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Should Total Plasma Drug Concentration Be Used to Predict Transporter Mediated Drug-Drug Interactions for Highly Protein Bound Drugs?

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Abstract

Despite the significant progress made in the drug transporter field, predicting clinical Drug-Drug Interactions (DDIs) based on in vitro transporter studies still remains a major challenge. Specifically, the extent of DDIs in terms of changes in Cmax and AUC of victim drugs is often significantly underestimated using in vitro transporter inhibition constants (Ki/IC50), as exemplified by the well-studied OATP mediated DDIs between statins and rifampicin, and the recently reported dolutegravir-metformin interaction. Using OATP1B1 and OCT2 as examples, this talk will discuss the effect of protein binding on "in vivo" transporter inhibition and present a "binding equilibrium shift" hypothesis to explain our experimental findings. Dose-dependent OCT2 and OATP1B1 inhibitions by various drugs with both high and low plasma protein binding, were assessed in protein-free HBSS and protein-rich solutions including HBSS with 4% albumin, 100% human serum and 100% bovine serum. Strikingly, for highly protein bound drugs, unbound fraction (fu) adjusted IC50 values determined in protein-rich solutions were significantly lower than that obtained from assays using protein-free HBSS. For example, dolutegravir, a HIV integrase inhibitor with >99% protein binding in the human, was significantly more potent in inhibiting OCT2 mediated metformin transport in serum than in HBSS, with fu adjusted IC50 values of 87nM and 536nM, respectively. In addition, the fu adjusted permeability of dolutegravir tested in serum was also >5x higher than that assessed in HBSS. On the contrary, the fu adjusted IC50 for low protein bound Cimetidine (in vivo protein binding ~20%) was independent on assay matrix. These data suggest that for highly protein bound drugs, the "actual" free drug concentrations in presence of transporters and other drug binding membrane proteins could be substantially higher than the calculated free concentration based on fu measured in vitro, as high affinity binding to cell membrane proteins could effectively change the equilibrium of nonspecific binding between drug and serum proteins. Lastly, the talk will present a partial PBPK model incorporating total plasma drug concentration and in vitro transporter IC50s obtained with human serum, demonstrating improved IVIVC of dolutegravir and rifampicin associated DDIs. These studies, for the first time to our best knowledge, showed that for highly protein bound drugs, conventional approach based on unbound drug concentration may lead to underestimation of *in vivo* transporter inhibition. As such, we may consider conducting in vitro transporter studies in human serum and using total plasma drug concentration for modeling and prediction of transporter mediated DDIs.

Background on Protein Binding

- At binding equilibrium between Albumin (A) and Drug (D) *in vitro*, the binding dissociation constant $K_d = k_{off}/k_{on}$.
- Unbound fraction at equilibrium is given, $f_u = \frac{[D]}{[AD]+[D]}$
- However, in the presence of additional disposition mechanism, is fu measured under equilibrium condition appropriate for estimating the effective unbound drug concentration in vivo?

$$A + D \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} AD$$

Binding shift in presence of transporter?

- Traditional way of *in vitro – in vivo* extrapolation (IVIVE) and DDI prediction goes

1. Measure IC50 in protein free assay buffer (e.g. HBSS or Krebs-Henseleit buffer)
2. Measure drug *fu* at equilibrium *in vitro* (e.g. rapid equilibrium dialysis)
3. Use *fu* to adjust plasma concentration *in vivo* (C_{total} x *fu*) to predict substrate clearance and DDI potentials for perpetrators (e.g. $R = 1 + fu \cdot [C_{max}]/Ki$)

- Nevertheless, this process required drug-dependent scaling factors for uptake transporters to correlate the observed drug clearance (Jones, H. et al, 2012 DMD). Particularly, Duan, P. et al. reported that such approach under-predicted OATP1B1 inhibition potency of certain perpetrators, e.g. rifampicin-pitavastatin DDI.

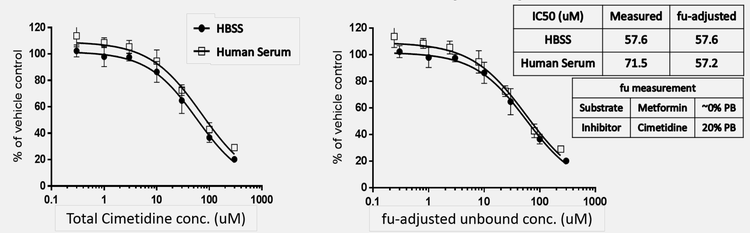
Pitavastatin – Rifampicin DDI Prediction on AUCR (95% CI) (Modified from P., Duan, P. Zhang and L. Zhang, US FDA, ASCPT 2015, PT-17)		
Predicted AUCR	Predicted AUCR with adjusted OATP1B1 Ki (10 fold less)	Observed AUCR
1.89 (1.69-1.98)	5.96 (4.69-6.01)	6.74 (4.73-8.74)

