Reassessing Models of Hepatic Extraction

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Abstract. The aim of this investigation is to compare different mathematical models of the liver in the context of *in vitro-in vivo* correlation. We reanalyze drugs from the Houston reviews [1, 2], and compare the mathematical models. For the well-stirred model, a particular form of the distributed tubes model, and the dispersion model, fits are done to *in vitro* and *in vivo* intrinsic clearance data from microsomal and hepatocyte experiments. The distributed and dispersion models have decreased residuals as compared to the well-stirred model, but neither is to be clearly preferred over the other. It seems likely that drug-specific factors have a major impact on the quality of IVIVC correlations. While new experiments are needed to validate IVIVC models, our results indicate that improved correlation of *in vitro* and *in vivo* data is possible for high clearance drugs by using either a dispersion or distributed tube model rather than a well-stirred model.

Key words: *in vivo-in vitro* correlations, multi-compartment models, pharmacokinetics

1. Introduction

Following administration of a given dose of pharmacologically active drug to a human or an animal model system, concentrations of that drug appear in various body organs and tissues, and ultimately a physiological effect is produced. Because of the relationship between the intensity and duration of the action of a drug and the concentration of the drug present, plasma concentrations of the drug are usually evaluated over time in pharmacokinetic studies [3]. A variety of descriptive pharmacokinetic parameters characterize the behavior of the drug, such as clearance, bioavailability, absorption rate, volume of distribution, etc. [4]. Estimates of these parameters are provided by the plasma concentration-time data as a function of time $C(t)$ [5].

The volume of distribution of a drug V_d is the apparent volume necessary to account for the total amount of drug in the body, X_{body} , if it were present throughout the body at the same concentration found in the plasma: $V_d = X_{body}/C_{plasma}$. Factors which tend to keep the drug in the plasma or increase C_{plasma} , such as escalated plasma protein binding, reduce the volume of distribution; while factors which decrease C_{plasma} , such as increased tissue binding or lipid solubility, increase the volume of distribution.

$$V_d(t) = \frac{Dose \cdot AUC|_t^\infty}{C(t) \cdot AUC|_0^\infty}, AUC|_t^\infty = \int_t^\infty C(t)dt \quad (1)$$

where $AUC|_0^\infty = AUC$ is called the Area Under the Curve. The clearance CL can be imagined as the intrinsic ability of the body and its organs of elimination to remove drug from the plasma, expressed as a volume per time. The total amount of drug removed depends on the plasma concentration of drug as well as the clearance. Clearance can be measured as a proportionality constant at steady state [6].

$$R_{i.v.} = R_{\text{elimination}} = CL \cdot C_{\text{steady state}}, \quad (2)$$

where systemic clearance is equal to the sum of all organ clearance processes:

$$CL = CL_{\text{hepatic}} + CL_{\text{renal}} + CL_{\text{other}}. \quad (3)$$

Plasma concentration data following oral doses of the drug in solution and common dosage forms provides additional pharmacokinetic parameters related to absorption and intrinsic clearance. These data permit assessment of bioavailability (F) and of the mean absorption time (MAT). Bioavailability refers to the fraction of an oral dose that actually reaches the systemic circulation of the biological model system. Since the availability of an intravenous dose is usually unity, bioavailability can be estimated by comparison, adjusted for differences in the oral and intravenous doses, as follows [7]

$$F = \frac{AUC_{\text{oral}}/D_{\text{oral}}}{AUC_{i.v.}/D_{i.v.}}. \quad (4)$$

Physiologically-based pharmacokinetic models envisage the organism as a network of compartments representing individual organs or tissue groups interconnected by the arterial and venous blood. Pharmacokinetic models contain a number of physiological, physico-chemical and biochemical parameters (as many as twenty or more) each of which is subject to error and uncertainty. These parameters are also subject to constraints. For example, the total volume of all body tissues must equal the total body volume, and the sum of the blood flow rates in different tissue groups must equal the rate of cardiac output. Most of the physiological quantities are estimated prior to modeling by *in vitro* experiments and are subject to a certain degree of uncertainty, depending on the precision and accuracy of the methods used for their determination.

Absorption of the drug from outside the model system can occur via several routes of administration: ingestion, which involves the digestive system, inhalation, which involves the respiratory system, dermal absorption, which involves the cutaneous structure, or i.v. injection. Once absorbed, the drug is distributed throughout the body to various organs and tissues. A routine assumption regarding the transport of the drug between the various defined compartments, is that, movement is constrained to be limited within the blood [8].

