Effect of Plasma Protein Binding on Kinetics of Capillary Uptake and Efflux

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We examined the effect of plasma protein binding on the kinetics of organ accumulation and washout of drugs using the single-pass Kety–Renkin–Crone capillary model. An equation relating the accumulation and washout rate constant (k) with the plasma unbound fraction (f_u) was derived. Simulations showed that k was highly dependent on f_u if capillary permeability was high but was independent of f_u if permeability was low. The effect of plasma protein binding was to increase the rate of tissue accumulation and washout of drug but to decrease the equilibrium amount of drug taken up by the tissue, both effects mediated via a decrease in the volume of distribution. This model was used to analyze published data on the effect of plasma protein binding on the kinetics of accumulation and washout of isradipine and propafenone in the isolated perfused heart preparation. The relationship between k and f_u and the directly measured volume of distribution were in accordance with the model. Although more complex models relating k and f_u could be proposed, taking into account unequal flows in capillaries, slow dissociation of ligand from protein, and unstirred layer constraints, this simple model appears adequate for describing the effect of f_u on myocardial accumulation and washout of isradipine and propafenone.

KEY WORDS: protein binding; capillary clearance; capillary permeability; isolated perfused heart preparation; isradipine; propafenone; rate constant for accumulation and washout.

INTRODUCTION

Several factors affect the accumulation of drugs and other substances from the circulation into areas such as muscle, lung, brain, and heart. These factors include the permeability (P) and surface area (S) of the blood vessel membrane and the blood flow rate (Q). The relationship among these and the fraction (E) of the substance extracted from the blood at equilibrium in a single pass is defined in the Kety–Renkin–Crone equation (1–4):

$$E = 1 - e^{-PS/Q} \tag{1}$$

Plasma protein binding of drug is also an important factor because only unbound drug is thought to diffuse across membranes. Baker and Bradley (5) incorporated the effect of protein binding on extraction into Eq. (1) by reducing the permeability of the membrane in proportion to the unbound fraction of drug in the plasma (f_u):

$$E = 1 - e^{-f_u PS/Q} \tag{2}$$

According to Eq. (2), the effect of plasma protein binding on the equilibrium extraction ratio will depend on the permeability of the drug, being negligible for a highly permeable drug and substantial for a lowly permeable drug. Regardless of drug permeability, plasma protein binding will influence the amount of drug taken up by the tissue or organ at equilibrium. The volume of distribution of the tissue (referenced to total drug) (V_T) is related to the plasma unbound fraction according to (6)

$$V_T = V_{T,u} \frac{f_u}{f_{T,u}} \tag{3}$$

where $V_{T,u}$ is the tissue volume of distribution referenced to unbound drug and $f_{T,u}$ is the unbound fraction in tissue. This shows that V_T, and therefore the amount of drug taken up by the tissue, is directly proportional to the unbound fraction of drug in plasma. This is supported by data obtained from experiments with single-pass isolated perfused heart (7,8) and lung (9,10) preparations.

Whereas the effect of plasma protein binding on equilibrium accumulation has been well defined, there is less known about the effect of plasma protein binding on the time course of accumulation and, also, on the time course of washout of drug from the tissue. As it is the unbound drug concentration that is thought to produce the response at the effector site, the time course of tissue drug accumulation and washout will, therefore, influence the time course of the drug's action.

In this report we examine the theoretical relationship between plasma protein binding and the time course of drug accumulation and washout. We then test the validity of this theoretical examination with published data describing the effect of plasma protein binding on the time course of myocardial accumulation and washout of the calcium antagonist isradipine and the antiarrhythmic propafenone in the isolated perfused rat heart (7,8).

THEORY

Equation (2) describes the equilibrium extraction ratio for accumulation of drug by an organ or tissue from blood flowing through a single capillary or group of capillaries with equal blood flow rates. The capillary clearance (CL) is given by (1–4)

$$CL = QE = Q(1 - e^{-f_u PS/Q}) \tag{4}$$

The first-order rate constant (k) for equilibration is given by

$$k = \frac{CL}{V} = \frac{CL}{f_u V_u} \tag{5}$$

where V is the volume of distribution for the system (i.e., blood and tissue combined) referenced to total drug and V_u is the volume of distribution referenced to unbound drug.

Combining Eqs. (4) and (5) yields

$$k = \frac{Q}{f_u V_u}(1 - e^{-f_u PS/Q}) \tag{6}$$

Assuming a one-compartment model, the time course of the output drug concentration in blood (C_{out}) from an organ or

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tissue receiving a constant input drug concentration (C_{in}) is given by

$$C_{out} = C_{in}\,(1 - e^{-kt}) \quad (7)$$

Output concentration for washout of drug is given by

$$C_{out} = C_0\, e^{-kt} \quad (8)$$

where C_0 is the initial output drug concentration. In this case, the rate constant, k, is given by Eq. (6) (11). In terms of a classical compartmental model analysis, in which this system would be viewed as diffusion between two closed compartments (i.e., blood and myocardium), k is a hybrid of the intercompartmental rate constants k_{12} and k_{21}, which govern uptake and efflux, respectively (12).

