XENOTECH
A BioIVT Company

Application of Hepatocytes for In Vitro Uptake Studies

David Buckley, Ph.D. - CSO

February 6th, 2013

dbuckley@xenotechllc.com

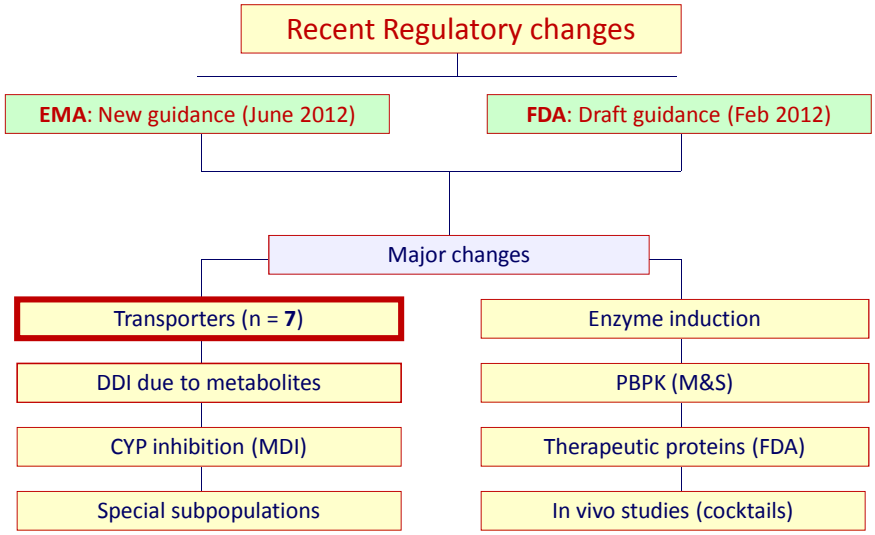
XENOTECH
A BioIVT Company

Presentation Overview – Today's Topics

- A basic review of concepts: a structure-based approach
- Selection of appropriate in vitro study type and design
- Description of the basic in vitro methodologies
  - oil-spin method and whole-system *vs* medium loss
  - uptake in to plated hepatocytes
- Discussion of the practical challenges
- Basic review on the estimation of active and passive uptake
- Estimation of fraction unbound in hepatocytes
- Prediction of in vivo hepatic clearance and scaling techniques

XENOTECH
A BioIVT Company

Recent regulatory changes

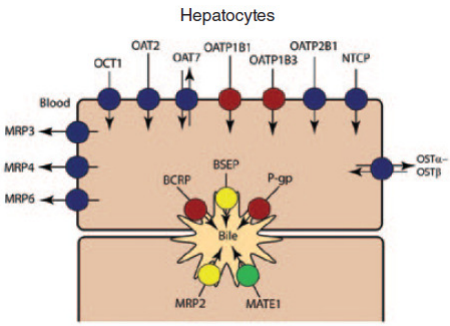


XENOTECH A BioIVT Company

3

Recommended hepatic transporters

Hepatic uptake and efflux transporters



FDA draft and EMA guidance (uptake)
- OATP1B1
- OATP1B3
- OCT1

FDA draft and EMA guidance (efflux)
- BCRP
- P-gp

ITC, others to consider
- BSEP (evidence of DILI)
- MRP2 (hyperbilirubinemia)
- MATE1 (cationic drugs)

XENOTECH A BioIVT Company

Adapted from Zamek-Gliszczynski et al., ITC Workshop, *Clin Pharm Ther* 92(5): 2012

4

Structure and physiochemical properties

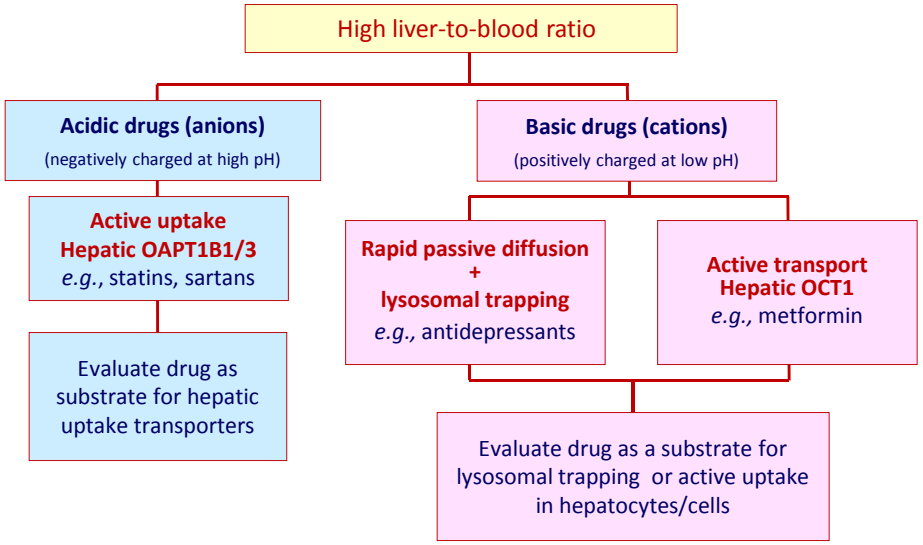
Physiochemical properties relevant to hepatic uptake

LogP (or $logD_{7.4}$)	The higher the value of lipophilicity, the more likely a drug will have a high rate of passive diffusion and require one or more biotransformation reactions to be eliminated
Polar surface area	If total PSA (TPSA) is 50 A^2 or less, the drug candidate will likely be completely absorbed, whereas if it's more than 150, none of it will be absorbed by passive diffusion.
pKa	This determines how high the pH must be for anionic drugs (acids, tetrazoles, phenols) to be negatively charged and how low the pH must be for cationic (basic) drugs to be positively charged.

