Title

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Are All Measures of Liver Kp_{uu} a Function of F_H, as Determined Following Oral Dosing, or Have We Made a Critical Error in Defining Hepatic Drug Clearance?

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Running Title: Is liver Kp_{uu} a function of F_H? Present theory says it is. (59 characters including spaces)

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Abbreviations:

C_{Blood}, steady-state concentration of total drug in blood; $C_{Blood,u}$, steady-state concentration of unbound drug in blood; $C_{H,u}$, average unbound drug concentration within the liver; C_{in}, blood concentration entering the liver; CL_{Blood}, blood clearance; $CL_{Blood,u}$, unbound blood clearance; CL_H, hepatic clearance; CL_{int}, intrinsic hepatic clearance; $CL_{reabsorption}$, renal reabsorption clearance; $CL_{secretion}$, renal secretion clearance; C_{out} and $C_{Hepatic\ vein}$, blood concentration exiting the liver; DM, dispersion model; ECM, Extended Clearance Model; *ER*, hepatic extraction ratio; F_H, hepatic bioavailability; f_{uB}, fraction of drug unbound in the blood; GFR, glomerular filtration rate; KL, Kirchhoff's Laws; Kp_{ss}, ratio of total liver drug concentration to total drug concentration exiting the liver; Kp_{uu}, *in vivo* ratio of unbound liver drug concentration to unbound systemic blood concentration; $Kp_{uu,ss}$, ratio of

unbound liver drug concentration to unbound drug concentration exiting the liver; OATP, organic anion transporting polypeptide; PS_{efflux}, intrinsic basolateral efflux clearance; PS_{influx}, intrinsic basolateral influx clearance; PTM, parallel tube model; Q_H, hepatic blood flow; WSM, well-stirred model

Abstract

We recently recognized, as presented here, utilizing universally accepted relationships for hepatic clearance at steady state that the ratio of unbound liver drug concentration to unbound systemic blood concentration, Kp_{uu}, for all models of hepatic elimination is a function of or related to the hepatic bioavailability for that drug, F_H. According to the derivation for the well-stirred model, Kp_{uu} can never exceed unity, can frequently be a function of hepatic blood flow, and is equivalent to the value of F_H as determined following oral dosing. For the parallel tube model, Kp_{uu} will not equal F_H but will be a function of F_H, and will also never be greater than 1. When hepatic clearance is rate limited by basolateral transporters, Kp_{uu} will be less than 1. We believe that most of these outcomes are highly unlikely, and that the error arises from a basic assumption concerning hepatic clearance that leads to the mechanistic models of hepatic elimination, the well-stirred, parallel tube and dispersion models. That basic concept is that the steady-state systemic concentration multiplied by the hepatic systemic clearance is equal to the product of the average unbound liver steady-state concentration and the intrinsic hepatic clearance. We present arguments as to why this universally accepted relationship is not correct. Alternatively, we have shown in recent publications that hepatic clearance may be adequately determined based on Kirchhoff's Laws where no assumption of the equality above concerning hepatic intrinsic clearance is required and where Kp_{uu} is independent of hepatic extraction ratio and F_H.

Significance Statement

We demonstrate that the basic assumption that the product of the steady-state systemic blood concentration multiplied by blood clearance is equal to the product of the unbound drug concentration in the liver multiplied by the liver intrinsic clearance leads directly to results showing that Kp_{uu} is a function of F_H. There is no reason to believe that such an outcome is true. The model independent clearance-intrinsic clearance relationships can be defined using Kirchhoff's Laws for rate defining processes in series.

