Determination of Low Intrinsic Clearance Values using Primary Human Hepatocytes and the HepaRG® Cell Line - A Comparison of Methods

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Background

Oral drugs typically require effective half lives in the region of 10 – 20 h for once or twice daily dosing. For candidate drugs with low distribution volumes it is necessary to define intrinsic clearance (CLint) values of 0.1 - 1µL/min/million human hepatocytes (Grime et al., 2013). We wanted to compare in vitro systems that potentially can provide a solution to the problem of robustly defining low CLint values in human hepatocytes. Recently the HepatoPac™ Platform and a novel relay suspension method (Di et al., 2012) have shown promising results producing reliable low CLint values. XenoTech have made a well characterised platable pool of cryopreserved human hepatocytes (5 donors) commercially available, which makes plated heptocytes methods attractive to evaluate. Also of interest is the HepaRG® human hepatoma cell line, since it offers stable expression of drug metabolising enzymes (DMEs) (Kanebratt et al., 2008, Aninat et al., 2006). We are evaluating all four methods but this poster focuses on HepaRG & plated primary hepatocytes since data from Hepatopac and Relay at present are inconclusive.

Methods

CLint for a set of DME substrates (Table 1) with known low turnover was determined in each in vitro system.

Plated human hepatocytes

The platable pooled human hepatocytes (lot 1310168) were purchased from XenoTech, thawed and plated according to vendors protocol for 4 hours. Culture media with 1µM substrate was added and repeated samples were withdrawn during incubations. Formation of 1OH-Midazolam and 4OH-Diclofenac and depletion of Naloxone were assessed to determine DME activity change over time.

HepaRG ®

Cryopreserved differentiated HepaRG® cells were plated in 96-wells plates for 5 days prior to start of incubation for 24 hours. Substrate was added to the cells and media samples withdrawn from one well per timepoint.

Substrates or metabolites were quantified using LCMSMS.

Results and discussion

Both HepaRG® and plated hepatocytes produced CLints for low turnover substrates in the expected range (Table 1).

The plated primary hepatocytes gave a linear decline for 2D6 and 2C19 substrates (Fig. 1). Moreover 80 minute metabolite formation and CLint-studies with specific DME-substrates (Diclofenac (2C9), Midazolam (3A4) and Naloxone (UGT2B7)) at different timepoints during culture shows sustained activity for 9 hours (Fig. 2).

Diclofenac shows lower CLint in this evaluation than in literature (Table 1), we also couldn't determine S-Warfarin CLint, which may indicate 2C9 is lower in activity in this batch or the ten hours incubation is not enough to produce a reliable CLint. The same holds true for Theophylline and CYP1A2. For in-house 3A4 substrates and Disopyramide we were able to determine Clints down to 0.2-0.4 uL/min/million cells (Table 1). Further plated experiments are needed to fully characterize DMEs in the plated system.

CLint values correlate well between hepatocytes run in suspension and plated (Fig. 4) with the important difference that major DMEs seem to retain same activity up to 10 hours of plating giving possibilities to determine lower CLints.

Linear depletion of Metoprolol (2D6), Diazepam (2C19/3A4) and Naloxone in plated hepatocytes.

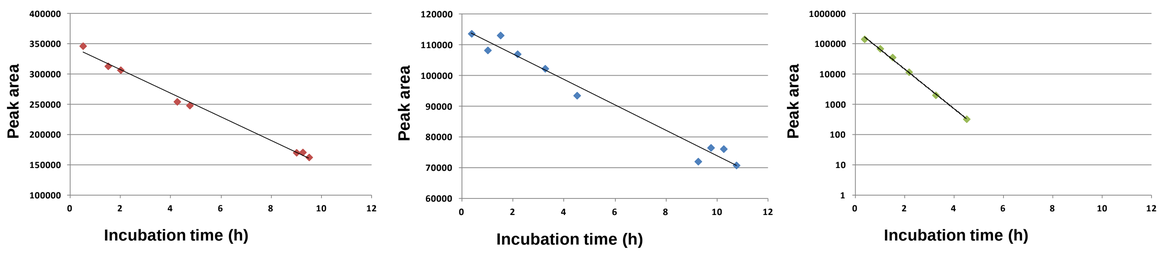

Figure 1. *Depletion profiles for CYP2D6-substrate Metoprolol (left), CYP2C19/3A4-substrate Diazepam (middle) and Naloxone (right).*

Metabolite formation for Diclofenac (2C9) and Midazolam (3A4). Glucuronidation of Naloxone (UGT2B7) in plated hepatocytes.

