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COMMENTARY

UTILITY OF *IN VITRO* DRUG METABOLISM DATA IN PREDICTING *IN VIVO* METABOLIC CLEARANCE

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In recent years, there has been a large expansion in both the range and use of *in vitro* systems to study drug metabolism and metabolism-related phenomena. Purified enzymes, subcellular fractions (microsomal, 9000 *g* supernatant), whole cells (freshly isolated or in culture), and precision tissue slices are in widespread use. Understandably, investigations have centred mainly, but not exclusively, on the liver as this tissue represents the major site of metabolism for most drugs. In addition to investigating hepatic drug metabolism at all levels of structural integrity, there is also the option of employing customized *in vitro* preparations by using molecular biological techniques. The use of heterologous expression systems for particular drug-metabolizing enzymes, or for isoforms from within a particular enzyme family, provides an extremely valuable supplement to the *in vitro* armament. Furthermore, the increased availability of human tissue has provided additional impetus to the current high level of interest in *in vitro* methodology for drug metabolism.

These advances in *in vitro* methodology when taken together with the sensitivity of modern analytical chemistry provide opportunities to detail drug metabolism in a manner not previously possible. The overall increase in the level of sophistication in study design has led to a greater expectation from the data generated. A case in point is the utility of these *in vitro* systems in generating data to allow prediction of *in vivo* behaviour.

Discussion of theoretical aspects important in correlating *in vitro* and *in vivo* quantitative data on drug metabolism commenced more than 20 years ago [1], and in 1977 a seminal study by Rane *et al.* [2] was published. These latter investigators used *in vitro* data, mainly from 9000 *g* supernatant incubations, to predict *in vivo* disposition. The good correlation reported between predicted and observed hepatic extraction ratios of seven drugs by isolated rat livers was the first demonstration of the potential of *in vitro*:*in vivo* predictions. More recently, there has been further subsequent analysis of largely the same data base [3, 4] with a view to comparing pharmacokinetic models for describing liver extraction efficiency. However, with the exception of a small number of specific studies [5–10], there has been little attempt to substantiate and advance the early work of 1977.

The aim of this commentary is to present a strategy to achieve the prediction of *in vivo* clearance from *in vitro* data based on our current understanding of metabolite kinetics *in vitro* and *in vivo*. Hepatic microsomes and hepatocyte suspensions are the main *in vitro* systems addressed. The theoretical basis of the strategy is outlined and its general applicability evaluated, using a data base of 25 drugs drawn from the literature. Finally, the prospective value of the approach is discussed.

Theoretical basis

The cornerstone for the extrapolation of *in vitro* data to the *in vivo* situation is the parameter intrinsic clearance (CL_{int}). This term was coined in the 1970s to describe the rate of metabolism of a drug under *in vivo* conditions [1, 2]. Intrinsic clearance is a pure measure of enzyme activity towards a drug and is not influenced by other physiological determinants of liver clearance such as hepatic blood flow or drug binding within the blood matrix. As with all clearance terms, it has units of volume rate (e.g. mL/min) and acts as a proportionality constant to describe the relationship (equation 1) between rate of metabolism of a drug and its concentration at the enzyme site (C_E).

$$\text{Rate of metabolism} = CL_{int} \cdot C_E. \qquad (1)$$

The free concentration of drug (unbound to macromolecules) within the liver is assumed to equate with C_E.

From a biochemical viewpoint, CL_{int} can be considered in terms of the enzyme parameters of the Michaelis–Menten relationship shown in equation 2.

$$\text{Rate of metabolism} = \frac{V_{max} \cdot C_E}{K_m + C_E} \qquad (2)$$

where V_{max} is the maximum rate of metabolism and K_m is the Michaelis constant for the drug–enzyme interaction.

Under linear conditions when C_E is 10% or less of the K_m, equation 2 reduces to

$$\text{Rate of metabolism} = \frac{V_{max} \cdot C_E}{K_m} \qquad (3)$$

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which is analogous to equation 1. Hence

$$CL_{int} = V_{max}/K_m \quad (4)$$

$$= \text{Rate of metabolism}/C_E \quad (5)$$

Thus, the commonly reported Michaelis–Menten parameters can be used to obtain an *in vitro* intrinsic clearance. In the case of hepatic microsomes, CL_{int} will be expressed in terms of μL/min/mg microsomal protein and in the case of hepatocytes μL/min/10^6 cells since rates of metabolism are usually measured as pmol/min for micromolar drug incubation concentrations.

Determination of CL_{int} *in vivo* is more problematic due to two major reasons. First, although total body clearance can be easily partitioned to provide hepatic metabolic clearance, this term is an index of overall efficiency of hepatic removal of drug and is not solely a consequence of CL_{int}. Other major determinants of hepatic clearance are hepatic blood flow and the extent of binding of drug to the blood components, principally albumin [11]. Second, *in vivo* clearance terms relate to circulating plasma or blood concentration of drug. For example, for hepatic clearance (CL_H) and blood concentration (C_b)

$$CL_H = \frac{\text{Rate of metabolism}}{C_b} \quad (6)$$

where

$$CL_H = fm \cdot CL \quad (7)$$

and fm is the fraction of the dose subjected to hepatic metabolism and CL is total body clearance.