Introduction

In 2018, our laboratory (Benet et al., 2018b) began questioning the relevance and accuracy of the mechanistic models of hepatic elimination, i.e., the well-stirred model (WSM), the parallel tube model (PTM) and the dispersion models (DM). Our position was vigorously countered (Rowland and Pang, 2018; Pang et al., 2019). We continued to examine the issue based on experimental data generated in our laboratory (Wang and Benet, 2019) and numerous studies from other laboratories (Sodhi et al., 2020), as well as from theoretical perspectives (Benet and Sodhi, 2020; Benet et al., 2021; Benet and Sodhi, 2022; Benet and Sodhi, 2023). These presentations were also vigorously countered (Jusko and Li, 2022; Rowland and Pang, 2022; Rowland et al. 2022; Rowland et al., 2023). At the September 2022 ISSX/MDO meeting a debate was organized between Professors Benet and Pang under the title “Perspectives on Long-Held Clearance Concepts”, however, this still did not settle the question in the minds of many. Here we present a previously unrecognized relationship for the mechanistic models of hepatic elimination between Kp_{uu}, the ratio of unbound liver drug concentration to unbound systemic blood concentration and F_H, the model independent measure of the fraction of an oral dose that escapes elimination on a drug’s first pass through the liver. Given that such a relationship between Kp_{uu} and F_H and in certain cases the limitations it imposes are not realistic, we present here a compelling argument for the invalidity of using mechanistic models of hepatic elimination when only systemic drug concentrations are measured.

Beginning in the 1970s, hepatic clearance equations were developed based on a specific mechanistic model, initially the WSM (Rowland et al., 1973; Wilkinson and Shand, 1975). However, the conditions for the WSM have been believed to be non-physiological. Yet, when seemingly more physiologically sound models were proposed, initially the PTM (Pang and Rowland, 1977) and DM (Roberts and Rowland, 1986), the WSM-derived equation continued to best represent the experimental data (Pang et al., 2019; Sodhi et al., 2020). We reasoned that the equations following the WSM might be valid for reasons other than those assumed in the WSM derivation. We then reported that we could simply derive the equation believed to be the WSM using Kirchhoff’s Laws (Patcher et al., 2022) independent of any mechanistic model of hepatic elimination, where for rate defining processes in series, i.e., hepatic blood flow (Q_H) and the intrinsic clearance of unbound drug (CL_{int}) multiplied by the fraction of drug unbound in blood (f_{uB}), the addition of the inverse of each rate defining process would be equal to the inverse of hepatic clearance (CL_H) .

$$\frac{1}{CL_H} = \frac{1}{Q_H} + \frac{1}{f_{uB} \cdot CL_{\int ¿}¿}$$

(1)

Solving Eq. 1 gives

$$CL_H = Q_H \cdot f_{uB} \cdot \frac{CL_{\int ¿}}{Q_H + f_{uB} \cdot CL_{\int ¿}¿}¿$$

(2)

Simply we have shown that it is possible to derive the coefficient of proportionality between the rate of reaction and the driving force for that reaction, concentrations or amounts, independent of differential equations, where the coefficients of proportionality (clearance for concentration driven reactions and rate constants for amount driven reactions) are added for parallel processes and where the addition of the inverse of the of the individual in series coefficients of proportionality equals the inverse of the overall clearance or rate constant. It is critical to recognize that the inclusion of the coefficients of proportionality within the Kirchhoff's Laws derivations are valid only for rate defining processes, that is, processes that under certain conditions can be singly equal to the total clearance parameter. For example, in hepatic elimination: hepatic blood flow, metabolic intrinsic clearance or basolateral transport; in kidney elimination: kidney blood flow, glomerular filtration or renal tubular transport. This is the reason that the recent publication of Korzekwa and Nagar (2022) questioning our approach is not valid. None of the multicompartment distribution and elimination parameters analyzed by Korzekwa and Nagar (2023) ever can be considered rate defining processes. We argued that Eq. 2 was not the WSM, but rather the general equation describing clearance when only systemic concentrations are measured and basolateral transporters are not considered. We further proposed that mechanistic models of hepatic elimination provide no valid explanation for hepatic clearance values based on systemic concentration measurements. Although we have introduced the Kirchhoff's Laws approach, this manuscript only details the unusual outcomes relating to Kp_{uu} and F_H when considering the present mechanistic models of hepatic elimination.