XENOTECH A BioIVT Company

5

Physchem properties - hepatic uptake



XENOTECH A BioIVT Company

6

Methods for evaluation of uptake in hepatocytes

Three common *in vitro* methods

Oil-Spin Method	Medium loss method	Plated hepatocytes
Rapid (15-90+ sec) uptake of drug in hepatocyte suspension	Medium loss *vs* whole system loss of drug in hepatocytes suspension or plated	Rapid (15-90+ sec) uptake of drug in plated hepatocytes
Hepatocytes isolated from medium by centrifugation through silicone oil	Centrifuge hepatocytes to determine loss of drug from medium or whole system	Hepatocytes washed and then evaluate uptake of drug into cells or loss from medium

All methods can be employed to evaluate active uptake by transporters

Lysosomal trapping can be evaluated by medium loss or plated cell uptake methods

XENOTECH A BioIVT Company

7

Evaluation of uptake with various methods

Utility of various hepatocyte formats

- Product characterization
- Uptake (active/passive)
- Transporter phenotyping
- Inhibition potential
- Intrinsic clearance
- Metabolism vs. Transport

Hepatocyte Suspensions
Oil spin method
HepatoSure or CryostaX

Hepatocyte Suspensions
Medium-loss methods
HepatoSure or CryostaX

Plated Hepatocytes
Attaching hepatocytes

Selection of test system and methodologies will dependent on the application/purpose of the experiment.

XENOTECH A BioIVT Company

8

Basic methods for evaluation of uptake in hepatocytes

Basic methods – uptake? (yes/no)

Oil-Spin Method	Medium loss Method	Plated hepatocytes
Suspension hepatocytes incubated with single [S] at one or more times (15-90 sec)	Centrifuge hepatocytes to determine loss of drug (single, low [S]) from medium and evaluate metabolic stability	Hepatocytes washed and then evaluate uptake of drug into cells and loss from medium – similar to oil-spin design

Evaluate with either 'hot' or 'cold' drug

Active *vs.* Passive Uptake

Radioactive 'hot' test article: Evaluate hot TA +/– a molar excess of cold TA

Cold test article by LC-MS/MS: 37° *vs.* 4 °C ***OR***

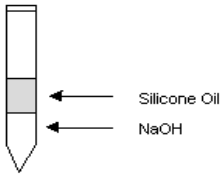
Presence or absence of specific transporter inhibitors (*e.g.* rifampin)

XENOTECH A BioIVT Company

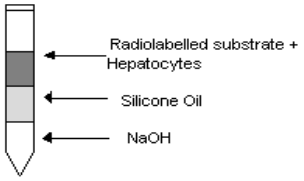
9

Hepatocyte uptake assays – Oil-spin method

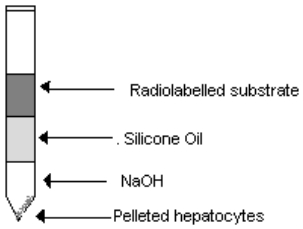
1) Oil tube setup (prior to uptake assay)



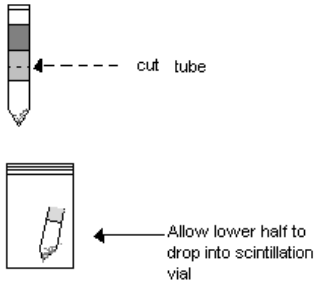
2) Prior to centrifugation



3) After centrifugation



4) Cutting the tube



XENOTECH A BioIVT Company

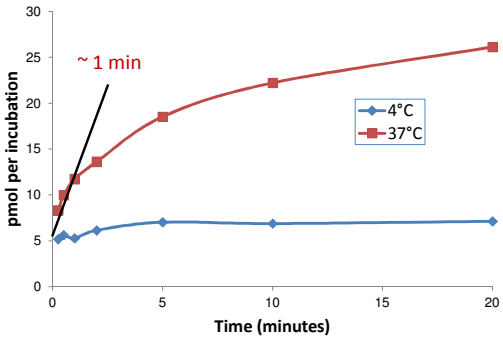
10

Hepatic uptake with the oil-spin method

Active uptake with pooled human hepatocytes (*HepatoSure*)

1) Determine active *vs.* passive uptake
Single, low [substrate] at several time points (0-90+ sec)
37 °C vs. 4 °C

Estrone-3-sulfate (1 µM)



2) Determination initial rate conditions – linearity with time

Note: Linearity with time is often lost in as little as 60 sec.

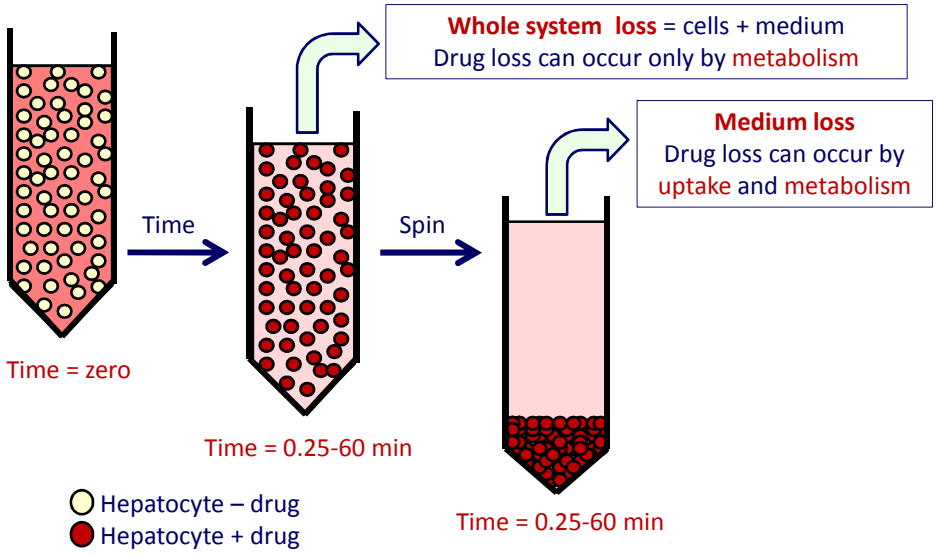
3) Calculate uptake rate under initial rate conditions (*e.g.* 1 min) (pmol/min/10^6 cells)