The quantitative interrelationships between hepatic clearance, hepatic blood flow, unbound fraction of drug in blood and CL_{int} are complex [11] and cannot be defined fully without knowing the relationship between circulating drug concentrations and C_E. As this cannot be determined practically, liver models are necessary to relate these two drug concentrations and are an implicit part of obtaining an *in vivo* CL_{int} [12]. Thus, the use of the term CL_{int} requires a knowledge and an acceptance of the assumptions of a particular liver model.

Several liver models have been proposed and these include: the venous equilibration model, the sinusoidal perfusion model, and the dispersion model [12]. The properties of these models have been reviewed in depth and so only essential features are outlined here. Common features are: (a) rate of distribution of drug from blood to the enzyme site is perfusion rate limited; there are no diffusion barriers, (b) only unbound drug crosses membranes, (c) there is good mixing of hepatic arterial blood and hepatic portal blood prior to transport down the sinusoids of the liver, and (d) there is a homogenous distribution of enzymes within the liver. Whereas the first three of these assumptions can be accepted without too much concern, the disregard of the well-known heterogeneity in the distribution of drug-metabolizing enzyme systems along the liver unit is of particular note.

In global terms, all models provide similar predictions with respect to the overall effects on CL_H of changes in CL_{int}, blood flow and blood binding of drug. They have been extremely valuable in defining broad and useful characteristics of pharmacokinetic behaviour [11]. However, the models differ in the manner in which they relate the processes of delivery of drug to the hepatic milieu (blood flow and unbound fraction) and the actual drug–metabolizing event (CL_{int}). For drugs with low hepatic clearance relative to hepatic blood flow and therefore with a low extraction ratio by the liver, the differences between the models are minimal [13]. This is because the concentration difference across the liver is minimal and unbound blood concentration probably approximates C_E. When extraction ratios reach 0.7, the concentration difference across the liver is substantial, and liver model differences are evident. For drugs of high extraction ratio (>0.9), considerable differences arise between models [13].

For the purposes of this commentary, the venous equilibration model has been adopted. This is the most used liver model due to its simplicity and the lack of consensus on whether the more elaborate models, which are more in keeping with hepatic anatomy and physiology but more mathematically complex, provide any better description of hepatic events.

The interrelationship between hepatic clearance, CL_{int}, blood flow (Q_H) and unbound fraction of drug in the blood (fu) using the venous equilibrium model is shown in equation 8.

$$CL_H = \frac{Q_H \cdot fu \cdot CL_{int}}{Q_H + fu \cdot CL_{int}}. \quad (8)$$

This equation may be rearranged to define CL_{int}

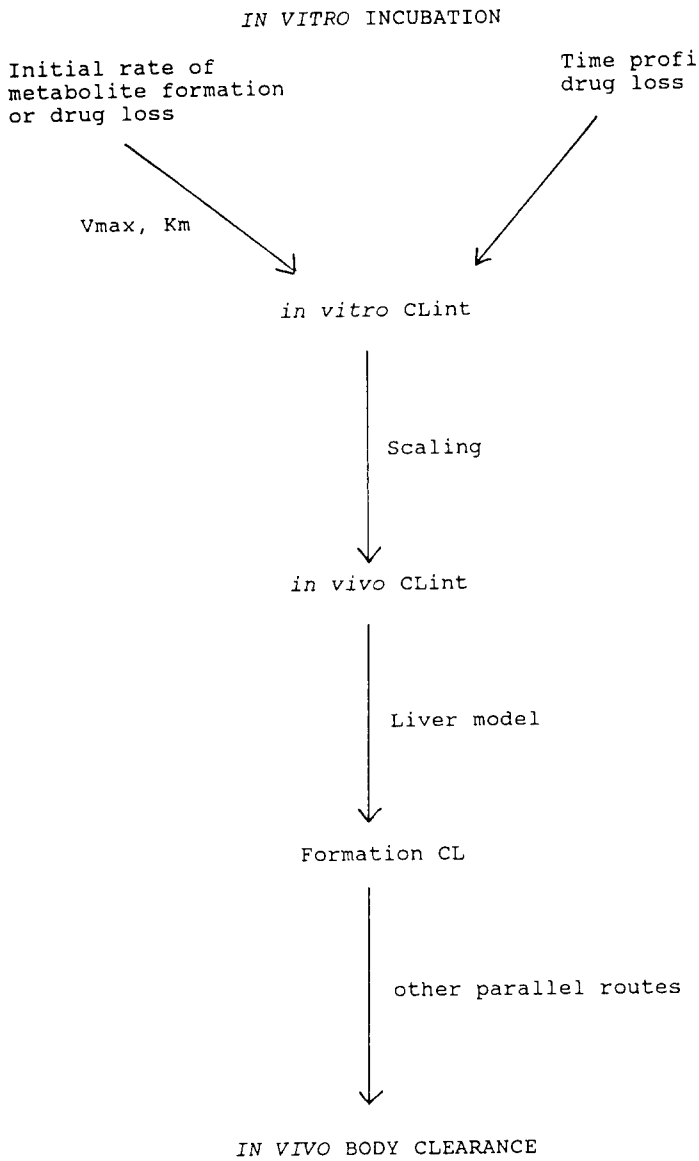
$$CL_{int} = \frac{CL_H}{fu(1 - E)} \quad (9)$$

where E is the hepatic extraction ratio defined by CL_H/Q_H.

