Supporting Information

Estimation of volume of distribution in humans from high throughput HPLC-based measurements of human serum albumin (HSA) binding and immobilized artificial membrane (IAM) partitioning

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		number	L/kg		%	PPB		acid class	base class	(VD)	(PPB)	
1	ACECAINIDE	32795-44-1	1.5	ss, IV	10	0.38	1.64	0	7	63		61
2	ACETAMINOPHEN	103-90-2	0.95	ss, IV	1	0.14	0.49	1	0	72		63
3	ACETANILIDE	103-84-4	0.7	a, IV	na	na	1.16	0	0	59	Na	
4	ACETAZOLAMIDE	59-66-5	0.2	a, IV	95	3.32	-1.13	2	0	59		61
5	ALCLOFENAC	22131-79-9	0.1	a, IV	99	5.44	2.73	7	0	59		61
6	ALOSETRON HCl	122852-69-1	1.1	a, IV	na	na	1.74	0	2	61	Na	
7	AMANTADINE	768-94-5	6.6	ss, IV	67	1.34	2.00	0	7	72		61
8	AMILORIDE	2609-46-3	5.0	a, IV	40	0.83	-0.55	0	5	59		59
9	AMOXAPINE	14028-44-5	16.0	a, PO, 36	na	na	4.62	0	6	61	Na	
10	AMOXICILLIN	26787-78-0	0.27	ss, IV	18	0.51	-1.87	7	2	72		61
11	AMPICILLIN	69-53-4	0.29	ss, IV	18	0.51	-1.20	7	3	72		61
12	APOMORPHINE HCl	314-19-2	2.0	a, IV	na	na	2.49	1	2	61	Na	
13	ASPIRIN	50-78-2	0.15	ss, IV	70	1.42	1.02	7	0	45		61
14	BAMETHAN	3703-79-5	3.7	a, IV	na	na	1.50	1	7	59	Na	
15	BETAMETHASONE	378-44-9	1.3	ss, IV	64	1.27	1.79	0	0	72		66
16	BROMAZEPAM	1812-30-2	0.9	ss, IV	52	1.03	1.70	0	1	72		61
17	BROMOCRIPTIN	25614-03-3	3.0	a, IV	90	2.48	6.59	1	1	59		61
18	BUDESONIDE	51333-22-3	3.9	ss, IV	88	2.29	2.91	0	0	72		61
19	BUMETANIDE	28395-03-1	0.14	ss, IV	96	3.61	3.37	7	1	72		61
20	CARBAMAZEPINE	298-46-4	1.4	a, PO, 70	75	1.58	1.98	0	0	63		61
21	CEFAZOLINE	25953-19-9	0.10	ss, IV	89	2.39	-1.14	7	1	63		67
22	CEFIXIME	79350-37-1	0.24	ss, IV7	67	1.34	0.45	7	1	72		67
23	CEPHALEXIN	15686-71-2	0.30	ss, PO, 90	14	0.45	-1.64	7	3	72		67
24	CHLORPHENIRAMINE	132-22-9	3.2	ss, IV	72	1.48	3.15	0	6	72		59
25	CHLORPROPAMIDE	94-20-2	0.19	ss, IV	90	2.49	2.35	7	0	72		59
26	CHLORPROTHIXENE	113-59-7	15.0	ss, IV	na	na	5.48	0	6	72	Na	
27	CINOXACIN	28657-80-9	0.33	ss, IV	63	1.25	1.50	7	0	61		63
28	CLONAZEPAM	1622-61-3	2.8	ss, IV	85	2.07	2.38	0	1	72		63
29	CLONIDINE	4205-90-7	2.1	ss,IV	20	0.54	1.43	0	5	72		63
30	CLOXACILLIN	61-72-3	0.12	ss, PO, 43	95	3.32	2.52	7	1	72		63
31	COLCHICINE	64-86-8	5.20	ss, IV	31	0.70	1.20	0	0	72		58
32	CYTARABINE	147-94-4	2.5	a, IV	13	0.44	-2.20	0	1	61		59
33	DIAZEPAM	439-14-5	1.3	ss, IV	98	4.55	3.17	0	1	72		63
34	DIAZOXIDE	364-98-7	0.24	ss, IV	94	3.09	1.20	1	7	72		63
35	DICLOFENAC	15307-86-5	0.17	ss, IV	99	5.44	4.73	7	0	72		59
36	DIFLUNISAL	22494-42-4	0.1	ss, IV	99	5.44	4.40	7	0	72		63
37	DILTIAZEM	33286-22-5	3.1	ss, IV	98	4.55	3.65	0	5	72		63

