The Corrected Traditional Equations for Calculation of Hepatic Clearance that Account for the Difference in Drug Ionization in Extracellular and Intracellular Tissue Water and the Corresponding Corrected PBPK Equation

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Received 17 May 2010; revised 15 July 2010; accepted 16 July 2010

Published online 31 August 2010 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jps.22324

ABSTRACT: The estimation of hepatic clearance, Cl_h, using *in vitro* data on metabolic stability of compound, its protein binding and blood–plasma equilibrium concentration ratio is commonly performed using well-stirred, parallel tube or dispersion models. It appears that for ionizable drugs there is a difference of the steady-state concentrations in extracelluar and intracellular water (at hepatocytes), where metabolism takes place. This occurs due to the different pH of extra- and intracellular water (7.4 and 7.0, respectively). The account of this fact leads to the novel equations for Cl_h. These equations include the additional parameter named ionization factor, F_I, which is the ratio of the unionized drug fractions in plasma and intracellular tissue water (or the ratio of the unbound drug concentrations in intracellular tissue water and plasma at equilibrium). For neutral drugs $F_I = 1$ and the novel equations coincide with the traditional ones. It is shown that the account of this factor may yield the calculated Cl_h up to 6.3-fold greater than that obtained by the traditional equations for the strong diprotic basic compounds, and up to 6.3-fold smaller for the strong diprotic acidic compounds. For triprotic acids and bases the difference could be as much as 15-fold. The account of pH difference between extra- and intracellular water also results in the change of the term commonly used to describe drug metabolic elimination rate in physiologically based pharmacokinetic (PBPK) equation. This consequently may lead to a noticeable change of drug concentration-time profiles in plasma and tissues. The effect of ionization factor is especially pronounced for the low-extraction ratio drugs. The examples of significant improvement in the prediction of hepatic clearance due to the account of ionization factor are provided. A more general equation for hepatic clearance, which accounts for ionization factor and possible drug uptake and efflux, is obtained. J Pharm Sci 100:1167–1183, 2011

Keywords: hepatic clearance; well-stirred model; parallel tube model; dispersion model; drug ionization; extra- and intracellular water distribution; physiological model; PBPK equation; hepatic uptake and efflux; metabolic elimination; pharmacokinetics

INTRODUCTION

Hepatic metabolism is recognized as one of the major routes of drug elimination. Determination of hepatic clearance is commonly done using the scaled data on compound metabolic stability in liver microsomes or hepatocytes. Its accuracy is very important for the consequent quantitative prediction of the new drug disposition in human using appropriate simulation model. Hepatic clearance is most often calculated using well-stirred, Eq. 1, parallel tube or dispersion models.[1]

$$Cl_{\mathrm{h}} = rQ\frac{Cl_{\mathrm{int}}f_{\mathrm{up}}}{rQ + Cl_{\mathrm{int}}f_{\mathrm{up}}} \quad (1)$$

where r is the equilibrium blood–plasma concentration ratio, Q is the rate of liver blood flow, Cl_{int} is the intrinsic hepatic clearance, f_{up} is the unbound drug fraction in plasma. All these models implement the assumption of instantaneous equilibration between

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Journal of Pharmaceutical Sciences, Vol. 100, 1167–1183 (2011)

blood and liver tissue concentrations inside the organ, and apply the common equation for the tissue-plasma partition coefficient, P_{t-p}, defined as tissue–plasma drug concentration ratio at equilibrium