A strategy for in vitro–in vivo *extrapolation*

A four-stage strategy to extrapolate data from an *in vitro* incubation to provide *in vivo* clearance is outlined in Scheme 1. This strategy will be discussed in terms of its applicability to hepatic microsomal and hepatocyte data; however, the principles will apply equally to other *in vitro* systems.

To be of value for *in vivo* extrapolation, *in vitro* data must be obtained under conditions that are linear with respect to time and enzyme concentration. For drug-metabolizing enzymes, rate data are usually expressed per unit of microsomal protein or hepatocyte number. The traditional method is to measure specific metabolite production rate, although "total" metabolite production may be equally useful and in certain circumstances more appropriate (e.g. when there is sequential metabolism of a primary metabolite to a secondary or even tertiary metabolite). The additional pathways must be quantified either specifically or in an overall form in order to conserve mass balance conditions. Complex pathways involving several primary metabolites and secondary and tertiary metabolites that may arise from a number of precursors are clearly best measured by the "total" metabolism option.



Scheme 1. A four-stage strategy for the extrapolation of *in vitro* drug metabolism data to estimate *in vivo* clearance.

An alternative variant is to measure the rate of drug disappearance from the incubation medium. However, it is of importance to substantiate that loss of drug equates with metabolism rather than some other process, such as physical adsorption or incorporation into cellular components. In principle, there is no reason why data on drug disappearance under initial rate conditions should not be processed by the same Michaelis–Menten-type analysis used for metabolite production under initial rate conditions.

Thus, the first stage is to obtain the $V_{max}(s)$ and $K_m(s)$ for the metabolism of the drug in question. Whether a K_m expressed in terms of the concentration of substrate added is equivalent to the concentration of substrate available to the enzyme is a matter for conjecture. Ideally, any futile binding of a non-specific nature which is independent of the enzyme binding process should be accounted for. This has been measured by some workers [5, 10, 14] using microsomal preparations, and the degree of futile binding has been found to be low (fu 0.4 to 0.9) when compared to the value for plasma albumin unbound fractions that are known to be of importance in restricting liver uptake *in vivo*. This probably results from the low protein concentration used in microsomal preparations (usually 1 mg/mL) compared with that of plasma albumin (40 mg/mL), as well as the different type of proteins in both media. However, this particular issue remains to be fully explored.

An alternative method for obtaining *in vitro* data

Table 1. Physiological and biochemical parameters important for scaling *in vitro* drug metabolism data

	Parameter value	Scaling factor used for SRW*
Liver weight	4.5 g/kg body weight	11 g
Liver blood flow	1.8 mL/min/g liver	20 mL/min
Hepatocyte number	1.35×10^8 cells/g liver† [19,20]	1.5×10^9 cells
Microsomal protein yield	45 mg protein/g liver† [5, 9, 10, 21]	500 mg protein

* SRW, standard rat weight of 250 g.
† Literature average.

does not involve measurement of initial rates but characterizes the time course of metabolism. Within the context of extrapolation to *in vivo*, this method has certain attractions as it involves the same type of data treatment as is carried out in a pharmacokinetic analysis of plasma concentration–time profiles. Analysis of a progress curve for metabolite production from a drug is unlikely to be straightforward for a whole variety of reasons, which have already been discussed thoroughly in the metabolite kinetics literature [15]. However, the use of drug disappearance time profiles to obtain direct assessment of metabolic clearance *in vitro* is theoretically feasible and has been carried out by a small number of investigators with isolated hepatocytes [7, 16–18]. This particular *in vitro* system is appropriate for such an approach which requires relatively lengthy incubations and hence longevity in enzyme activity. In contrast, hepatic microsomes probably do not maintain full activity over a sufficiently long incubation period to allow the temporal profile of most drugs to be measured with any accuracy.

The second stage of the strategy involves the scaling of the *in vitro* clearance to an *in vivo* intrinsic clearance. The scaling factors employed will depend upon the *in vitro* systems initially used, and Table 1 summarizes literature values for microsomal protein recovery, and cell number, which can be used for microsomal and hepatocyte data, respectively. Each scaling factor is expressed per gram of liver; therefore, when these are used with liver weight measurements, *in vitro* clearance units are converted to an *in vivo* basis.

