Application of MetMax™ Pooled Donor Human Hepatocytes in a Higher Throughput Assay for Human Hepatic Metabolic Stability Screening

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Scientific Rationale

- **Screening for metabolic stability is routinely performed in drug development**
- **Metabolic stability screening with human hepatocytes provide a more comprehensive evaluation than that with human liver microsomes due to the presence of all key drug metabolizing enzyme pathways, especially phase 2 conjugative metabolism**
- **High throughput screening with human hepatocytes is challenged by the sensitivity of the cells to robotic manipulation**
- ***MetMax™ human hepatocytes, a novel in vitro drug metabolism system, with the complete drug metabolizing enzyme pathways and the robustness and ease-of-use of liver microsomes, represent an ideal systme for metabolic stability screening.***
- **Our study compares results of metabolic stability screening with drugs with known in vivo hepatic clearance between pooled donor human hepatocytes and MetMax™ pooled donor human hepatocytes**

The MetMax™ Advantage

MetMax™ Hepatocytes

Freezer to Incubation:
<5 minutes

1. *Retrieve from -80 C freezer*
2. *Thaw in a 37 C water bath*
3. *Add equal volume to 2X test article*
4. *Incubate*

Intact CryoHepatocytes

Freezer to Incubation:
>30 minutes

1. *Retrieve from LN2 Freezer*
2. *Thaw in a 37 C water bath*
3. *Add to recovery medium*
4. *Centrifuge*
5. *Microscopically quantify viability and cell number*
6. *Adjust to 2X final cell density*
7. *Add at equal volume to 2X test article*
8. *Incubate*

Materials & Methods

Hepatocytes: Pooled Donor Cryopreserved Human Hepatocytes Lot# PHS9001 and MetMax™ Pooled Donor Human Hepatocytes Lot# PHHX 8001 were used in the study (In Vitro ADMET Laboratories, Inc. (Columbia, MD).

Metabolic Stability Assay: Cryopreserved hepatocytes, PHS9001 was thawed, recovered and adjusted to a density of 2 x 10^6 viable cells/mL, using Dulbecco's Modified Eagle Medium, 1X, high glucose. MetMax cells were provided at a concentration of 2 x 10^6 viable cells/mL (2.5 mL per vial) and were thawed in a water bath and used directly without further manipulation. A 20-µL aliquot of test compound was added to each test well of a 96-well polypropylene plate immediately followed by the addition of 20 µL of the hepatocyte suspension. At each time point, the appropriate incubation plate was removed from the incubator and a solution containing IS (200 µL, 0.2 µM labetalol in 60% acetonitrile) was added to each well follow by centrifugation to remove solid precipitates and the supernatent transferred from each well to a 96-well shallow plate (Costar) for storage and analysis.

Analytical Chemistry: The LC-MS/MS system was comprised of an HTS-PAL autosampler, an HP1200 HPLC, and an API4000 triple quadrupole mass spectrometer. Chromatographic separation of the analyte and internal standard was achieved at room temperature using a Phenomenex C18 column in conjunction with gradient conditions using mobile phases A (aqueous 0.1% formic acid with 1% isopropyl alcohol) and B (0.1% formic acid in acetonitrile). Mass spectrometric detection of the analytes was accomplished using the ESI^+ ionization mode. Analyte responses were measured by multiple reaction monitoring (MRM) of transitions unique to each compound. Data were acquired and peak areas were calculated for test compounds and the internal standard using Analyst 1.6.1 software (Sciex).

Results

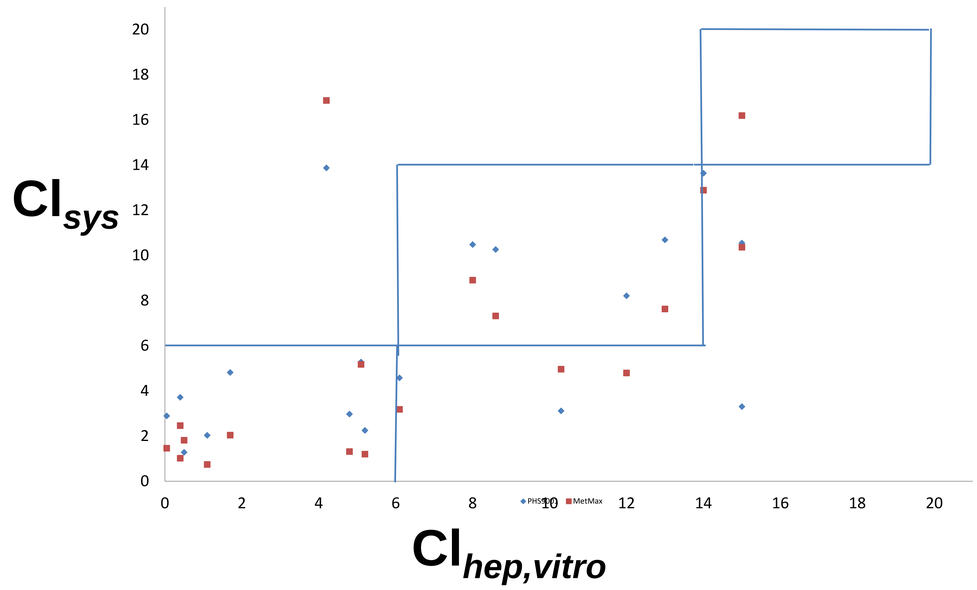
Predicting Systemic Clearance from *In Vitro* Data

