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Transporter-Induced Protein Binding Shift (TIPBS): Hypothesis and Modeling

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OPTIVIA BIOTECHNOLOGY

Abstract

Transporters are key modulators of drug disposition. Therefore, predicting transporter mediated drug clearance and potential drug-drug interactions (DDI) is crucial. Currently, as a surrogate method for *in vitro-in vivo* correlation (IVIVC), cell-based assays adopt the '**equilibrium model**' where transporter kinetics are measured in protein-free buffers *in vitro* (e.g. HBSS without albumin), then adjusted by drug unbound fraction at equilibrium, f_u (e.g. measured by rapid equilibrium dialysis), for *in vivo* prediction. However, we observed that this approach tends to under-estimate the disposition of certain highly-protein bound drugs, often requiring large scaling factors for IVIVC.

Hence, we hypothesized that transporters may shift drug-serum protein binding from equilibrium by creating a sink condition for its high-affinity substrates. Using a mathematical model, we aimed to quantitatively demonstrate the substrate's higher binding affinity towards a transporter can influence the binding equilibrium between the drug and serum protein (SP). '**Dynamic models**' with a series of differential equations describing cellular uptake of a substrate and its competitive inhibition were constructed, in order to simulate the contribution of SP on drug transport and its inhibition, as well as to contrast the results to that of an equilibrium model calculated based on f_u-adjustment. Scenarios were, 1) uptake of a highly SP-bound substrate +/- SP, with varying transporter abundance and binding affinity toward transporter; 2) competitive inhibition of a substrate uptake without protein binding, by a highly protein bound inhibitor, +/- SP.

As a result, the dynamic model for a highly protein bound substrate (f_u=0.015 or 98.5% bound) showed the transport rate in the presence of 660 uM of SP can be much higher than the equilibrium model when the substrate had markedly higher affinity for the transporter than for SP (K_{d_tp}/K_{d_ad}=0.1 or less). Also, the extent of such shift depends on transporter abundance and substrate uptake rate constant k_{uptake}. Expectedly, when a highly protein bound inhibitor was also a transporter substrate, such a shift became apparent, impacting the apparent inhibition potency (e.g. IC_{50}). The dynamic model with highly bound inhibitor (f_u=0.015) in presence of SP showed that the conventional f_u adjustment method could underestimate up to 42 fold (as if it was less potent).

Substantiated by our previously reported *in vitro* observations[1], the current theoretical simulation showed that disposition kinetics may appear differently for highly protein bound transporter substrates and inhibitors assessed with or without serum protein in medium (e.g. serum, HBSS with albumin). Such phenomena, namely, **Transporter-Induced Protein Binding Shift (TIPBS)**, may cause underestimation of kinetic parameters acquired by application of f_u-adjustment and could contribute to the discrepancies in predicting transporter-mediated drug clearance and DDIs *in vivo*.

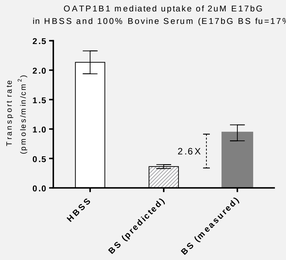
Background

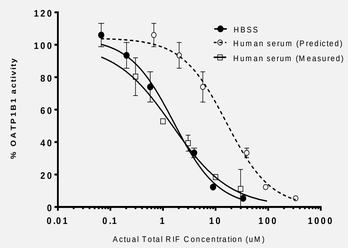
- Transporter kinetic parameters (Vmax, Km, and K_i /IC_{50}) are typically measured *in vitro* in protein free buffers (e.g. HBSS). Current methods for calculating active transport and its inhibition in serum or serum-like media requires the use of fraction unbound (f_u) as the approach is based on the free-drug hypothesis and the assumption of rapid serum protein binding. In other words, drugs are in binding equilibrium with proteins in serum and only the free fraction (f_u) is transported or has inhibitor effect. This method is known for requiring drug-dependent scalar in predicting *in vivo* clearance[2], it also underestimates *in vivo* inhibition potency of some highly protein bound transporter inhibitors[3].

$$J_{uptake,serum} = C_{total} \times f_u \times \frac{V_{max}}{K_m}, \qquad IC_{50,serum} = IC_{50,in\ vitro}/f_u$$

- By comparing *in vitro* transport and inhibition data measured in protein free buffer and in 100% serum, we found the f_u adjustment may not be compatible with some highly protein bound compounds.

Example. Effects of serum protein binding on OATP1B1 mediated E17bG uptake (left) and its inhibition by Rifampicin (Right). The conventional f_u adjustment approach underestimated both E17bG uptake and rifampicin IC_{50} in serum.