The third stage of the strategy requires the use of a liver model that allows intrinsic clearance to be used to estimate the formation clearance for a particular pathway(s). As outlined earlier, the use of a liver model accounts for the influence of non-enzymatic factors in determining the clearance process *in vivo*. Thus, a hepatic blood flow measurement (Table 1) and information on the binding of drug in plasma/blood is required. The net effect of applying a liver model is to express the scaled kinetic data in terms of circulating plasma/blood concentration rather than the concentration within the liver.

The final stage in predicting *in vivo* hepatic clearance involves consideration of the other elimination routes available to the drug within the liver. This would include metabolic pathways not assayed *in vitro* and biliary excretion. In the majority of cases, hepatic clearance terms are additive, and hence the formation-clearance term can be augmented by similar terms describing the parallel routes for elimination of drug. In a similar fashion, the contribution of non-hepatic organs may need to be accounted for to provide a total body clearance. When the organs are anatomically arranged in parallel (e.g. kidneys and liver), the additivity principle applies. However, when the organs are in series (e.g. gastrointestinal tract and liver), a different approach is required [22].

While being comprehensive, the strategy outlined in Scheme 1 may not be required in its fullest form. For example, drugs like caffeine and antipyrine are metabolized slowly and essentially completely by the liver. Their binding to macromolecules is trivial, and hence the use of scaled V_{max}/K_m ratios for each pathway allows direct prediction of *in vivo* metabolic clearance; stages 3 and 4 are redundant. Other drugs like warfarin and tolbutamide show pharmacokinetic and metabolic characteristics similar to those of the former examples with the exception of high plasma protein binding. Therefore, a protein-binding correction is required at the modelling stage. A third class of drugs with rapid metabolism would require the modelling stage because of the substantial blood concentration difference across the liver for these high extraction drugs. Examples of this class of drugs include lignocaine and phenacetin.

An essential stage for all drugs, regardless of the level of complexity of their pharmacokinetics and metabolism, is the scaling requirement. Thus, it is crucial to establish scaling factors that are both biologically meaningful and empirically useful if strategies of the type outlined are to be widely applied.

A survey of comparative in vitro *and* in vivo *studies*

As a result of surveying the literature for comparative *in vitro–in vivo* studies, only a relatively small number of drugs were identified for which both *in vitro* and *in vivo* metabolism was sufficiently well defined to allow evaluation of the proposed strategy. Although there is widespread use of *in vitro* methods, most of the effort has been expended on delineating metabolic pathways. Whereas these data are often compared with the *in vivo* metabolite pattern, the comparison is limited to a qualitative assessment since there is often little attempt to balance the dose/incubation concentrations used.

In certain cases, the kinetics of a particular reaction have been characterized fully with an *in vitro* system, and these data may be scaled and modelled to generate a formation clearance. However, this is to no avail if the importance of this particular metabolic pathway is not known. Often there is incomplete recovery of the dose administered *in vivo* due to unidentified secondary and tertiary metabolites or unidentified parallel primary metabolites. Thus, the prediction from *in vitro* data cannot be made due to a lack of information to complete the final stage in Scheme 1.

A second common problem encountered is inadequacy of the *in vivo* pharmacokinetic study. Frequently at the dose used nonlinearity is evident. In other cases the intravenous route of administration is used for a high clearance drug, and hence the clearance reported merely reflected a blood flow estimate.

The largest body of information retrievable from the literature by the author relates to drug metabolism by rat cytochromes P450. For this enzyme family there is a sufficient body of information both *in vivo* and *in vitro* from hepatic microsomes and/or freshly isolated hepatocytes to allow evaluation of the strategy proposed above. Although additional data on other enzyme systems were found (e.g. monoamine oxidase and glucuronyl transferase), the number of studies were insufficient to assist the present evaluation. Table 2 lists clearance values for 25 drugs. *In vivo* intrinsic clearance values are shown together with microsomal clearance and/or hepatocyte clearance. Many of the 25 are old drugs that are no longer therapeutically useful but are frequently employed as drug probes.

In vivo CL_{int} values were taken directly from the literature sources indicated or the venous equilibration liver model was used when CL_{int} was not explicitly reported. Blood/plasma ratios were assumed to be unity unless there was evidence to the contrary. To standardize clearances, data were expressed per standard rat weight (SRW) of 250 g. All *in vitro* data were taken directly from the literature sources cited. In virtually all the microsomal studies on 19 drugs, V_{max} and K_m were obtained from linearized plots of the Michaelis–Menten relationship. In 14 cases, metabolite appearance was analysed, and in the remaining five cases initial rates refer to loss of drug substrate. Hepatocyte data on 17 drugs were obtained from V_{max} and K_m reports based on metabolite production in 10 cases and drug loss in one other case. For the remaining six drugs, clearances were obtained from time profiles. V_{max} and K_m values were determined for more than one pathway for six drugs, and in these cases the CL_{int} reported in Table 2 refers to the sum of the *in vitro* V_{max}/K_m ratios. There were 11 drugs identified for which useful data could be abstracted for all three systems. In most of the cases listed in Table 2, *in vitro* data were not obtained in the same laboratory as the pharmacokinetic studies. Also, different rat strains were used in certain *in vitro* and *in vivo* studies. In all but one case, male rat data were analysed.

