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The Universally Unrecognized Assumption in Predicting Drug Clearance and Organ Extraction Ratio

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Abstract

For almost a half-century clearance concepts have been utilized in pharmacokinetics to understand the relationship between the dose administered and the time course of systemic concentrations to predict efficacy and safety, as well as how dosing should be modified in disease states. Various models of organ clearance/elimination have been proposed and tested. Surprisingly, however, the theoretical basis for the appropriate data collection to test these models has never been evaluated. Here we show that in vivo data collection limitations and the extraction ratio concept itself are only consistent with the well-stirred model of hepatic elimination. Evaluating measures of drug concentrations entering and leaving an organ will appear to best fit the well-stirred model, since driving force concentrations within the organ of elimination cannot be measured.

Keywords

Hepatic clearance; Extraction Ratio; Well-Stirred Model

Introduction

More than 65,000 publications have appeared in the scientific literature addressing drug clearance as used in pharmacokinetics, with a significant number of these papers evaluating the appropriate model to be used to describe organ elimination of drug. The great majority of these papers address hepatic elimination, utilizing predominantly what is called the well-stirred model[1, 2]. The mathematical mass balance relationship as used in pharmacokinetics for the well-stirred model, as far as we can tell, has not been explicitly defined, but we do so here. This simple model adequately describes drug clearance under most conditions, but it is believed to be deficient for high extraction ratio drugs where the clearance approaches blood flow to the organ of elimination. In those cases, alternate models of hepatic elimination, such as the parallel tube model[3] and the dispersion model[4], are expected to more accurately describe the hepatic elimination process. However, most experimental studies beginning

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Author Contributions

LZB drafted the manuscript. All authors contributed to the theory and hypotheses presented and participated in many revisions.

Conflict of Interest/Disclosure

The authors declared no conflict of interest.

with the analysis of lidocaine pharmacokinetics in 1977[5] have been unable to demonstrate that these alternate models do, in fact, better describe the elimination process. Here we show that the testing of these alternate models for the past 40 years has been flawed in that the investigators have not recognized that the form of the data they use in the analysis assumes the well-stirred model. We conclude that at present, although these alternative models should theoretically more adequately describe hepatic drug elimination, there is little support for these alternate models. But of even greater relevance, we show that the common universally accepted definitions of organ clearance and extraction ratio are based on the well-stirred model and that attempting to apply alternate models hampers our ability to predict in vivo clearance values from in vitro measures of elimination. The analysis here only considers the evaluation of hepatic metabolic clearance, ignoring any potential transporter effects, since the correct analysis of metabolism must be understood prior to extending the clearance concept to encompass transporter effects. Additionally the importance of uptake and efflux transporters in the liver was not recognized when hepatic clearance was first defined.

In a 1972 publication[6], Rowland defined organ clearance (here hepatic, CL_H) as the fraction of the entering drug concentration (C_{in}) that is lost multiplied by the organ blood flow (Q_H)

$$CL_H = Q_H \cdot \frac{C_{in} - C_{out}}{C_{in}} \quad (1)$$

Rowland wrote, “The instantaneous clearance [CL_H], units volume per unit time, of a drug by an eliminating organ is given by [Equation 1] and may be defined as the volume of blood entering the organ which is cleared of drug per unit time. Often the fraction [$\frac{C_{in} - C_{out}}{C_{in}}$] is referred to as the extraction ratio of the substance by the organ.” Further in the 1972 Rowland paper and in all subsequent treatments of the models of hepatic clearance, the in vivo definition of clearance has universally been taken as Eq. 1. In 1977, Pang and Rowland wrote that Eq. 1 “by definition, at steady state” is hepatic clearance[7].