Methods, Theoretical Analysis and Results

The primary mass balance equation that serves today and for the past 50 years as the basis for the derivation of the hepatic clearance-intrinsic clearance relationship for the WSM, PTM and DM, as well as for the characterization of hepatic elimination in physiologic based pharmacokinetic (PBPK) models at steady-state is

$$CL_{Blood} \cdot C_{Blood} = Q_H \cdot (C_¿ - C_{out}) = CL_{\int ¿ \cdot C_{H,u} ¿} \quad (3)$$

where C_{Blood} is the steady-state concentration of total drug in the blood, CL_{Blood} is blood clearance, C_{in} and C_{out} are the blood concentrations of total drug (unbound plus bound) entering and leaving the liver, respectively, and $C_{H,u}$ is the average concentration of unbound drug within the liver, as recently reviewed by Li and Jusko (2022). Equation 3 may also be written in terms of unbound steady-state blood concentration ($C_{Blood,u}$) and unbound hepatic blood clearance ($CL_{Blood,u}$).

$$CL_{Blood,u} \cdot C_{Blood,u} = Q_H \cdot (C_{¿} - C_{out}) = CL_{\int ¿ \cdot C_{H,u} ¿} \quad (3a)$$

When mechanistic models of hepatic elimination are considered, Eq. 3 can be rewritten as proposed by Pang and Rowland (1977) and Roberts and Rowland (1986) for all mechanistic models of hepatic elimination as

$$CL_{Blood,u} \cdot C_{Blood,u} = CL_{\int,WSM} \cdot C_{H,u} = CL_{\int,PTM} \cdot C_{H,u} = CL_{\int,DM} \cdot C_{H,u} \quad (3b)$$

with each mechanistic model assuming a differing $C_{H,u}$.

Deriving Kp_{uu} For the WSM Equation of Hepatic Clearance When Basolateral Transporters Are Not Considered

It is possible to derive the *in vivo* steady-state relationship between Kp_{uu} ($i.e., \frac{C_{H,u}}{C_{Blood,u}}$) and the clearance-related parameters for each model by substituting the unbound blood clearance into Eq. 3b. For the WSM derivation (equivalent to Eq. 2)

$$Kp_{uu,WSM} = \frac{C_{H,u}}{C_{Blood,u}} = \frac{CL_{Blood,u,WSM}}{CL_{\int ¿}}$$

$$CL_{\int ¿} = Q_H \cdot \frac{\frac{Q_H + f_{uB} \cdot CL_{\int ¿}}{Q_H}}{CL_{\int ¿} = \frac{Q_H}{Q_H + f_{uB} \cdot CL_{\int ¿} ¿}} ¿¿¿ \quad (4)$$

Thus, for the WSM hepatic clearance, based on Eq. 3, *in vivo* Kp_{uu} can never be greater than unity and except for very low clearance drugs will be a function of hepatic blood flow.

But there is a further outcome. Independent of the model of hepatic elimination, the fraction of an oral dose that escapes first pass hepatic elimination, F_H, is calculated by Eq. 5,

$$F_H = 1 - ER = 1 - \frac{CL_{Blood}}{Q_H} \quad (5)$$

where ER is the hepatic extraction ratio. For the WSM, the expression for F_H can be calculated as

$$F_H = 1 - \frac{CL_{Blood,WSM}}{Q_H} = 1 - Q_H \cdot \frac{\frac{f_{uB} \cdot CL_{\int ¿}}{Q_H + f_{uB} \cdot CL_{\int ¿}}}{Q_H} = ¿¿¿ \frac{Q_H}{Q_H + f_{uB} \cdot CL_{\int ¿}} ¿$$

(5a)

Thus, based on Eq. 3, for the WSM hepatic clearance derivation for a drug only eliminated by hepatic processes, $Kp_{uu} = F_H$. Although not previously reported, the outcome is consistent with the WSM, where $C_{H,u}$ is assumed to equal $C_{out,u}$. Thus, for the WSM both Kp_{uu} and F_H equal $\frac{C_{out}}{C_¿}$.

Deriving Kp_{uu} For the Parallel Tube Model Equation of Hepatic Clearance

Recognizing that the steady-state average concentration within the liver for the PTM is $\frac{C_¿ - C_{out}}{\ln \frac{C_¿}{C_{out}}}$ and $ER = \frac{C_¿ - C_{out}}{C_¿} = 1 - F_H$, independent of mechanistic hepatic elimination models, the equation for $Kp_{uu,PTM}$ can be written directly

$$Kp_{uu,PTM} = ¿ \quad \frac{C_{H,u}}{C_{Blood,u}} = \frac{f_{uB} \cdot \frac{C_¿ - C_{out}}{\ln \frac{C_¿}{C_{out}}}}{f_{uB} \cdot C_¿} = \frac{\frac{C_¿ - C_{out}}{C_¿}}{\ln \frac{C_¿}{C_{out}}} = \frac{1 - F_H}{\ln \frac{1}{F_H}} \qquad (6)$$

