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Complete List of Authors:	MacFaul, Philip; AstraZeneca, Oncology DMPK Colclough, Nicola; AstraZeneca, Oncology DMPK Ruston, Linette; AstraZeneca, Oncology DMPK Wood, Matt; AstraZeneca, Oncology DMPK

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ARTICLE TYPE

Species differences in drug plasma protein binding.

Nicola Colclough,[a] Linette Ruston,[a] J. Matthew Wood[a] and Philip A. MacFaul,*[a]

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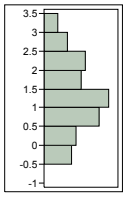
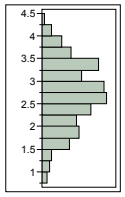
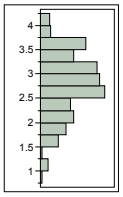
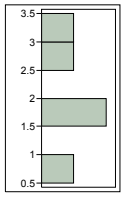
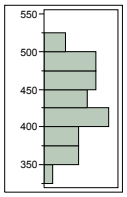
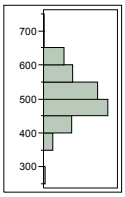
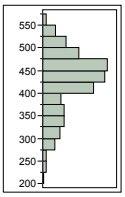
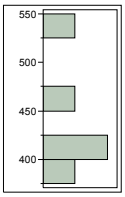
Comparison of the human plasma protein binding data for a variety of drug discovery compounds indicates that compounds tend to be slightly more bound to human plasma proteins, than compared to plasma proteins from rats, dogs or mice. However, the majority of measurements from the pre-clinical species fall within 5-fold of the human plasma value, although there are some compounds that do show significantly different interspecies plasma protein binding.

Drug plasma protein binding is a critical parameter that is measured during drug discovery as it generally accepted that only free drug in plasma is available to elicit a pharmacological effect, and from a pharmacokinetic point of view, only free drug is available to be cleared.[1-3] Rather than drive compound optimisation on fraction unbound, lead optimisation programmes gain from understanding the relationship between unbound drug concentration and pharmacodynamic effect.[4,5] Therefore to assess the free drug concentration in species used for pharmacokinetic (e.g. rat and dog) and pharmacodynamic (e.g. mouse) studies, the plasma protein binding needs to be measured in plasma from each of the relevant species. In addition, the human plasma protein binding value needs to be measured to facilitate the prediction of human pharmacokinetics and pharmacodynamics. In order to reduce the requirement for measurements across plasmas from multiple species, the question was asked if human plasma protein binding was measured; will this value be the same as that in other species?

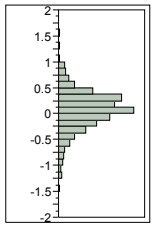
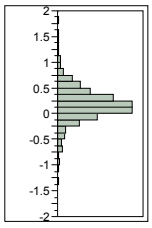
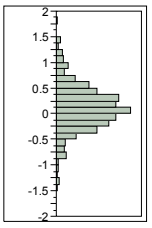
Previous analysis on a GSK compound set concluded that compounds tend to be slightly more bound to human plasma proteins compared to rat plasma proteins.[6] A similar analysis has therefore been conducted on AstraZeneca (AZ) compounds measured at Alderley Park, and extended to cover dog and mouse plasma protein binding. Consequently, the rat, dog and mouse plasma protein binding data measured at Alderley Park over the last 14 years has been analysed and compared to that for human.[7] All the data used in this current analysis has been collected employing the equilibrium dialysis technique.[8] All results with off-scale indicators were excluded from the analysis, resulting in a dataset comprising 574 compounds that had measured data in all four species. The compounds covered the three main compound ion classes (acids, bases and neutrals), in addition to a small number of zwitterions, and spanned a wide range of lipophilicity and molecular weight – see table 1.[9] All the % free values have been converted to $\log K_{bound/free}$ values, where

Table 1 Division of ion class and distribution of corresponding measured logD values and molecular weights for AZ compounds

Distribution of logD values[a]				
Distribution of molecular weights				
Ion class	Acid	Base	Neutral	Zwitterion
Number of compounds	72	206	291	5

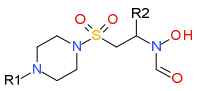
[a] Measured logD octanol at pH 7.4.[10]

Table 2 Comparison of human plasma protein values to those obtained in rat, dog and mouse plasmas for various AZ compounds

