Lysosomes – falling into a trap of our own making?

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Background

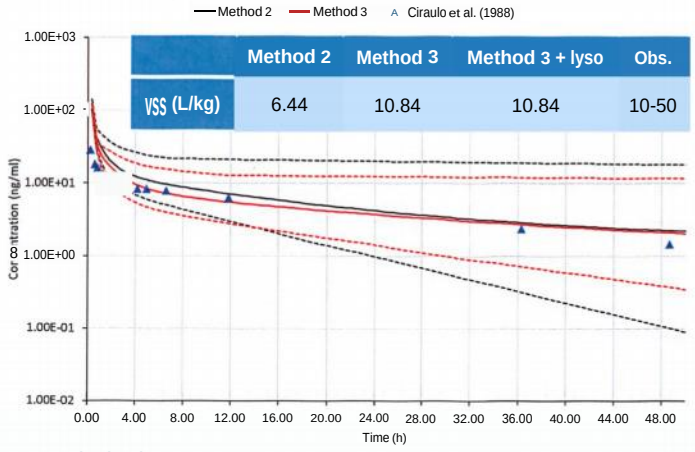
Cationic amphiphilic drugs (CADs) are used within a wide range of therapeutic areas (antidepressant, antipsychotic, antimalarial) and many are known to distribute extensively into lysosomes. Indeed, the extensive distribution into acidic organelles can be critical to their mechanism of action (e.g. chloroquine). As such there is great interest in these mechanisms and their impact on drug distribution, efficacy and safety (e.g., phospholipidosis).

Recent publications highlight that V_{SS} predictions using the established Rodgers and Rowland method (Rodgers *et al.,* 2005; Simcyp Method 2) under-predict V_{SS} since lysosomal distribution is not accounted for. Assmus *et al* propose an extension of Rodgers and Rowland incorporating an intracellular compartment representing the lysosome, thus allowing for the differential pH between the intracellular water and lysosome to be represented within the model. Citing desipramine as an exemplar compound, Samant *et al* correct this under-prediction using an optimized blood-to-plasma ratio in the back-calculation of the acidic phospholipid association constant (KaAP). A significant proportion of intracellular acidic phospholipids are localised to endosomes and thus compounds binding to acidic phospholipids (APs) extensively distribute into endosomal compartments including lysosomes.

However, neither of these approaches address fundamental distribution mechanisms and over-stress the impact of the lysosome on *in vivo* whole body distribution of CADs. The modified Rodgers and Rowland method (Simcyp Method 3), implemented in Simcyp v16, revises assumptions of Method 2 accounting for the permeability of the ionised fraction of drug into tissues and taking into account the impact of membrane potential on ion permeability into the tissue and into subcellular organelles, as well as accounting for pH gradients. Here we review the approaches proposed in these publications.

Results

Desipramine (pKa 10.26) is known to be a lysosomotropic compound and this is thought to contribute to its high, and variable, volume of distribution (V_{SS}; 10-50 L/kg). The distribution of desipramine to the lysosome is not mediated through an 'ion-trapping' effect, but through extensive binding to APs (Daniel *et al.,* 1995). While Rodgers and Rowland does slightly under-predict the V_{SS} of desipramine, the significant under-prediction identified by Samant *et al* arises primarily from incorrect modelling of the observed data, the authors simulate an IV bolus rather than an IV infusion used in the clinical study. The desipramine compound library file (v17) was modified to a full-PBPK model using Method 2 or 3 to predict V_{SS}; no other parameters were modified. Simulations were run to reproduce a single dose IV infusion study as reported (Ciraulo *et al.,* 1988; Figure 1).



	Method 2	Method 3	Method 3 + lyso	Obs.
V_{SS} (L/kg)	6.44	10.84	10.84	10-50

Figure 1. Simulated concentration time profiles IV desipramine infusion (12.5mg over 30mins) using a full-PBPK model and alternate approaches to predict V_{SS}; 5th and 95th percentile plotted as dashed lines.

Simulations using Method 3 show better recovery of the reported concentration time profile than with Method 2, with predicted V_{SS} within the reported range. However, V_{SS} predictions with Method 3 accounting for subcellular distribution in lysosome rich tissues (kidney, liver and lung) showed no significant difference to Method 3 alone. Indeed, reviewing liver-plasma partition coefficients ($K_{p\,Mver}$) predictions using Method 3 with and without lysosomal distribution shows a small reduction when accounting for lysosomal distribution (Table 1).

