Short Communication

Robust Assessment of Statistical Significance in the Use of Unbound/Intrinsic Pharmacokinetic Parameters in Quantitative Structure–Pharmacokinetic Relationships with Lipophilicity

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ABSTRACT:

The optimization of pharmacokinetic properties remains one of the most challenging aspects of drug design. Key parameters, clearance and volume of distribution, are multifactorial, which makes deriving structure-pharmacokinetic relationships difficult. The correction of clearance and volume of distribution for the unbound fraction in plasma is one approach taken that has enabled quantitative structure-pharmacokinetic relationships to be derived.

Optimization of pharmacokinetic properties is an important part of the drug discovery process. This optimization is aided by an understanding of the ways in which physicochemical properties affect drug distribution, metabolism, and excretion (Smith, 1997). These relationships have been commonly established by simple correlation analysis and key physicochemical properties for controlling drug disposition appear to be lipophilicity, as measured by n-octanol-water partition of un-ionized drug (LogP) or distribution at pH 7.4 (logD) for ionizable compounds, and extent of ionization as described by the pKa. Such structure-pharmacokinetic relationships have been established for clearance (Bernareggi, 1990), renal clearance (Toon and Rowland, 1983), volume of distribution (Smith et al., 1996), adipose storage (Barton et al., 1997), brain penetration (Young et al., 1988; Rowley et al., 1997), and tissue affinity (Nestorov et al., 1998).

Plasma protein binding limits the concentration of drug available for metabolism and distribution in vivo (Rowland et al., 1973). A common approach in analyzing pharmacokinetic parameters is to correct for this factor, and derive so-called “unbound” or intrinsic clearance and unbound volumes. Several authors have reported correlations between lipophilicity and unbound pharmacokinetic parameters (Toon and Rowland, 1979, 1983; Arendt et al., 1983; Hiura et al., 1984; Hinderling, 1988; Smith, 1988; Bernareggi, 1990; Ohkouchi et al., 1990; Blakey et al., 1997). Although this is a rational approach, a statistical ambiguity may be introduced by using pharmacokinetic parameters corrected for fraction unbound in plasma (fu) in correlations with lipophilicity. These correlations might be artifactual, as fraction unbound in plasma is itself correlated with lipophilicity (Hinderling, 1988; Bernareggi, 1990). The problem of statistical significance is explored using three literature data-sets where unbound clearance and unbound volume have been found to be highly correlated to lipophilicity.

Three published data-sets where unbound parameters have been correlated with lipophilicity have been reanalyzed. The reanalysis has shown that high correlation coefficients can be achieved without any true correlation in the data and can lead to misinterpretation of the ways in which lipophilicity influences pharmacokinetics. Randomization procedures are proposed as a more robust method of assessing significance.

Optimization of pharmacokinetic properties remains one of the most challenging aspects of drug design. Key parameters, clearance and volume of distribution, are multifactorial, which makes deriving structure-pharmacokinetic relationships difficult. The correction of clearance and volume of distribution for the unbound fraction in plasma is one approach taken that has enabled quantitative structure-pharmacokinetic relationships to be derived.

Results

Toon and Rowland (1983) measured physicochemical and metabolic data for a series of barbiturates in rat. Unbound volumes of distribution at steady state were calculated by dividing by fu, eq. 1:

\[ V_{ss} = \frac{V_{ss}}{f_u} \]

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fu is also correlated to logD, Fig. 2. Inspection of the significance of this linear correlation would lead you to assume this correlation was highly correlated to lipophilicity.

Abbreviations used are: fu, fraction unbound drug in plasma; Vuss, unbound steady-state volume of distribution; Vss, steady-state volume of distribution; Clint, intrinsic clearance; Clh, hepatic plasma clearance; Qh, hepatic blood flow.