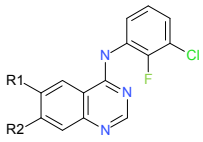
Comparator species to human	Rat	Dog	Mouse
Distribution of $\Delta\log K_{b/f}$[a]			
Mean difference	0.07	0.18	0.12
Standard deviation	0.39	0.36	0.46
% within 3-fold	83	87	76
% within 5-fold	92	93	88

[a] $\Delta\log K_{b/f} = \log k_{b/f,\ human} - \log K_{b/f,\ rat,\ dog\ or\ mouse}$

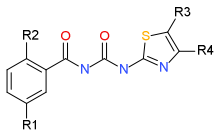
$\log K_{b/f} = \log_{10}(100\text{-}\%free/\%free)$, and the distributions shown in Table 2 are for compounds where the human data has been compared directly to that from rat, dog and mouse plasmas, and expressed as $\Delta\log K_{b/f}$ ($\log k_{b/f,\ human} - \log K_{b/f,\ rat,\ dog\ or\ mouse}$).[11] The



(1)



(2)



(3)

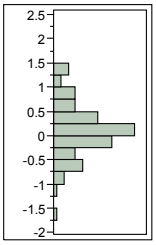
mean difference in each case was found to be positive, indicating compounds tend to be more bound to human plasma proteins than those from the pre-clinical species. Matched pair analysis of the data from the pre-clinical species compared to human plasma indicated that the means are significantly different at the 95% confidence interval.[11] Paired t-test analysis of the means from the rat, dog and mouse data also indicated that they were statistically different to that observed for the human data.[11]

When comparing human to rat plasma protein binding, the mean difference in the $\log K_{b/f}$ values was found to be 0.07 log units, and the difference in the binding appeared to have a normal distribution around this mean difference. Analysis of the difference in the paired $\log K_{b/f}$ values showed that 83% of the measurements lie within a factor of three of each other, and 92% lie within a factor of 5. However, it is evident that there are several compounds that are significant outliers with certain compounds exhibiting a greater than an order of magnitude difference between the human and rat protein binding. Analysis of the outliers showed that there were no clusters or series of compounds that showed much higher binding to human plasma than rat plasma, and close analogues of these compounds do not such marked differences between the species. In contrast, there were small clusters of compounds within the outliers that exhibited higher binding to rat plasma than to human plasma, and examples include a series of reverse hydroxamates (**1**). However, it should be noted that not all reverse hydroxamates show this behaviour.

In terms of the comparison between human and dog plasma protein binding, the mean difference in the $\log K_{b/f}$ values was found to be 0.18 log units, indicating on average compounds are 1.5 times less bound to dog plasma proteins than to human, although the distribution of differences is narrower than that for the comparison of human and rat data, with 87% of the measurements lying with a factor of three of each other, and 93% within a factor of 5. Again, there are several compounds that are significant outliers with some compounds exhibiting greater than an order of magnitude difference between the human and dog plasma protein binding. Visual analysis of the outliers showed that there was no real grouping of compounds that show much higher binding to human plasma than to dog plasma, although there was a group of quinolines (**2**) that exhibited higher binding to dog plasma than to human plasma. Again, not all of the compounds in this class demonstrate this behaviour.

In terms of the comparison between human and mouse plasma protein binding, the mean difference in the $\log K_{b/f}$ values was found to be 0.12 log units, indicating on average compounds are 1.3 times less bound to mouse than human plasma proteins. The distribution of differences is more widespread than the rat and dog comparisons above with only 76% of the measurements lying within a factor of three of each other, and 88% within a factor of 5. This greater spread of differences between the species is also reflected in the standard deviation for the $\Delta\log K_{b/f}$ values for mouse being higher at 0.46, compared to 0.39 and 0.36 for the comparison to rat and dog measurements respectively. Visual analysis of the outliers again showed that there was no real grouping of compounds that show much higher binding to human plasma than to mouse plasma, although there was a group of acyl ureas (**3**) that appeared to be significantly less bound in human plasma compared to mouse plasma. However, it should be again noted that not all acyl ureas exhibit this behaviour and indeed the addition of basic centre into the R4 of (**3**) results in compounds that are up to 8-fold less bound in mouse plasma compared to human plasma, reversing the trend.

Table 3 Comparison of literature human plasma protein values to those obtained in rat plasma.

