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Letter to the Editor

Misuse of the Well-Stirred Model of Hepatic Drug Clearance

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The "well-stirred" model of hepatic drug clearance was first proposed by Gillette (1971) and established by Rowland et al. (1973) and Wilkinson and Shand (1975). In the form that it is commonly used, net hepatic drug clearance based on whole-blood drug concentration ($CL_{H,B}$) is derived as a function of hepatic blood flow ($Q_{H,B}$), the free fraction of drug in blood (fu_B), and the intrinsic metabolic clearance in the liver based on unbound drug concentration ($CLu_{int,H}$):

$$CL_{H,B} = \frac{Q_{H,B} \cdot fu_B \cdot CLu_{int,H}}{Q_{H,B} + fu_B \cdot CLu_{int,H}} \quad (1)$$

This assumes that the drug is distributed instantly and homogenously throughout liver water and that the unbound concentrations in plasma and liver water are identical. Effectively, this means that drug distribution into the liver is perfusion-limited with no diffusion delay and that no active transport systems are involved. The latter possibilities have generally been disregarded in most applications.

A key feature of this model is that it equates whole-blood drug clearance, rather than plasma drug clearance, to liver blood flow, because the organ is potentially capable of extracting the drug from both plasma and blood cells. Most reported clearance values are referenced to plasma rather than blood, since it is more common to measure drug concentration in plasma. If plasma drug clearance (CL_H) is to be estimated, then eq. 1 must be modified to take into account the free fraction in plasma (fu) and the total blood to total plasma drug concentration ratio (C_B/C_P):

$$CL_H = \frac{Q_{H,B} \cdot fu \cdot CLu_{int,H}}{Q_{H,B} + fu \cdot CLu_{int,H}/(C_B/C_P)} \quad (2)$$

As expected conceptually, eq. 1 indicates that hepatic blood clearance cannot exceed liver blood flow. However, from eq. 2, it is possible that hepatic plasma clearance may exceed hepatic blood flow (and hepatic plasma flow) when $fuCLu_{int,H} \gg Q_{H,B}$ and $C_B/C_P > 1$ (Hinderling, 1997). Some confusion over the definition of fu_B and a failure to appreciate the conversion between blood and plasma clearance are evident in some of the recent literature, confounding the ability to extrapolate between in vitro and in vivo drug clearance.

The Definition of fu_B

The free fraction of drug in blood is given by eq. 3:

$$fu_B = Au_B/A_B \quad (3)$$

where Au_B is the amount of unbound drug in whole blood and A_B is the total amount of drug in blood. Thus:

$$fu_B = (Cu_{RBC} \cdot V_{RBC} + Cu \cdot V_P)/(C_B \cdot V_B)$$
$$= (Cu_{RBC} \cdot Hc + Cu \cdot (1 - Hc))/C_B \quad (4)$$

where Cu_{RBC} is the unbound drug concentration in cells (essentially red blood cells; RBC); V_{RBC} is the volume of RBC per milliliter of blood; Cu is unbound drug concentration in plasma water; V_P is the volume of plasma per milliliter of blood; and Hc is the hematocrit.

In the original development of the well-stirred model, the assumption was made that the unbound concentration of drug in blood cells equates to the unbound concentration in plasma ($Cu_{RBC} = Cu$). Hence, eq. 4 becomes:

$$fu'_B = Cu/C_B = fu \cdot C_P/C_B \quad (5)$$

In contrast, Masimirembwa et al. (2003) defined the free fraction in blood as:

$$fu'_B = fu(C_P/C_B)(1 - Hc) \quad (6)$$

However, this only applies where no drug associates with the cells ($Cu_{RBC} = 0$ in eq. 4) when, to provide a correct value of hepatic blood clearance, eq. 1 would need to be modified to eq. 7:

$$CL_{H,B} = \frac{Q_{H,B} \cdot fu'_B \cdot CLu_{int,H}}{Q_{H,B}(1 - Hc) + fu'_B \cdot CLu_{int,H}} \quad (7)$$

In the special case of the drug not associating with blood cells at all, it is preferable to report plasma, rather than blood, clearance, using fu and hepatic plasma flow ($Q_{H,B}(1 - Hc)$).

