Title Page

Commentary

In defense of current concepts and applications of clearance in drug development and therapeutics

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Running Title Page

Clearance models: the case for status quo

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Significance statement

Clearance concepts are widely applied in drug development and drug therapy. Historically, hepatic clearance has been defined as the ratio of the rate of elimination divided by the ingoing blood concentration. Recently, this approach has been challenged arguing that clearance should be referenced to the concentration within the liver, a feature that corresponds to the intrinsic clearance of the chosen clearance model, a widely accepted parameter applied to PBPK and IVIVE. There is no need for additional clearance terms, which are confusing and offer no material benefit.
Abstract

Clearance is one of the most widely quoted and applied pharmacokinetic concepts in drug development and therapy. Its foundations and associated models of drug elimination are well embedded and accepted within the scientific community. Recently, however, the prevailing views that have held us in good stead for the past 50 years have been challenged with the argument that organ clearance should not be based on elimination rate, now defined by extraction across the liver divided by incoming or systemic concentration, as in current practice, but rather by the mean concentration of drug within the blood in the organ, which is model dependent. We argue that all needed parameters already exist, and that the proposed new approach to organ clearance is confusing and unnecessary.
Introduction

Clearance is one of the most widely quoted and applied pharmacokinetic concepts in drug development and therapy. Its foundations and associated models of drug elimination are well embedded and generally accepted within the scientific community and have served it well over the past 50 years.

Recently, however, Benet et al. (2021) have written a provocative paper rejecting one of the prevailing views, which states that clearance, calculated by relating rate of elimination across the eliminating organ to the systemic or incoming concentration (rate / Cin), is independent of any mechanistic models. Rather, they claim, based on chemical engineering principles that the value of organ clearance should be given by dividing the rate of elimination of substance by the mean concentration of drug within the tissue water space within the organ, and advocate that it is model dependent. In particular they derive relationships for the common models of organ clearance, the well stirred model (WSM) and the parallel tube model (PTM). In this short commentary, we briefly review the background to Benet’s clearance equations and show what these authors are calculating is the intrinsic clearance of the liver associated with the particular chosen model, a parameter already widely accepted and applied in IVIVE, PBPK and drug development. There is no need for additional clearance terms.
Analysis and Discussion

Clearance ($CL$) is a steady-state concept: rate of elimination = $CL \cdot C$, where the in vivo $C$ is typically the systemic concentration of a drug or solute, in a plasma or blood sample taken from a peripheral venous site, with dimensions of volume per unit time. It is most commonly applied when the kinetics of the drug is linear, that is, when parameters of the system are independent of concentration and time. In such circumstances, $CL$ derived from the systemic exposure (represented by area under the curve from time of dosing to infinite time $AUC_{\infty}$) after single dose intravenous administration ($CL = \text{Dose} / AUC_{\infty}$) can serve to predict plasma or blood concentrations at steady state, $C_{ss}$, such as following constant rate input, $R_0$ and ($C_{ss} = R_0 / CL$) is a useful and heavily applied property. In such applications, total body clearance is a global estimate of the efficiency of all the eliminating sites within the entire body to remove drug. But inherently, there is no requirement to know where or how elimination occurs, nor is any structural or mechanistic model of clearance implied. However, to progress further, attention needs to move from global events in the body to relate events occurring in individual organs of elimination. There is also increasing recognition that to ensure mass balance and correctly estimate organ extraction ratio, reference must be given to measurement of drug in whole blood and not plasma, as many drugs partition into blood cells, some extensively, and potentially be available for elimination. A correction could be made easily by dividing by the blood-to-plasma concentration ratio ($R_b$) to convert plasma to blood concentrations.