The time course of myocardial accumulation and washout of several drugs has been found to conform to Eqs. (7) and (8) in single-pass experiments with the isolated perfused heart preparation (7,8,13). Therefore, the relationship between the rate constant, k, and the plasma unbound fraction should be described by Eq. (6) for these experiments.

METHODS

The relationship between k and f_u according to Eq. (6) was simulated for several values of the ratio PS/Q. The k values corresponding to f_u values of 1, 0.5 and 0.05 were then used to simulate the effect of f_u on the C_{out} versus time profile during accumulation and washout of drug according to Eqs. (7) and (8), respectively.

We then examined the applicability of Eq. (6) to previously published data describing the effect of f_u on k for accumulation and washout of isradipine and for accumulation of propafenone in the single-pass isolated perfused heart (Langendorff) preparation (7,8). In those experiments, perfusion with a constant perfusate inflow concentration (1 nM (±)-isradipine, 293 nM (±)-propafenone) was performed for sufficient time to allow the perfusate output concentration to reach equilibrium. In the case of isradipine, a drug-free perfusate was then used, and washout of drug from the myocardium was monitored for the next 20 min. The effect of f_u on pharmacokinetics was determined by using a range of perfusate bovine serum albumin concentrations (0–40 g/L) in the case of isradipine (one albumin concentration per heart), which produced a range of f_u values of 1.0–0.0846, as determined by equilibrium dialysis. In the propafenone experiments, α_1-acid glycoprotein (0–0.2 mg/L) produced f_u values of 1.0–0.08. Total myocardial content of drug at equilibrium was either assayed directly or calculated, which allowed determination of the effect of f_u on myocardial drug content. For each experiment, k was estimated by fitting Eq. (7) or (8) to the accumulation or washout C_{out} versus time data, respectively. We then computer-fitted Eq. (6) to these k versus f_u data by nonlinear least-squares regression to assess the applicability of the extended Kety–Renkin–Crone equation. Simulations and nonlinear least-squares regression analysis were performed using Sigmaplot 4.1 (Jandel Scientific, Corte Madera, CA, USA).

RESULTS

Simulations

Figure 1 shows the relationship between k and f_u according to Eq. (6) for a range of permeability values corresponding to a range of PS/Q values of 0.1–100. The relationship between k and f_u is highly dependent on PS. For a high permeability (e.g., PS/Q = 100; Fig. 1A), k decreases with increasing f_u. At this high permeability extreme, Eq. (6) approximates to

$$k \approx \left(\frac{Q}{V_u}\right)\frac{1}{f_u} \quad (9)$$

and thus, k is inversely proportional to f_u, as shown in Fig. 1A. It also follows in this case that k is inversely proportional to the volume of distribution of the system referenced to total drug, V, since $V = V_u f_u$. For a low permeability (e.g., PS/Q = 0.1; Fig. 1B), k is independent of f_u. At this low permeability extreme, Eq. (6) approximates to

$$k \approx \frac{PS}{V_u} \quad (10)$$

Figure 2 shows the influence of f_u on the C_{out} versus time profile during accumulation of drug by the tissue, according to Eq. (7), for the same PS/Q values examined in Fig. 1. The k values used in Eq. (7) were obtained for each value of f_u from Fig. 1. Equilibration of drug with the tissue is slowest when plasma protein binding is absent (f_u = 1), and the rate of equilibration increases as f_u decreases (Fig. 2). Figure 3 shows the influence of f_u on the C_{out} versus time profile during washout of drug from the tissue, according to Eq. (8). Washout of drug is slowest in the absence of plasma protein binding and increases as binding increases (Fig. 3).

Myocardial Accumulation and Washout of Isradipine and Propafenone

Machard and Chaumet-Riffaud (7) determined k during myocardial accumulation and washout of isradipine for a range of f_u values, and their data are shown in Fig. 4. Equation (6) was fitted to the data for accumulation (Fig. 4A) and washout (Fig. 4B). The best fit was obtained when a weighting factor of $(1/k^2)$ was used. The fitted value for PS was 21,500 mL/min/g heart for accumulation and 9437 mL/min/g heart for washout, indicating extremely high permeability. The values of V_u obtained from the fittings were 79.3 and 96.2 mL/g for the accumulation and washout data, respectively. Machard and Chaumet-Riffaud (7) determined V for each f_u value in their experiments as the ratio of myocardial

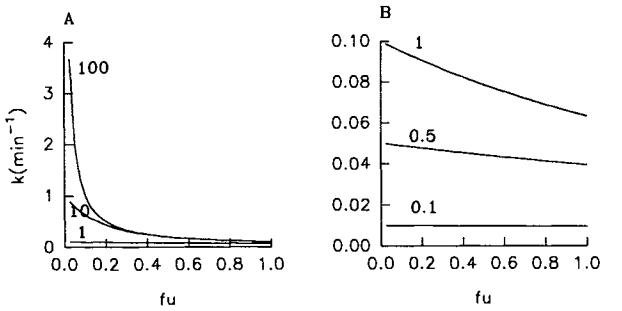

Fig. 1. Relationship between k and f_u, according to Eq. (6), for a range of PS/Q values from 0.1 to 100.

