FUTURE PROSPECTS FOR TOXICOKINETICS: PREDICTION OF DRUG DISPOSITION AND ADVERSE EFFECTS IN HUMANS FROM IN VITRO MEASUREMENTS OF DRUG METABOLISM, TRANSPORT AND BINDING

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Pharmacokinetic parameters such as the area under the plasma concentration-time curve (AUC) and the steady-state plasma concentration (Css) are usually considered to be directly related to the pharmacological and/or adverse effects of a drug. Therefore, it is very important to predict these parameters in humans to avoid the unexpected adverse effects of a drug. Taking account of the recent increasing availability of human liver samples such as microsomes and P450 isozyme expression systems, successful prediction of in vivo drug disposition from in vitro data in humans may be realized. Therefore, by combining in vitro data on drug metabolism, transport and binding with prediction of renal clearance by animal scale-up methods etc., it may become possible to predict human optimal doses and the therapeutic index in clinical situations (Iwatsubo et al., 1996; Iwatsubo et al., 1997; Iwatsubo et al., impress).

**Prediction of in vivo metabolic clearance from in vitro data.**

The intrinsic clearance (CL_int) was calculated for 25 metabolic reactions using the values reported in literature. CLint determined from in vitro data (CL_int, in vitro) was compared with that determined from in vivo data (CL_int, in vivo) assuming a dispersion model (Fig.1). A good correlation is observed between CLint, in vitro and CL_int, in vivo, with around 50% and more than 70% of all the reactions exhibiting no greater than 3-fold and 5-fold differences, respectively (Fig.2) (Iwatsubo et al., 1997). This finding suggests the possibility of predicting in vivo hepatic clearance in humans from in vitro metabolic data if appropriate experimental conditions are used to determine metabolic rates in vitro. For the five metabolic reactions, however, differences greater than 5-fold were observed. Since most information used to calculate CL_int, in vitro and CLint,

![Diagram](image)

**Fig. 1.** Procedure for the calculation of CL_int, in vitro for the metabolism catalyzed by enzyme 1 (E1). In the analysis using the dispersion model, a Δv of 0.17 was used. Mototal, total amounts of all metabolites.

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Fig. 2. Comparison of CLint, in vitro with CLint, in vivo humans. The methods for calculating intrinsic clearances both in vivo and in vitro are described in the text. The solid line indicates a 1:1 correspondence. The numbering of the drugs is as follows: 1, alprazolam; 2, diazepam; 3, doxepin; 4, imipramine; 5, lidocaine; 6, loxetine; 7, α-hydroxymetoprolol; 8, O-demethylmetoprolol; 9, hydroxymethylmexetine; 10, p-hydroxymexetine; 11, phenacetin; 12, quinidine; 13, 1,3-dimethylyric acid + 1-methylxanthine; 14, 3-methylxanthine; 15, tolbutamide; 16, norverapamil (R-form); 17 D-617 (R-form); 18, D-703 (R-form); 19, norverapamil (S-form); 20, D-617 (S-form); 21, D-703 S(form); 22, 6-hydroxywarfarin (R-form); 23, 7-hydroxywarfarin (R-form); 24, 6-hydroxywarfarin (S-form); 25, 7-hydroxywarfarin (S-form).

in vivo comes from a variety of different literature experiments, not carried out for the purpose of comparing both clearances, various factors can cause the differences in CLint, in vitro and CLint, in vivo.

First possibility is the incorrect assumption of rapid equilibrium of drugs between blood and hepatocytes. When the intrinsic metabolic clearance of drugs in hepatocytes is much greater than the efflux clearance from hepatocytes to blood, CLint, in vitro calculated from in vivo information based on the blood concentration-time profile of parent drugs is rate-limited by the influx process from blood to hepatocytes, resulting in the underestimation of CLint, in vivo (Yamazaki et al., 1996).

Secondly, when active transport through the sinusoidal membrane of hepatocytes is operating, the unbound concentration of drugs in hepatocytes is different from that in blood at equilibrium. The unbound concentration of drug in hepatocytes should be higher or lower than that in blood when active transport is involved in the influx or efflux process, resulting in a corresponding overestimation or underestimation of CLint, in vivo.

Third possible reason may be that the contribution of metabolism in tissues other than liver needs to be taken into consideration. Bearing in mind that the drug concentration is very high in the gut after oral administration and that considerable amounts of CYP3A4 distribute throughout the small intestine, a significant contribution of metabolism in the small intestine is possible for the substrates for CYP3A4.