Evaluation of scaling factors

As previously outlined in the proposed strategy, up to four stages may be required to extrapolate *in vitro* data to the *in vivo* situation depending upon the particular metabolic and pharmacokinetic characteristics of the drug in question. One stage that is always required is the scaling process, which acts as a bridge between the two kinetic disciplines—enzymology and pharmacokinetics. As the identification of suitable scaling factors (SF) is pivitol for the general applicability of the strategy proposed, such factors should ideally be based on biological information and should preferably operate as simple product functions

$$\textit{In vitro}\ \mathrm{CL} = \mathrm{SF}(\textit{in vivo}\ \mathrm{CL}). \qquad (10)$$

Appropriate first estimates for scaling factors for microsomal and cell data extrapolation are shown in Table 1. Recovery of microsomal protein following differential centrifugation is known to be incomplete. Joly and coworkers [21] demonstrated a 30% recovery by standard procedures. The actual microsomal yield will vary according to the precise experimental conditions and hence will tend to be laboratory dependent. However, a survey of literature[5, 9, 10, 21]provides a range of microsomal protein recoveries of 33–54 mg microsomal protein/g liver and, therefore, a value of 45 mg microsomal protein/g liver can be used to obtain a biochemically based SF for microsomes of 500 mg/SRW.

For hepatocyte preparations, the analogous parameter would be hepatocyte number per gram of liver. The standard method of hepatocyte isolation involves steps that discard other liver cell types and subsequent incubations are essentially pure hepatocytes [19]. Literature reports [19, 20] for the number of hepatocytes harvested per gram of liver quote a value of 1.35×10^8, which provides an anatomical SF of 1.5×10^9 cells/SRW.

The utility of these biologically based factors has been assessed using the data on the 25 drugs listed in Table 2. Figure 1 shows the relationship between *in vivo* CL_{int} and CL_{int} determined in freshly isolated hepatocyte suspensions. The corresponding relationship between *in vivo* CL_{int} and microsomal CL_{int} is shown in Fig. 2. The range of intrinsic clearances obtained for all three systems extends over four orders of magnitude and necessitates the use of logarithmic scales; hence, equation 10 becomes

$$\log(\textit{in vitro}\ \mathrm{CL}) = \log(\textit{in vivo}\ \mathrm{CL}) + \log \mathrm{SF}. \qquad (11)$$

If a linear scaling factor is suitable, then the data would be expected to be distributed along a line of identity with an intercept equivalent to the SF. Figures 1 and 2 show these particular scaling lines for cells and microsomes, respectively.

Figure 1 demonstrates that the cell data are particularly useful in predicting the *in vivo* clearance and indicate that the SF based on cellularity is perfectly adequate for 16 of the 17 drugs. There is no apparent difference between hepatocyte clearance data derived from metabolite production (solid symbols) and substrate loss (open symbols). Considering the wide spread of data and the numerous sources used to obtain the data, the relationship is impressive and endorses the value of the SF proposed.

The microsomal data (see Fig. 2) are less successful

Table 2. Intrinsic clearances for 25 drugs determined *in vivo*, in hepatic microsomes and in isolated hepatocytes

Drug	Intrinsic clearance			Specific comments on *in vitro* data
	In vivo (mL/min/SRW)	Microsomes (μL/min/mg protein)	Hepatocytes (μL/min/10^6 cells)	
Carbamazepine	0.8* [2]	2.2 [2]		Refers to epoxidation only
Tolbutamide	1.5 [23]	2.1 [24]	0.6 [25]	Hepatocyte data at one concentration
Antipyrine	1.5 [23]	3.6 [26]	0.8 [27]	Refers to three pathways, assumes 4'hydroxylation to be a secondary pathway
Butylbarbitone	2.1 [28]		1.9† [16]	
Aminopyrine	2.2 [29]	6.6 [30]	4.1 [29]	Refers to N-demethylation only
Caffeine	3.4 [31]	4.3 [31]	1.7 [31]	Refers to total metabolites
Ethoxybenzamide	4.9 [5]	4.5 [5]	1.3 [32]	
Oxodipine	5.5 [33]	5.3† [33]		
Loxtidine	7.3 [34]		11 [34]	
Hexylbarbitone	34 [28]		21† [16]	
Phenytoin	54 [35]	80 [35]	46 [36]	
Ibuprofen	61 [37]	29† [37]		
Heptabarbitone	70 [38]		29† [16]	
Hexobarbitone	80 [38]	330† [14]		
Heptylbarbitone	99 [28]		33† [16]	
Felopidine	110 [9]	500† [9]		Binding in microsomes reported to be equal to binding in plasma
Phenacetin	130* [39]	33 [40]	78† [7]	
Ethoxycoumarin	140 [35]	50 [35]	26 [41]	
Diazepam	160 [42]	66 [42]	83 [42]	Refers to three pathways
Metoprolol	170* [43]	44 [43]		Refers to two pathways, female rats
Codeine	320 [44]		290† [17]	
Propranolol	425* [45]	3120 [46]		Refers to four oxidative pathways, glucuronidation insignificant in rat [47]
Lignocaine	625* [48]	200 [49]		Refers to two pathways
Alprenolol	980* [50]	80† [50]	36† [18]	
Imipramine	1870 [10]	4500 [10]	990 [51]	Refers to two pathways, product inhibition taken into account

* Refers to isolated perfused liver data.
† Refers to data on substrate loss.