- **Human Physiological and Biochemical Parameters**
  - **Liver Weight: 25.7 g liver/kg body weight**
  - **Hepatic blood flow rate (Q_h): 20.6 mL/min/kg body weight**
  - **Hepatocyte number: 120 x 10^6 cells/g liver**
  - **Scaling factor: 3084**
- **Half-life calculation:** $t_{1/2} = \frac{\ln 2}{\text{slope of } [\ln(\%\text{ remaining}) \text{ vs time}]}$
- **Intrinsic clearance calculation:**

$$Cl_{int,h} = \frac{\left(\frac{\ln 2}{av\ t_{1/2}(\text{min})}\right)}{\left(\frac{10^6\ cells}{mL\ incubation}\right)}\left(\frac{120\ x\ 10^6 cells}{g\ liver}\right)\left(\frac{25.7\ g\ liver}{kg\ human}\right)$$

- **Calculation of hepatic clearance and hepatic extraction ratio assuming a w-stirred model and low protein binding:**

$$Cl_h = \frac{Q_h \cdot f_u \cdot Cl_{i,h}}{Q_h + f_u \cdot Cl_{i,h}} = \frac{Q_h \cdot Cl_{i,h}}{Q_h + Cl_{i,h}} \qquad ER = \frac{Cl_h}{Q_h}$$



Drug	Enzymes Responsible for Metabolism	Observed *in vivo* Cl_{sys} (mL/min/kg)	Predicted *in vivo* Hepatic Clearance (mL/min/kg)			
			Hepatocytes PHS9001		*MetMax™, PHX8001*	
			Average	STDEV	Average	STDEV
Acebutolol	Acetylation	5.2	2.2	0.5	*1.2*	*0.3*
Afatinib	FMO/GST	15	3.3	1.7	*16.2*	*0.7*
Antipyrine	CYP1A2	0.5	1.3	0.9	*1.8*	*0.3*
Betaxolol	P450	4.8	3.0	0.9	*1.3*	*0.7*
Carbamazepine	CYP3A4 > 2C8/9	0.4	1.0	0.5	*1.0*	*0.3*
Chlorzoxazone	CYP2E1	5.1	5.3	1.4	*5.2*	*1.3*
Clozapine	CYP1A2 >> UGT, 2D6, 3A4	6.1	4.6	1.4	*3.2*	*3.1*
Desipramine	CYP2D6 > UGT	10.3	3.1	0.1	*5.0*	*1.4*
Dextromethorphan	CYP2D6 > 3A/2C19	8.6	10.3	0.6	*7.3*	*0.7*
Diazepam	CYP2C19 > 3A	0.4	3.7	0.5	*2.5*	*0.9*
Diclofenac	CYP2C9	4.2	13.9	0.8	*16.9*	*0.5*
Diltiazem	CYP3A4	12	8.2	0.5	*4.8*	*1.8*
Furosemide	P450	1.7	4.8	2.4	*2.0*	*1.7*
Imipramine	CYP2D6/1A2/2C19/3A/UGT1A4	8	10.5	0.3	*8.9*	*0.6*
Lorazepam	UGT	1.1	2.0	1.0	*0.7*	*0.6*
Nifedipine	CYP3A4	15	10.5	0.2	*10.4*	*1.0*
Propranolol	CYP2D6 > 1A2/2C19/UGT	13	10.7	0.4	*7.6*	*0.7*
Verapamil	CYP3A4	14	13.6	0.5	*12.9*	*1.0*
Warfarin	CYP2C9, 3A4	0.05	2.9	0.9	*1.5*	*0.8*

Conclusions

- Both MetMax™ and Intact Human Hepatocytes yielded data comparable to clinical systemic clearance
  One interesting finding was that MetMax™ hepatocytes were superior to pooled cryopreserved human hepatocytes in the evaluation of afatinib, a drug that is known to be subjected to FMO and GST.

The excellent in vitro-in vivo correlation plus the simplicity of the application of MetMax™ hepatocytes (storage in -80 deg. C, thaw-and-use (elimination of centrifugation and cell counting), are features supporting routine application of this novel experimental system in the high throughput screening of hepatic metabolic stability