Methods: Assessing Transporter Inhibition in HBSS and Human Serum

1. MDCK cells were seeded at 60K cells/well and cultured on PCF porous membrane inserts (0.4 µm pore size, Millipore) using standard culture conditions.
2. Cells were transiently transfected by proprietary Opti-Expression™ using cDNAs encoding OATP1B1 or OCT2 alone, or five transporters shown below.
3. Radioisotope-labeled transporter substrates were applied to the basal side of the cell membrane for uptake assays in presence of various inhibitor concentrations. Actual inhibitor concentrations were measured with LC/MS.
4. Transport assays were conducted in HBSS only or in 100% human serum (HS).

Result 1: Protein Binding Effect on IC50

1. Cimetidine inhibition on Metformin transport by OCT2

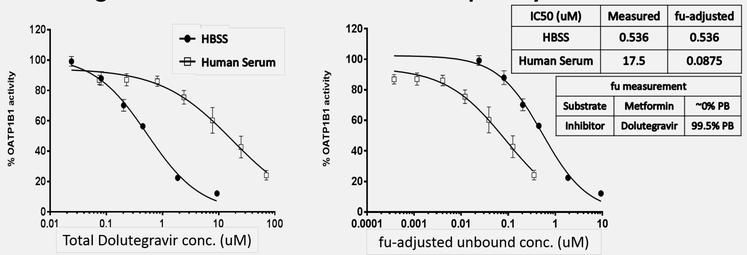


IC50 (uM)	Measured	fu-adjusted
HBSS	57.6	57.6
Human Serum	71.5	57.2

fu measurement		
Substrate	Metformin	~0% PB
Inhibitor	Cimetidine	20% PB

- IC50 of the low protein binding Cimetidine was not affected by serum protein after fu adjustment.

2. Dolutegravir inhibition on Metformin transport by OCT2

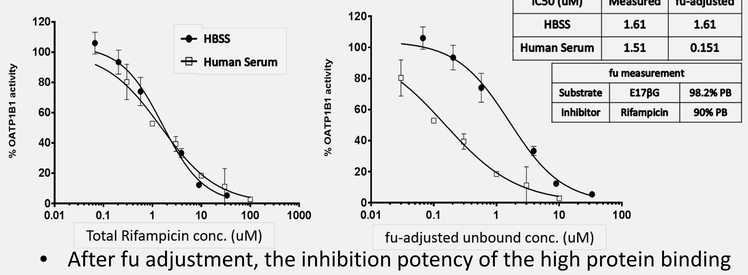


IC50 (uM)	Measured	fu-adjusted
HBSS	0.536	0.536
Human Serum	17.5	0.0875

fu measurement		
Substrate	Metformin	~0% PB
Inhibitor	Dolutegravir	99.5% PB

- After fu adjustment, the inhibition potency of the high protein binding Dolutegravir in human serum was 6.2x higher than that assessed in HBSS

3. Rifampicin inhibition on E17βG transport by OATP1B1



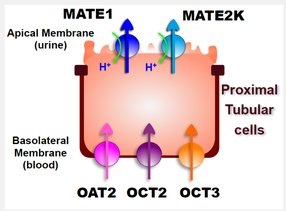
IC50 (uM)	Measured	fu-adjusted
HBSS	1.61	1.61
Human Serum	1.51	0.151

fu measurement		
Substrate	E17βG	98.2% PB
Inhibitor	Rifampicin	90% PB

- After fu adjustment, the inhibition potency of the high protein binding Rifampicin in human serum was 10x higher than that assessed in HBSS

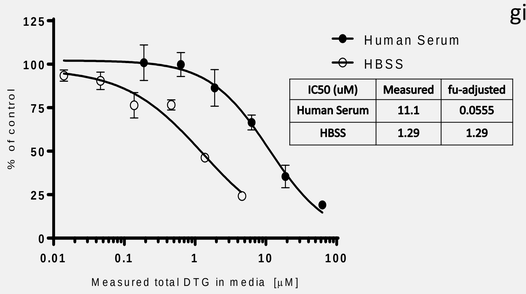
Result 2: Dolutegravir IC50 measurement for IVIVE (Quintuple model)

- MDCK-II monolayers expressing a combination of five transporters known as "Quintuple model" with basal OCT2, OAT2, OCT3 and apical MATE1 and MATE2K.
- Radiolabeled Metformin was measured after B>A transcellular transport.



