VIVD: a Virtual *In Vitro* Distribution Model for Predicting Intra- and Sub-Cellular Concentrations in Toxicity Assays

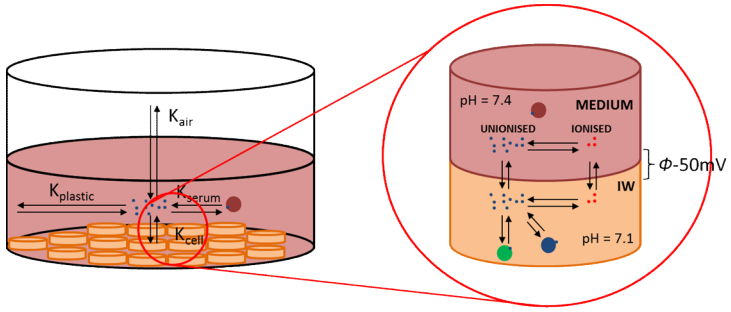
Ciarán Fisher, Masoud Jamei, Iain Gardner
Simcyp Ltd. (a Certara company), Blades Enterprise Centre, John Street, Sheffield S2 4SU, United Kingdom

Background

In vitro testing routinely uses nominal treatment concentrations as the driver for measured toxicity endpoints. However, test compounds can bind to the plastic of culture vessels or interact with culture media components, such as lipids and albumin. Additionally, compounds can partition into the air above culture media. Together these processes reduce free concentrations of compound to which cells are exposed and could lead to an over-prediction of toxic concentrations determined *in vitro*. Here we present a steady-state model for predicting free media concentrations of test compounds as well as intra-cellular and intra-organelle concentrations.

Methods

Figure 1. Schematic representation of the *in vitro* distribution model



The virtual *in vitro* distribution (VIVD) model predicts the unbound concentration in culture media by calculating a hypothetical volume of distribution accounting for binding to serum components, binding to plastic, distribution into the air in the headspace above media and distribution into the cultured cells (figure 1; equation 1). Distribution into the headspace is described by the Henry's law constant and binding to plastic is defined by a previously described model based on $logP_{ow}$ (Kramer, 2010).

$$C_{media,dissolved,u} = \frac{C_{nominal} fu_{FBS,dilu} V_{media}}{V_{media} + k_{air} fu_{i} V_{air} + k_{cell} V_{totalcell} + k_{plastic} SA_{media} \cdot 10^{3}}$$

Equation 1

Partitioning into cultured cells is described based on the Rodgers and Rowland approach adapted to incorporate ion permeability, the impact of membrane potential and subcellular organelles (equation 2; Rodgers *et al.*, 2005).

$$k_{cell,u} = \frac{C_{cell}}{C_{media_{unbound}}} = \begin{pmatrix} (1 - f_{lyso+}f_{mito}) \left(\begin{matrix} f_{iw}X_{lw} + P_{nl}f_{nl} + P_{np}f_{np} \\ +K_{aAP}[AP^{-}](Y_{1,iw} + Y_{2,iw}) \end{matrix} \right) \\ +f_{lyso} \left(\begin{matrix} f_{iw}X_{lyso} + P_{nl}f_{nl} + P_{np}f_{np} + \\ K_{aAP}[AP^{-}](Y_{1,lyso} + Y_{2,lyso}) \end{matrix} \right) K_{\substack{uu,uu \\ iw^{lyso}}} \\ +f_{mito} \left(\begin{matrix} f_{iw}X_{mito} + P_{nl}f_{nl} + P_{np}f_{np} + \\ K_{aAP}[AP^{-}](Y_{1,mito} + Y_{2,mito}) \end{matrix} \right) K_{\substack{uu,uu \\ iw^{mito}}} \end{pmatrix} \frac{1}{X_{ew}} k_{cell\ uu,uu}$$

Equation 2

Cellular composition is described in terms of intracellular water (f_{iw}), neutral lipids (f_{nl}), neutral phospholipids (f_{np}) and acidic phospholipids ([AP^{-}]). Binding to protein and lipids in foetal bovine serum (FBS) can also be described using a similar approach (equation 3) and corrected for dilution in media.

Results

Figure 2 shows the behaviour of the FBS binding prediction model relative to $logP_{ow}$ and the fraction unionised, assuming that only unionised compound is able to bind serum lipids. Albumin and triacylglycerol (TAG; specifically trioleate) are taken to be representative of FBS proteins and lipids, respectively. Using experimentally determined TAG and albumin concentrations, and their respective partial specific volumes, the model can predict binding to serum components in a batch specific manner.

Figure 2. Predicting fraction unbound in foetal bovine serum

$$fu_{FBS} = \frac{f_{aq,FBS}}{f_{aq,FBS} + K_{protein} f_{protein} + \frac{P_{nl} f_{nl,FBS}}{X_{FBS}}}$$

$$f_{nl,FBS} \approx f_{TAG} = \frac{[TAG] \cdot 10^{-3} \cdot MW_{TAG} \cdot PSV_{TAG}}{1000}$$

$$f_{protein} \approx f_{alb,FBS} = \frac{mass\ albumin \cdot PSV_{albumin}}{1000}$$