Distribution of $\Delta\log K_{b/f}$[a]	
Number of compounds in set	87
Mean difference	0.09
% within 3-fold	64
% within 5-fold	69

[a] $\Delta\log K_{b/f} = \log k_{b/f,\ human} - \log K_{b/f,\ rat}$

Analysis of literature protein binding data, again measured using equilibrium dialysis, also shows that compounds tend to be slightly more bound to human plasma proteins than to those from rat. As with the in-house data, all the %free values have been converted to $\log K_{b/f}$ ($\log_{10}$(100-%free/%free) and the distribution of $\Delta\log K_{b/f}$ ($\log k_{b/f,\ human} - \log K_{b/f,\ rat}$) is shown in Table 3. The mean difference between human and rat plasma protein binding for these literature compounds is very similar to that observed for the in-house compounds above, although in this latter case the difference in the means is not statistically different at the 95% confidence interval.[9] Again, there is a distribution of differences in the bindings between the species with 64% of results within a factor of 3 of each other, and 69% within a factor of 5. As with the in-house compounds, there are examples of compounds that do show significant variation in the amount of unbound drug in plasma from the pre-clinical species compared to that in human plasma, and several examples of the literature data for marketed compounds are shown in Table 4. Additional examples of marketed compounds with significant species differences in protein binding measured in-house are also included in Table 4.

In trying to understand the cause of the different extents of

Table 4 Comparison of human plasma protein binding to a second species for example literature and marketed compounds.

Compound	% free in human plasma	% free in second species plasma	Second species
Amiodarone[a]	<0.01	0.12	Dog
Cefotetan[12]	9.0	70	Rat
Cefotetan[12]	9.0	61	Dog
Cefpiramide[13]	1.6	60	Dog
Diazepam[a]	2.0	15.2	Rat
Etoposide[14]	4.9	52	Rat
Etoposide[14]	4.9	63	Dog
Ibuprofen[a]	<0.23	0.68	Rat
Phenprocoumon (S)[15]	0.7	11	Mouse
Pimozide[a]	0.16	<0.04	Dog
Prazosin[a]	5.3	35	Rat
Prazosin[a]	5.3	37	Dog
Sildenafil[a]	6.4	26	Dog
Tasulosin[16]	1.0	20	Rat
Valproate[17]	5.2	88	Mouse
Valproate[18]	5.2	37	Rat
Zamifenacin[18]	0.01	0.2	Rat

[a] In-house measured data for marketed compounds.

binding to the plasma proteins from the various species, the composition of plasma must be considered. The main component of plasma is albumin with concentrations ranging from about 400 to 700μM; the remainder being made up of α_1-acid glycoprotein (9-23μM) and various lipoproteins.[2] In the majority of cases, drug binding is therefore likely to be dominated by the interaction with albumin. This is supported by Figure 1 which shows that the binding measured in human and rat plasmas, correlates extremely well with that for human serum albumin (HSA) and rat serum albumin (RSA) respectively, again as measured using the equilibrium dialysis technique where, in the latter cases, plasma has been replaced by the corresponding albumin solution prepared at physiologically relevant concentrations.

From the above analysis, it is assumed that the majority of drug binding in plasma samples is to albumin. The basis of the non-subtle species differences may therefore be explained in terms of the differences in the amino acid sequence of the corresponding albumins.[19] Largely, the amino acid sequences of the albumins are well conserved across mammals with 80% of the amino acids conserved in dog, 73% in rat, and 72% in mouse relative to human.[20] Although the overall albumin amino acid sequences are relatively well conserved between the species, changes in the amino acid sequence in the drug binding sites may well indicate why some compounds bind differently to albumins from various species. It is known that there are numerous drug binding sites on albumin with the main two being known as site I (located in subdomain IIA) and site II (located in subdomain IIIA).[21] Differences in the drug binding sites of albumins from different species have been highlighted using binding, fluorescence and hydrolysis experiments where significant differences in the drug binding site I were observed for RSA, compared to other mammalian albumins, with smaller, less significant, differences noted for site II.[22] Of course certain compounds will bind strongly to α_1-acid glycoprotein in