Table 1b Literature and calculated data for drug molecules in the test set. (VD: literature volume of distribution; PPB: literature plasma protein binding; $\log K_{PPB}$: logarithm of association constant for plasma protein binding calculated from the % binding data as $e^{\log(\%PPB/(101-\%PPB))}$; ClogP: calculated octanol/water partition coefficient; pKa acid class and pKa base class: classification based on the pKa values of acidic and basic groups – higher number means higher % of ionization)

No	DRUG	CAS number	VD L/kg	Comment	PPB %	logKPPB	ClogP	pKa acid class	pKa base class	Ref (VD)	Ref (PPB)
1	ACYCLOVIR	59277-89-3	0.71	ss, IV	15	0.47	-2.42	0	1	72	58
2	AMINOGLUTETHIMIDE	125-84-8	1.4	a, IV	24	0.6	0.77	1	1	60	67
3	ANTIPYRINE	60-80-0	0.6	ss, IV	13	0.43	0.20	0	1	72	61
4	CEFTAZIDIME	78439-06-2	0.43	ss, IV	21	0.56	-3.26	7	1	72	68
5	CHLORPHENTERMINE	461-78-9	2.4	A, IV	na	na	2.85	0	7	60	na
6	CIMETIDINE	51481-61-9	1.0	ss, IV	20	0.54	0.35	0	2	72	61
7	DAUNORUBICIN	20830-81-3	23	a, IV	na	na	0.06	1	6	72	na
8	DEXAMETHASONE	50-02-2	0.82	ss, IV	77	1.66	1.79	0	0	72	60
9	DICLOXACILLIN	3116-76-5	0.89	ss, IV	98	4.55	2.98	7	1	59	67
10	DOMPERIDONE	57808-66-9	5.7	ss, IV	na	na	4.27	0	6	62	na
11	DOTHIEPIN	113-53-1	70	ss, IV	na	na	4.53	0	6	72	na
12	FLUNISOLIDE	03/03/3385	1.8	a, PO, 42	80	1.79	2.41	0	0	72	58
13	GANCICLOVIR	82410-32-0	0.7	ss, IV	1	0.14	-2.55	0	1	72	58
14	GLYBURIDE	10283-21-8	0.14	a, IV	99	5.44	4.24	7	0	72	61
15	HALOPERIDOL	52--86-8	18	ss, IV	90	2.49	3.85	0	5	72	61
16	IBUPROFEN	15687-27-1	0.15	ss, IV	99	5.44	3.68	7	0	72	61
17	INDOPROFEN	31842-01-0	0.1	ss, IV	98	4.55	2.74	7	1	72	65
18	MEPHOBARBITAL	115-38-8	2.5	A, PO, 70	na	na	1.55	0	0	60	na
19	METOCLOPRAMIDE	364-62-5	3.4	ss, IV	40	0.83	2.23	0	7	72	61
20	NIMODIPINE	66085-59-4	1.5	a, IV	95	3.32	4.14	0	0	72	61
21	PHENACETIN	62-44-2	1.5	a, IV	33	0.73	1.77	0	0	60	61
22	PHENOBARBITAL	50-06-6	0.63	ss, IV	50	0.99	0.67	1	0	72	61
23	PHENYLBUTAZONE	50-33-9	0.17	a, IV	99	5.44	3.65	1	0	60	61
24	PROPAFENONE	54063-53-5	3.6	a, IV	95	3.32	3.64	0	7	72	61
25	PYRIMETHAMINE	58-14-0	0.43	ss, IV	85	2.07	3.00	0	2	72	61
26	RIFAMPIN	13292-46-1	0.38	ss, IV	80	1.79	3.77	1	7	72	61
27	SAQUINAVIR	127779-20-8	10	ss, IV	98	4.55	4.73	0	3	72	61

28	TETROXOPRIM	53808-87-0	0.8	a, IV	15	0.47	0.73	0	3	60	61
29	VANCOMYCIN	1404-90-6	0.39	ss, IV	30	0.69	-1.14	0	0	63	61
30	VERAPAMIL	52-53-9	4.7	ss, IV	90	2.49	4.47	0	5	72	601

na = not available
a = VD area
ss = VD ss
IV = intravenous admin.
PO = oral admin.
number = percent of bioavailability