Absorption or transport into or out of a particular organ is described by a mass balance around that compartment. Diffusion within the compartment could be illustrated by either a lumped-parameter or a distributed-parameter model. The lumped-parameter approach assumes that the concentration of drug within a compartment is homogeneous and that the physical variables describing the transport processes in the compartment are only time dependent. This approach results in a system of ordinary differential equations (ODEs) with initial conditions required to describe the data. The distributed-parameter approach assumes that the physical processes change both spatially and temporally, yielding a system of partial differential equations, such that both the boundary and initial conditions are needed to seek a meaningful solution. Most of the pharmacokinetic models assume that all of the chemical delivered by the flow of blood rapidly equilibrates and is taken up by the various tissues under the control of a 'partition coefficient' $P_{ij} = C_j/C_i$.

Another complication arises due to drug binding to the proteins in blood or membranes, since it is often assumed that only the fraction of drug in the circulating blood which is free or unbound in the plasma is available for transport, metabolism, or excretion [7]. When partition or binding occurs within any of the compartments, the mass balance must include the effects of the partition coefficient and binding constants, to account for both the free and bound fractions of drug.

Elimination of the drug can occur in the body via several processes, but the two most important are by enzymatic metabolism in the liver and by first order clearance in the kidney. Enzymatic mechanisms are often complex, resulting in nonlinear kinetic equations.

Pharmacokinetic models usually employ a system of first order differential equations in terms of the concentration variable C_i in the i -th compartment. The rate of change in the concentration of the drug in an eliminating or metabolizing organ is

$$\frac{dC_j}{dt} = \frac{Q_j}{V_j} \left(C_{in} - \frac{C_j}{P_j} \right) - \frac{1}{V_j} \frac{dX_j}{dt}, \quad (5)$$

where Q_j , V_j , and P_j are blood flows, tissue volumes, and partition coefficients which indicate the relative solubility of a circulating drug in a specific tissue, for the j^{th} compartment, respectively. C_{in} is the concentration of the blood entering the compartment. Here, X_j is the amount of drug eliminated or metabolized in the j -th compartment. The rate of metabolism is typically given by the Michaelis-Menten equation governing enzymatic reactions

$$\frac{dX_j}{dt} = \frac{V_{maxj} C_j / P_j}{K_j + C_j / P_j}, \quad (6)$$

where V_{maxj} and K_j are the maximum velocity and the Michaelis-Menten constant for the metabolizing organ, respectively.

While it is often expressed in recent pharmacokinetic literature that compartmental modelling is unrealistic and difficult to justify physiologically [5, 9], the practical usefulness of such models and a lack of better alternatives have resulted

in a continued popularity of multi-compartment models of pharmacokinetic phenomena [6, 10–13]. In this article we compare and contrast several most popular models that utilize the concept of interconnected compartments. Our objective is to evaluate their usefulness from the point of view of providing a correlation between *in vitro* and *in vivo* data sets for various drugs studied.

Three particular models are compared here: the well-stirred, the parallel-tube and the distributed model.

2. *In Vitro-In Vivo* Correlation

In recent years, interest in quantitatively predicting *in vivo* pharmacokinetic parameters from purely *in vitro* data has grown. This process, known as *in vitro-in vivo* correlation (IVIVC) of drug metabolism, requires quantitating the metabolism kinetics *in vitro*, understanding the scaling factors associating the *in vitro* measurement with liver metabolism, and applying an accurate model of the liver [1, 2]. IVIVC promises the possibility of assessing drug kinetics *in vitro* at an earlier stage of development, allowing problematic substances to be discarded or modified earlier and reducing the need for expensive animal testing. However, *in vitro* techniques and their match to *in vivo* behavior are not yet sufficiently well understood or controlled to replace *in vivo* testing. This paper presents a new assessment of the validity of popular mathematical models of the liver as they are used in IVIVC. Although this paper restricts itself to hepatic metabolism, similar considerations could apply to other organs.

In vitro-in vivo correlation begins with kinetic data, such as the $V_{\max}$ and K_m values obtained from a microsomal or hepatocyte preparation. This kinetic data gives a CL_{int} value for the *in vitro* process, which must then be scaled up to the full metabolic capacity of the liver. Next, a liver model is used to predict the total organ clearance from the total metabolizing capacity. Most IVIVC work assumes a well-stirred model for the liver, such that all metabolic enzymes in the liver are exposed to the same drug concentration. The well-stirred model is easy to understand and work with, and produces results for many drugs which are reasonably good, especially given that they belie the actual anatomy of the liver as a complex network of branching and anastomosing sinusoids. The focus of the present paper is on the liver modeling step of IVIVC, and on two specific questions: (a) What accounts for the acknowledged success of the well-stirred model in capturing many pharmacokinetic phenomena? and (b) Are there situations under which a more physiologically plausible model provides better agreement with the published data?