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original data. This randomization exercise showed that 34% of the randomized correlations gave an \( r^2 > 0.86 \), the observed coefficient of determination for the published data-set. As significance at the 5% level is the normally accepted upper limit of safety to protect from spurious correlations (\( P < .05 \)), the correlation between unbound volume and logD was clearly nonsignificant. It is also clear that the nonlinearity of the logVuss versus logD correlation, Fig. 1 is solely due to the nonlinear relationship between logf_u versus logD, Fig. 3. (The relationship between protein binding and logD can be linearized by representing protein binding as a pseudo binding constant by correlating log (fraction unbound/fraction bound) versus logD). Analysis of the complete data-set gives similar conclusions 16% of randomized correlations gave an \( r^2 \geq 0.73 \) the observed coefficient of determination from the complete data-set.

In a subsequent study (Blakey et al., 1997) of 5-substituted barbituric acids (limited to the 5-alkyl homologous series, alkyl = methyl to n-nonylethyl), unbound volume and “unbound” clearance were correlated with logP. From the data in the original publication, unbound parameters were calculated by applying eq. 1, and randomization studies were carried out as we have just described. This showed the correlation between log unbound volume and logP to be not significant and the correlation between log unbound clearance and logP to be highly significant. The actual observed \( r^2 = 0.94 \) was not achieved after randomization of the clearance data 10,000 times. This result was expected as the observed in vivo clearances themselves were significantly correlated with logP even before correction to the unbound data, \( r^2 = 0.84 \).

Table 1 shows physicochemical and metabolic data for a series of cytochrome P-450 3A4 substrates studied by Smith (1997). This data table is not included in the original publication and is compiled in Table 1 from the reference source quoted by the authors. The clearances in humans were corrected to intrinsic hepatic clearance by application of the well stirred model, eq. 2:

\[
CL_{\text{int}} = \frac{CL_h}{f_u(1 - CL_d/Q_h)}
\]

where \( CL_{\text{int}} \) = intrinsic clearance

\( CL_h \) = hepatic plasma clearance

\( f_u \) = fraction drug unbound in plasma

\( Q_h \) = hepatic blood flow = 20 ml/min/kg in humans

For this set of diverse drugs, a very clear correlation is found between log-intrinsic clearance and logD, Fig. 5. However, the plasma clearance values are not correlated with logD, Fig. 6 and protein binding is found to be highly correlated with logD, Fig. 7. The correlation of logCL_{\text{int}} with logD had an \( r^2 = 0.877 \), which would suggest a correlation as good as this could only occur by chance less than 1/1000 times. The randomization experiment suggests that this correlation is indeed significant, and that a correlation as good as this would only occur by chance 12 times in 1000 (\( P = .012 \)), Fig. 8.

It is believed that CYP3A4 has a large, open, and hydrophobic active site. Although these drugs have differing sites of oxidation involving N-dealkylation of the bases and oxidation at allylic and benzylic positions for the neutral compounds, it has been suggested that binding is dominated by hydrophobic interactions. As hydrophobic interactions are relatively weak and nonspecific, different binding orientations of similar energy are allowed in the receptor, which partly explains its lack of specificity (Smith et al., 1997). The fact that the correlation of logCL_{\text{int}} with logD is indeed significant supports this hypothesis.
Discussion

The problem of statistical significance arises in these and other publications using unbound pharmacokinetic parameters because of two factors. Firstly, and most importantly, protein binding across a series of structures is often highly correlated to lipophilicity. Secondly, pharmacokinetic parameters generally have limited numerical range compared with the large ranges of protein binding/lipophilicity measurements. Essentially, in the lipophilicity-unbound pharmacokinetic relationships published, correcting $y$ for the fraction unbound has resulted in the inclusion of a variable on the $y$-axis, which is already known to be highly correlated to lipophilicity. In a typical compound series clearance varies from 1 to 20 (human) or 1 to 100 (rat) ml/min/kg, volume from 0.3 to 20 l/kg whereas the fraction unbound and logP varies over three to six orders of magnitude. In correcting clearance or volume for fraction unbound in the plasma, the variance of the original parameter gets swamped by the correction. Even when $CL_{int}$ is calculated using the well stirred model the effect is observed. Hence the variance of the unbound pharmacokinetic parameter will have markedly increased compared with the original data, and the information that was contained in the original pharmacokinetic parameter now only exists as minor residual variance on the protein-binding-lipophilicity correlation.

