A Mechanistic Framework for In Vitro – In Vivo Extrapolation of Liver Membrane Transporters: Prediction of Drug-Drug Interaction between Rosuvastatin and Cyclosporine.

M. Jamei[1]*, F. Bajot[1†], S. Neuhoff[1], Z. Barter[1], J. Yang[1,‡], A. Rostami-Hodjegan [1,2] and K. Rowland-Yeo[1]

[1]Simcyp Limited, Blades Enterprise Centre, Sheffield, UK; [2] Centre of Applied Pharmacokinetic Research, the School of Pharmacy and Pharmaceutical Sciences, the University of Manchester, Manchester, UK

[†] Current position: Computational Toxicology, British American Tobacco, Southampton, UK
[‡] Current position: Clinical Pharmacology Modelling and Simulation, GSK, Shanghai, China;

Supplementary Materials 1

1. PBPK typical tissue/organ values

Reference tissue volumes for an adult male (20 years old, 81 kg and 176.5 cm height) as simulated by the Simcyp Simulator (V12, Release 2) are shown in Table S1.

Table S1 – The reference tissue volumes (L) for an adult male

Organ	Tissue volume (L)
Blood volume	5.75
Red blood cells (erythrocytes)	2.25
Plasma	3.55
Skin	3.08
Heart	0.35
Spleen	0.15
Kidneys	0.33
Brain	1.34
Lungs	0.53
Adipose*	24.83
Skeletal muscle	30.58
Bone	2.32
GI organs	1.19
Liver	1.61

* Adipose = Total Adipose – interstitial adipose – Yellow marrow

In the case of Adipose, interstitial adipose tissue and the yellow marrow are subtracted from adipose tissue volume to give the necessary value for the partition coefficient calculations. The venous blood volume is assumed to be 66% of the total blood volume assuming the same ratio reported in rats [1].

Table S2 provides the specific density of different organs and tissues which can be used in calculation of volume of distribution.

Table S2 – The organs and tissues density in adults

Organ or Tissue	Density (g/cm3)	Reference
Adipose	0.923	[2]
Blood	1.058 (Adult)	[3] pp 34
Brain	1.04	[4] pp 209
Heart	1.04	[2] pp 481
Intestine (average of different segments)	1.042	[3] pp 144
Kidney	1.05 (Adult)	[4] pp 149
Liver	1.08	[2] pp 482
Lung free of air but with blood	1.05	[4] pp 98-96
Muscle	1.04	[2] pp 479
Skin (Epidermis and Dermis)	1.1	[4] pp 201
Spleen	1.06	[4] pp 223
Wet Bone	1.85 (Adult)	[2] pp 490
Yellow marrow	0.98	[2] pp 490

Table S3 provides tissue blood flow rates in male adults as fraction of cardiac output [4]. On average the cardiac output (CO) is 356 (L/h).

Table S3 – Tissue blood flows as fraction of cardiac output in male adults

Organ or tissue	Blood flow rate (fraction CO) [4] pp 21
Adipose	0.05
Bone	0.05
Brain	0.12
Heart	0.04
GI tract	0.15
Kidney	0.19
Liver (arterial)	0.065
Liver (portal)	0.19
Lung	1
Muscle	0.17
Skin	0.05
Spleen	0.02
Villi	0.06

The gut flow rate in this table is the sum of the blood flow rate to stomach and oesophagus, small intestine and large intestine which include the villous blood flow too.

The erythrocyte–to-plasma ratio (E:P) can be calculated based on the blood-to-plasma ratio (B:P) and the individual level of haematocrit (Ht).

$$\mathrm{E:P} = \frac{\mathrm{B:P} - (1 - \mathrm{Ht})}{\mathrm{Ht}} \qquad (1)$$