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

Assay buffer	IC50 (uM)
HBSS (protein free)	1.61
Human serum (calculated)	16.1
Human serume (measured)	1.51

Transporter-Induced Protein Binding Shift (TIPBS) Hypothesis

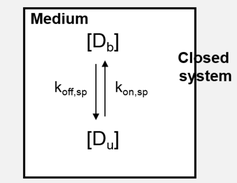
We hypothesize that,

- Higher binding affinity of a drug to plasma-facing uptake transporters may displace drug binding to serum proteins, "pulling" more drug molecules to be transported or to inhibit the transporter.
- The extent of TIPBS would depend on drug binding affinities to both serum proteins and transporters, transporter abundance and transport rate.
- TIPBS may not directly influence an efflux transporter whose binding site(s) are inside cells; however, it may indirectly impact efflux activity through affecting intracellular drug concentration via acting on a uptake transporter.

Modeling: Equilibrium vs. Dynamic

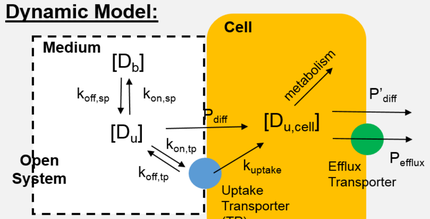
In an equilibrium dialysis tube used for measuring f_u, the medium is a **closed system**, where rapid equilibrium of drug-protein binding can be reached. However, in cell-based assay or in clearance organs (liver or kidney), the medium is an **open system**, where drug binding and transport may stay dynamic without reaching binding equilibrium.

Equilibrium Model:



$$D_u + SP_u \underset{k_{off,sp}}{\overset{k_{on,sp}}{\rightleftharpoons}} SPD_b$$

Dynamic Model:



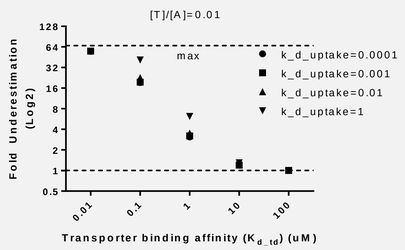
$$SPD_b \underset{k_{on,sp}}{\overset{k_{off,sp}}{\rightleftharpoons}} SP_u + D_u + TP_u \underset{k_{off,tp}}{\overset{k_{on,tp}}{\rightleftharpoons}} TPD_b$$

$$P_{diff}: D_{u,cell}, \quad k_{uptake}$$

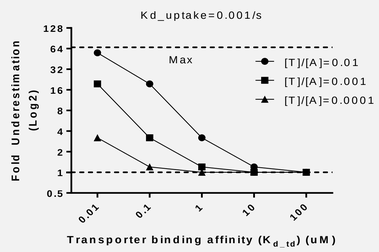
Scenario 1. Substrate Protein Binding Shift

- Assumptions: For both transporter and serum protein (SP), dissociation constants k_{off} are fixed at 0.01/s. SP binding affinity (K_{d_ad}) is 10 uM (f_u approximating 0.015). No passive diffusion (P_{diff}=0).
- Simulation: the effects of [TP] and drug affinity towards the transporter (K_{d_td}), in presence or absence of 660 uM SP. The latter represents the buffer only (HBSS) condition. Intracellular concentration over time represents rate of uptake transport, $[TPD_b]*k_{uptake}$.
- Results are demonstrated as Fold Underestimation, an indicator to describe the extent of protein binding shift, defined by the fold difference of the intracellular concentration of $D_{u,cell}$, at two conditions: uptake rate with SP vs. f_u-adjusted uptake rate without SP.

$$\textbf{Uptake Fold Underestimation} = \frac{uptake_{w}SP}{fu \times uptake_{w/o}SP}$$



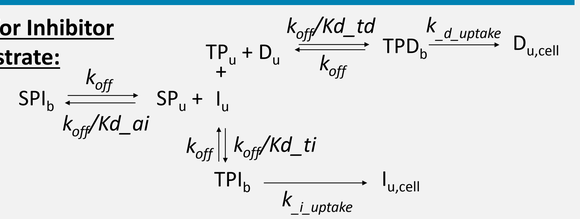
Result 1-1. Effect of rate constant k_{uptake} is relatively small after parameter sensitivity analysis. However, the shift is most influenced by the affinity towards the transporter (K_{d_td}) compared to affinity towards the serum protein (K_{d_ad}). Therefore, for future simulation, we fixed the k_{uptake} to be 0.001/s and expect only a minor influence from this parameter.



Result 1-2. Effect of transporter abundance ([T]) is relatively large. When [T]/[A] ratio = 0.01 was scanned for parameter sensitivity, a 15% shift was observed at K_{d_td}=10 uM. However, at [T]/[A] ratio = 0.0001, such shift was delayed until K_{d_td}=0.1 uM.