Bradley et al. (1945) estimated hepatic blood flow $Q_H$ in man using bromosulfophthalein, BSP, a compound exclusively eliminated via the liver. They infused BSP at a known constant rate $R_0$ to steady state when this rate is matched by the rate of elimination across the liver, given by the difference between the rate in, $Q_H \cdot C_{in,ss}$, and rate out $Q_H \cdot C_{out,ss}$, i.e. $Q_H \cdot (C_{in,ss} - C_{out,ss})$, an application of Fick’s Principle of Perfusion, where $C_{in,ss}$ and $C_{out,ss}$ are the (measured) hepatic input and output blood concentrations at steady state, respectively. The basis of this calculation is that at steady state the only reason for a drop in concentration across the organ is elimination; prior to then output is due to a mixture of elimination and tissue uptake/binding. Given that clearance is rate of elimination divided by concentration, adopting a similar approach as Bradley et al. (1945) and keeping to directly measurable quantities, Rowland (1972) propose Eq. 1 for the estimation of organ (hepatic) clearance.

$$CL_{H,b} = \frac{Q_H \cdot (C_{in,ss} - C_{out,ss})}{C_{in,ss}} = Q_H \cdot E_H$$

where $CL_{H,b}$ is hepatic clearance based on measurements in whole blood, and $E_H$ is the hepatic extraction ratio, the fraction of the inputted drug that is eliminated during a single passage through the liver at steady state, a measure of the efficiency of elimination of the process. The difference, $1 - E_H$, is the fraction escaping single pass elimination, often referred to as the organ (hepatic) availability (to the systemic circulation) $F_H$. This concept of clearance may be applied to other eliminating organs.
Eq. 1 has been widely adopted consistently and almost universally by the pharmaceutical community as the definition of organ clearance. It is to be noted that the equivalent of $F_H$ in the chemical reaction engineering literature is the fraction of compound emerging unchanged in the exit stream from a chemical reactor $F_c$. Importantly, this literature shows that, for a given amount of catalyst (enzyme) and first order kinetics, $F_c$ varies with the flow characteristics within the reactor (Levenspiel, 1999; Fogler, 2016). Given that the flow characteristics inside the liver are not well stirred in nature (Roberts and Rowland, 1986a), Eq. 1 does not, as repeatedly proposed by Benet et al. (2018; 2021) confer a WSM property to hepatic clearance (discussed later). Also, this property was not, as claimed, required for the definition of clearance as implied in the paper on clearance concepts in pharmacokinetics by Rowland et al. (1973). The only requirement stated in the 1973 paper, which deals with the WSM and applies to other flow models too, is that the rate of elimination across the perfused organ at steady state associated with the chosen model must equal that obtained directly from measurement of drug entering and leaving the organ. More recently, we have restated that Eq. 1 is not based on the WSM (Rowland and Pang, 2018; Pang et al., 2019). However, Eq. 1 is a helpful representation of hepatic clearance on at least two counts. First, it indicates that there is a physical upper limit to the value of clearance, that is blood flow, when the extraction ratio approaches its upper limit of 1. This feature is not apparent from the general formulation, rate of elimination = $CL \cdot C$.

Second, in most cases peripheral venous blood, the most common sampling site in pharmacokinetic studies, drains from non-eliminating tissues, typically skin and muscle, in which case the venous concentration of drug at steady state (or the integral of the concentration over time after a single bolus dose, AUC$_\infty$) equals that in the entering arterial concentration, which is generally the same as that entering the eliminating organ. Accordingly, the value of organ clearance obtained by applying Eq. 1 adds directly as part of total body clearance, estimated following intravenous administration.