2. Derivation of extracellular and intracellular unbound fraction

2.1. Extracellular compartment

As described by Rodgers and co-workers [5], the plasma unbound concentration, Cu_P, is the sum of the ionised (BH^+) and unionised (B) species:

$$Cu_p = [B]u_p + [BH^+]u_p \quad (2)$$

For a monoprotic base, these species are related to the pK_a of the drug and the pH of the environment using Henderson–Hasselbalch equation (3):

$$pH = pK_a + \log\frac{[B]}{[BH^+]} \quad (3)$$

Hence, the unbound concentration of drug in the vascular space and the extracellular water (Cu_{EW}), can be written as:

$$Cu_p = [B]u_p\left(1+10^{pK_a-pH_p}\right) \quad (4)$$

$$Cu_{EW} = [B]u_{EW}\left(1+10^{pK_a-pH_{EW}}\right) \quad (5)$$

Respectively, where pH_p and pH_{EW} are the pH values of plasma and extracellular water. Since the unbound unionised species are in equilibrium in the vascular space and extracellular water:

$$[B]u_p = [B]u_{EW} \quad (6)$$

Combining the last three equations gives:

$$Cu_{EW} = Cu_p\frac{\left(1+10^{pK_a-pH_{EW}}\right)}{\left(1+10^{pK_a-pH_p}\right)} \quad (7)$$

Equation (7) applies to a monoprotic base and it can be generalized for all compound charge types as follows:

$$Cu_{EW} = Cu_p\frac{W}{Y} \quad (8)$$

where Y and W determine the ionisation ratio in plasma and extracellular water respectively. For a monoprotic base Y= $1+10^{pK_a-pHp}$ and W=$1+10^{pK_a-pH_{EW}}$ where pH_{EW} and pH_p are the pH in the extracellular water and plasma respectively. The concentration of drug in the extracellular water is the sum of the unbound concentration (Cu_{EW}) and concentration associated with extracellular proteins ($C_{PR,EW}$). In general, the dominant binding protein in plasma for acids and albumin for very weak bases [6]. Thus, albumin is assumed to be the dominant binding protein in tissues for these compound classes. Several binding sites have been reported for albumin, two of the major ones being the benzodiazepine and warfarin sites. Benzodiazepines are very weak bases that are predominately unionised at physiological pH, whereas warfarin, being reasonably acidic (pK_a 5.1), is essentially ionised. This indicates that both ionised and unionised acids and very weak bases are capable of interacting with albumin. Therefore, this assumption has been incorporated into the equations. Regarding neutral drugs, lipoproteins are assumed to be the dominant binding protein. The association constant for proteins (Ka_{PR}) can be calculated using:

$$Ka_{PR} = \frac{[PR\_Drug]_{EW}}{[PR]_{EW}\, Cu_{EW}} \tag{9}$$

where $[PR\_Drug]_{EW}$ represents the protein-drug complex concentration in the extracellular water space of the tissue, that is $C_{PR,EW}$, and $[PR]_{EW}$ represent the concentration of sites still available for binding on the protein. Assuming non-saturating conditions prevail, which occurs when few of the binding sites are occupied, then $[PR]_{EW}$ can be approximated to be the total concentration of binding protein in the tissue extracellular water. Rearranging (9) and using (8) generates the following expression for $C_{PR,EW}$.

$$C_{PR,EW} = Ka_{PR}[PR]_{EW}\, Cu_p \frac{W}{Y} \tag{10}$$

Now, the total concentration in the extracellular water space (C_{EW}) is:

$$C_{EW} = Cu_{EW} + C_{PR,EW} = Cu_p \frac{W}{Y}\left(1 + Ka_{PR}[PR]_{EW}\right) = Cu_{EW}\left(1 + Ka_{PR}[PR]_{EW}\right) \quad (11)$$

Since $[PR]_{EW}$ is calculated by dividing the concentration of binding protein (albumin or lipoprotein) in the liver ($[PR]_{Liv}$) by f_{EW}, then we can write:

$$\mathrm{C_{EW}} = \mathrm{Cu_p}\,\frac{\mathrm{W}}{\mathrm{Y}}\left(1 + \frac{\mathrm{Ka_{PR}}[\mathrm{PR}]_{\mathrm{Liv}}}{\mathrm{f_{EW}}}\right) \quad (12)$$

$$\mathrm{C_{EW}} = \mathrm{Cu_{EW}}\left(1 + \frac{\mathrm{Ka_{PR}}[\mathrm{PR}]_{\mathrm{Liv}}}{\mathrm{f_{EW}}}\right) \quad (13)$$

Hence, the unbound fraction of drug in the extracellular water becomes:

$$\mathrm{fu_{EW}} = \frac{\mathrm{Cu_{EW}}}{\mathrm{C_{EW}}} = \frac{1}{\left(1 + \frac{\mathrm{Ka_{PR}}[\mathrm{PR}]_{\mathrm{Liv}}}{\mathrm{f_{EW}}}\right)} \quad (14)$$

For strong bases (pKa >7.0) usually Ka_{PR} is assumed to be zero, hence fu_{EW} becomes 1.