Supporting information on the literature volume of distribution data

We have checked each and every value of volume of distribution data to the original references. When the data were obtained after oral administration the bioavailability data were taken into account for the estimate of the volume. When using pharmacokinetics to make drug dosing decisions, the difference between VDarea and VDss is not usually clinically significant. (Wilkinson, G. R. (2001) Pharmacokinetics: the dynamics of drug absorption, distribution, and elimination. In: Hardman, J. G. Limbird, L. E., Gillman, A. G. (eds) Goodman& Gillman's the pharmacological basis of therapeutics. 10th edn. McGraw-Hill, New York, pp 20 22). However, we have recalculated the equations for two subsets of compounds with the VDarea and the VDss data. The coefficients and the statistics are not significantly different as is shown below.

Model for compounds with the steady state volume of distribution (VDss) data:

$$\log VDss = 0.42 \log K(IAM) - 0.19 \log K(HSA) - 0.74 \quad (1)$$

$n = 65$ $r^2 = 0.62$ $s = 0.35$ $F = 51$

Model for compounds with VDarea values:

$$\log VDarea = 0.41 \log K(IAM) - 0.22 \log K(HSA) - 0.54 \quad (2)$$

$n = 114$ $r^2 = 0.80$ $s = 0.32$ $F = 217$

Model for the combined data set:

$$\log VD = 0.42 \log K(IAM) - 0.21 \log K(HSA) - 0.61 \quad (3)$$

n=180 r^2 = 0.76 s=0.33 F=274

Based on the above equations there were no significant differences between the models for the steady state and the apparent volumes of distribution.

The effect of plasma protein binding other than albumin on the volume of distribution

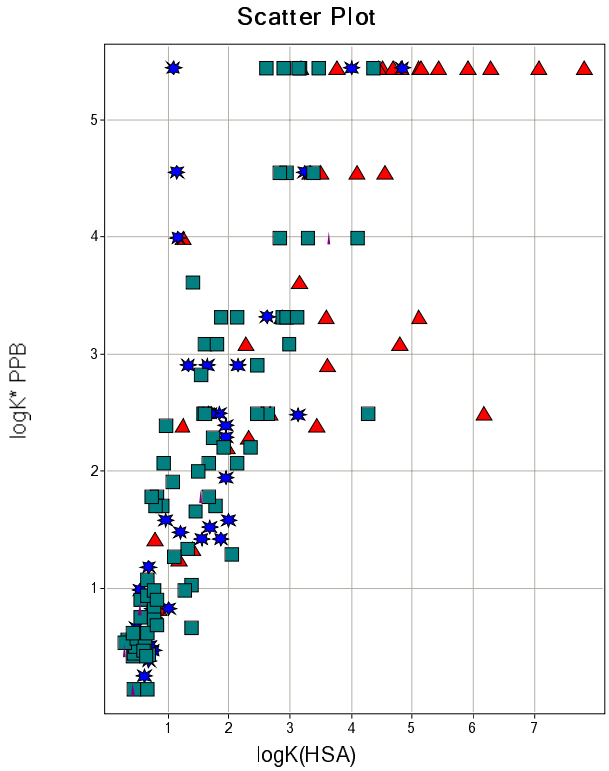
We have tried to use directly the literature plasma protein binding data in the model as well, to check whether HSA binding alone is enough to take into consideration in modeling volume of distribution. The statistical characteristic of the regression equation was slightly worse as shown by equation 6 in the paper.

$$LogVD_{ss}=0.44(\pm0.02)logK(IAM)-0.19(\pm0.02)logK^{*}(PPB)-0.65 \quad (4)$$

N=152 r^2 = 0.72 s= 0.36 F= 182

It is worth mentioning that the regression coefficients were not statistically different from our model. When we compared our measured HSA binding data with the literature plasma protein binding, it was found that plasma protein binding is normally similar or higher than HSA binding (see figure below). The higher plasma protein binding is probably due to compound's binding to other than albumin, such as alpha-1-acid glycoprotein (AGP) or globulins.

The plot of logK (HSA) vs logK*(PPB).



The worse statistical characteristic of the model when plasma protein binding was included can also be explained by the observation that compounds binding primarily to HSA were restrictive binders, while compounds binding to other plasma proteins (for example alpha-1-acidglycoprotein) showed non-restrictive binding. We have observed that the IAM binding and AGP binding showed good correlation with each other (positively charged lipophilic compounds tend to bind strongly for both), thus reducing the difference between the tissue and plasma protein binding.