However, Rowland did not derive the relationship, and until today, it has neither been derived nor questioned. In the mid-1970s, a further clearance term, the intrinsic clearance[1, 2], was introduced, which represents the clearance ability of an organ independent of blood flow and protein binding. This intrinsic clearance term combined with organ blood flow and fraction of drug unbound in blood was mathematically related to organ clearance in the well-stirred model as defined in Eq. 1, resulting in

$$CL_H = Q_H \cdot \frac{f_{u,B} \cdot CL_{int,H}}{Q_H + f_{u,B} \cdot CL_{int,H}} \quad (2)$$

where $CL_{int,H}$ is the intrinsic hepatic clearance and $f_{u,B}$ is the ratio of unbound plasma drug concentration to the whole blood concentration.

Results

Consider an isolated perfused liver at steady-state with blood flow, Q_H, and blood drug concentrations C_{in} and C_{out} entering and exiting the liver, respectively. The mass difference between these two products of flow and concentration will then be the mass of drug lost in the liver at steady-state per unit time. Following mass balance criteria, upon which pharmacokinetics is based, this rate of loss will be equal to the product of the hepatic clearance, CL_H, multiplied by the steady-state concentration of drug within the liver that is driving elimination, $C_{H,ss}$, as given in Eq. 3.

$$Q_H \cdot C_{in} - Q_H \cdot C_{out} = CL_H \cdot C_{H,ss} \quad (3)$$

Equation 3 has a form similar to all pharmacokinetic equations at steady-state where rate of elimination is set equal to the product of a driving force concentration somewhere within the body multiplied by a clearance reflective of that measurement. For example, with a constant rate zero-order infusion the steady-state concentration of drug in the venous blood ($C_{VB,ss}$) will not equal the concentration of drug in the arterial blood ($C_{AB,ss}$) and the respective clearances for these measurements (CL_{VB}, CL_{AB}) will also not be equal, but $CL_{VB} \cdot C_{VB,ss} = CL_{AB} \cdot C_{AB,ss}$. Here for the isolated perfused liver, although the concentrations of drug in the blood going into and out of liver can be measured, it is not possible to measure the driving force concentration at steady-state within the liver, a heterologous organ containing various water and lipid components. It would be possible to stop the flow, grind up the liver, quantify the total amount of drug in the liver and divide this by the mass/volume of the liver to determine a measure of steady-state concentration. However, this would probably not be equivalent to the $C_{H,ss}$ term in Eq. 3 that reflects the concentration of drug driving elimination. We are unaware of any attempt to determine $C_{H,ss}$ by this methodology. Thus, since we cannot measure $C_{H,ss}$ within this perfused liver, it is not possible to determine CL_H. Dividing both sides of Eq. 3 by $C_{H,ss}$, as shown in Eq. 4, allows clearance to be defined as the product of hepatic blood flow and the ratio of the change in the blood drug concentration entering and leaving the liver at steady state to the driving force concentration for elimination within the liver.

$$CL_H = Q_H \cdot \frac{(C_{in} - C_{out})}{C_{H,ss}} \quad (4)$$

Since organ clearance can never exceed blood flow to the organ, the value of $C_{H,ss}$ can never be less than C_{in}-C_{out}, but different models of hepatic elimination will allow different values for $C_{H,ss}$ and CL_H to be assumed and simulated, although the product of $C_{H,ss}$ and CL_H for the different models will all be equal to the left hand side of Eq. 3.

Comparing Eq. 1 and Eq. 4, it is obvious that although Rowland did not derive Eq. 1[6], he defined CL_H by setting $C_{H,ss}$ (a concentration within the liver that is not measured) equal to

C_{in}. This has been universally accepted for the past 45 years as the definition of hepatic clearance. What we show now is that by doing this and accepting Eq. 1 as the definition of clearance in a perfused organ, the field has defined clearance in terms of the well-stirred model, and more importantly restricted the concept of the extraction ratio to only be consistent with the well-stirred model