Finally, the livers used for the estimation of in vitro and in vivo CLint were completely different so that

Fig. 3. Correlations between receptor dissociation constant (Kd) and maximum plasma concentration (Cmax) or maximum unbound plasma concentration (Cunbound) of benzodiazepine anxiolytics after oral administration of their therapeutic doses (Ito et al., 1997).
Interindividual variability can affect the difference between CLint, *in vitro* and CLint, *in vivo*. If the effect of extrinsic variability is large rather than that of intrinsic variability, it may be necessary to predict metabolic activity for the study drug based on the concept of a "scaling factor" by measuring the metabolic activity of typical substrates for the P450 isozymes involved instead of their contents in liversamples. By selecting some reference compounds with well established pharmacokinetic parameters to calculate the CLint, *in vivo*, measuring the metabolic activity of this reference compound in the same enzyme source as that used to measure the metabolic activity of the study drug, multiplying the ratio of CLint, *in vivo* to CLint, *in vitro* for the reference compound as a scaling factor by the CLint, *in vitro* for the study drug, it may be possible to predict the CLint, *in vivo* for the study drug.

**Importance of prediction of free drug concentrations.**

The occurrence of toxic effects may be related to AUC, *C*<sub>max</sub>, or mean residence time (MRT), depending on the type of drug involved. Furthermore, many of the PK/PD studies have so far emphasized the need to measure concentrations of free drug, i.e. not bound to plasma proteins. In practice, plasma protein binding can be measured noninvasively in humans only by blood sampling. As the interspecies and interindividual variability in protein binding is large, this is especially important for drugs which are highly protein bound (more than 80-90%).

For example, a significant log-linearity was observed in the relationship between receptor dissociation constant (K<sub>d</sub>) obtained from *in vitro* binding studies and maximum plasma concentration of various benzodiazepine anxiolytics after oral administration of their therapeutic average doses (Fig. 3) (Ito et al., 1997). The correlation coefficient was larger for unbound plasma concentration (r=0.961) than for total plasma concentration (r=0.768), indicating that unbound drug concentration is more important as a determinant of drug effects compared with total concentration. This may be explained by the assumption that the unbound drug in the plasma is in equilibrium with that in the target tissue, brain.

![Diagram of a hybrid model](image)

**Fig. 4.** Schematic representation of a hybrid model.

- C<sub>a</sub>: Drug concentration in the arterial blood.
- C<sub>v</sub>: Drug concentration in the venous blood.
- C<sub>e</sub>: Drug concentration in the extracellular fluid (ECF).
- C<sub>r</sub>: Drug concentration in the cell.
- V<sub>e</sub>: Extracellular volume.
- V<sub>r</sub>: Intracellular volume.
- f<sub>b</sub>: Blood unbound fraction.
- f<sub>r</sub>: Tissue unbound fraction.
- PS<sub>inf</sub>: Influx clearance for unbound drug.
- PS<sub>eff</sub>: Eflux clearance for unbound drug.
- CL<sub>int</sub>: Intrinsic metabolic or excretory clearance for unbound drug.

\[
\text{AUC}_{0-t} = \text{AUC}_{0-t} \times \frac{\text{PS}_{\text{eff}}}{\text{PS}_{\text{inf}}}
\]

![Graph of drug concentration profiles](image)

**Fig. 5.** Simulation of the time profiles of drug concentrations in the cell as a function of membrane permeability (S). AUC in the cell is kept constant irrespective of the PS value.
Prediction of drug concentrations in the target site.

In many cases, the pharmacological and/or toxic effects of a drug is most directly associated with the drug concentration in the target site. Drug concentrations in the tissue can be estimated using the hybrid model (Fig.4) if the information is available for the membrane permeability, tissue unbound fraction, and so on (Sugiyama and Ooie, 1993). When the drug effect depends on the AUC for the unbound drug in the tissue (AUCu,T), the prediction should be more simple because AUCu,T is equal to the AUC for the unbound drug in the blood (AUCu,b) if the drug transport between the blood and tissue is by the passive diffusion. If the active transport system is involved in the membrane transport, AUCu,T can be calculated as AUCu,T = AUCu,b × (PSeff / PSref) where PSref and PSeff represents the membrane permeation clearance of unbound drugs for the influx from blood to tissue and the efflux from tissue to blood, respectively. Therefore, in the case of a drug which is actively transported through the membrane, interindividual and interspecies differences in PSref and PSeff in the target tissue as well as those in AUCu,b should be taken into consideration in the prediction of the pharmacological and/or toxic effects.

How can the pharmacological and/or toxic effects be predicted if they depend not only on the AUCu,T but also on the maximum unbound concentration (Cmax) and/or the mean residence time (MRT) in the tissue (Fuse et al., 1995; Suzuki et al., 1996)? Assuming the absence of the active transport (PSref = PSeff = PS) for simplification, it is theoretically demonstrated that the increase in the PS elevates the Cmax and shortens the MRT without influencing the AUCu,T (Fig.5) (Sugiyama and Ooie, 1993). Therefore, it is clear that the effect of the change in PS on the change in pharmacological and/or toxic effects depends greatly on which kinetic parameter (AUCu,T, Cmax, or MRT) governs the pharmacological and/or toxic effects of the drug in question.