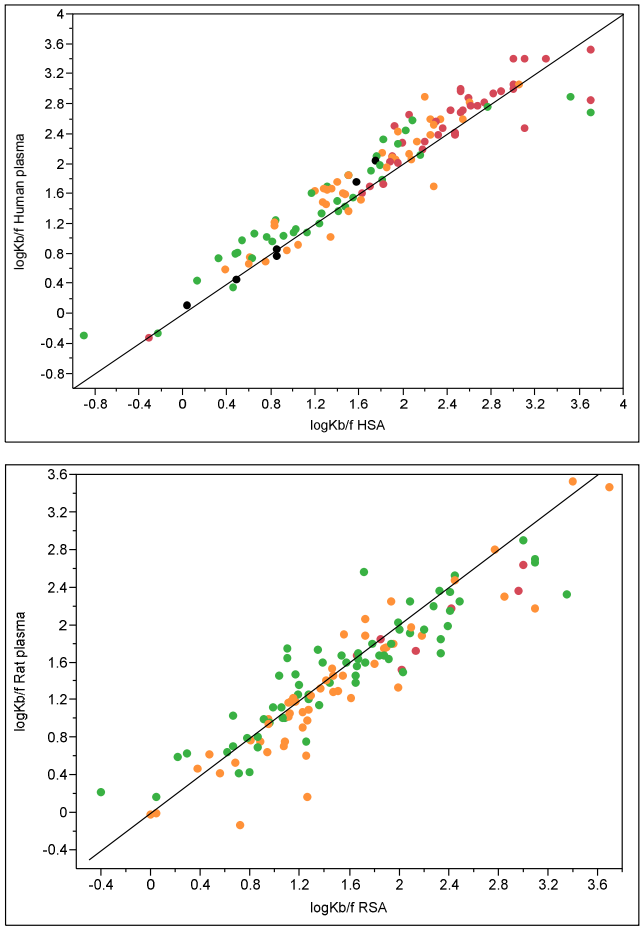


Fig. 1 Correlations of drug binding to plasma proteins and serum albumin; top = human and bottom = rat. The line is slope 1 and intercept of zero: RMSE = 0.30 for human; 0.32 for rat). The colour denotes the charge type of the compounds: red = acid, green = base, orange = neutral and black = zwitterion.

preference to albumin.[23] It is interesting to note that where compounds have been studied in terms of binding to α_1-acid glycoprotein from various species, compounds tend to be more bound to the human form, than compared to the form from the pre-clinical species, and that significant species differences can be observed.[24]

Conclusions

In general, there is a good correlation between the protein binding observed for drug molecules in human plasma and that for rat, dog and mouse plasma, although compounds tend to be slightly more bound to human plasma proteins compared to those from the pre-clinical species. This phenomenon has been observed for data from a large set of diverse internal compounds, as well as from a small set of literature data. There are, however, examples of compounds that do show significant variation in the amount of unbound drug in plasma from pre-clinical species compared to that in human plasma. These outliers are difficult to rationalise or predict in the absence of further studies, for example albumin binding site identification work or

crystallography.[10,21] This is in agreement earlier work comparing human plasma protein binding to that in rat plasma proteins,[6] and also with a recent review of plasma protein binding where it was commented that although not very common, large differences in binding between species can exist and can have major implications.[3] For general screening purposes human or rat measurements can be used as a single parameter but it is recommended that for compounds of greater interest, plasma protein binding is always measured in the species of interest whether it is for understanding pharmacokinetics in pre-clinical species, understanding pharmacodynamics in disease models and generation of dose to human predictions.

Notes and references

[a] *AstraZeneca, Mereside, Alderley Park, Macclesfield, Cheshire, SK10 4TG, UK.; Tel: 44 1625 516080; E-mail: philip.macfaul@astrazeneca.com*

† Electronic Supplementary Information (ESI) available: [Details of plasma protein binding measurements for the AZ library of compounds, along with measured logD, molecular weight and basic group and physical property information. Details of the literature data where human plasma protein binding values have been compared to those in rat, dog or mouse plasmas are available together with the corresponding references]. See DOI: 10.1039/b000000x/

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7. The analysis presented here has been limited to these species since although measurements of plasma protein binding are conducted in plasmas from other species during the drug discovery process for compound progression or problem solving activities, for example guinea pig and cynomologous monkey, and bovine serum albumin (BSA) or foetal calf serum (FCS), these measurements are much less frequent.
8. The extent of plasma protein binding was measured using equilibrium dialysis at 37°C. The relevant plasma was filled into one half of the dialysis cell, while the corresponding half-cell was filled with pH7.4 phoshpate buffer. After 18 hours incubation the compound concentration was measured in both the protein containing and protein free sides of the dialysis cell. The standard deviation of the assay is 0.16 log units.
9. Further details of the library including the plasma protein binding measurements, ion class and selected physical property data, along with a description of some of the scaffolds and group types encountered is available in the Supplementary information.
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