The well-stirred model was introduced into pharmacokinetics by Rowland et al. in 1973[1], with the present first author of this manuscript serving as a coauthor of that paper. Following the model used in chemical engineering referred to as the steady-state mixed flow reactor or the continuous stirred tank reactor[8], Rowland et al. wrote the following equation in terms of unbound concentrations, but modified here to the terms utilized above and assuming steady-state

$$Q_H \cdot (C_{in} - C_{out}) = k_m \cdot K_p \cdot V_E \cdot f_{u,B} \cdot C_{out} \quad (5)$$

where k_m is the rate constant for elimination of drug from the liver, K_P is an apparent partition coefficient between C_E, the steady-state concentration of drug within the liver and C_{out}, and V_E is the volume of distribution of the liver. C_E in the Rowland et al. derivation is not $C_{H,ss}$, the steady-state concentration driving liver elimination as defined in Eq. 3, but rather the steady-state concentration in the liver after elimination has occurred. Subsequently, the liver intrinsic clearance was defined as

$$CL_{int,H} = k_m \cdot K_p \cdot V_E \quad (6)$$

where $CL_{int,H}$ is assumed to be the intrinsic ability of the liver to eliminate drug independent of blood flow and the fraction of drug unbound in the blood. Rowland et al.[1] gave no justification for Eq. 5, and Eq. 5 seems to violate mass balance considerations. That is, how can the amount lost on the left hand side of Eq. 5 be equal to a clearance term multiplied by the concentration that leaves the liver? Pang and Rowland[7] define intrinsic clearance as a parameter “which relates the rate of hepatic elimination to the concentration of drug surrounding the hepatic enzymes” but why should unbound drug concentration after elimination has already occurred be related to intrinsic clearance? The well-stirred model in chemical engineering was proposed to facilitate a construct that would allow a kinetic process to be evaluated within a black box reactor in terms of the measured concentrations entering and exiting the reactor, where the rate of the kinetic process could not exceed the rate of delivery to the reactor. This simplest chemical engineering steady-state mixed flow reactor model[8] assumes that every reaction to occur takes place instantaneously as the reactants enter the black box driven by the concentration entering the reactor, and that the concentration in the reactor after the initial reaction has occurred equals the measured exiting concentration. It allows chemical reactions to be modeled without reference to the actual concentrations within the reactor. The pharmacokinetics field has attributed a physiologic meaning to these models and we now recognize that when attempting to characterize models of hepatic elimination, the liver unbound drug clearance multiplied by the average steady-state driving force concentration in the liver equals the liver intrinsic

clearance multiplied by the average steady-state concentration in the liver after elimination has occurred (i.e., downstream from the zone of the reaction). Thus, for the well-stirred hepatic model:

$$CL_{int,H} = \frac{CL_H}{f_{u,B}} \cdot \frac{C_{in}}{C_{out}} \quad (7)$$

We believe this principal has never previously been enunciated in pharmacokinetics, but with the equality in Eq.7, we see that mass balance in Eq. 5 is maintained. One can readily determine that substituting the Eq. 7 ratio for $\frac{C_{out}}{C_{in}}$ into Eq. 1 will give Eq. 2. That is, Eq. 1 is only consistent with the well-stirred (WS) model where the steady-state driving force concentration within the liver, $C_{H,ss}$, is assumed to equal C_{in}.

An alternative model in pharmacokinetics, the parallel tube (PT) model (the steady-state plug flow reactor[8] in chemical engineering) assumes that the liver is composed of a number of cylindrical tubes, arranged in parallel, with enzymes uniformly distributed in cells surrounding the tubes[3]. In the PT model the average steady-state driving force concentration cannot be C_{in} and

$$CL_{int,H} = \frac{CL_H}{f_{u,B}} \cdot \frac{C_{H,ss}}{C_{avg,ss,PT}} \quad (8)$$

where

$$C_{avg,ss,PT} = \frac{C_{in} - C_{out}}{in\frac{C_{in}}{C_{out}}} \quad (9)$$

Substituting Eq. 9 into Eq. 8, and recognizing the numerator on the right side of Eq. 8 equals $Q_H(C_{in}-C_{out})$ one many readily derive a parallel tube relationship

$$\frac{C_{out}}{C_{in}} = e^{-\frac{f_{u,B} \cdot CL_{int,H}}{Q_H}} \quad (10)$$