We first review the relationship between various models of substrate transport and clearance: including the well-stirred model, the parallel tube model, the distributed tube model, and the dispersion model. Next, we explain how models may be used to predict intrinsic clearance from *in vivo* data, including the effects of administration location. We apply our technique to drugs taken from two Houston review articles [1, 2].

Figure 1. Five representative liver models: (a) the well-stirred or venous equilibrium model, (b) the parallel tube model, (c) the distributed tube model, (d) the dispersion model, and (e) the interconnected tube model.

3. Methods

3.1. LIVER MODELS

There are a substantial number of mathematical models of the liver in the literature, some of which are reviewed in Saville and Tam [14]. We discuss four models in detail: the well-stirred model, the parallel tube model, the distributed model, and the dispersion model (see Figure 1). These models, in one form or another, can closely mimic the results of a broad range of simulations obtained in various popular models of transport and metabolism, including models such as the series compartment model and the dispersion model.

The liver model provides a mapping from physiological liver parameters, such as the hepatic flow Q and the intrinsic clearance CL_{int} , to *in vivo* kinetic parameters: the hepatic availability, the clearance, and so on. Different models of the

Figure 2. Liver models: well-stirred, distributed, and dispersion, at $\epsilon^2 = 2 * D_N = 0.28$.

liver produce different results. The common models can all be specified by giving their predicted availability F as a function of the non-dimensional ratio of the total intrinsic clearance, $f_u CL_{int}$ to the hepatic flow Q . This ratio defines the so-called efficiency number R_N as

$$R_N = \frac{f_u CL_{int}}{Q} \quad (7)$$

The models being considered are shown in Figure 2 for a range of clearances.

The well-stirred model assumes that the liver is a single, homogeneous, well-stirred compartment. The ensuing mathematical formulation follows from Eq. (5). Taking a liver volume V , equal input and output flows Q , and an intrinsic clearance CL_{int} , the evolution of the concentration inside the liver $C(t)$ will be described by

$$V \frac{d}{dt} C(t) = Q(C_{in} - C) - f_u CL_{int} C \quad (8)$$

where f_u is the unbound fraction available to be metabolized. At steady state, the effluent concentration will be

$$C = \frac{QC_{in}}{Q + f_u CL_{int}} = C_{in} F \quad (9)$$

where the hepatic availability F is

$$F = \frac{1}{1 + f_u CL_{int}/Q} = \frac{1}{1 + R_N} \quad (10)$$

The total hepatic clearance CL_H is

$$CL_H = Q(1 - F) = QR_N/(1 + R_N) = Q \frac{f_u CL_{int}}{Q + f_u CL_{int}} \quad (11)$$

Some general features are worth pointing out in the context of this model, but hold true in general.

1. For high flow rates / low internal clearance, i.e. $Q \gg f_u CL_{int}$, or $R_N \ll 1$, the availability F is close to 1, the drug concentration inside the liver is close to the drug concentration outside the liver, and the hepatic clearance approaches $f_u CL_{int}$. In this case, a drug is being metabolized under *enzyme-limited* conditions, and the hepatic clearance is relatively independent of the details of the liver model.
2. For low flow rates / high internal clearance, i.e. $Q \ll f_u CL_{int}$, or $R_N \gg 1$, the availability F is close to zero, and the drug concentration inside the liver is much lower than outside. The hepatic clearance approaches the total organ blood flow: $CL_H = Q$ and this case is referred to as *flow-limited* conditions. The availability is quite dependent on details of liver model.
3. For the well-stirred model, if some pathways are saturated, then we simply replace $CL_{int} = CL_{int}(C)$, and the proceeding formulas for availability and clearance are unaltered. The kinetics will be different, however.