Data generated with ***HepatoSure***, XenoTech's 100 donor pooled hepatocytes

XENOTECH A BioIVT Company

11

Whole-system versus medium drug loss



Whole system loss = cells + medium
Drug loss can occur only by metabolism

Medium loss
Drug loss can occur by uptake and metabolism

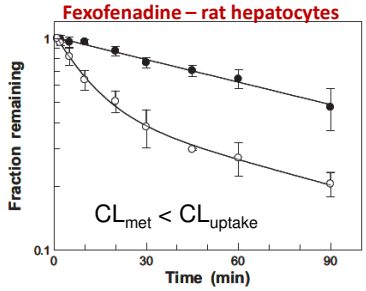
XENOTECH A BioIVT Company

12

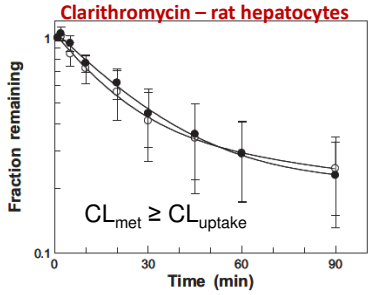
Drug depletion profiles (medium loss method)

Identifying rate-limiting processes and the role of passive diffusion

Fexofenadine – rat hepatocytes



Clarithromycin – rat hepatocytes



Metabolic clearance (black circles)
Relatively slow - minor

Medium clearance (open circles)
Relatively rapid - major

Metabolic clearance (black circles)
Relatively fast – metabolism clearance greater than uptake clearance

Medium clearance (open circles)
Rate-limiting step

XENOTECH A BioIVT Company

Figure adapted from Jigorel and Houston *et al., DMD*. 40:1596-1602, 2012

13

Hepatic uptake with plated hepatocytes

Active uptake with plated human hepatocytes

Test System:
Plated hepatocytes (6-48+ hr adaptation)
Serum-free medium (hrs)

Evaluate Uptake into cells
Single, low [substrate] at several time points (1-2 min)
+/- inhibitors, +/- excess cold compound

↓

Remove media at each time point and wash cells
Lyse cells and measure drug

↓

Determination initial rate conditions – linearity with time

↓

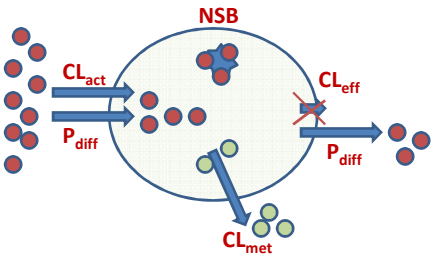
Calculate uptake rate under initial rate conditions (*e.g.* 1 min)
(pmol/min/10^6 cells)

XENOTECH A BioIVT Company

14

Approaches to estimating uptake clearance

Parameters and assumptions



Note: Typically assumes absence of efflux transport

Test System
Hepatocytes Suspension (human pooled)
Plated hepatocytes (attaching)

Uptake mechanisms
Active (CL_{act}) or Passive (P_{diff})

Cellular properties and metabolism
Non-specific binding or partitioning
Metabolism (CL_{met}) +/- 1-ABT

Efflux mechanisms
Active efflux (CL_{eff}) – Assumed negligible (internalization of efflux transporters)
Passive diffusion (P_{diff})

XENOTECH A BioIVT Company

15

Methods for evaluating uptake in hepatocytes

Methods for estimating clearance from hepatocytes

Oil-Spin Method	Medium loss Method	Plated hepatocytes
Suspension hepatocytes incubated with multiple [S] at multiple times (0-90 sec)	Centrifuge hepatocytes to determine loss of drug (single, low [S]) from medium plus and evaluate metabolic stability	Hepatocytes washed and then evaluate uptake of drug into cells or loss from medium
Plot velocity (y-axis) vs. substrate concentration (x-axis)	Plot concentration remaining (y-axis) vs. time (x-axis)	Plot velocity (y-axis) vs. substrate concentration (x-axis)
Total uptake intrinsic clearance Active uptake clearance (CL_{act}) Passive diffusion (P_{diff}) Fraction unbound in cell (fu_{cell})	Medium loss: Clearance (CL) from parent disappearance and metabolic CL Metabolism vs. transport	Total uptake intrinsic clearance Active uptake clearance (CL_{act}) Passive diffusion (P_{diff}) Fraction unbound in cell (fu_{cell})

+/- metabolism inhibitors (*e.g.* 1-ABT)

XENOTECH A BioIVT Company

16

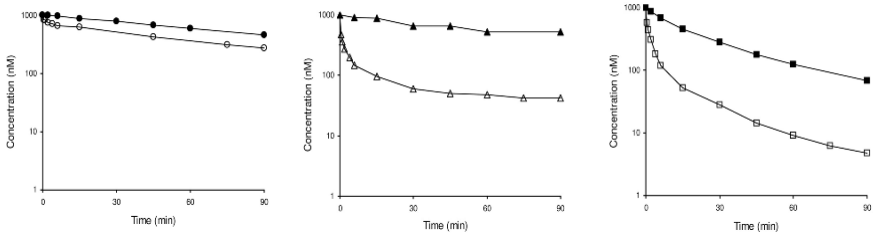
Application of the medium loss method

Medium loss is a better predictor of *in vivo* hepatic clearance than whole-system loss

Soars et al., (2007) tested 36 compounds for metabolic stability in rat hepatocytes (mostly anions and zwitterions)

Examined loss for the cell culture medium (open symbols) and the whole system (closed system)

A AZ10 (acid) B AZ14 (acid) C AZ20 (zwitterion)



