a safe dosing range in early clinical studies. Primary hepatocytes in suspension are routinely used for determination of metabolic clearance. However, hepatocytes in suspension are only viable for several hours, which is not long enough to appropriately evaluate the metabolic stability of low clearance compounds. Using HepatoPac, a recently developed micropatterned hepatocyte-stromal cell co-culture system which can be used for continuous incubations for up to 7 days, we have successfully determined the metabolic stability of 17 coμMercially available low clearance drugs (CL < 5 μL/min/kg). Intrinsic clearance was calculated using the *in vitro* half-life determined for each compound and hepatic clearance was subsequently calculated using the well-stirred model with and without a correction for plasma protein binding. Without correction for plasma protein binding, hepatic clearance was accurately predicted for only 2 compounds, as determined by prediction of clearance within 2-fold of published clinical CL values. However, with correction for plasma protein binding, hepatic clearance was accurately predicted for 15 compounds. These results clearly demonstrate the utility of HepatoPac for the prediction of *in vivo* hepatic clearance of low-clearance compounds.

INTRODUCTION

- Metabolic stability assessment using suspended human hepatocytes has been variably successful for predicting human clearance of drug candidates. One key limitation of this system is the short incubation times due to rapid loss of cell viability. This precludes the ability to predict the clearance of compounds which are slowly metabolized by hepatocytes.
- HepatoPac is a new format of hepatocyte culture that achieves superior longevity in viability to suspended cultures and is not affected by losses in drug metabolizing enzyme function associated with hepatocytes in sandwich or monolayer cultures[1,2].

MATERIALS AND METHODS

1. Selection of compounds

- Preference for compounds with ≤ 5 μL/min/kg human *in vivo* clearance (or ≤ 25% of human hepatic blood flow)
- Preference for compounds mainly cleared by hepatic metabolism (See Table 1)

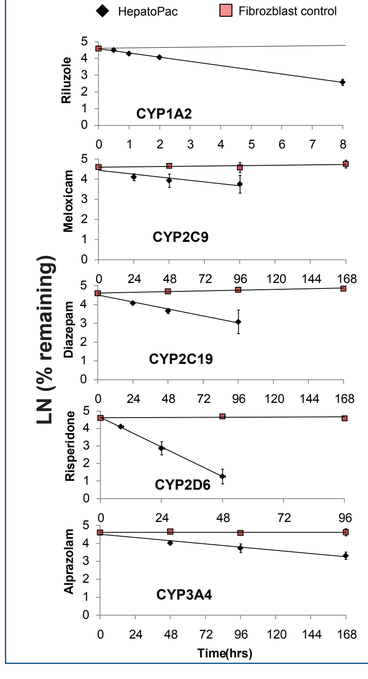
2. Methodology

- 96-well plate format consisting of approximately 5000 hepatocytes per well from one donor
- Each incubation consisted of 64 μL of serum-free proprietary HepatoPac media containing 0.1 μM drug (0.5 μM for atomoxetine).
- Incubations were stopped by the addition of 3 volumes of a solution consisting of 60% acetonitrile, 39.9% water and 0.1% acetic acid with internal standard (nevirapine or 1-naphthyl glucuronide).
- Filtered samples were injected into an LC/MS where parent compound depletion from the incubation was followed using multiple reaction monitoring (MRM).
- Clearance prediction was accomplished using the well-stirred model.