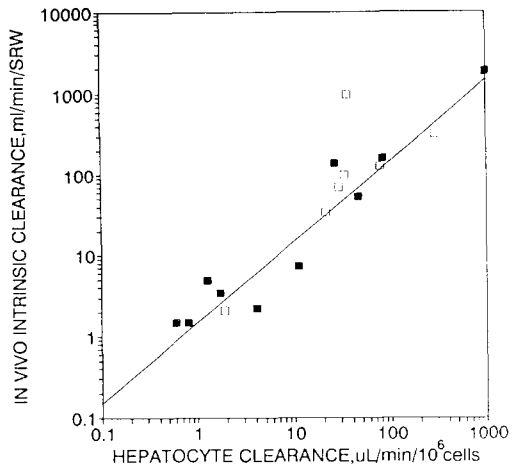


Fig. 1. Relationship between *in vivo* intrinsic clearance and hepatocyte clearance for 17 drugs metabolized by cytochromes P450 in the rat. Solid symbols refers to *in vitro* data obtained from metabolite production, whereas open symbols refers to *in vitro* data obtained from loss of substrate. The line shown represents a scaling factor of 1.5×10^9 cells/standard rat weight (SRW).

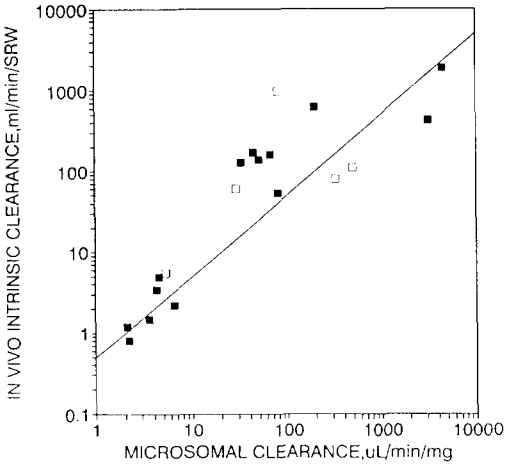


Fig. 2. Relationship between *in vivo* intrinsic clearance and hepatic microsomal clearance for 19 drugs metabolized by cytochromes P450 in the rat. Symbols are defined as in Fig. 1. The line shown represents a scaling factor of 500 mg microsomal protein/SRW.

overall than the hepatocyte data in predicting *in vivo* CL_{int} as judged by the uneven distribution of data around the SF line. Once again *in vitro* clearances from metabolite production (solid symbols) and substrate loss (open symbols) data show similar behaviour. At low microsomal clearances (<10 μL/min/mg microsomal protein), prediction is reasonable; however, there is a tendency to underestimate *in vivo* clearance when using microsomal data. In the region of 100 μL/min/mg microsomal protein,

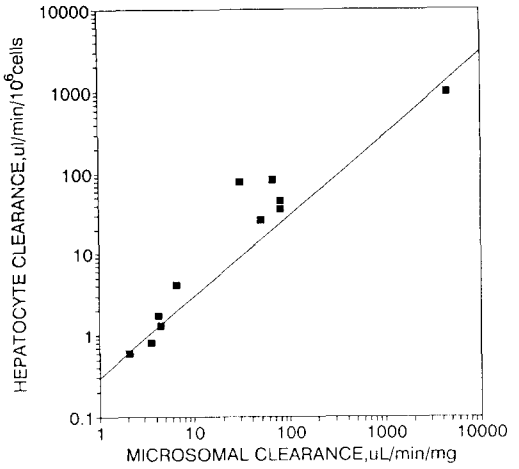


Fig. 3. Relationship between hepatic microsomal clearance and hepatocyte clearance for 11 drugs metabolized by cytochromes P450 in the rat. The line shown represents a scaling factor of 0.3 mg microsomal protein/10^6 cells.

this disparity may indicate that the linear scaling factor is not adequate and that the relationship between *in vivo* and microsomal clearances has a slope >1. However, there is no theoretical basis for employing a power relationship to describe the association between the two clearances. Microsomal preparations are open to considerable interlaboratory variability due to differences in rat strain, diet and specific techniques. Microsomal protein yield (and hence the microsomal SF) is calculated from the ratio of cytochrome P450 in homogenate to that in microsomes and is, therefore, inversely related to the efficiency of the differential centrifugation procedure. The SF indicated corresponds to 70% losses [21], and to accommodate the degree of disparity observed for certain cases in Fig. 2 this factor would need to be increased approximately 5-fold. Thus, other factors must be contributing.