XENOTECH A BioIVT Company

Adapted from Soars et al. *DMD* 36:695, 2008 and 35:859, 2007

17

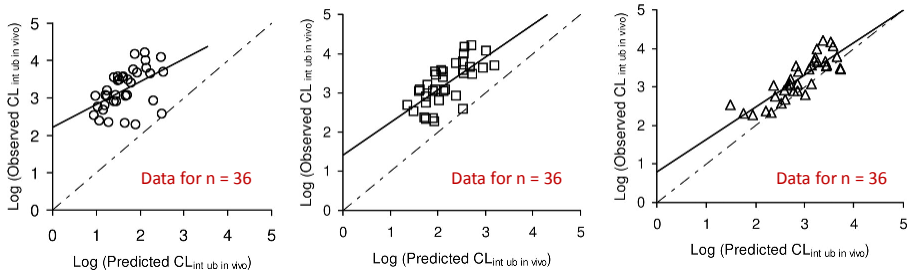
Application of the medium loss method

Medium loss is a better predictor of *in vivo* hepatic clearance than whole-system loss

A. Whole system loss (conventional method)
In vitro under-predicts

B. Medium loss from t = 0 s
Cl_{int} = Dose/$AUC_{0-\infty}$
Better prediction

C. Medium loss from t = 30 s
Cl_{int} = V x k_{el}
Even better prediction



Method C: Estimating rates beginning with the 0.5-min timepoint improves the prediction for drug candidates that undergo non-specific binding or biphasic kinetics

XENOTECH A BioIVT Company

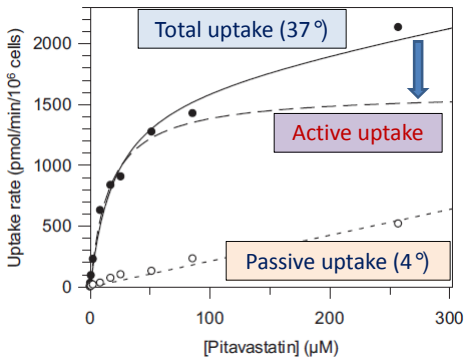
Adapted from Soars et al. *DMD* 36:695, 2008 and 35:859, 2007

18

Active uptake with oil-spin or plated methods

1) Uptake clearance with a conventional two-step approach

1) Determine active *vs.* passive uptake
Multiple [substrate] at several time points (0-2 min)
37 ℃ vs. 4 ℃



2) Determine uptake rates
(under initial rate conditions)

$$v = P_{diff} \times S$$

3) Determine uptake kinetics from initial uptake velocities (e.g. Grafit)

$$V = \frac{V_{max} \times S}{K_m + S} + P_{diff} \times S$$

4) Determine active uptake intrinsic clearance from active component
$CL_{act} = V_{max}/K_m$

XENOTECH A BioIVT Company

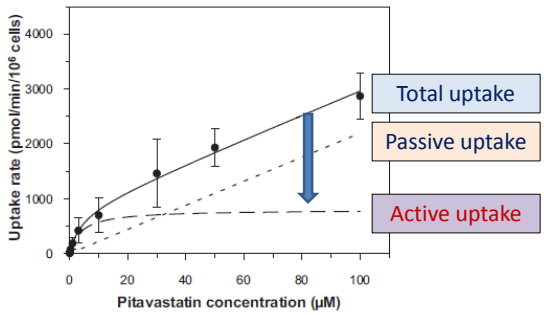
Figure adapted from Menochet *et al., JPET*. 341:2-15, 2012

19

Active uptake with oil-spin or plated methods

2) Mechanistic two compartment model – simultaneous fit

1) Determine active *vs.* passive uptake
Multiple [substrate] at several time points (0-2 min)
37 °C Only (4 °C optional)
+/- metabolism inhibitor (e.g. 1-ABT)



2) Cell and medium concentrations at steady-state
(ratio at equilibrium)
30-90 min time points

3) Processing – fit data to model with simultaneous fitting
(e.g. Matlab)

4) Establish multiple parameters
K_m, V_{max}, P_{diff}, CL_{act}, fu_{cell}, $[D]_{cell}$, $[D]_{med,u}$, (CL_{met})

XENOTECH A BioIVT Company

Figures adapted from Yabe *et al., DMD*. 39:1808-1814, 2011

20

Active vs. passive uptake into hepatocytes

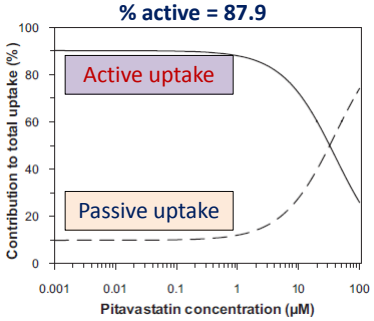
Impact of uptake mechanisms with increasing drug concentration

Pitavastatin

CL_{act} = 197 µL/min/10^6 cells

P_{diff} = 18 µL/min/10^6 cells

% active = 87.9

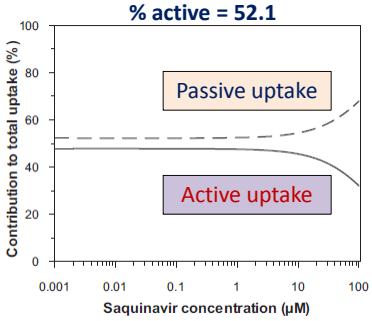


Saquinavir

CL_{act} = 239 µL/min/10^6 cells

P_{diff} = 191 µL/min/10^6 cells

% active = 52.1



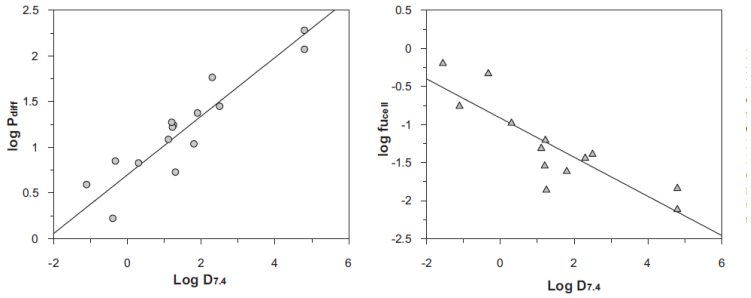
XENOTECH A BioIVT Company

Figure adapted from Yabe *et al., DMD*. 39:1808-1814, 2011

21

Influence of physiochemical properties

Passive diffusion and unbound fraction of drug in hepatocytes



Passive uptake increases proportionally with $logD_{7.4}$

↑permeability

Fraction of unbound drug decreases proportionally with $logD_{7.4}$

↑non-specific binding (and metabolism)

