Toxicology in Vitro 27 (2013) 11–15

Contents lists available at SciVerse ScienceDirect

Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit

Brief communication

Deriving an explicit hepatic clearance equation accounting for plasma protein binding and hepatocellular uptake

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ARTICLE INFO

Article history:
Received 28 June 2012
Accepted 8 October 2012
Available online 17 October 2012

Keywords:
Hepatic clearance
Plasma protein binding
Steady-state equations
Fraction unbound
Dissociation/association
Active efflux rate constants

ABSTRACT

High throughput in vitro biochemical and cell-based assays have the promise to provide more mechanism-based assessments of the adverse effects of large numbers of chemicals. One of the most challenging hurdles for interpreting in vitro toxicity findings is the need for reverse dosimetry tools that estimate the exposures that will give concentrations in vivo similar to the active concentrations in vitro. Recent experience using *IVIVE* approaches to estimate in vivo pharmacokinetics (Wetmore et al., 2012) identified the need to develop a hepatic clearance equation that explicitly accounted for a broader set of protein binding and membrane transport processes and did not depend on a well-mixed description of the liver compartment. Here we derive an explicit steady-state hepatic clearance equation that includes these factors. In addition to the derivation, we provide simple computer code to calculate steady-state extraction for any combination of blood flow, membrane transport processes and plasma protein–chemical binding rates. This expanded equation provides a tool to estimate hepatic clearance for a more diverse array of compounds.

1. Introduction

Toxicity testing appears at a crossroads, with growing emphasis on developing mode-of-action based in vitro assays for more rapidly assessing adverse responses to chemicals (Andersen and Krewski, 2010; Houck et al., 2009; Huang et al., 2011; Judson et al., 2010, 2011; Knight et al., 2009; Knudsen et al., 2011; Krewski et al., 2011; Martin et al., 2011). These assays will provide measures of concentrations that cause responses in vitro. Pharmacokinetic (PK) modeling can relate the in vitro point of departure concentration to in vivo human exposure. In vitro–in vivo extrapolation (IVIVE) is the process by which a PK model permits calculation of the in vivo exposure that will cause tissue or plasma concentrations equivalent to the active in vitro concentration (Yoon et al., 2012).

Recently, IVIVE showed promise for applications with higher throughput (Rotroff et al., 2010; Wetmore et al., 2012) by measuring intrinsic hepatic clearance and fraction unbound in blood for hundreds of compounds with primary human hepatocytes and human plasma, respectively. In these studies, estimation of in vivo hepatic clearance used an Eq. (1) first described by Gillette (1971):

$$\mathrm{Clh} = Q \cdot fu \cdot Clint/(Q + fu \cdot Clint) \quad (1)$$

Here Q is liver blood flow; *Clint* is intrinsic metabolic clearance by liver; and, *fu* is fraction unbound (i.e., fraction free) in the plasma. This relationship holds for specific conditions where all compound bound in blood can dissociate during passage of blood through the liver. However, it does not explicitly account for the kinetic processes of association and dissociation of chemical with the plasma protein or for transport limitations in liver uptake or active efflux from liver to blood. As the IVIVE field moves to higher throughput PK studies, the chemicals under study show a more diverse suite of physical chemical properties than those commonly seen with drugs. An even wider range of behaviors with respect to binding and transport limitations is likely as the diversity of chemical structures increases during testing of larger and larger chemical libraries, such as ToxCast Phase II (http://www.epa.gov/ncct/toxcast/) and the expanded 8000 plus chemical library with a more limited set of assays (http://epa.gov/ncct/Tox21/).

To enhance the range of applicability for IVIVE, it is necessary to enhance Eq. (1) to account for a broader set of binding and transport processes. Toward this end, we developed an explicit steady-state hepatic clearance equation, including blood flow, tissue transport processes and association and dissociation rate constants for chemical binding to plasma protein. In this paper, we describe the derivation of a more complete clearance equation, provide a straightforward program for estimating hepatic clearance for any combination of parameters, and show how our explicit steady state clearance equation gives Eq. (1) as a limiting case. This expanded clearance equation may also augment current IVIVE

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http://dx.doi.org/10.1016/j.tiv.2012.10.003

methods by refining algorithms in commercial software such as Simcyp (Jamei et al., 2009) that predict expected clearance in diverse populations.

2. Methods

2.1. Model structure

The basic PK model used in this work (Fig. 1) included compartments for liver blood and liver tissue and accounted for the exchange between bound and unbound forms of chemical in the blood compartment. The association and dissociation rate constants were, respectively $k1$ and $k2$, both described as first-order rate constants (s^{-1}). Although the association reaction is a second order process, we assumed that the amount of protein available for binding is not a limiting factor and thus $k1$ was a first order rate constant in the current analysis. In addition, only unbound chemical diffuses from the liver blood compartment into the liver tissue. We also included the possibility for asymmetric active transport with uptake clearance from blood ($K3$; l/h) and an efflux clearance ($K4$; l/h). Note that these distribution clearance terms include passive permeability, which scales the same as blood flow and is usually expressed as a permeability-surface area product (l/h), in addition to active transport. In this way, the model can simulate uptake/efflux clearances regardless of the mechanisms for cross-membrane fluxes. The tissue to blood partition coefficient (Pl) was used to define the free concentration in the liver tissue as Cl/Pl based on thermodynamic equilibration of the compound between the plasma and tissue matrix at steady-state (Clewell et al., 2007). Model parameters are in the legend to Fig. 1. The Supplemental material includes the model code.