For the PTM, $Kp_{uu,PTM} \neq F_H$, but it is a function of F_H. When a drug exhibits a very low extraction ratio, $Kp_{uu,PTM}$ is just less than unity (e.g., F_H = 0.99 then $Kp_{uu,PTM} = ¿$ 0.995) and when the extraction ratio is very high, $Kp_{uu,PTM}$ must be less than one to a greater degree (e.g., F_H = 0.01 then $Kp_{uu,PTM} = ¿$ 0.215). If one accepts Eq. 3 as valid, then the relationship between Kp_{uu} and F_H for the WSM and PTM are depicted by the solid lines in Fig. 1, and Kp_{uu} for both models are always ≤ 1.0. Above, we indicated that the finding of $Kp_{uu,WSM} = F_H$ may be justified for the WSM, but there appears to be no justification for the Eq. 6 relationship.

The Definition of Kp_{uu}

Above, we have defined Kp_{uu} as the *in vivo* ratio at steady-state of the unbound concentration within the liver to the unbound systemic blood concentration, the usual measurement (e.g., Di et al., 2021). Recently Li and Jusko (2022) have proposed that Kp_{ss}, the ratio of total drug concentrations, should not be total drug concentration in the liver to total drug concentration in the systemic blood at steady-state, but rather total drug concentration in the liver to total drug concentration in the blood exiting the liver,

$C_{Hepatic\ vein} = C_{out}$. Although they did not address this directly, we assume that they would then calculate $Kp_{uu,ss}$ in the same manner (i.e., $Kp_{uu,ss,Li \wedge Jusko} = \frac{C_{H,u}}{C_{out,u}}$)

For such an assumption the $Kp_{uu,ss,Li \wedge Jusko}$ values for the WSM with and without transporters would equal 1.0 for all drugs, since the WSM assumes $C_{H,u} = C_{out,u}$. It is difficult to see how the Li & Jusko assumption provides any value.

Since $C_{out} = F_H \cdot C_{¿}$ the Kp_{uu} relationship for the PTM, independent of whether basolateral transporters are included or not, would be:

$$Kp_{uu,PTM,Li \wedge Jusko} = ¿ \quad \frac{C_{H,u}}{C_{Hepatic\ vein,u}} = \frac{f_{uB} \cdot \frac{C_{¿} - C_{out}}{\ln \frac{C_{¿}}{C_{out}}}}{f_{uB} \cdot C_{out}} = \frac{\frac{C_{¿} - C_{out}}{C_{¿}} \cdot \frac{C_{¿}}{Cout}}{\ln \frac{C_{¿}}{C_{out}}} = \frac{\frac{1 - F_H}{F_H}}{\ln \frac{1}{F_H}} \tag{7}$$

For the Li and Jusko (2022) assumption, $Kp_{uu,PTM}$ is always ≥ 1.0. For a low extraction ratio drug, there is essentially little change in $Kp_{uu,PTM}$ between Eqs. 6 and 7 but now it is slightly greater than unity (e.g., F_H= 0.99 then $Kp_{uu,PTM,Li \wedge Jusko} = ¿$ 1.005), and for a high extraction ratio drug $Kp_{uu,PTM,Li \wedge Jusko}$ now becomes much greater than 1.0 (e.g., F_H= 0.01 then $Kp_{uu,PTM,Li \wedge Jusko} = ¿$ 21.5). If one accepts Eq. 3 as valid, and the proposal of Li and Jusko (2022) that partition coefficients should be liver steady-state concentration to hepatic vein steady-state concentration, then the relationship between Kp_{uu} and F_H for the WSM and PTM are depicted by the dashed lines in Fig. 1.