The parallel tube model of the liver constructs a number of identical parallel tubes representing the sinusoids [15]. Each tube has plug flow of the blood carrying the drug and metabolites.

$$S \frac{\partial C}{\partial t} + Q \frac{\partial C}{\partial x} = -\rho(x)g(C) \quad (12)$$

Introducing a new variable $z = x - (Q/S)t$, and changing to coordinates $C(z, x)$, we have (using the chain rule to do the coordinate transformation)

$$\begin{aligned} S \frac{\partial C}{\partial t} + Q \frac{\partial C}{\partial x} &= S \left[\frac{\partial C}{\partial z} \frac{dz}{dt} + \frac{\partial C}{\partial x} \frac{dx}{dt} \right] + Q \left[\frac{\partial C}{\partial z} \frac{dz}{dx} + \frac{\partial C}{\partial x} \frac{dx}{dx} \right] \\ &= Q \frac{\partial C}{\partial x} \end{aligned} \quad (13)$$

In this way, the PDE is separated, and solutions for arbitrary initial conditions can be found from the solution to the ODE in $C(x)$:

$$Q \frac{dC}{dx} = -\rho(x)g(C) \quad (14)$$

Here, $\rho(x)$ is the local linear enzyme density, and $g(C)$ encompasses the reaction kinetics, and generalized Michaelis-Menten kinetics. For linear reactions, we can write

$$Q \frac{dC}{dx} = -\frac{f_u C L_{int}}{L} C \quad (15)$$

This appears to assume homogeneity of enzyme distribution, but in fact it does not: the general solution involves only $\int_0^L dx \rho(x)$ and no higher moments. This equation is solved by

$$C(x) = C_{in} e^{-x R_N / L} \quad (16)$$

where R_N is as before $f_u C L_{int} / Q$ and L is the length of the sinusoid.

Under the condition of linear kinetics we may solve for the hepatic availability

$$F = e^{-R_N} = e^{-f_u C L_{int} / Q} \quad (17)$$

and hepatic clearance

$$C L_H = Q(1 - F) = Q(1 - e^{-R_N}) \quad (18)$$

As before, for poorly eliminated drugs, $C L_H = f_u C L_{int}$, while for highly eliminated drugs $C L_H = Q$. Note that the tube model does not assume a homogeneous axial distribution of metabolic activity: the enzymes may be arbitrarily distributed along the tube without affecting the availability, assuming linear kinetics. For partially or completely saturated kinetics, these results will change.

Neither the well-stirred model nor the tube model represents accurately the distribution of times which drug molecules spend in the liver. This is due to their simplified picture of the flow through the liver, resulting in a transit time distribution of an exponential decay (for the case of the well-stirred model) or a delayed delta function (for the case of the tube model). Each model is parametrized by the lowest moment of the transit time distribution function. The discrepancy of these models can be seen by comparing their predicted transit time profiles to experimental transit time distributions, but can also be seen in *in vitro-in vivo* correlation data, since, for linear homogenous kinetics, the hepatic availability is related to the transit time distribution as $F = \int_0^\infty f(t) e^{-R_N t / MTT} dt$ where $f(t)$ is the transit time distribution, and MTT is the mean transit time [16]. Several more sophisticated models have been put forward, of which we consider the distributed model, the dispersion model, and the interconnected tubes model.

The distributed model extends the parallel tube model by considering a distribution of tubes, each with its own flow and metabolic capacity. From the distribution of individual tubes i , each with flow Q_i and clearance $C L_{int_i}$, we can compute the flow weighted distribution:

$$F = \langle F_i \rangle = \langle e^{-R_{Ni}} \rangle = e^{-\langle R_{Ni} \rangle} \left(1 + \frac{1}{2} \langle (R_{Ni} - \bar{R}_N)^2 \rangle + \text{higher order terms} \right) \quad (19)$$

where $\langle \cdot \rangle$ indicates a flow-weighted average. In this form, the model is both completely general and completely free of predictive value, as no particular form of the actual distribution is assumed. To reach a model with predictive value, we drop the higher order terms and compute the averages as follows

$$\langle R_{N_i} \rangle = \left\langle \frac{CL_{int_i}}{Q_i} \right\rangle_Q = \frac{\sum_i Q_i \frac{CL_{int_i}}{Q_i}}{\sum_i Q_i} = \frac{\sum_i CL_{int_i}}{\sum_i Q_i} = \frac{CL_{int}}{Q} \quad (20)$$