TABLE 1: Sampling time points and LC/MS/MS transitions

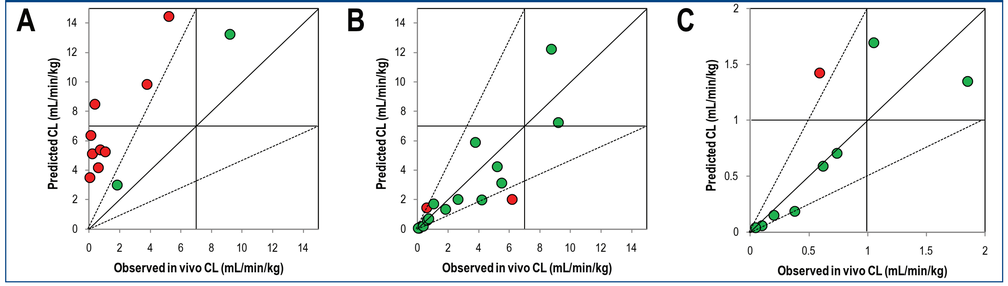
Major CYP450	Compound name	Sampling time points (h)	MS/MS Transition
CYP1A2	Lidocaine	0,2,8, 24, 48, 72, 96	235-86 (+)
	Riluzole	0,0.5, 2, 8, 24	235-166(+)
	Theophylline	0, 24, 48, 96, 144	181-124(+)
CYP2C9	Diclofenac	0, 0.5, 1, 2	298-180(+)
	Glimipiride	0, 0.5, 1, 2, 8	489-376 (-)
	Meloxicam	0, 3, 24, 48, 72, 96, 120, 144	352-184(+)
	Tolbutamide	0, 3, 24, 48, 72, 98, 120, 144, 168	271-155(+)
	Warfarin	0,3,24,48, 72, 96, 120, 144, 168	307-250(-)
CYP2C19	Diazepam	0, 24, 48, 72, 96	285-193(+)
	Lansoprazole	0, 0.5, 2, 8, 24, 48, 72, 96	370-252(+)
	Voriconazole	0, 2, 8, 24, 48, 72, 96	350-281(+)
CYP2D6	atomoxetine	0, 0.5, 2, 8, 24	256-148(+)
	Flecainide	0, 24, 48, 72, 96, 144	415-301(+)
	Risperidone	0, 2, 8, 24, 48, 72, 96	411-191(+)
CYP3A4	Alprazolam	0, 0.5, 1, 2, 8, 24, 48, 72, 96, 168	309-281(+)
	Atazanavir	0, 0.5, 1, 2, 8, 24, 48, 72, 96, 168	705-335(+)
	Prednisolone	0, 0.5, 1, 2, 8, 24, 48, 72, 96, 168	361-343(+)

Figure 1: Parent Depletion



RESULTS

Figure 2: Accurate prediction of low clearance compounds using the well-stirred model.



Panel A: Overprediction of in vivo clearance values without incorporation of plasma protein binding in the well-stirred model.

Panel B: Accurate prediction of in vivo clearance following the inclusion of plasma protein binding.

Panel C: Magnification of the lowest clearance values in Panel B.

Inclusion of previously-reported plasma protein binding data leads to an accurate prediction of the in vivo clearance of 88% of the compounds tested as defined by a predicted CL within 2-fold of the in vivo value. For the 2 compounds outside of the inclusion criteria, the prediction was still relatively close (0.3 and 2.4 fold for atomoxetine and alprazolam respectively).

Table 2: Human clearance prediction in HepatoPac using the Well-Stirred model

Compound	f_u (plasma unbound fraction)	$CL_{(obs)}$ (*in vivo* hepatic CL)	Predicted CL (Well-Stirred)		Predicted CL/ CL_{obs}	
			Without f_u	With f_u	Without f_u	With f_u
Lidocaine	0.3 [a]	10.3 [b]	13.2	7.2	1.3	0.7
Riluzole	0.02 [c]	5.5 [c]	18.6	3.1	3.4	0.6
Theophylline	0.6 [d]	1.05 [e]	5.2	1.7	5.0	1.6
Diclofenac	0.005 [c]	4.2 [c]	19.7	2.0	4.7	0.5
Glimipiride	0.005 [c]	0.62 [c]	17.7	0.6	28.5	1.0
Meloxicam	0.006 [f]	0.1 [f]	6.4	0.6	63.6	0.6
Tolbutamide	0.022 [g]	0.2 [h]	5.1	0.15	25.5	0.8
Warfarin	0.01 [c]	0.045 [c]	3.5	0.04	77.3	0.9
Diazepam	0.013 [c]	0.38 [c]	8.5	0.2	22.3	0.5
Lansoprazole	0.03 [i]	4.0 [i]	16.1	2.0	6.1	0.5
Voriconazole	0.42 [c]	3.8 [c]	9.8	5.9	2.6	1.6
Atomoxetine	0.013 [c]	6.2 [c]	18.4	2.0	3.0	0.3
Flecainide	0.39 [c]	1.85 [c]	3.0	1.3	1.6	0.7
Risperidone	0.11 [c]	5.2 [c]	14.4	4.2	2.8	0.8
Alprazolam	0.29 [c]	0.59 [c]	4.2	1.4	7.1	2.4
Atazanavir	0.14 [j]	6.38 [j,k]	18.8	12.2	2.9	1.9
Prednisolone	0.1 [c]	0.74 [c]	5.4	0.7	7.3	1.0