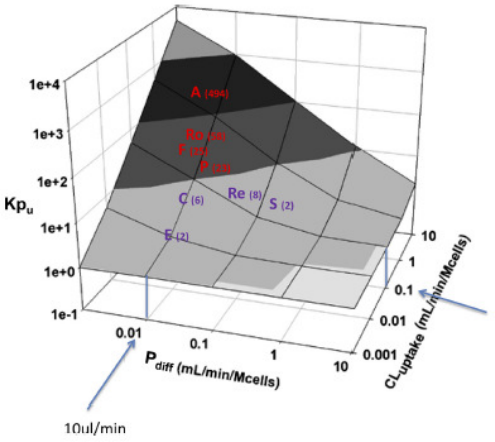
XENOTECH A BioIVT Company

Figure adapted from Yabe *et al., DMD*. 39:1808-1814, 2011

22

Drug concentration in hepatocytes

Relationship between passive diffusion and active uptake on hepatocyte drug concentrations



Hepatocytes-to-medium ratio - Unbound drug

$Kp_u = (CL_{act} + P_{diff})/P_{diff}$

Assumes negligible metabolism

High passive diffusion
P_{diff} dominates
[Hepatocyte] ≅ [Medium]

High active uptake
CL_{act} dominates
[Hepatocyte] >> [Medium]

XENOTECH A BioIVT Company

Figure adapted from Jigorel and Houston *et al., DMD*. 40:1596-1602, 2012

23

Drug concentration in hepatocytes – metabolism

Determining fraction of unbound drug in hepatocytes

Cell-to-medium ratio (total)

$$Kp_{total} = \frac{[D_{cell}]}{[D_{medium}]}$$

Cell-to-medium ratio (unbound)

$$Kp_u = \frac{(CL_{act} + P_{diff})}{(CL_{met} + P_{diff})}$$

Fraction unbound in hepatocytes

$$Kp_u / Kp_{total} = 1/fu_{cell}$$

Hepatic uptake and metabolism characteristics of eight drugs

Values are taken from Yabe et al., 2011.

Compound	CL_{active} ($\mu l \cdot min^{-1} \cdot 10^6 cells^{-1}$)	P_{diff}	Kp_u[a]	fu_{cell}[a]	Metabolism
Atorvastatin	1500	5.35	494	0.056	Extensive
Clarithromycin	50.7	10.9	6.05	0.024	Moderate
Erythromycin	11.4	12.2	2.00	0.05	Moderate
Fexofenadine	167	6.75	25.5	0.10	Minor
Pitavastatin	197	18.8	22.9	0.028	Minor
Repaglinide	299	58.2	7.94	0.036	Extensive
Rosuvastatin	418	7.08	58.2	0.48	Minor
Saquinavir	239	191	2.34	0.0075	Extensive

Metabolism can reduce the unbound drug in hepatocytes with high cell-to-medium ratios (*e.g.* atorvastatin)

High passive permeability vs. active uptake leads to low cell-to medium ratios (e.g. saquinavir)

XENOTECH A BioIVT Company

Eqns adapted from Yabe *et al., DMD* 39, 2011 and Nordell *et al., DMD* 2013 Epub
Figure adapted from Jigorel and Houston *et al., DMD*. 40:1596-1602, 2012

24

Effect of hepatocyte concentration in incubation

High hepatocyte concentrations in incubation decreases observed metabolic clearance for transported drugs

Result: Unbound cell-to-medium ratio decreases for actively transported drugs with increasing hepatocyte concentration

Lower [Drug] in hepatocytes (unbound) due to increased non-specific binding and partitioning (sink conditions)

Most notable for drugs with high unbound cell-to-medium ratio (*i.e.* uptake transporter substrates – atorvastatin)

Result: Decreased free [drug] = decreased $CL_{int,met}$

XENOTECH A BioIVT Company

Equations adapted from and Nordell *et al., DMD* 2013 Epub

25

Scaling in vitro clearance data to in vivo clearance

In vitro uptake clearance data typically under-predicts in vivo hepatic uptake clearance

Watanabe et al., (2009, 2010) recommended a '*scaling factor*' from rat hepatocyte uptake studies to predict *in vivo* intrinsic clearance data

This scaling factor determined in rat for each compound can then be used to scale human hepatocyte uptake to *in vivo* hepatic intrinsic clearance

	Uptake Clearance		Metabolic Clearance	Overall Intrinsic Clearance
	$PS_{inf,MID}$ [a]	$PS_{inf,vitro}$ [b]	$CL_{met,int,vitro}$ [c]	$CL_{int,all,vivo}$ [d]
	ml/min/g liver			
Pravastatin	6.48 ± 0.06	2.59 ± 0.14	0.793[e] ± 0.020	6.9–11
Pitavastatin	91.5 ± 8.5	53.3 ± 8.3	0.619 ± 0.434	42–53
Atorvastatin	42.1 ± 5.3	23.1 ± 2.5	0.910 ± 0.056	26–38
Fluvastatin	126 ± 12	29.2 ± 2.0	2.71 ± 0.24	85–154

[a] Determined by the MID analysis.
[b] Determined using rat hepatocytes at 0.1 μM.
[c] Determined using rat liver microsomes.
[d] Determined using the dispersion model with F_H, f_B, and Q_H (1.21–1.94 ml/min/g liver).
[e] Determined using rat liver S9 (Watanabe et al., 2009).