The dispersion (Disp) model (the plug flow reactor model with axial dispersion[8] in chemical engineering) yields more complicated relationships[4] for equations comparable to Eqs. 9 & 10 containing $C_{avg,ss,Disp}$. Note that Eq. 10 defines the relationship for the PT model in terms of $\frac{C_{out}}{C_{in}}$, and one may derive the relationship for any of the models of organ

elimination in terms of measured $\frac{C_{out}}{C_{in}}$ values. Thus, one may calculate the $CL_{int,H}$ values for the three models since only measures of C_{in} and C_{out} are required. For the lidocaine rat liver perfusion studies of Pang and Rowland where measured $\frac{C_{out}}{C_{in}}$ was 0.0021 at a flow rate of 10 ml/min and an $f_{u,B}$ of 0.95[5], $CL_{int,WS}$ = 5002 ml/min, $CL_{int,PT}$ = 64.9 ml/min and $CL_{int,Disp}$ =160.8 ml/min (for a dispersion number of 0.3). But $\frac{C_{out}}{C_{in}}$, 1 minus the extraction ratio, is a well-stirred model concept, so any attempt to use these $CL_{int,H}$ values from the PT and Disp models, when either Q_H or $f_{u,B}$ are varied, will not be consistent with the experimental data since it is not possible to calculate CL_H for those models.

Discussion

With such a wide range of intrinsic clearance values for different models of hepatic elimination how can any of these models be validated, and why do pharmaceutical scientists want to know the appropriate model to use and the value of the intrinsic clearance? Initially, Pang and Rowland attempted to differentiate the well-stirred from the parallel tube model for their lidocaine rat liver perfusion studies at different flow rates[5]. They did not recognize that calculation of what is called the extraction ratio $\left(\frac{C_{in}-C_{out}}{C_{in}}\right)$ is only consistent with the well-stirred model. Thus, it is very obvious why the many analyses in the literature attempting to differentiate the well-stirred model from incremental models of liver metabolism (i.e. the parallel tube and dispersion models) for high clearance/extraction ratio drugs (e.g., lidocaine[5, 9]; meperidine[9]; propranolol[10]) were unsuccessful. Rowland's 1972 definition of extraction ratio[6], i.e., the fraction of the entering concentration that is lost, is only consistent with the well-stirred model. Investigators[3-5, 7, 9-14] define the extraction ratio (*ER*) as the middle term in Eq. 11 and then try to equate this to one of the incremental metabolism models.

$$ER = \frac{C_{in}-C_{out}}{C_{in}} < \frac{C_{in}-C_{out}}{C_{H,ss,PT}} \text{or} \frac{C_{in}-C_{out}}{C_{H,ss,Disp}} \quad (11)$$

However, for the definition of extraction ratio as given above, the term is only consistent with the well-stirred model. Attempts to evaluate measures of C_{out} and C_{in} with a model will appear to best fit the well-stirred model, as has been reported in the literature. And as seen in Eq. 11, this well-stirred model extraction ratio will be less than the PT and Disp ratios since $C_{H,ss}$ will always be less than C_{in}.

The second, more relevant, reason for knowing the appropriate hepatic model and the value of intrinsic clearance is to predict in vivo measures of organ clearance from in vitro measures, i.e., IVIVE. Investigators assume that an in vitro measure of drug elimination, for example in a microsomal mixture, can be scaled up based on enzyme quantity in the liver versus the microsomal mixture, to an in vivo measure of intrinsic clearance[11-14]. This value

is then used to predict in vivo hepatic clearance. This is potentially possible for the well-stirred model where there is no incremental clearance within the liver and all clearance is driven by the entering concentration, C_{in}. For the parallel tube and dispersion models, however, incremental metabolism is inferred throughout the liver and the value of $C_{H,ss}$ is required to be able to predict hepatic clearance (Eq. 4). Thus, CL_H values for these models cannot be extrapolated from experimental in vitro $CL_{int,H}$ values. Not only cannot one determine the value of $C_{H,ss}$ within the liver, even as a value relative to C_{in}, but one has no knowledge of the fraction of drug unbound within the liver. Thus, comparisons of different models of hepatic metabolism are not possible and IVIVE predictions of drug clearance can only be potentially valid for the well-stirred model. Lack of recognition of these relationships leads to wasted efforts, impeding progress in the field, when investigators attempt to use in vitro measures of metabolism to predict in vivo clearance using different models of hepatic elimination.