Table 1. Calculated liver-plasma partition coefficients, $K_{p\,,ver}$ for desipramine

	Method 3	Method 3 + Subcellular	Ratio
$K_{p\ liver}$	37.81	37.73	0.998

Results

This reduction in Kp can be attributed the fact that at physiological pH the majority of desipramine is ionised in all physiological spaces (Table 2). While Method 3 does account for the permeability of ions, this permeability is orders of magnitude lower than the unionised form. The lysosomal permeability of the ionised form is also limited by the positive membrane potential maintained by lysosomes (+10 mV) acting against movement of the cationic fraction.

Table 2. Desipramine (pKa = 10.26) fraction unionised in plasma, intracellular water and lysosomes was calculated using the Henderson-Hasselbalch equation

	Plasma (pH 7.4)	Intracellular water (pH 7.0)	Lysosome (pH 5.0)
	1.38E-03	5.49E-04	5.50E-06

Given this it becomes apparent that a major contributor to the under-prediction of V_{SS} for CADs using Rodgers and Rowland is not the absence of the lysosome, but the assumption that only the unionised fraction of compound can passively diffuse across biological membranes. Critically the impact of membrane potential on the intrinsic permeability of the ionised fraction must also be considered. Neither of the published approaches address this critical assumption and so do not mechanistically account for mechanisms resulting in distribution to lysosomes due to binding to APs, **since if the majority of compound cannot enter the cell it cannot extensively bind cellular APs.**

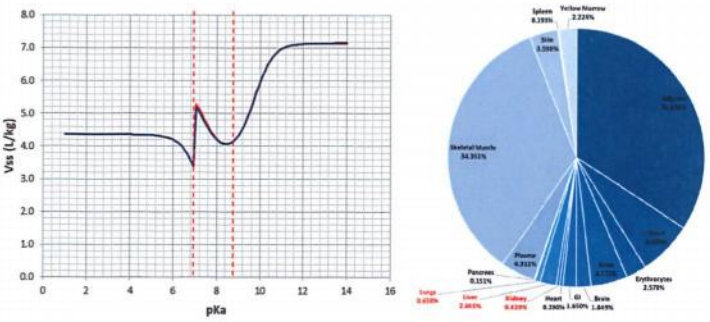


Figure 2. Automated sensitivity analysis of the impact of changing pKa on predicted V_{SS} using Method 3 (blue) and Method 3 with lysosomal distribution (red). Pie chart shows percentage contributions of individual tissues to total physiological body volume; lysosome rich tissues (kidney, liver, lung) are highlighted in red.

Using diltiazem as an exemplar compound known to sequester in lysosomes (Mateus *et al.,* 2013) through the 'ion-trapping' mechanism (monoprotic base, pKa = 8.06, logP = 2.8), Figure 2 shows the sensitivity of V_{SS} predictions to changing pKa with and without subcellular distribution being accounted for in prediction. This demonstrates that not only does distribution into lysosomes have a minimal effect on V_{SS}, but the impact is limited to a narrow pKa window. Considering the relative contribution of the lysosomal rich tissues to total physiological body volume (~3%), it is no surprise that accounting for distribution into organelles that comprise ~1% of the cell/tissue has little effect on V_{SS} predictions. The narrow pKa range of sensitivity is also expected given that a high enough fraction of compound must be unionised in the intracellular water to extensively distribute in to the lysosome and subsequently ionise at the acidic pH of the lysosome interior, thus limiting the permeability of the ionised, internalised compound to leave the lysosome.

Conclusions

Published approaches to address the under-prediction of V_{SS} for CADs have over emphasised the role that the lysosome has to play in the large volume of distribution observed for this class of compound. Incorrect modelling of reported studies, empirical correction, and revision of existing prediction methods with only partial consideration of the mechanisms involved exaggerate the role of the lysosome and perpetuate misunderstanding of the underlying mechanisms. Method 3 more mechanistically revises the assumptions of the Rodgers and Rowland approach, demonstrating that while lysosomal 'ion-trapping' may have localised effects with respect to efficacy and toxicity, it has only a limited effect on V_{SS}.

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