However, as written, Eq. 1 does not describe the likely causes for change in hepatic clearance, and hence in exposure of drug within the body, as a result of changes in hepatic blood flow, binding within blood, enzymatic activity and in permeability, situations that arise during drug therapy. To progress to this desired state of affairs, models have been developed incorporating postulated blood flow behavior and transport within liver sinusoids and Disse space linking output to input, and in particular disposition in hepatocytes where elimination occurs. Historically, there was also the common assumption that elimination is driven by unbound concentration of drug at the site of elimination. This led to the concept of intrinsic clearance, $CL_{int}$ (Wilkinson and Shand, 1975), with dimensions of flow per unit time, so named because it describes the intrinsic ability of the hepatocyte to eliminate substrate (rate of elimination = $CL_{int} \cdot C_{ucell}$, where $C_{ucell}$ is the unbound drug concentration in hepatocyte water), which in the context of the model is considered independent of such external factors as blood flow and binding within blood. When elimination occurs via metabolism that is characterized by Michaelis-Menten kinetics operating under linear conditions $CL_{int} = \sum v_{max}/K_m$, where $v_{max}$ is the maximum velocity of the reaction(s) and $K_m$ is the Michaelis-Menten constant of the respective enzymes involved. In principle, intrinsic clearance may also involve biliary excretion and active transport processes. At steady state, it should be readily apparent that rate of elimination occurring globally within the liver equals that determined externally across the liver.
Physiologically oriented clearance models that are particularly applied to hepatic elimination have been widely discussed (Roberts et al., 1988; Hung et al., 2001; Pang et al., 2019). The first, least physiologic, but still the most widely applied in pharmacokinetics, is the well-stirred model, WSM (Pang and Rowland, 1977), alternatively called the continuously stirred tank model. Assumptions of this model, shared in common with other models of elimination described below, are that only unbound drug permeates cells and that permeation of the drug is so fast that radial distribution is blood flow rate-limited, such that unbound drug in hepatocyte is in equilibrium with that unbound in plasma of blood within the organ. Specific assumptions of the WSM are that the organ is well-mixed such that there is no concentration gradient of drug within the organ in the direction of bulk (vascular) flow, and that the unbound concentration of drug in the venous outflow \((C_{\text{out}})\) equals the unbound concentration within the organ, including the aqueous space of the hepatocyte \((C_{\text{cell}})\), a condition also known as venous equilibration. Accordingly, the steady state equalities of the WSM are:

\[
CL_{\text{int,WSM}} \times C_{\text{cell,ss}} = CL_{\text{int,WSM}} \times C_{\text{out,ss}} = f_{ub} \times CL_{\text{int,WSM}} \times C_{\text{out,ss}} = Q_H \times (C_{\text{in,ss}} - C_{\text{out,ss}}) \tag{2}
\]

where \(CL_{\text{int,WSM}}\) is the intrinsic clearance associated with the WSM and \(f_{ub} = C_{\text{u}} / C\) is the ratio of unbound plasma concentration to whole blood concentration. On collecting terms and applying the definition for hepatic clearance expressed into Eq. 1 one obtains the familiar solution of the WSM.

\[
CL_{H,b} = Q_H \cdot E_H = Q_H \left[ \frac{f_{ub} \cdot CL_{\text{int,WSM}}}{Q_H + f_{ub} \cdot CL_{\text{int,WSM}}} \right] \tag{3}
\]

To re-emphasize, as defined here, for a given \(E_H\) and \(Q_H\) the value of \(CL_{H,b}\) is the same in Eqs. 1 and 3, but whereas the value of \(E_H\) in Eq. 1 is based solely on observations without reference to a structural or mechanistic model of events within the liver, in Eq. 3 \(E_H\) is a model-dependent relationship based on the WSM.

We and others have also described physiologically based models of hepatic elimination that will be addressed in more depth in a separate communication. Here, we will confine ourselves to two simple, reasonably realistic models of hepatic elimination that take into account the continuous decline in drug concentration along the sinusoidal flow path within the liver (Gumucio, 1983). These are the dispersion model (DM) (Roberts and Rowland, 1986b), which also accommodates the observed spread in transit times of compound as it is conveyed by blood traveling through liver sinusoids, and a limiting case in which no such dispersion is assumed - frequently called the parallel-tube model (PTM) (Bass et al., 1976; Pang and Rowland, 1977). As pointed out by Pang and Rowland (1977) and Benet et al. (2021), the latter model is classified as a plug flow reactor. In contrast to the WSM, where the unbound concentration is assumed constant throughout the liver, in the PTM although the enzymes (and associated intrinsic clearance) is assumed to be distributed uniformly along identical lengths and hence parallel tubes within the liver, the unbound blood...
concentration within the liver, the unbound hepatocyte concentration and the associated rate of elimination all incrementally decline monoexponentially with distance along the sinusoidal flow path from input to output along the length of the sinusoid. The corresponding overall rate of elimination across the organ is the integral of the rate-distance profile within the organ, for which there is a corresponding average rate. Accordingly, the equalities for the PTM globally within and across the liver at steady state are:

\[
CL_{\text{int,PTM}} \times C_{\text{logav,cell,ss}} = CL_{\text{int,PTM}} \times C_{\text{logav,ss}} = f_{ub} \times CL_{\text{int,PTM}} \times C_{\text{logav,ss}} = Q \times (C_{\text{in,ss}} - C_{\text{out,ss}})
\]