One of the most important subjects to be studied in the near future is to develop the method predicting PSref and PSeff in each human tissue from the in vitro experiments using human tissues, cells, or transporter expression systems.

Prediction of in vivo drug-drug interactions from in vitro data.

Serious adverse effects caused by drug-drug interactions have been paid special attention and have become a social problem since an interaction between sorivudine and fluorouracil resulted in fatal toxicity. Drug-drug interactions involving metabolism are one of the principal problems in the clinical practice to evaluate the pharmacological and adverse effects of drugs. In order to prevent the toxic drug-drug interactions, therefore, it is very important to quantitatively

\[ \text{Scheme 1. Proposals for the prediction of inhibitory effects of co-administered drugs on the metabolism of a novel drug in vivo.} \]

1. Confirmation of the involvement of P-450 (in vitro metabolism studies using human hepatocytes and microsomes);
   - Inhibition by SKF-525A and CO.
2. Identification of P-450 isozyme (in vitro metabolism studies);
   - Inhibition studies using human P-450 expression systems, antibodies, or specific inhibitors.
3. Estimation of the maximum plasma unbound concentration of the coadministered inhibitor (lin, max, u);
   - Searching the in vivo pharmacokinetic data for the co-administered inhibitor.
4. Evaluation of the active transport into hepatocytes;
   - (i) Measurement using hepatocytes isolated from experimental animals (rat).
     - 2-fold margin should be considered for the concentration ratio obtained by rat studies.
   - (ii) Measurement using human hepatocytes and human liver slices.
   - (iii) If no information available: 10-fold margin for the concentration ratio.
5. Measurement of Ki for the co-administered inhibitor (using human livermicrosomes or human P-450 expression systems).
6. Judging the possibility of metabolic inhibition;
   - Comparison of the values of lin, max, u and Ki (lin, max, u/Ki>0.2)

*represents the smallest limit for the in vivo interaction. This value should depend on the pharmacodynamic and/or toxicodynamic features of the drug investigated.
predict pharmacokinetic changes caused by co-administration of drugs which are known to inhibit the hepatic metabolism of the drug under study (Sugiyama et al., 1996).

Based on the physiological pharmacokinetic analysis, we have tried to predict in vivo drug-drug interactions from in vitro data on drug metabolism obtained from the literature. In order to avoid a false negative prediction due to underestimation of the inhibitor concentration (Iu), the plasma unbound concentration at the entrance to the liver, where the blood flow from the hepatic artery and portal vein meet, was considered to be the maximum value of Iu and was used in the prediction (Fig.6). The procedures for estimating the possibility of in vivo drug-drug interactions are summarized in Scheme 1.

The prediction was successful for some of the drug combinations but not for others (Table 1), suggesting that alterations in in vivo pharmacokinetic parameters which have been attributed to the certain metabolic

Table 1. Summary of the prediction of in vivo drug-drug interaction based on in vitro data in literature<sup>a</sup>.

<table>
<thead>
<tr>
<th>Inhibitor-Inhibited drug</th>
<th>Inhibition ratio</th>
<th>AUC ratio&lt;sup&gt;b&lt;/sup&gt;</th>
<th>I&lt;sub&gt;max&lt;/sub&gt;/K&lt;sub&gt;i&lt;/sub&gt;&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)Successful cases:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(i) sulfaphenazole-tolbutamide (C29)</td>
<td>×5.3 (X5.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(ii) erythromycin-cyclosporin (C4A)</td>
<td>×1.6 (X1.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(iii) erythromycin-theophylline (C4A)</td>
<td>×1.1 (X1.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(2)Successful for the metabolic pathway but unsuccessful for the total:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(iv) quinidine-sparteine (C2D)</td>
<td>×2.9 (X1.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(v) ketoconazole-terfenadine (C4A)</td>
<td>×10 (X1.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(3)Unsuccessful for the metabolic pathway:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(vi) fluoxetine-imipramine (C2D)</td>
<td>×1.9 (X1.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(vii) ciprofloxacin-caffeine (C1A2)</td>
<td>×1.6 (X1.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(viii) omeprazole-diazepam (C2C19)</td>
<td>×2.0 (X1.0)</td>
<td></td>
<td></td>
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</tbody>
</table>

<sup>a</sup> Predictions were based on the inhibition of the P-450 isozyme which is mainly related to the metabolism of the corresponding drug.<br>
<sup>b</sup> Change in AUC induced by the drug-drug interaction (observed value).<br>
<sup>c</sup> Change in AUC induced by the drug-drug interaction (predicted value).<br>
<sup>d</sup> Index for the extent of the drug-drug interaction. I<sub>max</sub>/K<sub>i</sub> was calculated using pharmacokinetic parameters of the drug.
inhibition may be caused by other factors such as an interaction involving other metabolic pathways, carrier-mediated biliary and urinary excretions, and/or the intestinal absorption process.

REFERENCES