On first inspection, the values for microsomal CL and cellular CL in Table 2 suggest a similar range of values, indicating that 1 mg of microsomal protein metabolizes drugs at rates approximately equivalent to 10^6 cells. This observation is surprising when one considers the P450 content of the two incubation mixtures. Taking 10^6 cells and 1 mg of microsomal protein as typical 1-mL enzyme incubation concentrations, it would be anticipated that microsomes would metabolize drugs approximately three times faster than cells. The P450 concentration is 0.8 μM in the microsomal incubation [7, 21] and 0.3 μM in the hepatocyte incubation [7, 52]. These concentrations give similar estimates of 36 and 40 nmol P450/g liver when multiplied by their respective scaling factors and are in agreement with reported P450 content determined from liver homogenates of 43 nmol/g liver [10, 14, 21, 41].

The relationship between the two *in vitro* systems is examined more closely in Fig. 3 using data for eleven drugs from both systems. The line shown represents a slope of 0.3 mg microsomal protein/

10^6 cells, which corresponds to the expected difference between clearance in the two systems based on the ratio of the SFs. Whereas the low CL data are consistent with the line (values for hepatocyte CL being less than microsomal CL), certain of the larger CL values for hepatocytes are comparable with microsomal CL. Thus, for this latter group of data, hepatocyte CLs are significantly higher than would have been anticipated from microsomal behaviour.

Collectively, the information presented in Table 2 indicates that microsomal data for high clearance drugs are substantially lower than the corresponding hepatocyte data and *in vivo* CL_{int}, and hence underprediction would result if these former data were used. The explanation for this behaviour is probably multifactorial. The disruptive nature of the microsomal preparation and the subsequent need to re-establish "optimal" conditions provide many possibilities. There may be some element of selectivity operating in terms of which particular isoforms of P450 retain full activity during the microsomal preparation. It is worth stressing that the sources of data for the high microsomal clearance group are no more varied than the lower microsomal clearance group or the corresponding hepatocyte clearance group.

It is of interest that in order to describe the kinetics of imipramine, the drug with the highest clearance in Table 2, it is necessary to include product inhibition considerations [10, 51]. Despite its high CL_{int}, the correlations between *in vivo*, microsomal and hepatocyte clearances of imipramine are excellent (see Figs 1–3). Product inhibition within microsomes as a consequence of the lack of active conjugating enzymes has been discussed by Gillette [53], who has also speculated on other potential problems with using the artificially generated microsomal system. Product inhibition is one of several phenomena that may prove to be of relevance in explaining inconsistency between microsomes and hepatocytes for high clearance drugs.

Conclusions and prospective view

A systematic consideration of the factors differing between drug metabolism *in vitro* and *in vivo* provides a simple strategy for the extrapolation of *in vitro* data to predict *in vivo* metabolic clearance. The use of biologically based scaling factors for isolated hepatocytes and hepatic microsomes works satisfactorily. In particular, the hepatocyte data may be scaled to provide an excellent prediction of *in vivo* clearance over four orders of magnitude. With hepatic microsomes there is some concern over the accuracy of high clearance estimates, which requires further examination. It will be of interest to see whether similar biological scaling factors are as successful for liver slices and heterologous expression systems, two *in vitro* systems that are receiving increasing attention by drug metabolism investigators.

The precision of *in vivo* prediction is an issue for consideration if the strategy outlined is to be used in a prospective mode. The data illustrated in Figs 1 and 2 contain few examples of direct correspondence between *in vitro* and *in vivo* data, but do demonstrate clear general trends. Considering the differing sources of data and the range of different chemical structures and of P450 isoforms involved, it would be perhaps naive to expect a scaling factor to provide any better degree of accuracy.

One of the major conceptional aids of physiological pharmacokinetics is the classification of drugs into low and high hepatic extraction ratio groups [11]. This has been of considerable value for rationalizing a variety of pharmacokinetic situations, many of which are of direct concern in drug metabolism, e.g. the systemic availability of an orally administered drug, the consequences of enzyme induction and inhibition as drug–drug interaction phenomena, and the importance of intersubject variability (including genetic polymorphisms) in hepatic enzyme activity. Therefore, the use of *in vitro* studies to allow the classification of a drug as either high or low clearance without resorting to a full *in vivo* study would be of considerable value.

Table 3 provides the limiting values of CL_{int} for the different drug-metabolizing systems. Using the venous-equilibration liver model, there is a 5-fold difference in CL_{int} between the upper limit for a low extraction drug and the lower limit for a high extraction drug. This difference is maintained when scaling factors are used to calculate the corresponding limits for hepatic microsomes and hepatocytes. Figure 4 shows the data previously discussed with the low and high extraction ratio demarcation lines.

Table 3. Critical clearance values for *in vivo*, microsomal and hepatocyte models, which allow classification of low and high extraction drugs

	Upper limit for low extraction classification	Lower limit for high extraction classification
Extraction ratio	0.3	0.7
Hepatic clearance* (mL/min/SRW)	6	14
CL_{int}—*in vivo** (mL/min/SRW)	10	50
CL_{int}—hepatocytes† (μL/min/10^6 cells)	7	35
CL_{int}—microsomes† (μL/min/mg protein)	20	100

* Using a hepatic blood flow of 20 mL/min/SRW [54] and the venous equilibration model (equation 9).