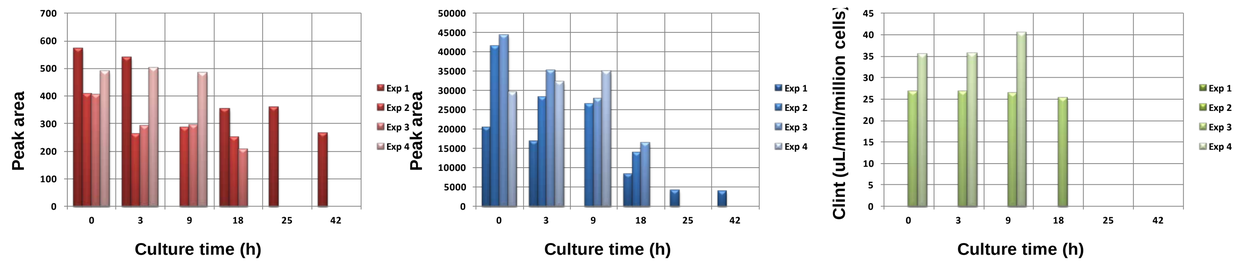

Figure 2. *Formation of metabolites at different timepoints during culture period, 4OH-Diclofenac to the left, 1OH-Midazolam in the middle. Each bar represent metabolite-formation (peak-area) during 60 minutes incubation. Depletion of Naloxone as measured by CLint at different timepoints during culture, to the right.*

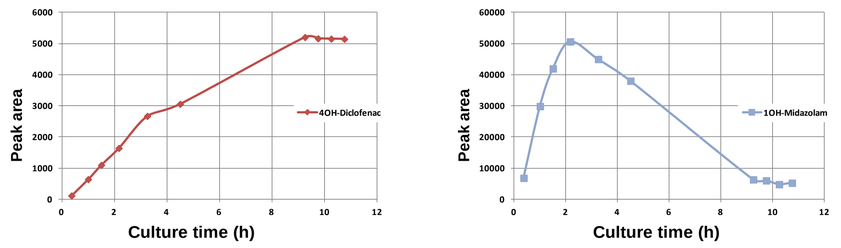

Figure 3. *Formation of metabolites during 10 hours. Left panel ;1-OH-Diclofenac, right panel; 1OH-Midazolam.*

Table 1. Clint values and compound information

Compound	Ion class	Drug metabolising enzyme	CLint [e] Literature data	CLint [e] Plated hepatocytes	CLint [e] HepaRG
Bufuralol	Base	2D6	17[a]	10.3	
Diazepam	Neutral	2C19>3A4	0.3[b], 3[c], 1.4[d]	0.9 ± *0.2*	0.2, 0.6
Diclofenac	Acid	2C9	38[a], 47[b]	5.8	
Disopyramide	Base	3A4	1[c]	0.2	0.2
Metoprolol	Base	2D6	7[b]	2.1 ± *0.7*	0.6 ± *0.3*
Midazolam	Neutral	3A4	7[d], 14[b]	7.3	
Naloxone	Base	UGT2B7	28[d], 216[b]	27.0, 38.0	
Sildenafil	Base	3A4>2C9,2C19	5[d]	9.7	
S-Warfarin	Neutral	2C9>3A4	1[a], 1[c], 1[d]	n.v.	0.1, 0.3
Theophylline	Neutral	1A2	0.6[c], 1[d]	n.v.	n.v.
Verapamil	Base	3A4	16[d], 18[b]	14.8	
AZ1	Neutral	3A4		0.9 ± *0.3*	0.4, 0.4
AZ2	Neutral			1.2 ± *0.3*	1.2 ± *0.2*
AZ3	Neutral	3A4		0.4 ± *0.2*	0.1, 0.2
AZ4	Base			0.9 ± *0.2*	0.3 ± *0.2*
AZ5	Acid	3A4		1.3 ± *0.2*	0.5 ± *0.2*
AZ8	Neutral			0.4, 0.5	0.4

[a] Brown et al., 2007, [b] McGinnity et al., 2004, , [c] Di et al., 2012, [d] Lau et al., 2002.
[e] uL/min/million cells
n.v. = no value

Intrinsic Clearance in Suspension versus Plated human hepatocytes

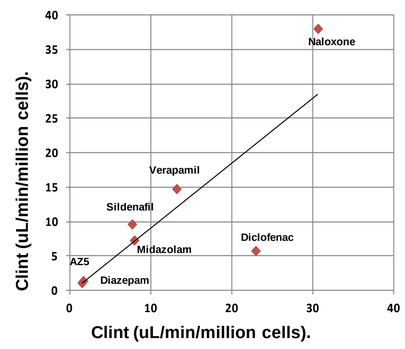

Figure 4. *CLint for 7 of the compounds when tested in Suspension method (2 hr incubation) compared to CLints from the Plated method (10 hr incubation). CLints from Plated on the y-axis, CLints from suspension on the x-axis.*
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Conclusions

Major drug metabolising enzymes seem to have sustained activity in XenoTech´s platable pool of human hepatocytes up to 10 hours directly after plating. The results in this evaluation demonstrates the use of both plated hepatocytes and HepaRG® in order to determine CLint values below 1 uL/min/million cells.

References

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