Similarly, the variance term is found as

$$\langle (R_{N_i} - R_N)^2 \rangle = \frac{\sum_i Q_i \left(\frac{CL_{int_i}}{Q_i} - \frac{CL_{int}}{Q} \right)^2}{\sum_i Q_i} \quad (21)$$

$$= \frac{\sum_i \frac{CL_{int_i}^2}{Q_i}}{Q} - \frac{CL_{int}^2}{Q^2} \quad (22)$$

$$= \epsilon^2 \frac{CL_{int}^2}{Q^2} \quad (23)$$

where ϵ^2 , a measure of the degree of functional heterogeneity of the liver, is given by Bass (1983):

$$\epsilon^2 = \sum_i \frac{(CL_{int_i}/CL_{int})^2}{Q_i/Q} - 1 \quad (24)$$

So the distributed model predicts

$$F = e^{-R_N} \left(1 + \frac{1}{2} \epsilon^2 R_N^2 \right) \quad (25)$$

with the nondimensional parameter ϵ^2 as defined by Equation (24) and whose meaning is to capture the amount of dispersion in the network. This dispersion is a function of both the enzyme and flow distribution, but it is invariant under shifts in the total enzyme quantity CL_{int} and the total flow rate Q , as long as the relative distributions among the different tubes are unaltered.

The dispersion model [17] captures variability in the transit time distribution by including a second order derivative in the PDE:

$$\frac{\partial C}{\partial t} = D_N \frac{\partial^2 C}{\partial x^2} - \frac{\partial C}{\partial x} - R_N C \quad (26)$$

The additional spatial derivative requires an additional boundary condition to specify a solution. The most appropriate form for this boundary condition has been subject to some debate [17–20]. This debate is not resolvable by reference to microscopic physiology, since the dispersion equation is only applicable macroscopically [19, 21]. A more nuanced approach is to develop finer-grained models of the flow and physiology in the liver, and derive the macroscopic model via some appropriate limit. The interconnected-tubes model (see Figure 1e) developed by Anissimov et al. is an important step in this direction [22, 23].

For mixed boundary conditions, the dispersion model gives

$$F = \exp \left[\frac{1 - \sqrt{1 + 4D_N R_N}}{2D_N} \right] \quad (27)$$

Expanding the exponent around $D_N = 0$ shows that this agrees with Equation (25) to second order in R_N if $\epsilon^2 = 2D_N$. Higher orders depend on what is assumed for the details of the transit time distribution for the distributed model; an inverse Gaussian distribution agrees exactly. When the enzyme density varies along the axis, this correspondance is altered to $\epsilon^2 = 2\bar{\rho}^2/\rho^{-2}D_N$ [24], where $\bar{\rho}$ and ρ^2 are the average and variance of ρ , the axial enzyme distribution. Note that axial inhomogeneity in individual tubes does not affect the form of the dispersion model, but heterogeneity between tubes does.

For linear kinetics and homogenous enzymes, the transit time distribution completely determines the hepatic availability [16]: $F = w(s)|_{s=R_N}$, where $w(s)$ is the transfer function of the transit time distribution. Inhomogenous enzymatic action can be handled by considering a contact time distribution, however this introduces drug dependence independent of R_N . If we expand $w(s)\exp(s)$ in a power series around $s = 0$, and substitute to find F , we have the following general form for liver models

$$F = e^{-R_N} \sum_{k=0}^{\infty} \alpha_k R_N^k \quad (28)$$

where $\alpha_0 = 1$ and $\alpha_1 = 0$ is required by the definition of R_N . In this sense, if R_N is sufficiently small to ignore terms larger than quadratic, the distributed model is completely general, with the value of ϵ^2 being set by the details of the model in question. For example, the parallel tube model (17) is just a distributed model with $\epsilon^2 = 0$. For this reason, we no longer consider the parallel tube model by itself, and include it with the distributed tube model. The well-stirred model does not correspond exactly with a two term expansion, but expanding (10) near $R_N = 0$ shows that $\epsilon^2 = 1$ corresponds approximately to the well-stirred model for low clearance drugs. As discussed above, the dispersion model also agrees up to second order in R_N with appropriate correspondance of parameters.

For our work below, we truncate the expansion Eq. (28) at R_N^2 . This choice reflects simply the desire to control the number of free parameters to be fit to the IVIVC data, and should not be construed as any statement about the nature of the higher order terms. Over the range $0 < \epsilon^2 < 1$ and $0 < R_N < 5$, the maximum discrepancy between the mixed boundary condition dispersion model Eq. (27) and Eq. (25) with $\epsilon^2 = 2D_N$ is about 30%. The well-stirred model differs from Eq. (27) with $\epsilon^2 = 1$ by nearly a factor of two at $R_N = 5$.

The assumption of the relationship between ϵ^2 and D_N is also of approximate nature and has been recently addressed in the literature based on more physiological models [22]. Note that whereas for $\epsilon^2 \ll 1$ and linear kinetics the dispersion model is close to the distributed model, this is not true for the range of values of ϵ^2 used in this work.