† Using the scaling factors from Table 1.

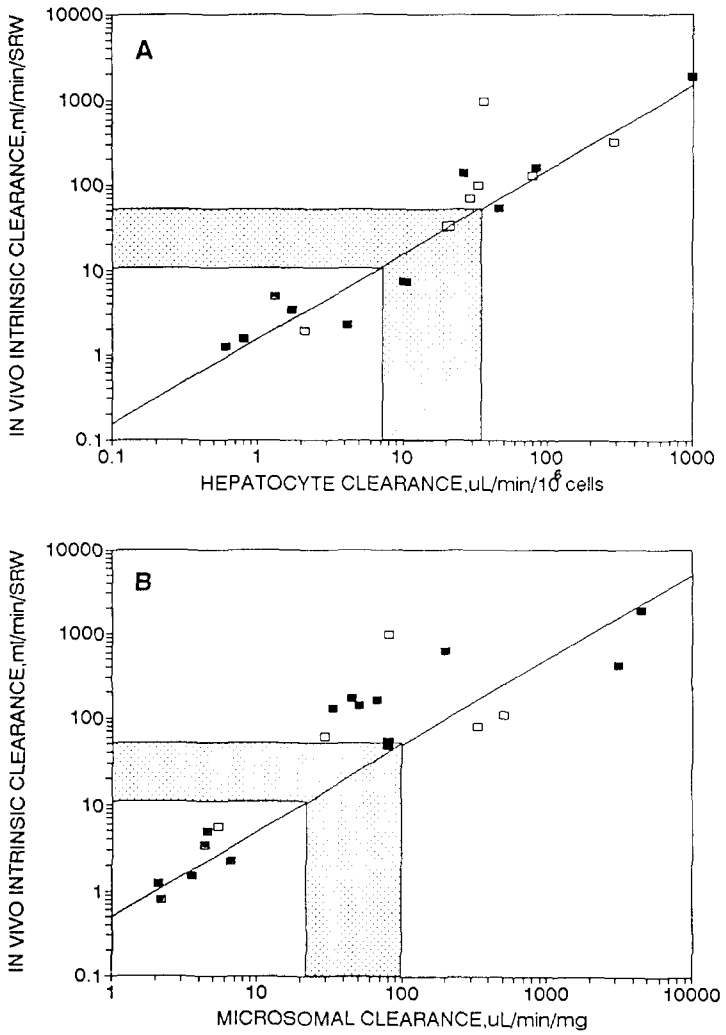


Fig. 4. Division of *in vivo* intrinsic clearance, hepatocyte clearance (A) and microsomal clearance (B) into low and high extraction groups. Demarcation lines are discussed in the text and presented in Table 3.

Both *in vitro* systems show the resolution, but once again the hepatocyte data provide a clearer picture. There is no problem in identifying low clearance drugs with either *in vitro* system. However, upon considering the higher clearance drugs the anticipated difference between hepatocytes and microsomes becomes evident. Nine high clearance drugs have been studied in hepatocytes; seven of these fall into the high clearance quadrant, whereas two are in the intermediate clearance band. In microsomes, 10 high clearance drugs have been investigated, and their microsomal clearances are equally divided between the high clearance quadrant and the intermediate clearance band. Thus, overall, the classification of drugs based on pharmacokinetic behaviour predicted from *in vitro* data alone would appear useful. Metabolic data together with other *in vitro* information (plasma protein binding and blood:plasma ratio) would provide valuable prospective information on new drug structures.

Any attempt to relate a simple incubation medium to a complex integrated system requires several compromises and assumptions. *In vitro*:*in vivo* scaling of drug metabolism data is no exception. The simplest liver model—the venous-equilibration model—has been employed, and despite all its simplifications, it would appear to be adequate for the present purpose. Similarly, the Michaelis–Menten model, which also represents the degenerative form of other more detailed models, appears to suffice for describing the action of cytochrome P450 despite the known complexities of this enzyme family. However, as we continue to explore *in vitro*:*in vivo* relationships in more depth, it is likely that more elaborate models will be required to rationalize the data in a comprehensive manner.

The present commentary represents the largest compilation of *in vitro* and *in vivo* drug metabolism data to date. Previous analyses [2–4] have used smaller data bases and have not been specific in

terms of either the drug-metabolizing enzyme system examined or the *in vitro* method assessed. The present investigation focuses on CL_{int} by cytochromes P450 in rat microsomes and hepatocytes. The data collated covers a range of four orders of magnitude and originates from a large number of varied sources. The fact that these data can be correlated by simple scaling factors provides good evidence for the validity of *in vitro*:*in vivo* extrapolations and provides encouragement for application beyond the cytochrome P450 system in rat microsomes and hepatocytes to other drug-metabolizing enzymes, to alternative *in vitro* systems, and to human tissue.

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