(4)

where \(C_{\text{logav,ss}}\) is the logarithmic average blood concentration within the liver, which for a monoexponential decline is given by

\[
C_{\text{logav,ss}} = \frac{C_{\text{in,ss}} - C_{\text{out,ss}}}{\ln(C_{\text{in,ss}} / C_{\text{out,ss}})}
\]

and \(C_{\text{logav,cell,ss}}\) is the corresponding log average unbound concentration in the sinusoid and hepatocyte, respectively (Winkler et al., 1973). On collecting terms and applying the definition for hepatic clearance given in Eq. 1 yields Eq. 5,

\[
CL_{H,b,PTM} = Q_H \cdot E_H = Q_H \cdot (1 - e^{-\left(\frac{f_{ub} \times CL_{\text{int,PTM}}}{Q_H}\right)})
\]

(5)

where \(CL_{\text{int,PTM}}\) is the intrinsic clearance for the whole liver that is associated with the PTM.

The WSM (infinite mixing, bulk flow) and the PTM (no mixing, plug flow) set limits for model prediction, and these models therefore serve as extremes of the boundary conditions of how blood flow, binding within blood and intrinsic clearance affect \(CL_{H,b}\) (Pang and Rowland, 1977; Pang et al., 2019). The DM predictions are, in principle, intermediate between these two extremes, noting DM predictions are also dependent on other physiological determinants such as hepatic flow rate induced dispersive changes in liver transit times.

In contrast to the above, Benet et al. (2021) argue that the only correct hepatic clearance associated with PTM, which they designated as \(CL_{H,b,PTM}'\), is given by Eq. 6.

\[
CL_{H,b,PTM}' = Q_H \times \ln\left(\frac{C_{\text{in,ss}}}{C_{\text{out,ss}}}\right)
\]

(6)

They derived this equation by dividing the rate of elimination across the organ by the mean concentration of drug in blood within the organ. Kochak (2020) has the same equation and uses the same terminology as Benet et al. (2021). A comparison clearly shows that the \(CL_{H,b,PTM}'\) term, as defined in Eq. 6, is numerically greater than the clearance given by Eq. 1 or Eq. 5 for the PTM, for a given \(E_H\) and \(Q_H\). The problem of defining hepatic clearance in this way, by referencing rate of organ elimination by the mean liver blood concentration, is
that it has the seemingly absurd value of approaching infinity as $E_H$ approaches a value of 1 because $C_{out,ss}$ approaches zero. This does not arise when referencing elimination to $C_{in,ss}$ as then clearance is limited at high $E_H$ values by organ blood flow rate. So, what exactly is the meaning of Eq. 6? This is shown by recognizing that,

$$\frac{C_{out,ss}}{C_{in,ss}} = 1 - E_H = F_H = e^{-\left(\frac{f_{u_b} V_{out,ss}}{Q_H} K_H\right)^{\frac{1}{n}}} = e^{-\left(\frac{f_{u_b} CL_{out,PTM}}{Q_H}\right)}$$

which upon inverting, taking logarithms, and rearranging can be seen to give

$$Q_{H,b} \cdot \ln\left(\frac{C_{in,ss}}{C_{out,ss}}\right) = f_{u_b} \cdot CL_{int,PTM}$$

This relationship (Eq. 7) has been shown previously by Keiding (1987) for $f_{u_b} = 1$. Thus Eq. 6 is just a direct way of calculating the value of intrinsic clearance, multiplied by the unbound fraction of drug in blood estimated from experimental data ($C_{in,ss}, C_{out,ss}$, and $Q_H$), and is not $CL_{H,b,PTM}$ as defined in Eq. 5. As such, it does not warrant the creation of a new clearance term; we already have defined it earlier in the form of intrinsic clearance, $CL_{int}$.