It should be mentioned in this context that recently models have been developed that account for both vascular and elimination heterogeneity in the liver [23] show-

Table I. CL_{int} (ml/min/SRW) for high clearance substrates. SRW refers to a 250 g rat body weight. The drugs are ordered by extraction ratio E , as derived from the reported *in vivo* CL_{int} . The columns labeled micro and hep give the CL_{int} estimated by scaling *in vitro* microsomal and hepatocyte results, respectively. The *in vivo* column gives the CL_{int} estimate from *in vivo* experiment assuming a well-stirred model. f_u is the unbound fraction in blood or plasma. The drugs marked (dex) and (bnf) are measured after pretreatment with dexamethasone and β -naphthoflavone, respectively

Substrate	E	f_u	micro	hep	<i>in vivo</i>
ethoxycoumarin	0.606	0.220	33	31	140
hexylbarbitone	0.630	1.000		25	34
diazepam	0.657	0.220	53	98	174
ondansetron	0.664	0.250	212	134	158
propranolol	0.695	0.107	2059		425
hexobarbitone	0.712	0.619	218		80
heptabarbitone	0.778	1.000		35	70
diazepam (dex)	0.780	0.220	317	259	323
tacrine	0.797	0.530		1200	148
phenacetin	0.867	1.000	22	94	130
metoprolol	0.882	0.880	29		170
ethoxycoumarin (bnf)	0.892	0.220	308	288	750
alprenolol	0.907	0.200	53	43	980
imipramine	0.909	0.107	2970	1188	1870
codeine	0.941	1.000		348	320
lignocaine	0.965	0.870	132		625

ing that appropriate elimination heterogeneity may lead to a lower dispersion heterogeneity than implied by the vascular dispersion alone.

3.2. SOURCES OF DATA AND SELECTION CRITERIA

A number of papers comparing *in vitro* and *in vivo* results have appeared in recent years. Here we reanalyze IVIVC data from Refs. [1] and [2]. The data in these papers are reported as unscaled *in vitro* CL_{int} and *in vivo* CL_{int} . Values for the unbound fraction f_u were taken from the literature [25–33]; for six drugs, we used human f_u values instead of rat, and for another 12 drugs, we pessimistically assume $f_u = 1$, reducing the correction due to our method. In accordance with Houston and Carlile [2], we use a hepatic flow rate of 20 ml/min/SRW, where SBW is a standard rat weight of 250 g.

3.3. DATA ANALYSIS

We wish to compare intrinsic clearances under the assumptions of different models for different individual drugs. To go from the reported *in vivo* measurements, which assume the well-stirred model, to *in vivo* CL_{int} assuming another model requires a two-step process. From the quoted *in vivo* CL_{int} , we may compute the actual *in vivo* hepatic availability F using Equation (10). Given the availability F , and an assumed value for ϵ^2 or D_N , the distributed model or dispersion model, respectively, can be solved for R_N , and then for the *in vivo* intrinsic clearance. Equation (25) is solved numerically for R_N , whereas the dispersion model is inverted analytically.

The best value of ϵ^2 or D_N is fit for each dataset, minimizing differences between *in vitro* and *in vivo* CL_{int} on a log scale without weighting. The chi-square is computed on this same log scale, and the number of degrees of freedom ν is computed as the number of datapoints minus either one (for the well-stirred model) or two (for the dispersion and distributed models).

4. Results

Figures 3, 4 and 5 shows hepatocyte and microsomal correlations fit to the well-stirred model, the distributed model, and the dispersion model, respectively. For the distributed model, the best fit values of ϵ^2 were 0.28 and 0.37 for hepatocyte and microsomal data, respectively. For the dispersion model, best fit values of D_N were 0.50 and 0.27 for hepatocyte and microsomal data, respectively.

For low clearance drugs, regardless of *in vitro* systems or mathematical liver model, the data show substantial scatter but balanced residuals, with similar numbers of drugs above and below the line. The well-stirred model with hepatocyte data shows a trend of under-prediction of the *in vivo* CL_{int} for high clearance drugs. There are a number of outliers regardless of method. For the hepatocyte data, the high clearance trend is improved by the distributed model, and for microsomal data, several outlying points are brought closer into line.

5. Discussion

Several general features of the models are worth reviewing. Referring to Figure 2, it is clear that under conditions of low clearance, all models give similar results. The reason for this is that the availability F is close to 1, so the drug concentration changes little during its passage through the liver. This nearly homogenous state is little different from the completely homogeneous conditions assumed by the well-stirred model, leading to only small differences between the models. This single observation is sufficient to explain much of the success of the well-stirred model. A large number of drugs exhibit low clearance, and the well-stirred model works well for low clearance drugs. In general, low clearance drugs are good drugs, and the well-stirred model works well for low clearance drugs.