This can also be used to obtain the ratio between the total extracellular water concentration and the total blood concentration in the tissue vascular space (V_{VS}) which mean two differential equations are enough to determine all three liver concentrations (C_{VS}, C_{EW} and C_{IW}).

$$\mathrm{K_{EW:B}} = \frac{\mathrm{C_{EW}}}{\mathrm{C_{B,VS}}} = \frac{\frac{\mathrm{W}}{\mathrm{Y}}\left(1 + \frac{\mathrm{Ka_{PR}}[\mathrm{PR}]_{\mathrm{Liv}}}{\mathrm{f_{EW}}}\right)}{\mathrm{B:P/fu}} \quad (15)$$

2.2. Intracellular Compartment

The concentration of the drug in the intracellular water space can be determined similarly.

The mass (A) of drug in the intracellular water space can be determined using the intracellular water concentration (C_{IW}) and its volume (V_{IW}):

$$A_{IW} = C_{IW} V_{IW} \tag{16}$$

As per [6] , it is assumed that the drug can distribute into the tissue constituents (neutral lipids (NL), neutral phospholipids (NP) and acidic phospholipids (AP), for drugs with pKa>= 7); therefore:

$$C_{IW} = \frac{A_{IW}}{V_{IW}} = \frac{Cu_{IW} V_{IW} + C_{B,NL} V_{NL} + C_{B,NP} V_{NP} + C_{B,AP} V_{B,REM}}{V_{IW}} \tag{17}$$

where both unbound and bound drug are considered. In order to use the liver composition fractions (f_{NL}, f_{NP} and f_{REM}, see [6]) we can rearrange equation (17) as follows:

$$C_{IW} = \frac{Cu_{IW} V_{IW} + C_{B,NL} V_{NL} + C_{B,NP} V_{NP} + C_{B,AP} V_{B,REM}}{V_{IW}} \frac{V_T}{V_T} \tag{18}$$

$$C_{IW} = \frac{Cu_{IW} V_{IW} + C_{B,NL} V_{NL} + C_{B,NP} V_{NP} + C_{B,AP} V_{B,REM}}{V_T} \frac{V_T}{V_{IW}} \tag{19}$$

$$C_{IW} = \frac{1}{f_{IW}} \left(Cu_{IW} f_{IW} + C_{B,NL} f_{NL} + C_{B,NP} f_{NP} + C_{B,AP} f_{REM} \right) \tag{20}$$

Distribution of drug into neutral lipids is assumed to equate to the partitioning of unbound unionised drug between intracellular water and neutral lipids. Then the affinity for neutral lipids equals the partition coefficient, where the *in vitro* surrogates for intracellular water and neutral lipid are assumed to be ‘pure’ water and n-octanol respectively, for all tissues except adipose. In adipose, vegetable oil was determined to be a better surrogate than n-octanol for neutral lipid as suggested in [7]. Partitioning into tissue neutral lipids can therefore be calculated using equation (21):

$$[B]_{NL} = P[B]u_{IW} \quad (21)$$

where $[B]_{NL}$ represents the concentration of unionised drug in the neutral lipid of tissue cells and P is the n-octanol:water partition coefficient of all tissues except adipose, where it represents vegetable oil:water partition coefficient. As suggested in [7] the neutral phospholipids can be represented by a mixture of 30% neutral lipid and 70% water the concentration of unionised drug in neutral phospholipids ($[B]_{NP}$) can therefore be calculated as follows:

$$[B]_{NP} = 0.3[B]_{NL} + 0.7[B]u_{IW} = 0.3P[B]u_{IW} + 0.7[B]u_{IW} \quad (22)$$