Deriving Kp_{uu} for the Extended Clearance Model of Hepatic Clearance When Basolateral Transport Is the Rate Limiting Step in Hepatic Elimination

We approach this topic based on the Extended Clearance Model (ECM) equation derivation presented in many papers as we reviewed (Benet et al., 2018a):

$$CL_{Blood,ECM} = Q_H \cdot PS_{influx} \cdot f_{uB} \cdot \frac{CL_{\int ¿}}{Q_H \cdot ¿¿} ¿ \tag{8}$$

where PS_{influx} and PS_{efflux} are the total hepatic (active plus passive) intrinsic basolateral influx and efflux clearances, respectively. Substituting Eq. 8 into Eq. 3 gives

$$C_{Blood} \cdot Q_H \cdot PS_{influx} \cdot f_{uB} \cdot \frac{CL_{\int ¿}}{Q_H \cdot ¿¿} ¿$$

which can be rearranged to:

$$\frac{C_{H,u}}{C_{Blood,u}} = Kp_{uu,ECM} = \frac{Q_H \cdot PS_{influx}}{Q_H \cdot ¿¿} \quad (9)$$

Equation 9 presents a potential conundrum that has not been previously addressed. Frequently it is proposed that when $Q_H \gg PS_{influx} \cdot f_{uB} \cdot CL_{\int ¿,¿}$ the more usual form for the $Kp_{uu,ECM}$ equation is seen (e.g., Di et al., 2021)

$$Kp_{uu,ECM} = \frac{PS_{influx}}{CL_{\int ¿ + PS_{efflux}} ¿} \quad (10)$$

However, this "significantly greater than" assumption is probably not possible. One knows that Q_H approximates 1500 ml/min. Now let's consider the literature data for atorvastatin where plasma clearance is approximately 625 ml/min (Lennernäs, 2003) from reported iv dosing data (Gibson et al., 1997). The blood clearance for atorvastatin acid will likely be greater since acids do not distribute well into blood cells, but for demonstration purposes, since the value is unknown, let's assume it is 625 ml/min. It is generally recognized that atorvastatin clearance is rate limited by hepatic uptake based on the studies where OATP uptake is inhibited (Lau et al., 2006; Maeda et al., 2011), therefore the total clearance value approximates $f_{uB} \cdot PS_{influx}$. Then for a drug where hepatic elimination is rate limited by hepatic uptake, $f_{uB} \cdot CL_{int}$ must be significantly greater than $f_{uB} \cdot PS_{influx}$. Taking the most likely underestimated clearance value of 625 ml/min and assuming that a 4-fold difference approximates significantly greater, $f_{uB} \cdot CL_{int} \approx$ 2500 ml/min. But f_{uB} for atorvastatin is ≤ 0.02, with the result that $CL_{int} \approx$ 125,000 ml/min. Thus, in the denominator of the final form of Eq. 9, the second term ($PS_{influx} \cdot f_{uB} \cdot \frac{CL_{\int ¿}}{Q_H} ¿$) is ≈ 52,000 and not negligible compared with the first term in the denominator ($CL_{\int ¿ + PS_{efflux} ¿}$), which is ≈125,000 ml/min (assuming negligible basolateral efflux). Therefore, it is questionable that analyses utilizing Eq. 10 adequately differentiate the rate limiting step since the assumption of the second term in the denominator of Eq. 9 being negligible is probably not true. However, this is not the major reason for this discussion. The analysis above recognizes that when Eq. 3 is utilized to derive Kp_{uu}, the values must always be less than 1. Yet, it is generally recognized (Di et al., 2021) that "When compounds accumulate intracellularly, Kp_{uu} can be greater than 1 due to uptake into the cells by active transport mechanisms, such as OATP (organic-anion-transporting polypeptide) uptake of its substrates into the hepatocytes", the situation for atorvastatin and other OATP substrates.

Substituting Eq. 8 into Eq. 5 gives

$$F_H = 1 - ER = 1 - \frac{CL_{Blood}}{Q_H} = 1 - Q_H \cdot PS_{influx} \cdot f_{uB} \cdot \frac{CL_{\int ¿}}{Q_H \cdot ¿¿} ¿¿ \; Q_H \cdot ¿¿ \quad (11)$$

Now by comparing Eq. 11 to Eq. 9, we see that for the ECM equation when hepatic basolateral transport is rate limiting, not only is Kp_{uu} always less than 1.0, but Kp_{uu} is always less than F_H.