Figure 3. Unscaled CL_{int} for all drugs in Houston database assuming the well-stirred model for hepatocyte data (top) and microsomal data (bottom). Straight lines are best fit to a single scaling factor. $\chi^2/\nu = 0.9755$ for hepatocyte data and 1.12 for microsomes.

Figure 4. Unscaled CL_{int} for all drugs in Houston database assuming the distributed model with $\epsilon^2 = 0.28$ for hepatocyte data (top) and $\epsilon^2 = 0.37$ for microsomal data (bottom). $\chi^2/v = 0.9721$ and 0.9516 , respectively, and $Q = 20$ ml/min/SRW.

Figure 5. Unscaled CL_{int} for all drugs in Houston database assuming the dispersion model with $D_N = 0.50$ for hepatocyte data (top) and $D_N = 0.27$ for microsomal data (bottom). D_N values selected by best fit. $\chi^2/\nu = 0.9664$ and 0.9781 , respectively.

For higher clearance drugs, the drug concentration is changing significantly as it passes through the liver, resulting in a greater difference between models. Some work comparing liver models only compares the well-stirred model with a parallel tube ($\epsilon^2 = 0$) model, which lie at the extremes of the range of models considered here. The micro-circulation of the liver exhibits substantial variability, suggesting that the most anatomically accurate model should be somewhere between a pure parallel tube and a well-stirred model. It should not be surprising that studies excluding the middle ground have produced varied results.

The effects of metabolic saturation are worth mentioning. Since the well-stirred model has homogeneous concentrations, saturable metabolism can be captured simply by replacing CL_{int} with $CL_{\text{int}}(C_{\text{out}})$, where C_{out} is the drug concentration exiting the liver. Tube geometries are not so simple, and under general conditions detailed prediction requires knowledge of the axial enzyme distribution. However, saturated conditions lessen the effect of drug concentration differences, resulting in less distinction between well-stirred and parallel or distributed tube models. For the extreme case of completely zero-order kinetics, the distinctions disappear, and the well-stirred model gives the same result as any other. However, it is important that saturation is considered in cases where it is relevant. For example, one of the biggest outliers is tacrine, a drug primarily metabolized by a high affinity, low capacity channel [27]. Unfortunately, the *in vivo* datapoint used appears to be in the saturated regime, resulting in a reduced extraction ratio, whereas the *in vitro* extrapolation assumes linearity.

The presence of binding has a strong effect on all calculations. Some work [34, 35] indicates that completely ignoring binding produces better correlations than accounting for it. If the primary binding is to microsomal or hepatocyte proteins, not plasma or blood cell proteins, then binding *in vitro* and *in vivo* would be identical, and not correcting would be appropriate.

Transport barriers are also a potential complication. If the transport of the drug from the space of Disse into the hepatocyte is both rapid and nonsaturable, then a transport barrier modifies the apparent intrinsic clearance as follows [17]:

$$CL'_{\text{int}} = \frac{PCL_{\text{int}}}{P + CL_{\text{int}}} \quad (29)$$

where P is the permeability coefficient of the hepatocyte to the drug, CL_{int} is the intrinsic clearance which would be observed in the absence of the transport barrier, and CL'_{int} is the apparent intrinsic clearance with the transport barrier in place. If the transport into the hepatocyte is saturable, or occurs on a timescale as slow as mixing and transport through the liver, then the internal concentration is no longer locally in equilibrium with the external concentration, and must be represented as an additional compartment in the model. Even where the transport barrier only modifies CL_{int} , it can cause difficulties in scaling *in vitro* values, because the nature of the transport barrier may be modified by the *in vitro* preparation process. This is particularly true for microsomal preparations, which do not preserve the integrity of the outer cell wall.