As it is discussed in [6] $[BH^+]u_{IW}$ preferentially interacts with the acidic phospholipids which are located within cell membranes with the ionised head groups predominantly protruding into the intracellular tissue water. Such an orientation suggests that the electrostatic interactions between unbound intracellular $[BH^+]u_{IW}$ and the charged head groups of the acidic phospholipids occur at cell membrane–cytosol interfaces. The association constant (Ka_{AP}) of strong bases (pKa >7.0) with these acidic phospholipids (AP^-) is given by:

$$Ka_{AP} = \frac{[AP^- BH^+]_{B,REM}}{[BH^+]u_{IW}[AP^-]_{REM}} \quad (23)$$

where $[AP^-BH^+]_{B,REM}$ represents the ionised base-acidic phospholipid complex concentration, that is, $C_{B,AP}$, and $[AP^-]_{REM}$ is the concentration of available binding sties on the acidic phospholipids. It Ka_{AP} is not known it can be determined as explained in [5, 6]. For weak bases (pKa <7.0), acids and neutral drugs Ka_{AP} is zero.

Assuming all acidic phospholipids are totally ionised and non-saturation conditions prevail, such that few of the total AP sites are occupied, $[AP^-]_{REM}$ is taken to be the total AP concentration (which is taken to be the sum of the acidic phospholipids concentrations in the residual tissue matter, f_{REM}). Rearranging equation (23) we obtain the drug concentration bound to acidic phospholipids.

$$C_{B,AP} = Ka_{AP}\,[AP]_{REM}\,[B]u_{IW}\,\alpha \tag{24}$$

Where α for a monoprotic base is: $10^{pKa-pH_{IW}}$ and for other charge types can be defined similarly based on the compound charge type using the Henderson–Hasselbalch equations.

For a monoprotic based compound, the unbound drug concentration in the intracellular water can be written as:

$$Cu_{IW} = [B]u_{IW}\left(1 + 10^{pK_a - pH_{IW}}\right) \tag{25}$$

where pH_{IW} is the intracellular water pH. Hence:

$$[B]u_{IW} = \frac{Cu_{IW}}{(1+\alpha)} \tag{26}$$

Putting all these together we can obtain:

$$C_{IW} = \frac{Cu_{IW}}{f_{IW}}\left(f_{IW} + \frac{Pf_{NL} + (0.3P + 0.7)f_{NP} + Ka_{AP}[AP^-]_{REM}\,f_{REM}\alpha}{1+\alpha}\right) \tag{27}$$

$$C_{IW} = \frac{Cu_{IW}}{f_{IW}}\left(f_{IW} + \frac{Pf_{NL} + (0.3P + 0.7)f_{NP} + Ka_{AP}[AP^-]_{T}\,\alpha}{1+\alpha}\right) \tag{28}$$

And finally:

$$fu_{IW} = \frac{f_{IW}}{\left(f_{IW} + \frac{Pf_{NL} + (0.3P + 0.7)f_{NP} + Ka_{AP}[AP^-]_{T}\,\alpha}{1+\alpha}\right)} \tag{29}$$

References

1. Hosseini-Yeganeh M, McLachlan AJ. Physiologically based pharmacokinetic model for terbinafine in rats and humans. Antimicrob Agents Chemother. 2002;46(7):2219–28.

2. Price PS, Conolly RB, Chaisson CF, Gross EA, Young JS, Mathis ET, et al. Modeling interindividual variation in physiological factors used in PBPK models of humans. Crit Rev Toxicol. 2003;33(5):469-503.

3. ICRP. Report of the task group on Reference Man (No. 23). Oxford: Pergamon Press; 1975.

4. ICRP. Basic anatomical and physiological data for use in radiological protection: reference values. A report of age- and gender-related differences in the anatomical and physiological characteristics of reference individuals. ICRP Publication 89. Ann ICRP. 2002;32(3-4):5-265.

5. Rodgers T, Rowland M. Physiologically based pharmacokinetic modelling 2: predicting the tissue distribution of acids, very weak bases, neutrals and zwitterions. J Pharm Sci. 2006;95(6):1238-57.

6. Rodgers T, Leahy D, Rowland M. Physiologically based pharmacokinetic modeling 1: Predicting the tissue distribution of moderate-to-strong bases. J Pharm Sci. 2005;94(6):1259-76.

7. Poulin P, Theil FP. Prediction of pharmacokinetics prior to in vivo studies 1. Mechanism-based prediction of volume of distribution. J Pharm Sci. 2002;91(1):129-56.