Scaling factor for uptake CL
$PS_{inf,MID}/PS_{inf,in\ vitro}$
(*i.e.* in vivo/in vitro)

Overall intrinsic clearance
Uptake clearance (hepatocytes)
+
Metabolic clearance (mcs)

XENOTECH A BioIVT Company

Figure adapted from Watanabe *et al., DMD.* 38:215-222, 2010
Watanabe *et al., JPET* 328: 652-662, 2009

26

Scaling in vitro clearance data to in vivo clearance

Use of an IVIV scaling factor determined from rat studies

Uptake clearance = scaling factor from rat *in vitro/ in vivo*
Metabolic clearance = microsomal data

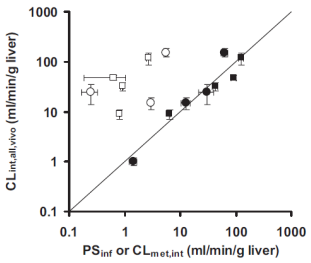


FIG. 4. Comparison of the hepatic overall intrinsic clearance of statins with the hepatic uptake clearance or metabolic clearance. In vivo hepatic overall intrinsic clearances of statins in rats and humans are plotted against the uptake clearance or metabolic clearance. ●, uptake clearance in humans predicted using scaling factor (Table 4); ○, metabolic clearance determined using human liver microsomes (Table 4); ■, uptake clearance in rats determined using a MID method (Table 3); □, metabolic clearance determined using rat liver microsomes (Table 3). The straight line indicates a 1:1 correlation. Each point represents the mean ± S.E. (n = 3).

Open symbols
Rat or human clearance scaled from metabolic clearance only (under-predicts)

Closed symbols
Rat or human clearance scaled from combined uptake and metabolic clearances (predicts well)

XENOTECH A BioIVT Company

Figure adapted from Watanabe *et al., DMD*. 38:215-222, 2010

27

Scaling in vitro clearance data to in vivo clearance

Application of scaling factors and inter-individual variability

Minochet et al., (2012) evaluated the variation in scaling factor needed to predict in vivo human hepatic clearance from hepatocyte uptake data

Empirical scaling factors applied to the active uptake clearances to recover the intrinsic uptake clearance in human livers

Drug	Scaling Factor			Mean
	HU4122	HU4199	HU8089	
Bosentan	16.1	13.0	24.8	17.9 ± 6.1
Pravastatin	6.67	ND	ND	6.67
Repaglinide	4.70	39.2	17.7	20.5 ± 17.4
Rosuvastatin	9.75	51.9	65.3	42.3 ± 29.0
Telmisartan	3.23	18.7	17.9	13.3 ± 8.7
Valsartan	0.740	2.00	2.20	1.64 ± 0.79
Mean	6.87 ± 5.46	25.0 ± 20.2	25.6 ± 23.7	17.1 ± 18.8

N.D., not determined, because $CL_{uptake,\ u}$ could not be measured accurately at a single concentration.

Scaling factor for uptake CL
Variable within a single donor based on drug
+
Variable between donors for a single drug

XENOTECH A BioIVT Company

Figure adapted from Minochet *et al., DMD*. 40:1744-1756, 2012

28

Inter-individual variability

In vitro uptake clearance can vary greatly between human hepatocyte donors

De Bruyn et al., (2011) observed 30-fold range in E3S uptake in 14 human hepatocyte donors

Menochet et al., (2012) reported variation in uptake clearance amongst three cultures of human hepatocytes from different individuals

Uptake kinetic parameters of rosuvastatin estimated in three human hepatocyte donors using a mechanistic two-compartment model

Uptake kinetics was measured in cryopreserved human hepatocytes plated for 6 h over 2 min at seven concentrations (0.1–300 μM).

Donor	$K_{m,u}$	V_{max}	$P_{diff,u}$	fu_{inc}	$CL_{active,u}$
	μM	pmol · min^{-1} · 10^6 cells^{-1}	μl · min^{-1} · 10^6 cells^{-1}		μl · min^{-1} · 10^6 cells^{-1}
HU4122	11.2	104	0.391	0.80	9.21
HU4199	9.80	16.6	0.148	1	1.69
HU8089	12.0	16.3	0.243	0.96	1.35
Mean ± S.D.	11.0 ± 1.1	45.5 ± 50.3	0.261 ± 0.123	0.93 ± 0.13	4.09 ± 4.44

V_{max} was variable amongst individuals
V_{max} ∝ transporter expression levels
Active uptake clearance was affected
Similar results were observed for repaglinide and rosuvastatin (next slide)

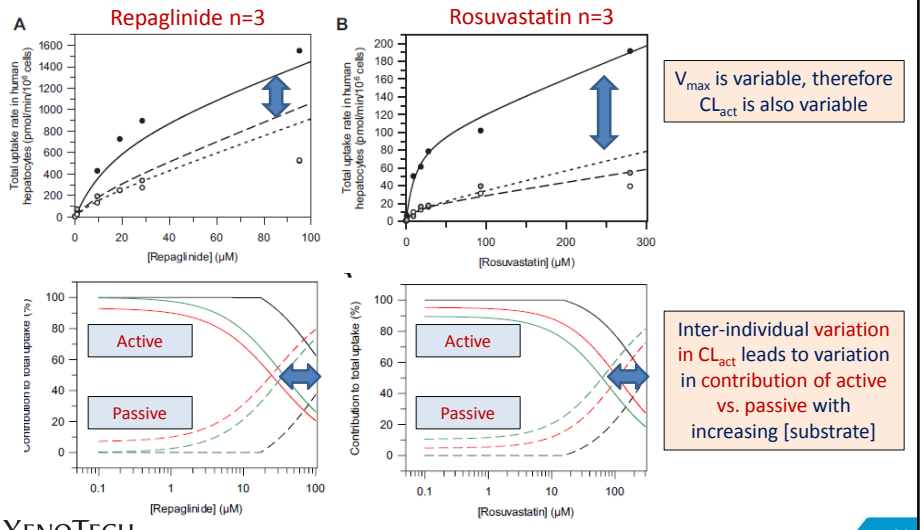
XENOTECH A BioIVT Company

Figure adapted from Minochet *et al., DMD*. 40:1744-1756, 2012

29

Inter-individual variability

Uptake clearance can vary greatly between human hepatocyte donors



V_{max} is variable, therefore CL_{act} is also variable

Inter-individual variation in CL_{act} leads to variation in contribution of active vs. passive with increasing [substrate]