"Quintuple Model" – Zhang Y. et al., 2015 DMD

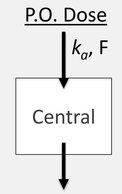
Dose dependent inhibition of B>A transport



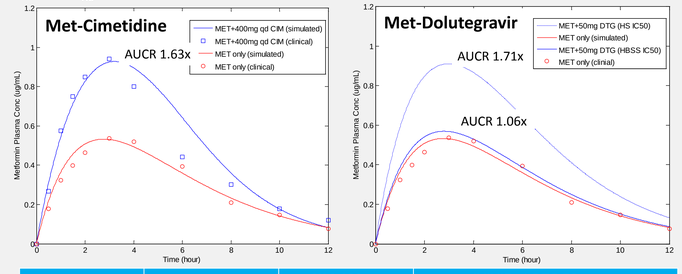
IC50 (uM)	Measured	fu-adjusted
Human Serum	11.1	0.0555
HBSS	1.29	1.29

Result 3: Transporter DDI Modeling

1. Metformin-Dolutegravir DDI as result of renal OCT/MATE inhibition



- Metformin 100% renally cleared, fitted to PK data from literature[1]
- CL_{ns} (net secretion clearance for control), is modified in presence of an inhibitor with [I] and Ki: $CL_{ns}(+I) = \frac{CL_{ns}}{1+\frac{[I]}{Ki}}$
- Inhibitor-independent In vitro to in vivo inhibition scaling factor was extrapolated from the metformin-cimetidine DDI data (Left)[1]
- Impact of DTG IC50 measured in HS or HBSS was shown (Right)



Predicted MET PK change	MET alone (fitted)	CIM 400mg (Single dose)	IVIVIE of DTG 50mg qd (Single dose)	
		in vitro assay in human serum	Using IC50 from human serum	Using IC50 from HBSS
Cmax Ratio	0.94	1.63 vs. 1.65[1]	**1.60 vs. 1.66[2]**	0.99 vs. 1.66[2]
$AUC_{(0-12)}$ Ratio	1.0	1.63 vs. 1.47[1]	**1.71 vs. 1.79[2]**	1.06 vs. 1.79[2]

2. Pitavastatin-Rifampicin DDI as result of hepatic OATP1B1 inhibition

- Rifampicin PK model: minimal PBPK with gut absorption, renal and hepatic clearances fitted to two sets of PK data in literature[3]
- Pitavastatin PK model: modified from extended clearance concept model with 5 extrahepatic compartment (EHC) model[4] with hepatic elimination
- Simultaneous simulation with only OATP1B1 mediated uptake clearance (PSinf) is inhibited by plasma rifampicin concentration. Two IC50s obtained from HBSS or 100% human serum were compared.

P.O admin (FaFg=1)	Pitavastatin 2mg only	Pitavasatatin 2mg with 600 mg Rifampicin	
Simulated Ki	No inhibitor	Ki = 16 uM (from IC50 in HBSS)	Ki = 1.5 uM (from IC50 in human serum)
Cmax Ratio	28.0	46.3 (1.7x)	124.2 (4.4x)
AUC Ratio	41.5	70.7 (1.7x)	319.7 (7.7x)

Conclusion

- For highly protein bound inhibitors, Dolutegravir and Rifampicin, the apparent IC50s measured in 100% human serum were significantly lower than what were calculated based on fu and the intrinsic transporter IC50s assessed in HBSS. For low protein binding Cimetidine, such difference was not observed.
- When IVIVE model was simulated with IC50 measured in human serum, Dolutegravir appeared to be a clinically relevant inhibitor of renal secretion of metformin, which is mediated primarily by OCT2 and MATEs.
- Pitavastatin-Rifampicin associated DDI was predicted to be similar to the previously observed AUCR data (background) only when applied with IC50 measured in human serum.
- For highly protein bound inhibitors, it may be useful to obtain their apparent IC50s by running *in vitro* assays in 100% human serum to predict their DDI potentials, e.g., given as ratio R in the presence of an inhibitor.

$$R = 1 + \frac{fu \cdot Ima_x}{Ki_{\_HBSS}} \Rightarrow R = 1 + \frac{fu \cdot I_{max}}{fu \cdot Ki_{\_Human\ Serum}} = 1 + \frac{I_{max}}{Ki_{\_Human\ Serum}}$$

Reference

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2. Zong, J. et al., J Int AIDS Soc. 2014 Nov 2;17 (4 Suppl 3):19584
3. Varma M.V. et al., Pharm Res., 2012 Oct;29(10):2860
4. Watanabe T. et al., J Pharmacol Exp Ther., 2009 Feb;328(2):652

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