Now, it should be readily apparent from a comparison between the WSM and PTM (Eq. 2 and Eq. 4) that the rate of elimination across the organ equals the product of the model-defined intrinsic clearance multiplied by its corresponding hepatocyte unbound concentration:

$$\frac{Q}{(C_{in,ss} - C_{out,ss})} = CL_{int,WSM} \cdot C_{out,ss} = CL_{int,PTM} \cdot C_{log,cell,ss}$$

and, since all unbound concentrations within the liver associated with the PTM (starting with $C_{u,in,ss}$ at the entry point) are greater than WSM, other than at the exit to the liver when they are equal, it follows that $CL_{int,PTM} < CL_{int,WSM}$. Restated, the PTM predicts that a lower amount of enzyme activity is needed than the WSM for a given extraction ratio or clearance.

So where does this analysis take the reader in the application of models of organ elimination, and particularly hepatic elimination, in drug development and clinical application? First, and most importantly, there is no need to invent new clearance parameters; all the needed ones have already been defined. Second, given the previously mentioned direct linkage with systemic events, commonly gained from peripheral venous measurements, it is preferable to keep to the simple definition of organ clearance proposed in Eq. 1 by Rowland (1972), which applies equally to all organ models, rather than invent new terminology for clearance dependent on the chosen structural model. Third, for those involved with IVIVE, which aims to obtain an in vitro estimate of $CL_{int}$ to predict organ clearance in vivo, the decision as to which model to apply, WSM, PTM, or DM, is the same as ever before, a topic of constant and vigorous discussion, often dependent on the characteristic of the drug (Roberts and Rowland, 1986a; Pang et al., 2019; Sodhi et al.,...
2020), drug interactions and pathophysiology (Hung et al., 2006). For example, if the in vitro $CL_{int}$ is very low, as is often the case in modern drug development, to help achieve low dosing rate and less frequent dosing, it is immaterial as to which model $CL_{int}$ is applied to predict clearance in vivo; all the above three models predict essentially the same value of clearance (error $<$ 5% between WSM and DM for $E_H \leq 0.4$) (Roberts and Anissimov, 1999). Even so, this does not guarantee accurate prediction for such compounds, with underprediction frequently encountered (Wood et al., 2017). Still, on the acquisition of in vivo data, the investigator then updates the value of $CL_{int}$ within the chosen model and proceeds forward in the usual manner. Fourth, when basolateral permeability of the compound is low, so that perfusion rate limited distribution no longer applies, the steady-state ratio of intracellular to extracellular unbound concentrations is impacted, when active transporters are involved, but equally for the WSM and PTM. Fifth, when dealing with clearance following iv administration, it is common practice to subtract renal clearance, and assign the remainder to hepatic clearance, estimate $E_H$ by dividing by the published mean value of $Q_H$, using Eq. 1, and subsequently chose the WSM, PTM or a related model to predict $CL_H$ for different scenarios, appreciating all assumptions made. Last, for a given rate of elimination, and therefore rate of administration when the organ is the primary site of elimination, the PTM (and DM) predicts a higher average steady-state unbound hepatic concentration than the WSM (Eq. 8), especially when $E_H > 0.7$, and therefore higher response, in the relatively unusual case of the target residing in the eliminating organ (such as statins acting on hepatic enzymes to lower cholesterol). Also, as a corollary, a lower rate of administration is expected to be needed before internal saturation (exceeding $K_m$) of enzymes or transporters occurs for such high extraction ratio compounds.

In summary, historically, organ clearance has referenced rate of elimination across an eliminating organ to its ingoing blood concentration based on experimental data. Recently, this approach has been challenged by Benet et al. (2021) arguing that clearance should be referenced to the mean blood concentration within the organ, such as the liver, whose value then depends on the choice of the model used to characterize postulating events occurring within the eliminating organ. We show that for the PTM this definition of organ clearance corresponds to the intrinsic clearance associated with that model, an already widely accepted parameter applied in PBPK and IVIVE. There is no need for additional clearance terms, which are confusing and offer no material benefit.
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Wrote or contributed to the writing of the manuscript: Rowland, Roberts, Pang

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References