XENOTECH A BioIVT Company

Figure adapted from Minochet *et al., DMD*. 40:1744-1756, 2012

30

Suspended hepatocytes vs. plated hepatocytes

Uptake clearance can be evaluated in suspended or cultured hepatocytes

Kimoto et al., (2012) evaluated the differences in uptake clearance in suspended and cultured hepatocytes from multiple donors *(pravastatin, CCK-8, and rosuvastatin…all largely cleared by OATP-mediated uptake)*

substrate	uptake (μL/min/mg protein) suspension	SCHH	percent of suspension (%)
lot Hu4163/ Hu4165			
pravastatin	1.95 ± 0.62	3.44 ± 1.34	177
CCK-8[b]	2.44 ± 0.24	1.06 ± 0.11	43
rosuvastatin	7.80 ± 3.1	8.15 ± 1.62	105
lot 109			
pravastatin	2.38 ± 0.95	2.45 ± 0.68	103
rosuvastatin	11.2 ± 0.43	10.8 ± 1.95	97

Uptake CL in suspension vs. plated hepatocytes
Can be variable between formats from the same donor
OR
Could be similar between formats from the same donor

XENOTECH A BioIVT Company

Figure adapted from Kimoto *et al., Molecular Pharmaceutics*. Epub, Nov. 2012

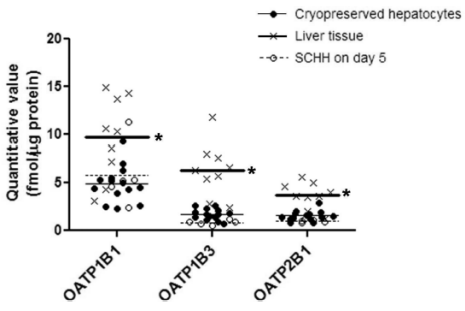
31

Suspended hepatocytes vs. plated hepatocytes

Uptake clearance can be evaluated in suspended or cultured hepatocytes

Kimoto et al., (2012) also evaluated the protein expression of multiple OATP transporters in the following formats:

1) Cryopreserved human hepatocyte suspensions
2) Sandwich cultured human hepatocytes (day 5)
3) Liver tissue from multiple donors



OATP protein expression
Generally higher in liver tissue than in either suspended or plated hepatocytes

Little difference between suspended and plated hepatocytes

XENOTECH A BioIVT Company

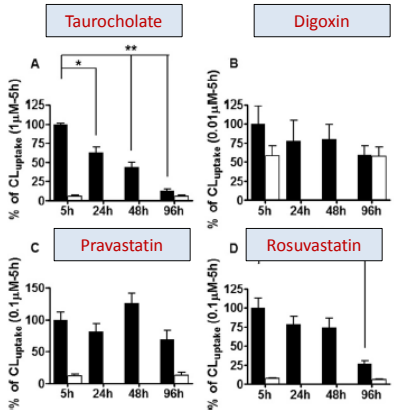
Figure adapted from Kimoto *et al., Molecular Pharmaceutics*. Epub, Nov. 2012

32

Time-dependent changes in transporter expression

Uptake clearance in plated hepatocytes varies over time in culture

Kimoto et al., (2012) evaluated the time-dependent changes in uptake clearance of various transporter substrates in cultured ***rat*** hepatocytes



Uptake of taurocholate (NTCP) and rosuvastatin (OATP) decreased over time in cultured rat hepatocytes

XENOTECH A BioIVT Company

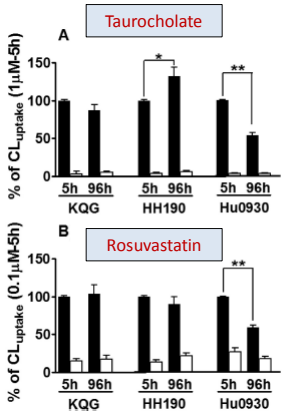
Figure adapted from Kotani *et al., DMD*. 39: 1503-1510, 2011

33

Time-dependent changes in transporter expression

Uptake clearance in plated hepatocytes varies over time in culture

However, transporter function in sandwich cultured human hepatocytes demonstrated less variation from 5 hr to 96 hr in culture



Uptake of taurocholate (NTCP) and rosuvastatin (OATP) decreased slightly over time in cultured human hepatocytes

However, the decrease over 96 hr was less pronounced than that observed in rat hepatocytes

XENOTECH A BioIVT Company

Figure adapted from Kotani *et al., DMD*. 39: 1503-1510, 2011

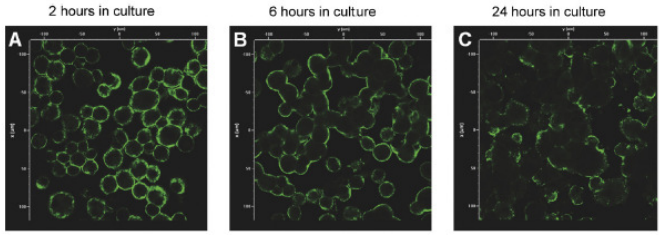
34

Time-dependent changes in transporter expression

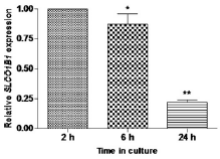
Uptake clearance in plated hepatocytes varies over time in culture

