Letter to the Editor

BINDING OF DRUGS TO HEPATIC MICROSOMES: COMMENT AND ASSESSMENT OF CURRENT PREDICTION METHODOLOGY WITH RECOMMENDATION FOR IMPROVEMENT

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In recent years the phenomenon of hepatic microsomal nonspecific binding of drugs has received considerable attention in this journal, as well as in others. There is general agreement on the need to correct kinetic constants (namely, \(K_m\) and \(K_i\) values) for the fraction of drug concentration unbound (fu\(_{inc}\)) to ensure optimal prediction of clearance and inhibition potential in vivo from in vitro hepatic microsomal studies (Tucker et al., 2001; Bjornsson et al., 2003). The ability to predict in silico the extent of microsomal binding from a drug’s physicochemical properties, as proposed by Austin et al. (2002), is an attractive option that has engendered much interest. Recently, the same authors have extended earlier principles from microsomes to intact hepatocytes (Austin et al., 2005) and have shown application to various sets of hepatocyte and microsomal clearance data (Riley et al., 2005). Having demonstrated a relationship between the charge-dependent lipophilicity parameter “\(\log P/D\)” and binding affinity to microsomes for a range of drugs, Austin et al. (2002) proposed that the fu\(_{inc}\) could be predicted using a simple linear relationship. This method was based on a theoretical combination of the above relationship and the ability to project fu\(_{inc}\) from one concentration of microsomes to another. As no evaluation of prediction outcome was provided, we have assessed the prediction of fu\(_{inc}\) using the Austin equation. A dataset including the 56 drugs used in the original study together with an additional 36 drugs both from other published studies (Obach, 1999; Naritomi et al., 2001; Soars et al., 2002) and from our own unpublished work was used.

First, we examined \(\log P\) values from several sources (including experimental and in silico) for as many drugs for which data were available but found no major influence of source; mean \(\log P\) values were therefore used, and these were converted to \(\log D\) values where appropriate. Binding affinity (\(K_i\)) values were calculated from experimental microsomal fu\(_{inc}\) values, and the latter were converted to values corresponding to 1 mg microsomal protein per ml, as required; when several experimental values were obtained for the same drug, a mean value was used. In demonstrating the relationship between binding affinity and \(\log P/D\), Austin et al. (2002) deliberately omitted drugs weakly bound (fu >0.9)—effectively excluding acidic drugs—and justified this based on the relative difficulty in determining minor binding and the fact that this degree of binding was not important. Figure 1A shows the relationship between binding affinity and \(\log P/D\) for all of the basic and neutral drugs in the extended database (n = 64). The fitted linear relationship is similar to that obtained by Austin et al. (2002), and the correlation \((r^2 = 0.68)\) is comparable despite the increase in the number of drugs used. The linear equation parameters obtained using this extended database analysis were substituted into the Austin equation, and the resultant predicted fu\(_{inc}\) values for all drugs were compared with observed values of fu\(_{inc}\). This prediction calculation typically resulted in underprediction of fu\(_{inc}\) across the range of binding (average fold error = \(-1.5\)); 65% of all fu\(_{inc}\) predictions were less than the observed fu\(_{inc}\) values. In terms of precision, 28% of the predictions were greater than 2-fold different from observed.

The charge-dependent parameter, \(\log P/D\), suggested by Austin et al. (2002) does show the most consistent trend between both drug type and individual drugs, including acidic drugs, when compared with \(\log P\) or \(\log D\). It does not seem justified to exclude acidic drugs in quantifying this relationship because they provide limiting values within this inclusive property. Figure 1B, which includes all the drugs, shows that the relationship between binding affinity and \(\log P/D\) is not linear. Any mechanistic model for microsomal binding would involve a polar interaction as well as a lipophilic/hydrophobic interaction, and so it is not surprising that the overall interaction is more complex than a simple linear relationship. As an alternative empirical approach, we fitted a quadratic relationship to all the data in the extended database and modified the fu\(_{inc}\) prediction to that given in the equation below.

\[
\frac{1}{fu_{inc}} = \frac{1}{C} + 10^{0.072 \cdot \log P/D + 0.067 \cdot \log P/D + 0.126}
\]

Despite a similar degree of correlation for the nonlinear affinity relationship \((R^2 = 0.75)\) compared with the linear relationship \((R^2 = 0.68)\), the resultant prediction calculation (average fold error = 1.1) successfully eliminated the negative bias found when applying the linear equation to prediction of fu\(_{inc}\) (Fig. 1B). This represents a useful improvement on the Austin prediction method, although the precision of prediction remains approximately the same, with 25% of the predictions outside 2-fold accuracy.

Drug amphiphilicity may be an important prerequisite for binding to microsomes, with strong binding being associated with a charged amine moiety in combination with a multiple aromatic/cyclic hydrophobic moiety. However, few drug molecules are this simple, and greater precision of prediction might only be achieved by mechanistic modeling of additional binding traits such as molecule shape, type and location of additional polar/functional groups, etc. Despite these caveats, there is a strong general trend in binding affinity with lipophilicity, which can be represented in a relatively simple nonlinear manner.

Consideration of neutral and basic drugs separately further illustrates the inadequacy of the linear relationship assumed in the Austin equation. In the case of neutrals, which are mostly moderately bound, inaccuracy in the fu\(_{inc}\) values predicted by the linear model can be explained simply by a constant overestimation of affinity. Figure 1C shows the predicted fu\(_{inc}\) (based on the linear equation) with a fitted bias for affinity. For bases, however, the fu\(_{inc}\) values predicted by the linear model seem to diverge from observed fu\(_{inc}\) in a disproportionate manner with increasing fu\(_{inc}\)
In other words, as binding affinity of bases decreases, increasingly biased estimates of affinity are provided; in both cases, this misrepresentation is resolved using the quadratic model [Fig. 1, E (neutrals) and F (bases)].

Austin and coworkers have also advocated a linear relationship for the intracellular binding of drugs within hepatocytes (Austin et al., 2005) and, hence, an \( f_{\text{u,in}} \) term for this in vitro system. For hepatocytes, although it seems that a major role for lipophilicity is reflected in the trend of logP/D with binding affinity, it would seem that inaccuracies similar to those we describe for microsomes may result. Furthermore, intracellular binding is not the sole reason for cell-to-medium concentration ratios differing from unity. The latter parameter is also affected by membrane transporter activity and is often concentration-dependent (Jones et al., 2004). Thus, for hepatocyte

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**Fig. 1.** Prediction of microsomal binding from logP/D relationships. Relationship between logP/D and binding affinity for neutral and basic drugs (\( n = 64 \)) with linear fit (A), and for acids, neutrals, and bases (\( n = 92 \)) with quadratic fit (B). Relationship between \( f_{\text{u,in}} \) observed and \( f_{\text{u,in}} \) predicted using the linear model for neutrals (C) and bases (D), and using a quadratic relationship (E and F, respectively). Dashed lines denote the line of identity; solid lines indicate the resultant relationship between observed and predicted from either arbitrarily using a power trend line (D-F) or by determination of \( f_{\text{u}} \) by applying a fitted bias on the affinity constant (C).
experiments, there is little support for the linear $f_{\text{unc}}$ assumption, and so its validity is questioned.

In summary, the relationship between $f_u$ in microsomes and the lipophilicity parameter $\log P/D$ can be best described by a nonlinear empirical equation which allows unbiased predictions unlike those based on an assumed linear relationship.

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References


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