We have demonstrated that the use of unbound pharmacokinetic terms in structure-pharmacokinetic correlations requires particular care when assessing significance. The usual significance tests reported by regression programs based on the F-test, or even internal validation procedures such as cross-validation (leave-one-out cross validation being the simplest and least robust internal validation test) cannot protect against chance effects brought about by transforming the $y$-variable. In this paper we have reanalyzed three literature data-sets where correlations between unbound pharmacokinetic parameters and lipophilicity have been reported. Irrespective of the statistical ambiguity that may be introduced by transforming the $y$ variable (by correcting for protein binding), we

### Table 1

<table>
<thead>
<tr>
<th>Name</th>
<th>Charge</th>
<th>LogD$_{7.4}$</th>
<th>Fraction Free in Plasma</th>
<th>CL$_{int}$ ml/min/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amiodarone</td>
<td>basic</td>
<td>6.22</td>
<td>0.0002</td>
<td>1.9</td>
</tr>
<tr>
<td>Felodipine</td>
<td>neutral</td>
<td>4.8</td>
<td>0.00401</td>
<td>12</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>neutral</td>
<td>2.87</td>
<td>0.042</td>
<td>7</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>basic</td>
<td>2.71</td>
<td>0.087</td>
<td>11.8</td>
</tr>
<tr>
<td>Bupivacaine</td>
<td>basic</td>
<td>2.54</td>
<td>0.052</td>
<td>7.1</td>
</tr>
<tr>
<td>Imipramine</td>
<td>basic</td>
<td>2.52</td>
<td>0.11</td>
<td>15</td>
</tr>
<tr>
<td>Trizolam</td>
<td>neutral</td>
<td>1.63</td>
<td>0.11</td>
<td>5.6</td>
</tr>
<tr>
<td>Diltiazem</td>
<td>neutral</td>
<td>2.05</td>
<td>0.28</td>
<td>12</td>
</tr>
<tr>
<td>Alfentanil</td>
<td>basic</td>
<td>2.11</td>
<td>0.086</td>
<td>6.7</td>
</tr>
<tr>
<td>Quinine</td>
<td>basic</td>
<td>2.11</td>
<td>0.07</td>
<td>1.9</td>
</tr>
<tr>
<td>Amlodipine</td>
<td>basic</td>
<td>1.05</td>
<td>0.075</td>
<td>5.9</td>
</tr>
<tr>
<td>Lidocaine</td>
<td>basic</td>
<td>1.56</td>
<td>0.42</td>
<td>9.2</td>
</tr>
<tr>
<td>Dofetilide</td>
<td>basic</td>
<td>0.96</td>
<td>0.46</td>
<td>10.2</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>neutral</td>
<td>0.96</td>
<td>0.19</td>
<td>9.1</td>
</tr>
<tr>
<td>Disopyramide</td>
<td>basic</td>
<td>-0.36</td>
<td>0.27</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Fig. 5. Plot of log (intrinsic human in vivo clearance) versus logD$_{7.4}$ for 14 CYP 3A4 substrates.

Fig. 6. Plot of clearance (millilitre per minute per kilogram) versus logD$_{7.4}$ for 14 CYP 3A4 substrates.

Fig. 7. Plot of log (unbound drug in plasma) versus logD$_{7.4}$ for 14 CYP 3A4 substrates.

Fig. 8. Distribution of random $r^2$ from log $CL_{int}$ versus logD$_{7.4}$ from 10,000 randomization of CL for 3A4 substrates.
have shown that randomization trials are a simple method of assessing true significance.

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References