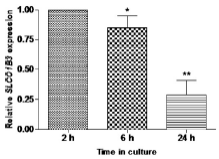
Ulvestad et al., (2011) demonstrated that OATP1B1 and OATP1B3 protein expression decreases from 2 hr to 24 hr in cultured human hepatocytes

Confocal microscopy of OATP1B1 in cultured human hepatocytes



OATP1B1





OATP1B3

XENOTECH A BioIVT Company

Figures adapted from Ulvestad *et al., Biochem Pharm*. 82: 1219-1226, 2011

35

Inter-system relative activity factors (RAFs)

RAF's can be employed to evaluate the contribution of specific uptake transporters based on *in vitro* hepatocyte and cell-line data

Hirano et al., (2004) evaluated the relative contribution of OATP1B1 and OATP1B3 to the hepatic uptake of pravastatin

OATP1B1

$$R_{\mathrm{act,OATP2}} = \frac{\mathrm{CL_{Hep,E-sul}}}{\mathrm{CL_{OATP2,E-sul}}}$$

OATP1B3

$$R_{\mathrm{act,OATP8}} = \frac{\mathrm{CL_{Hep,CCK-8}}}{\mathrm{CL_{OATP8,CCK-8}}}$$

$$\mathrm{CL_{hep,test,OATP2}} = \mathrm{CL_{OATP2,test}} \cdot R_{\mathrm{act,OATP2}}$$

$$\mathrm{CL_{hep,test,OATP8}} = \mathrm{CL_{OATP8,test}} \cdot R_{\mathrm{act,OATP8}}$$

$$\mathrm{CL_{hep,test}} = \mathrm{CL_{hep,test,OATP2}} + \mathrm{CL_{hep,test,OATP8}}$$

Selective substrate model to predict contribution of both OATP's

$$\mathrm{CL_{hep,test}} = \mathrm{CL_{OATP2,test}} \cdot R_{\mathrm{exp,OATP2}} + \mathrm{CL_{OATP8,test}} \cdot R_{\mathrm{exp,OATP8}}$$

Relative expression model (REF) to predict contribution of both OATP's

Data Required

Uptake CL of OATP1B1 and 1B3 specific probe substrates (E3S, CCK-8) in hepatocytes and cell lines

OR

Protein quantification data in cell lines and hepatocytes

Uptake CL of test drug in cell lines

XENOTECH A BioIVT Company

Figure adapted from Hirano *et al., JPET*. 311: 139-146, 2004

36

Estimation of free intracellular drug concentrations

Importance in predicting drug clearance and the potential for inhibition and induction of enzymes and transporters

Active uptake of drugs can be the rate-determining step in the systemic clearance of drugs, particularly anionic and zwitterionic drugs.

The free concentration of drug within the cell is available to inhibit drug metabolizing enzymes and transporters or cause enzyme induction via activation of nuclear receptors.

Growing regulatory interest

In their 2012 guidance, the EMA recommends estimation of unbound intracellular drug concentration when evaluating DDI potential with in vitro test systems (*e.g.* hepatocytes), especially for those drugs that undergo active uptake into cells or tissues.

XENOTECH A BioIVT Company

37

Approaches to estimating intracellular concentrations

There are a number of publications that estimate intracellular free fraction for clearance predictions (but not limited to the following publications)

Lu *et al*., *Drug Metab. Dispos*. 34:1600-1605, 2006
Paine *et al*., *Drug Metab. Dispos*. 36:1365-1374, 2008
Parker and Houston, *Drug Metab. Dispos*. 36:1375-1384, 2008
Yabe *et al*., *Drug Metab. Dispos*. 39:11808-1814, 2011
Menochet *et al*., *JPET*. 341:12-15, 2012
Nordell *et al., DMD* 2013 (epub)

Other publications estimate intracellular fraction unbound for CYP inhibition and transporter studies…potential surrogate endpoints.

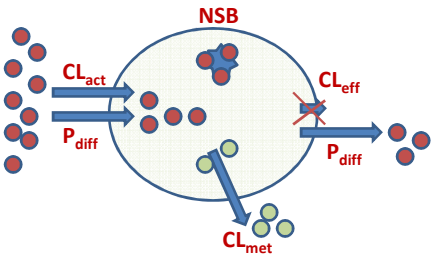
Grime *et al*., *Drug Metab. Dispos*. 36:1670-1678, 2008
Korzekwa *et al*., *Drug Metab. Dispos*. 40:865-876, 2012

XENOTECH A BioIVT Company

38

Approaches to estimating intracellular concentrations

The interplay between uptake and metabolism on the free concentration of drugs in hepatocytes



Note: Typically assumes absence of efflux transport

Metabolism >> Active or passive uptake
At low concentrations, the drug is metabolized rapidly and the free fraction is low, especially with high partitioning/binding (uptake-limited)
Hepatocytes are better predictors of clearance (e.g. midazolam[1])

Active uptake >> Metabolism
Drug can accumulate and ↑ free fraction.
Hepatocytes are better predictors of clearance due to uptake transporters (metabolism-limited)
(e.g. statins, acids, etc.)

Passive uptake >> metabolism
Drug concentration equilibrates with media
Subcellular fractions typically predict clearance

XENOTECH A BioIVT Company

Lu *et al., DMD*. 34:1600-1605, 2006[1]

39

XenoTech's Featured Human Hepatocyte Products

CryostaX™

Customizable, single-freeze pooled hepatocytes (n = 2 to 20)

HEPATOSURE™

Multi-freeze pooled hepatocytes (100 donor pool)

Attaching Human Hepatocytes

Single donor lots characterized for CYP induction and/or uptake transporters

XENOTECH A BioIVT Company

40


XENOTECH
A BioIVT Company
Thank You
Comments or Questions?
dbuckley@xenotechllc.com
XENOTECH
A BioIVT Company
www.xenotechllc.com | info@xenotechllc.com