Thus, in all cases detailed above for the WSM and PTM, whether they include basolateral transporter rate limitation or not, $Kp_{uu} \leq F_H$ when the relation between blood clearance and intrinsic clearance is based on Eq. 3 for the usual definition of in vivo Kp_{uu}(Di et al., 2021), and as a result, the value of Kp_{uu} cannot exceed unity.

Now this is not to say that Kp_{uu} cannot be greater than 1 when including basolateral hepatic transporters in the derivation. As a reviewer of this paper noted for a theoretical drug for which CL_{int} is the rate limiting step, if PS_{influx} = 3000 mL/min, CL_{int} = 100 mL/min, PS_{efflux} = 200 mL/min, f_{uB} = 0.1 and Q = 1500 mL/min, Kp_{uu} calculated in Eq. 9 is 9.375. However, for these parameters $CL_{Blood,ECM}$ = 93.75 ml/min (Eq. 8), a value very close to CL_{int}, which is the rate limiting step of hepatic elimination. Thus, when basolateral transport is not the rate limiting step in clearance, Kp_{uu} may be greater than 1. However, when basolateral hepatic transport is the rate limiting step, the relationship in Eq. 3 results in Kp_{uu} values always less than 1.

As we note, detail and ask in the Discussion, what knowledgeable drug metabolism/pharmacokinetics scientist would believe these outcomes showing that there is a relationship between Kp_{uu}, F_H and ER now that they have been explicitly presented and depicted for the first time?

Discussion

When we first began to question the practice of testing different models of hepatic metabolism for experiments that measure only concentrations entering and exiting an isolated organ (Benet et al., 2018b) the only equations available to relate clearance to hepatic blood flow were based on the derivation of the WSM, in which our laboratory had participated (Rowland et al., 1973), and the subsequent derivations of the PTM and the multiple variations of the DM in terms of organ blood flow, degree of protein binding and intrinsic clearance. Thus, at that time and until mid-2021, we incorrectly believed that the 1972 equation of Rowland,

$$CL_{organ} = Q_{organ} \cdot ER = Q_{organ} \cdot \frac{C_{¿} - C_{out}}{C_{¿}} \quad (12)$$

was the WSM. Rowland and Pang (2018) correctly responded that Eq. 12 was hepatic mechanistic model independent and not the WSM. Later in 2021 we concurred that Eq. 12 was hepatic model independent following re-examination of the chemical reaction engineering models upon which the

pharmacokinetic models of organ elimination were based (Benet et al., 2021). However, most notably, in 2022 we recognized that what had been previously universally believed to be the WSM, as given in Eq. 2, could be simply derived using Kirchhoff's Laws independent of any mechanistic model of hepatic elimination (Patcher et al., 2022). That is, Eq. 2, just like Eq. 12, is mechanistic organ model independent, and that when hepatic basolateral transporters are not considered, Eq. 2 defines hepatic clearance.

Until now, no recognized measures have been available to differentiate our position that the mechanistic models of hepatic elimination are not useful in defining clearance relationships for drugs when only systemic concentrations are measured. Our field's present analysis accepts Eq. 3 and then based on that equation, which characterizes the WSM without including transporter activity, leads to $Kp_{uu}=F_H$ with an outcome that Kp_{uu} can never exceed unity. A preposterous outcome is found using the potential Li and Jusko (2022) definition of Kp_{uu}, where the value is the ratio of unbound drug concentration in the liver to unbound drug concentration in the hepatic vein. Under that condition for the WSM relation, $Kp_{uu}=1.0$ for all drugs, as the WSM assumes $C_{H,u}=C_{out,u}$. Accepting the validity of Eq. 3, we also derived the relationship between $Kp_{uu}$and F_H for the PTM with outcomes that we believe no knowledgeable scientist will accept. That is, Kp_{uu} is a function of whether the drug is a low or high *ER* compound, independent of any structural molecule characteristics. We have not analyzed the much more complicated DM; the results would be numerically different than the PTM analysis depending on the dispersion number chosen, but the outcome will be the same; Kp_{uu}will be a function of F_H with lines intermediate those in Fig. 1 for the WSM and PTM.