For developing the steady-state relationships for this model, we start with a conventional clearance representation,

$$\mathrm{Clh} = \frac{Q \cdot \text{amount metabolized}}{\text{amount passing through the liver} + \text{amount metabolized}}$$

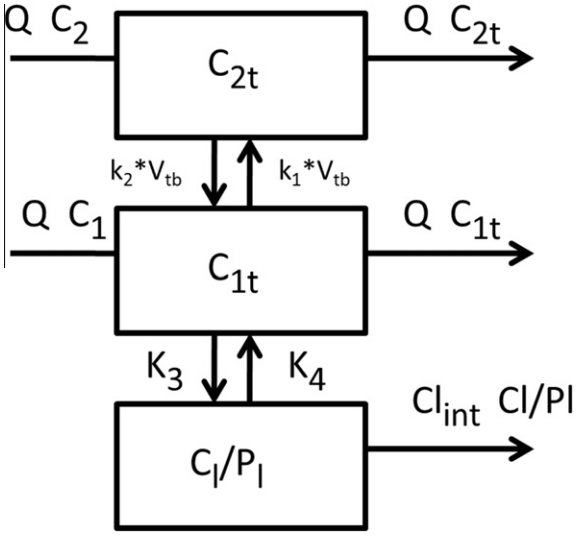

Fig. 1. The PK model for assessing influence of plasma protein binding and hepatic transport on hepatic clearance. There are three equations: one each for free compound in blood, for bound compound in blood and for compound in liver. The free concentration in the liver tissue is represented as Cl/Pl, where Cl is the total concentration in the liver tissue and Pl is the liver to blood partition coefficient. *Clint* (l/h), the intrinsic hepatic clearance is Vmax/(km + Cl/Pl), where Vmax is the maximum rate of metabolism (μmol/h) and km is the Michaelis–Menten constant (μM).

The term 'amount metabolized/(amount passing through the liver + amount metabolized)' represents the hepatic extraction (E); thus this expression is the equivalent to $\mathrm{Clh} = Q \cdot E$.

This clearance relationship for the current model (Fig. 1) is:

$$\mathrm{Clh} = \frac{Q \cdot \left(\frac{V_{max}}{km + \frac{Cl}{Pl}}\right)\frac{Cl}{Pl}}{Q \cdot C2t + Q \cdot C1t + \left(\frac{V_{max}}{km + \frac{Cl}{Pl}}\right) \cdot \frac{Cl}{Pl}} \quad (2)$$

Next, we sequentially expressed $C1t$ as a function of Cl/Pl and $C2t$ as a function of $C1t$ (see Appendix A). These steps give the denominator as,

$$\left(\frac{Q}{Q+K2}\right) \cdot Q \cdot C2 + Q \cdot A \cdot \frac{K4}{K3} \cdot \frac{Cl}{Pl} + \left(\frac{QA+K3}{K3}\right) \cdot \left(\frac{V_{max}}{km + \frac{Cl}{Pl}}\right) \cdot \frac{Cl}{Pl}$$

Here,

$$A = 1 + \frac{K1}{Q+K2}$$

Terms were grouped to provide *Clint*(Cl/Pl) in the denominator, as in Eq. (1). Then the denominator and numerator were divided by Cl/Pl. The resulting equation for hepatic clearance is:

$$\mathrm{Clh} = \frac{Q \cdot \left(\frac{K3}{Q \cdot A + K3}\right) \cdot Clint}{\left(\frac{Q}{Q+K2}\right) \cdot \left(\frac{K3}{Q \cdot A + K3}\right) \cdot Q \cdot \frac{C2}{\frac{Cl}{Pl}} + Q \cdot A \cdot \left(\frac{K4}{Q \cdot A + K3}\right) + Clint} \quad (3)$$

$K1$ and $K2$ are the association and dissociation binding clearances, respectively. These binding clearance terms were calculated as $K1 = k1 \cdot Vtb$ and $K2 = k2 \cdot Vtb$. In this way, $K1$ and $K2$ have units of volume/time. Note the use of upper case letters to denote clearance, whereas lower cases were used for rate constants.

For subsequent discussions, it is useful to examine the expected relationship for a case in which there is no binding (i.e., $k1 = 0$ and hence, $C2 = 0$ and $A = 1$). In that case, Eq. (3) reduces to

$$\mathrm{Clh} = \frac{Q \cdot \left(\frac{K3}{Q+K3}\right) \cdot Clint}{Q \cdot \left(\frac{K4}{Q+K3}\right) + Clint}$$

In this equation, the ratio, $K3/(Q + K3)$, is the proportion of chemical that is available for uptake into liver while the blood passes through the liver. $K4/(Q + K3)$ is the proportion of compound that returns from liver to blood.

2.2. Calculating Clh

With Eq. (3) we estimated hepatic clearance for various combinations of parameters, including ones where the dissociation rate was not large enough to allow all compound to be available while blood passed through the liver. These simulations were performed in Berkeley–Madonna (see Supplementary materials materials for the model code) starting with an equality: [amount in − (amount out + amount metabolized)] = 0. The rearranged equation gave Cl/Pl as a function of all other parameters (including Cl/Pl). We solved the equation using a ROOT-function relying on a Newton–Raphson approximation method to calculate Cl/Pl for given values of *C*in, where *C*in = *C*1 + *C*2. Clh was then calculated based on *C*2 and Cl/Pl. Although we derived the equation allowing for asymmetric transport ($K3$ differing from $K4$), the simulations in the paper were based on $K4C = K3C$.