Estimation of in Vivo Hepatic Clearance from in Vitro Data

Values of $CLu_{int,H}$ derived using human liver microsomes, hepatocytes, or recombinant enzymes are often scaled to in vivo hepatic clearance using the well-stirred model. However, in deriving hepatic plasma clearance, several recent studies have used eq. 8 instead of the correct form (eq. 2) (Blanchard et al., 2004; Foti and Fisher, 2004; Blanchard et al., 2005; de Graaf et al., 2006; Mohutsky et al., 2006; Strelevitz et al., 2006):

$$CL_H = \frac{Q_{H,B} \cdot fu \cdot CLu_{int,H}}{Q_{H,B} + fu \cdot CLu_{int,H}} \quad (8)$$

This would only be valid if C_B/C_P were equal to unity. For drugs with low values of $fuCLu_{int,H}$, estimates of CL_H would be similar using eqs. 2 and 8. Thus, when $fuCLu_{int,H} \ll Q_{H,B}$ (eq. 2 and 8), CL_H approaches $fuCLu_{int,H}$, and the factor C_B/C_P is not important. However, major discrepancies would result for higher clearance drugs when C_B/C_P does not equal unity.

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ABBREVIATION: RBC, red blood cells.

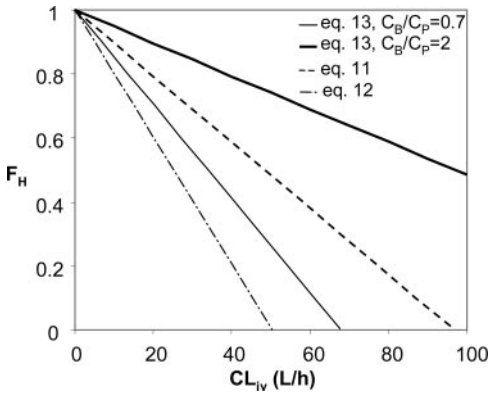

FIG. 1. Comparison of F_H values determined using eqs. 11 through 13 [$Q_{H,B}$ = 97 l/h; Hc = 0.48 (Valentin, 2002)] and different values of C_B/C (0.7 and 2). When $C_B/C_P = 1$, eqs. 11 and 13 give the same result; when $C_B/C_P = 1 - Hc$, eqs. 12 and 13 give the same result.

Estimation of Intrinsic Hepatic Clearance from in Vivo Data

To assess predictions of intrinsic hepatic drug clearance from in vitro data, estimates are often compared with values back-calculated from in vivo values of hepatic drug clearance using the well-stirred model. In this context, studies (e.g., Takanaga et al., 2000) have employed eq. 9 by rearranging eq. 8, instead of the correct form (eq. 10 from a rearrangement of eq. 2):

$$CLu_{int,H} = \frac{Q_{H,B} \cdot CL_H}{fu \cdot (Q_{H,B} - CL_H)} \quad (9)$$

$$CLu_{int,H} = \frac{Q_{H,B} \cdot CL_H}{fu \cdot (Q_{H,B} - CL_H/(C_B/C_P))} \quad (10)$$

Estimation of Hepatic Availability from in Vivo Data

Another common application of the well-stirred model is to estimate the fraction avoiding "first-pass" metabolism (hepatic availability; F_H) after oral drug administration from systemic clearance after intravenous administration. Assuming negligible extrahepatic clearance, several reports have used plasma drug clearance after intravenous administration (CL_{iv}) and eq. 11 (Lau et al., 2006; Strelevitz et al., 2006) or eq. 12 (Kharasch et al., 2004a,b) to estimate F_H:

$$F_H = 1 - \frac{CL_{iv}}{Q_{H,B}} \quad (11)$$

$$F_H = 1 - \frac{CL_{iv}}{Q_{H,B}(1 - Hc)} \quad (12)$$

Eq. 11 is only valid if C_B/C_P is unity, and eq. 12 is not valid unless C_B/C_P is equal to $(1 - Hc)$ (i.e., no drug associates with the cells). A more appropriate equation, based on the original assumptions of the well-stirred model, is given by eq. 13:

$$F_H = 1 - \frac{CL_{iv}}{Q_{H,B} \cdot (C_B/C_P)} \quad (13)$$

The different implications of eqs. 11 through 13 for the estimation of F_H are explored in Fig. 1.

Errors in the estimation of F_H may be further propagated into estimates of the fraction avoiding first-pass loss across the gut wall (F_G) from the relationship $F/(faF_H)$, where F is net oral bioavailability and fa is the fraction of the dose entering the intestinal wall (Takanaga et al., 2000; Kharasch et al., 2004a,b).

In summary, we emphasize that the mixing of plasma free fraction and hepatic blood flow is inadvisable and illogical when using the well stirred liver, or indeed any model of hepatic drug clearance, for in vitro/in vivo extrapolation.

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