If the numbers from the analysis here are considered, there must be an error in Eq. 3. Since Eq. 3 defines elimination in the liver, average $C_{H,u}$ must always be less than $C_{Blood,u}$. Therefore, $f_{uB}\cdot CL_{int}$ must always be greater than $CL_{Blood,u}$. One can easily confirm this. For Q_H = 1500 ml/min and any non-zero value of $f_{uB}\cdot CL_{int}$ in either what was considered the WSM (Eq. 2) or for the PTM (Eq. 13), $f_{uB}\cdot CL_{int} > CL_{Blood}$.

$CL_{Blood,PTM}=Q_H\cdot$¿ (13) And since $CL_{Blood,u} \le CL_{Blood}$, therefore according to Eq. 3, Kp_{uu} must be less than 1 (or equal to 1) for all drugs. Does our field really believe this?

The validity of Eq. 3 is often justified based on mass balance (Rowland and Pang, 2018 and 2022; Rowland et al., 2022 and 2023). However, mass balance is a necessary but not a sufficient validity criterion by itself. For example, Eq. 14 also maintains mass balance, just like Eq. 3.

$$CL_{Blood,u}\cdot C_{Blood,u}=Q_H\cdot(C_{¿}-C_{out})=Q_H\cdot C_{H,u} \quad (14)$$

where f_{uH} is the fraction of drug unbound in the liver.

Neither Eq. 14 nor Eq. 3 are valid for almost all drugs, but when systemic clearance is rate limited by hepatic blood flow the relationship cannot be differentiated from Eq. 14, and when systemic clearance is much, much smaller than hepatic blood flow the relationship cannot be differentiated from Eq. 3. As we demonstrated (Patcher et al., 2022; Benet and Sodhi, 2023), the Kirchhoff's Laws derivation of hepatic clearance makes no assumption concerning the mechanistic basis of liver elimination and it is not valid to define the clearance rate of drug as measured in the blood in terms of any intrahepatic relationship. There is no general validity to Eq. 3 and therefore the derivations of the WSM, PTM and DM also have no relevance when only systemic concentrations are measured. And thus, there is no valid relationship between Kp_{uu} and F_H, which had only resulted here by accepting Eq. 3 as valid.

How Should We Proceed?

Using Kirchhoff's Laws (KL), we have derived the equations for hepatic clearance when basolateral transporters are not (Eq. 2) and when they are (Eq. 15) clinically relevant, making no assumptions concerning mechanistic processes within the liver. The rationale for including the difference in influx and efflux transporter clearances is addressed in Benet and Sodhi (2023), where some of the rationale is also discussed here in the penultimate paragraph of the Discussion.

$$CL_{Blood,KL} = Q_H \cdot \left(PS_{influx} - PS_{efflux}\right) \cdot f_{uB} \cdot \frac{CL_{int}}{Q_H \cdot CL_{int}} \quad (15)$$

When Q_H is much greater than the difference between the liver basolateral transporter clearances multiplied by $f_{uB} \cdot CL_{int}$, Eq. 15 reduces to

$$CL_H = \frac{f_{uB} \cdot \left(PS_{influx} - PS_{efflux}\right)}{1 + \frac{\left(PS_{influx} - PS_{efflux}\right)}{CL_{int}}} \quad (16)$$

Independent of Eqs. 2 and 16, F_H may be calculated using Eq. 5 and has no relationship to Kp_{uu}.

When viewing Eqs. 12 and 16 for the first time, many scientists ask what if PS_{efflux} is greater than PS_{influx}? As we explain in Patcher et al. (2022), in our most recent publication (Benet and Sodhi, 2023) and here following Eq. 2, Kirchhoff's Laws relationships are only derived based on rate defining processes where it is possible that the clearance or the rate constant may singly be equal to that process. A common example of this concept is given in Eq. 2, where it is possible that clearance equals hepatic blood flow for a very high extraction ratio drug and for a very low extraction ratio drug that