Green highlighting represents predictions that were ≤ 2 fold and ≥ 0.5 fold of the in vivo CL (CLobs) value (predictive). Red highlighting indicates values that fell outside of this range (not predictive).

[a]Ujhelyi MR et. al. Clin Pharmacol Ther 53(1); 38-48
[b]Byrn S et. al. Clin Pharmacokinet 24(2); 183-186
[c]Goodman and Gilman's The Pharmacological Basis of Therapeutics 11th Edition.
[d]Ebden P et al. Thorax 39(5); 352-355.
[e]Mangione A et al. Chest 73(5); 616-622.
[f]Turck D et al Arzneimittelforschung 47(3); 253-258.
[g]Thiessen JJ et al. J Clin Pharmacol 16(7); 345-351.
[h]Veronese ME et al. Clin Pharmacol Ther 47(3); 403-411.
[i]Landes BD et al. Clin Pharmacokinet 28(6); 458-470.
[j]Le Tiec C et al. Clin Pharmacokinet. 44(10);1035-50.
[k]Piliero PJ et al. Drugs Today 40(11); 901-912.

DISCUSSION

- Interestingly, these results were acquired from only one donor while testing for the clearance by 5 of the major CYP450s. Preliminary data from hepatocytes from 2 other donors (data not shown) suggested that donor-dependent variability is present in HepatoPac, but markedly less than from cryopreserved hepatocytes[3].
- The accuracy of predicted CL_h for selected compounds with low CL_h from various *in vitro* systems is shown in the table below. HLM generally under-predicted the *in vivo* CL_h by 2- to 3- fold while cryopreserved human hepatocytes under-predicted the *in vivo* CL_h by more than 5- fold for 3 out of 6 compounds.

Table 3: CL prediction from HepatoPac vs. Predictions using cryopreserved human hepatocytes or HLM

Compound	Observed *in vivo* CL_h (mL/min/kg)	Predicted human CL_h from in vitro (mL/min/kg)		
		HepatoPac	cryopreserved human hepatocytes	HLM [4]
Diazepam	0.38	0.2	0.056 [5] or 0.054 [6]	0.2
Diclofenac	4.2	2.0	0.29 [7]	1.6
Lidocaine	10.3	7.2	1.9 [7]	No data
Prednisolone	0.74	0.7	2.6 [5]	No data
Theophylline	1.05	1.7	0.92 [5]	0.52
Tolbutamide	0.2	0.15	0.11 [5]	0.07
Warfarin	0.045	0.04	0.034 [5]	0.02

- *Green and red represent within 2-fold or > 2-fold of the in vivo CL values.*
- *Predicted CL_h was calculated from CLint obtained from various in vitro systems using well-stirred model considering fu in plasma.*

CONCLUSIONS

- HepatoPac cultures were able to demonstrate significant turnover of low-clearance compounds.
- In our current study with a limited number of compounds using HepatoPac, we have shown significant improvement in the accuracy of prediction of human *in vivo* CL in comparison to historical literature data.
- For most compounds, the prediction of human *in vivo* clearance using the well-stirred model relied on the incorporation of plasma protein binding.
- More compounds and more donors are being tested to generate a more comprehensive data set.

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