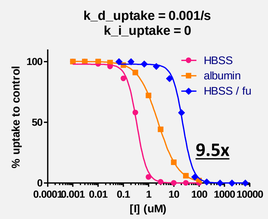
Scenario 2. Inhibitor Protein Binding Shift

Dynamic Model for Inhibitor that is also a substrate:

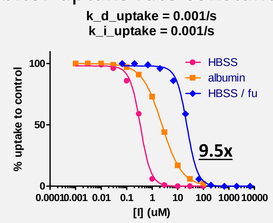


- Assumptions: inhibitor dissociation constant to SP (K_{d_ai}) was set to 10 uM (f_u approximating 0.015). Substrate has no protein binding. No passive diffusion (P_{diff}=0) for drug substrate and inhibitor.
- Simulation: the effects of [TP] and inhibitor binding affinity towards the transporter (K_{d_ti}), with or without 660 uM SP. IC50 curve at varying initial inhibitor concentration was demonstrated as % activity, representing relative amount of substrate uptake at given inhibitor concentration, normalized to control ([I]=0).

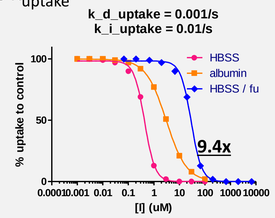
Result 2-1. Effect of inhibitor uptake rate constant k_{uptake}



	HBSS	albumin	HBSS / fu
IC50	0.3401	2.388	22.67

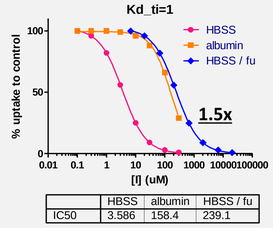


	HBSS	albumin	HBSS / fu
IC50	0.3462	2.425	23.08

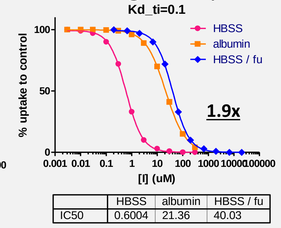


	HBSS	albumin	HBSS / fu
IC50	0.4339	3.064	28.93

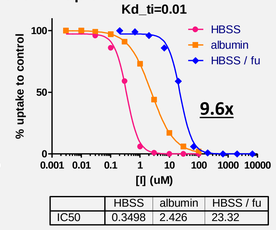
Result 2-2. Effect of inhibitor binding affinity to the transporter



	HBSS	albumin	HBSS / fu
IC50	3.586	158.4	239.1

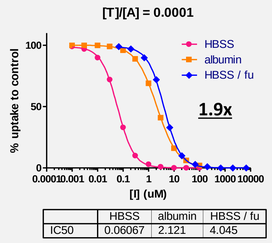


	HBSS	albumin	HBSS / fu
IC50	0.6004	21.36	40.03

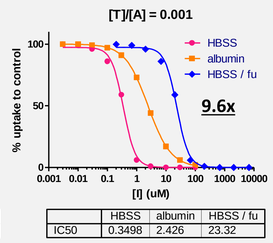


	HBSS	albumin	HBSS / fu
IC50	0.3498	2.426	23.32

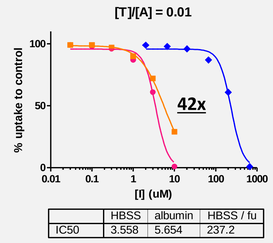
Result 2-3. Effect of transporter abundance



	HBSS	albumin	HBSS / fu
IC50	0.06067	2.121	4.045



	HBSS	albumin	HBSS / fu
IC50	0.3498	2.426	23.32



	HBSS	albumin	HBSS / fu
IC50	3.558	5.654	237.2

Conclusion

- A novel hypothesis of transporter-induced protein binding shift (TIPBS) is proposed as a possible theoretical basis for previously observed drug transport and inhibition in the presence of serum protein binding, which can not be explained by the conventional equilibrium model /fu adjustment method.
- We have constructed a dynamic model and simulation scheme that successfully validates the TIPBS hypothesis. Moreover, our parameter sensitivity analyses suggest that drug binding affinity to transporter and transporter abundance may be two dominant parameters that affect the extent of TIPBS.
- Without factoring in such transporter- and drug- dependent TIPBS effects, the conventional f_u adjustment method may substantially under-estimate *in vivo* clearance or DDI potential for certain highly protein bound drugs.

References

1. M. Jahic, J. Baik, et. al., 13th EU ISSX 2015, P164, Glasgow
2. Jones et al., DMD (2012) 40: 1007
3. P. Duan, P. Zhang and L. Zhang, ASCPT 2015, PT-17, New Orleans