clearance equals $f_{uB} \cdot CL_{\int ¿¿}$. Thus, for HMG-CoA reductase inhibitors, which are exemplary hepatic uptake transporter substrates, it is possible that hepatic clearance may be equal only to $(PS_{influx} - PS_{efflux})$ when that value is positive. However, if PS_{efflux} is greater than or equal to PS_{influx}, clearance cannot be equal to this negative value or zero value and thus the term would not appear in the clearance equation, and instead simplification of Eq. 15 when transporters are not elimination rate determining (Eq. 2) would be used. We see the same restriction in parallel rate defining processes for renal clearance. Renal clearance could be defined singly as ($CL_{secretion}$ - $CL_{reabsorption}$) or $f_{uB} \cdot GFR$ but renal clearance could never be described singly by ($CL_{reabsorption}$ – $CL_{secretion}$) if reabsorption is greater than secretion. Consider a drug such as codeine where the fraction excreted unchanged is negligible and yet the drug is only 7% bound to plasma proteins (Quiding et al., 1986). Certainly, there is significant glomerular filtration of codeine with such a small degree of protein binding and a half-life of 2.9 hr, and therefore $CL_{reabsorption}$ must be markedly greater than $CL_{secretion}$ but these renal clearance terms are not included in any clearance equation for codeine, since these are not rate defining processes. This concept is similar to the liver example discussed above; if PS_{efflux} is greater than PS_{influx}, this negative clearance value can never be the rate defining process for clearance (i.e., clearance can never be solely described by basolateral transporter processes that sum negative). Consider also the physiologic location of the influx and efflux transporters in the basolateral membrane. One would expect the efflux transporter to be active as soon as the drug enters the membrane, rather than wait until the drug is within the hepatocyte before it begins acting. Therefore, drug that ultimately enters into the hepatocyte will be a result of active influx that is not counteracted by active efflux, i.e., PS_{influx} - PS_{efflux}. Furthermore, Eq. 15 eliminates the inconsistencies of the ECM relationship, Eq. 16 when Q_H is much greater than the transporter clearances and $f_{uB} \cdot CL_{int}$, as we have previously described (Patcher et al., 2022; Benet and Sodhi, 2023).

$$CL_{Blood,ECM} = f_{uB} \cdot PS_{influx} \cdot \frac{CL_{\int ¿}}{CL_{\int ¿ + PS_{efflux}} ¿} \quad (17)$$

That is, for the ECM Eq. 17, PS_{efflux} must be zero or negligible for PS_{influx} to be the rate limiting process for clearance. But many of the drugs where basolateral transporters appear to be rate limiting are Extended Clearance Classification System (ECCS) Class 1B (Varma et al., 2015) and/or Biopharmaceutical Drug Disposition Classification System (BDDCS) Class 2 (Wu and Benet, 2005) drugs that exhibit high passive permeability. So how can PS_{efflux} be negligible or zero? Furthermore, in Eq. 17 how can CL_{int} become the rate defining process unless one assumes $PS_{influx} = PS_{efflux}$, where basolateral hepatic transporters have no effect on clearance. Equation 16 removes both of these limitations and shows that basolateral hepatic transporters can rate

limit clearance as long as influx permeability is greater than efflux permeability.

Conclusions

The present manuscript demonstrates that the basic assumption that the product of the steady-state systemic blood concentration multiplied by the systemic blood clearance is equal to the product of the unbound drug concentration in the liver multiplied by the liver intrinsic clearance (i.e., $C_{Blood} \cdot CL_{Blood} = C_{H,u} \cdot CL_{\int ¿¿}$ leads directly to the result that Kp_{uu} is a function of F_H. There is no reason to believe that such an outcome is always true, and since this equality serves as the basis for the WSM, PTM and DM, there is no rationale for defining drug clearance in terms of these hepatic mechanistic models when only systemic concentrations are measured. The model independent relationships between intrinsic clearance, hepatic blood flow and hepatic basolateral transporters can be adequately defined using Kirchhoff's Laws for rate defining processes in series.

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Data Availability

The authors declare that all the data supporting the findings of this study are contained within the paper.

Authorship Contributions

Participated in research design: Benet, Sodhi

Performed analysis: Benet, Sodhi

Wrote or contributed to writing of the manuscript: Benet, Sodhi

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Figure Legend

1. Theoretical relationship between Kp_{uu} and F_H for the WSM and PTM if one accepts Eq. 3 as valid. Solid lines when Kp_{uu} is defined as the steady-state ratio of average unbound drug concentration in the liver to the unbound systemic concentration. Dashed lines when Kp_{uu} is defined as the steady-state ratio of average unbound drug concentration in the liver to the unbound hepatic vein concentration. If similar calculations were made for the DM, the resulting lines would be intermediate those for the WSM and PTM dependent on the dispersion number.