if $logP_{ow} < 4.5$

$$logk_{protein} = 1.08 \cdot logP_{ow,Tsys} - 0.7$$

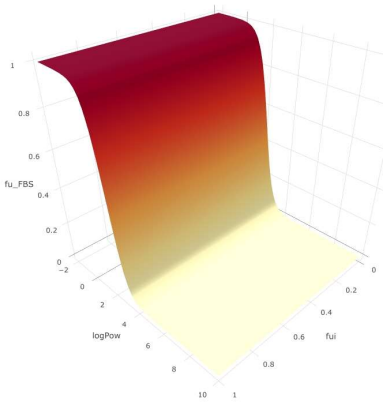
if $logP_{ow} \geq 4.5$

$$logk_{protein} = 0.37 \cdot logP_{ow,Tsys} + 2.56$$

$$fu_{FBS,dilu} = \frac{fu_{FBS}}{\frac{1}{D} \cdot (1 - fu_{FBS}) + fu_{FBS}}$$

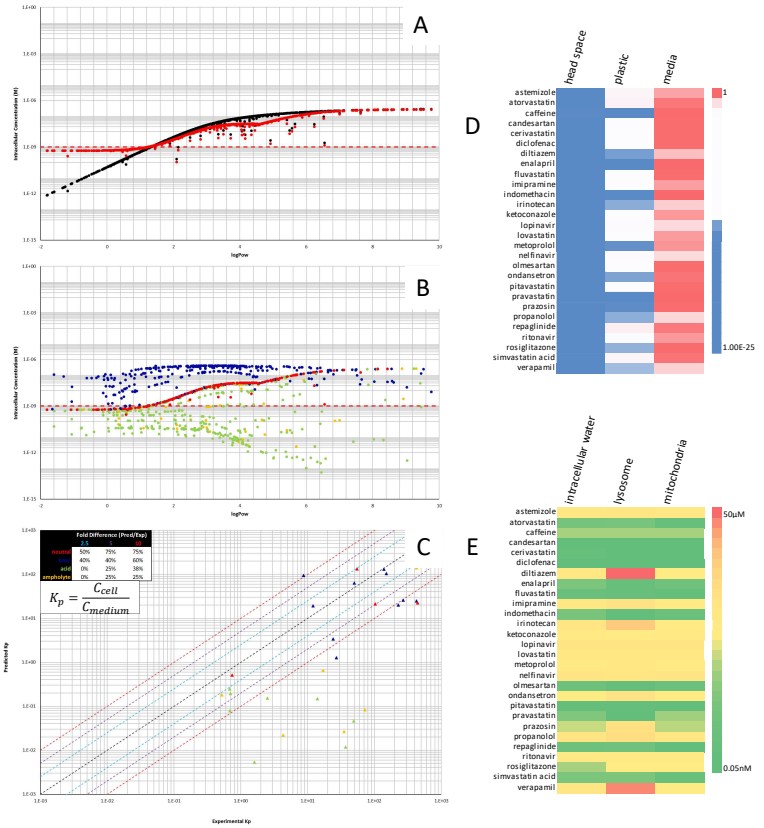
$$D = \frac{1}{f_{serum}}$$

Equation 3



Results (continued)

Figure 3. Model outputs; A) total intracellular concentrations determined using the VIVD model (red) and a published model (black; Armitage *et al.*, 2014) assuming no significant ionisation; B) intracellular concentrations determined using the VIVD model for acids (green), bases (blue), neutral (red) and ampholytes (yellow); C) comparison of experimental (Mateus *et al.*, 2013) and predicted ratios of unbound cell:unbound media concentrations; D) heatmap of predicted mole fraction distribution; E) heatmap of predicted intracellular concentration distribution.



Assuming neutrality, the VIVD model shows comparable performance to a published biokinetic model (Armitage *et al.*, 2014) at $logP_{ow} > 1.5$. At $logP_{ow} < 1.5$ the VIVD model predicts higher intracellular concentrations (figure 3A), resulting from its more mechanistic description of partitioning into cells. Incorporating ionisation and cell membrane potential demonstrates that the assumption of neutrality would result in an under-prediction of intracellular concentrations for significantly ionised bases and an over-prediction for ionised acids (figure 3B). To assess model performance, the VIVD model was parameterised to represent HEK293 (human embryonic kidney) cell assay conditions utilised in experimentally determining Kp for 28 pharmaceutical compounds with a range of physicochemical properties (Mateus *et al.*, 2013). Binding to FBS (10% v/v) was predicted and cell composition assumed to be the same as human kidney tissue (Simcyp v16). This first iteration of the VIVD model shows reasonable predictive power compared to experimental data for neutral and basic compounds. However, performance is less robust for acids and ampholytes, possibly due to poor prediction of protein binding in FBS for these compounds (figure 3C). The model allows an assessment of compound distribution across the total *in vitro* assay system. Of the 28 compounds assessed, none showed significant distribution into the headspace, with higher concentrations remaining in the media and binding to plastic (figure 3D). Intracellularly, the 28 compounds showed relatively uniform distribution; however, verapamil and diltiazem show high lysosomal concentrations associated with the 'ion-trapping' of weak bases (figure 3E) (Kubo *et al.*, 2016).

Conclusions

The VIVD model provides a steady-state, semi-mechanistic framework for predicting freely dissolved cellular and subcellular concentrations. Although such models have been previously published (Armitage *et al.* 2014; Fischer *et al.*, 2017), we incorporate differential ionisation and cell membrane potential as well as a more mechanistic model of intracellular lipid binding. Performance of the VIVD model will be further refined and verified within the EUToxRisk project, particularly with respect to lipophilic anions for which the current model under-performs.

References

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