Measures of protein binding are a critical issue for IVIVE and are a subject of ongoing research in attempting to explain the poor predictability of IVIVE methods[9-15]. As noted above, for incremental models of metabolism (e.g., parallel tube, dispersion) the appropriate unbound concentration is determined using the fraction unbound within the liver, but this is not an issue here for the well-stirred model where the relevant concentrations are those in the blood entering and exiting the liver. However, it may be worth mentioning that the extent of hepatic first-pass loss, calculated from the well-stirred model extraction ratio, is determined by hepatic clearance, which is a function of the product of fraction unbound and hepatic intrinsic clearance, not of either quantity separately. Thus, even for very highly protein bound drugs, hepatic clearance and first-pass hepatic loss can be significant since a sufficiently high intrinsic clearance can overcome the influence of any degree of protein binding.

Although experimental measures of $C_{H,ss}$ are not possible, it seems logical that the driving force steady state concentration for elimination would be less than C_{in}. Thus, it is reasonable when investigating physiologic based pharmacokinetic (PBPK) models for predicting drug concentrations in organs of the body to incorporate incremental models of organ elimination. Present PBPK models divide the liver into 6 or 7 compartments, usually with each compartment following a distributive incremental model. Here we have shown, however, that it will not be possible to prove that such a model is correct, only that it may provide a useful approximation. Since there are many assumptions in the PBPK models, there will be little difference in fitting human PK measurements with the various hepatic models, and no way to prove the superiority of a particular model, since driving force concentrations cannot be measured within the organs of elimination.

In conclusion, this study has several basic implications for clinical pharmacology. First, extraction ratio is a concept that is only consistent with the well-stirred model of organ elimination. Second, any calculations based on the extraction ratio are also only consistent with the well-stirred model. This will affect the measure of organ bioavailability such as F_H, the first pass hepatic bioavailability of the liver as given in Eq. 12.

$$F_H = 1 - ER_H = 1 - \frac{CL_H}{Q_H} \quad (12)$$

However, this only becomes an issue for high clearance compounds where C_{out} is very much smaller than C_{in}. For low clearance compounds the difference between $C_{H,ss}$, C_{in} and C_{out} is not great. Third, models of organ elimination using isolated organs and measuring only C_{in} and C_{out} can only be consistent with the well-stirred model of organ elimination. Perturbations of flow and protein binding will not allow one to differentiate the hepatic models, since when the extraction ratio is measured, the well-stirred model has been assumed. There is no justification in testing different models of hepatic metabolism for experiments measuring only concentrations entering and exiting an isolated organ.

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Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

For 45 years, organ clearance has been defined in terms of the extraction ratio, based on entering and exiting blood concentrations. Various models of hepatic elimination have been developed and used in modeling, but nearly all data best fits the well-stirred model, even for high extraction ratio drugs.

WHAT QUESTION DID THIS STUDY ADDRESS?

We identify an unrecognized assumption in calculating drug clearance: i.e., the drug concentration driving elimination always equals entering blood concentrations. This hampers our ability to understand model discrepancies.

WHAT THIS STUDY ADDS TO OUR KNOWLEDGE?

We address the theoretical basis for organ clearance calculations. Only the well-stirred model explicitly assumes that entering blood concentration drives elimination, while other models assume that internal liver concentrations, which are never measured, drive elimination.

HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE

There is no justification in testing different models of hepatic metabolism for experiments measuring only concentrations entering and exiting an isolated organ. Such calculations inherently assume the well-stirred model.