In order to compare intrinsic clearances from *in vitro* and *in vivo* data, it is necessary to invert the model being assumed in order to estimate the *in vivo* CL_{int} from the measured hepatic clearance. The following standard combination of experimental protocol and mathematical model of the liver make this inversion step trivial. If one models the systemic circulation as a single well-stirred compartment, one has the following relation for the full-body clearance

$$CL = \frac{\text{Dose}}{\text{AUC}} \quad (30)$$

where AUC indicates the area under the concentration-time curve, and the dose is the dose reaching the body compartment, i.e. the total quantity of drug reaching the site of measurement at least once. Now, if the administered dose is injected directly into the portal vein so that the entire administered dose is subjected to first-pass liver elimination, we have

$$CL = \frac{F \text{Dose}_{\text{ip}}}{\text{AUC}} \quad (31)$$

Assuming that the liver is the only eliminating organ, we may use

$$CL = Q(1 - F) \quad (32)$$

to compute F as

$$F = \frac{1}{1 + \frac{\text{Dose}_{\text{ip}}}{Q \cdot \text{AUC}}} \quad (33)$$

$$= \frac{1}{1 + \frac{CL_{\text{ip}}}{Q}} \quad (34)$$

This statement is independent of any liver model. In order to compute CL_{int} a liver model must be used which predicts the availability F . Considering first the the well-stirred model

$$F = \frac{1}{1 + R_N} \quad (35)$$

we may solve for R_N

$$R_N = f_u CL_{\text{int}} / Q = CL_{\text{ip}} / Q \quad (36)$$

$$f_u CL_{\text{int}} = CL_{\text{ip}} \quad (37)$$

We see that the hepatic flow rate cancels. This means that the i.p. clearance is a direct measurement of the intrinsic clearance, independent of the liver flow, and this is why i.p. administration is used in many IVIVC studies. However, this fortuitous cancellation occurs *only* for the well-stirred model, and is not a general feature of i.p. administration. In general, measuring CL_{int} from *in vivo* data requires a hepatic flow rate and inversion of whatever liver model is being used.

Unfortunately, the simplicity of this protocol masks the nature of the modeling assumption underlying it, and we have seen no references which invert models to directly measure an *in vivo* CL_{int} after assuming a non-venous equilibrium model.

The principal difficulty in comparing the well-stirred model to the more physiological distributed tube and dispersion models is the additional free parameter of those models.

The correct approach is to measure experimentally Q and ϵ^2 . As discussed in Bass et al. [36], ϵ^2 may be directly estimated using a perfused liver. If the value of F is measured at various flow rates, then a plot of $\log F$ vs. $1/Q$ will show a quadratic deviation from a linear trend, with ϵ^2 entering into the coefficient of $1/Q^2$. Specifically,

$$\log F = -R_N + \log \left(1 + \frac{1}{2} \epsilon^2 R_N^2 \right) \quad (38)$$

$$= -X + \frac{1}{2} \epsilon^2 R_N^2 \quad (39)$$

$$= -f_u CL_{\text{int}}/Q + \frac{1}{2} \epsilon^2 (f_u CL_{\text{int}})^2/Q^2 \quad (40)$$

Bass et al. [36] apply this technique to taurocholate data and estimate $\epsilon^2 = 0.12 \pm 0.05$ for this drug. One difficulty with this data is that the variation of the flow rates Q used in this work is out of the physiological range, which may lead to incomplete perfusion of the sinusoids. An alternative, is to vary the unbound fraction f_u by changing the concentration of proteins in the perfusate [15]. For a highly bound drug, f_u can be varied by up to a factor of 10 [37].

The liver model chosen has a significant effect for high clearance drugs, so appropriate attention should be paid to the choice of model when doing IVIVC for high clearance drugs. Since the hepatic flow rate cannot be eliminated for non-well-stirred models via the use of i.p. dosing, measurements or good estimates of Q are necessary for comparing models. Currently available data show a considerable amount of variability, nevertheless there is a marked tendency for the more physiologically sophisticated models, namely the distributed and dispersion models to better describe high extraction drugs than the well-stirred model.

In a broader context of earlier work done in this area we should mention that Roberts et al. [38] showed that the equivalence of the distributed model and dispersion model only applies at low hepatic extractions and that, as D_N increases, the distributed model shows an increased discrepancy from dispersion model predictions. These authors had earlier suggested that vascular dispersion should be a key premise for defining ϵ^2 . This work also suggested that the dispersion and tube models were asymptotic extremes of the dispersion model in which D_N was best described by using an adjustable parameter D_N – defining a D_N of 0.35 for flow variation i.e. ϵ^2 of 0.18. Furthermore, Roberts and Rowland [39] compared the *in situ* extraction of solutes with microsomal extraction and found the dispersion model more appropriate than the well-stirred model. The present study has conducted a similar evaluation using *in vivo* data but with ϵ^2 .

The overall correlation between *in vitro* and *in vivo* results seems to justify extrapolation of *in vitro* data to *in vivo*. However, using a well-stirred model risks underestimating the true hepatic clearance *in vivo* resulting in an optimistic assessment of the drugs clearance properties.

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