$$P_{t-p} = \frac{C_t}{C_p} = \frac{f_{up}}{f_{ut}} \quad (2)$$

where C_t and C_p are the total drug concentrations in liver tissue and plasma, respectively; $f_{up} = C_{up}/C_p$, $f_{ut} = C_{ut}/C_t$ are and the unbound drug fractions in plasma and tissue; and C_{up} and C_{ut} are the unbound drug concentrations in plasma and tissue water. It is important to note that extracellular water and intracellular tissue water at hepatocytes have different pH (7.4 and 7.0, respectively),[63] and f_{ut} in Eq. 2 meant to be calculated by using the unbound drug concentration in extracellular water. The intracellular space is not uniform with respect to pH. The interior of the lysosomes is acidic (pH 4.8) compared to the slightly alkaline cytosol (pH 7.2). The lysosome keeps this pH differential by pumping protons from the cytosol across the membrane via proton pumps and chloride ion channels. Though the pH of smooth endoplasmaic reticulum, which is the site where drugs are metabolized by microsomal enzymes, is maintained near neutral.[64] Actually, as considered further, for ionizable drugs an accurate account of the difference in drug ionization in extracellular and intracellular tissue waters leads to the correction of the traditional equations for hepatic clearance.

The goal of the article is to obtain the consistent equations for hepatic clearance that take into account the ionization properties of drug molecules. This leads to the addition of the correction factor F_I (Eqs. 24, 33, and 35 further), which is the ratio of unionized fractions of drug in plasma and intracellular tissue water. Eventually, it turns out that the traditional equations may overestimate or underestimate the consistently calculated hepatic clearance more than 2.5-fold for monoprotic acids or bases, more than 6-fold for diprotic acids or bases, and more than 15-fold for triprotic acids or bases, which is especially pronounced for highly ionizable compounds that have low extraction ratio.

RESULTS

Tissue–Plasma Partition Coefficient for Ionizable Compounds

One of the basic principles of pharmacokinetics, known as physiological concept of Gillette,[2] is that drug distribution in the body occurs through diffusion of unbound drug between plasma and tissue water. Thus, at steady state, assuming noneliminating tissue, the concentrations of free drug in plasma and tissue water are equal, so that $C_{up} = C_{ut}$, where C_{ut} is actually the concentration of unbound drug in extracellular tissue water. Since $C_p = C_{up}/f_{up}$ and $C_t = C_{ut}/f_{ut}$, this yields Eq. 2 for tissue plasma partition coefficient and also the commonly used equation for the steady state volume of distribution

$$V_{ss} = V_p + \sum_j P_{t-p,j} V_j = V_p + \sum_j \frac{f_{up}}{f_{ut,j}} V_j \quad (3)$$

where V_p is the plasma volume, and V_j, $P_{t-p,j}$, and $f_{ut,j}$ are, respectively, the volume, tissue–plasma partition coefficient and unbound drug fraction of jth compartment (blood cells, tissues, organs). Equation 3 can be rearranged and written as

$$V_{ss} = V_p + \frac{f_{up}}{f_{ut}} V_t \quad (4)$$

where $V_t = \Sigma V_j$ is the total volume of all compartments (except plasma), and f_{ut} is the averaged unbound fraction for all tissue compartments defined as $1/f_{ut} = \sum x_j/f_{ut,j}$, $x_j = V_j/V_t$.

For the tissue–plasma equilibrium of ionizable drugs, the concentrations of unbound unionized drug in tissue water and plasma are equal. This is valid because the solubility of unionized unbound drug S_{int} (intrinsic solubility) is pH independent and thus the same in plasma and tissue water. The extracellular tissue water pH is the same as of plasma. The intracellular water is more acidic than plasma. The difference in pH of plasma and intracellular tissue water is quite important for the consideration of tissue–plasma distribution of ionizable drugs. The unbound drug concentration in plasma comprises of two components

$$C_{up} = C_{up}^n + C_{up}^i$$

where C_{up}^n is the concentration of neutral (unionized) unbound drug in plasma and C_{up}^i is the plasma concentration of ionized unbound drug. The ionized, f_p^i, and neural, f_p^n, drug fractions in plasma are defined as

$$f_p^n = C_{up}^n/C_{up} \quad (5)$$

$$f_p^i = C_{up}^i/C_{up},\ f_p^i + f_p^n = 1.$$

Taking into that $C_p = C_{up}/f_{up}$ and by using Eq. 5 yields

$$C_p = \frac{C_{up}^n}{f_{up} f_p^n}. \quad (6)$$

Let us consider drug distribution in tissue water, which comprises of extracellular water and