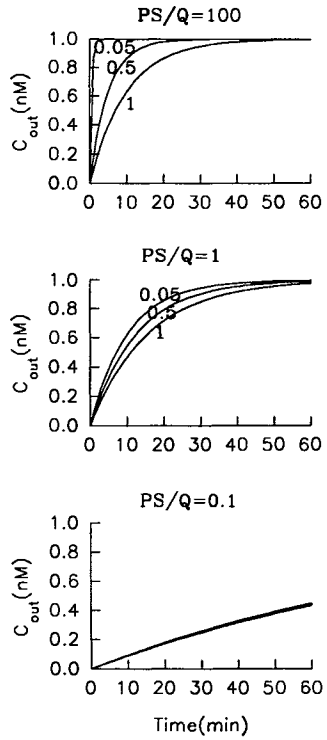

Fig. 2. Influence of protein binding on C_{out} versus time profile during single-pass perfusion with constant arterial drug concentration [according to Eq. (7)]. The value of f_u is shown next to each curve. For $PS/Q = 0.1$ (bottom), curves 1–3 are superimposable. The value of k for each value of f_u was obtained from Eq. (6).

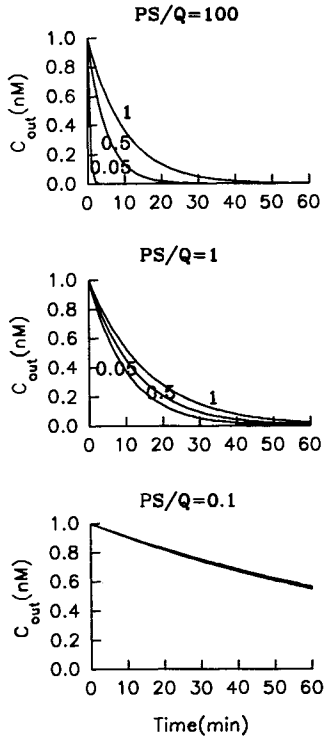

Fig. 3. Influence of protein binding on C_{out} versus time profile during single-pass perfusion with zero arterial drug concentration [according to Eq. (8)], showing washout from previously equilibrated tissue. The value of k for each value of f_u was obtained from Eq. (6).

drug content at equilibrium/C_{in}. If we calculate V_u for each f_u value as V/f_u, we obtain a mean value of 104 ± 23 (SD) mL/g.

The fit of Eq. (6) (with a weighting factor of $1/k^2$) to the k versus f_u data during accumulation of propafenone in perfused rabbit heart was also excellent (Fig. 4C). The value obtained for V_u was 494 mL and that for PS was 246 mL/min.

DISCUSSION

The time course of accumulation and washout of drug by an organ or tissue is important because it may influence the time course of the pharmacodynamic response elicited by the drug. Kety, Renkin, and Crone (1–4) developed Eq. (1) to relate transcapillary extraction, blood flow, and the permeability and surface area of the capillary wall. Experimental data from a variety of different organs support the validity of Eq. (1) (2,3,14,15). Baker and Bradley (5) developed Eq. (2) so as to describe the effect of plasma protein binding on the extraction of drugs by the liver. We have extended Eq. (2) to describe the relationship between the rate constant for accumulation and washout of drug for the organ or tissue and the degree of plasma protein binding of the drug [Eq. (6)].

Our simulations showed that, as is the case for the effect of blood flow rate on the rate constant (2,3), the effect of plasma protein binding on the rate constant increased with permeability (Fig. 1). Of particular interest was the prediction that plasma protein binding of drug hastens the attainment of equilibrium of drug between blood and tissue during accumulation and hastens the removal of drug during washout (Figs. 2 and 3). This does not imply a faster movement of drug between compartments (i.e., increased capillary clearance) with increasing protein binding because the rate constant depends on the volume of distribution (i.e., mass of drug moving between compartments) as well as the capillary clearance (rate of movement) [Eq. (5)]. Extensive protein binding reduces the volume of distribution, but, at a high permeability, does not affect capillary clearance, which is flow limited [Eq. (9)]. At a low permeability, the opposing effects of protein binding on volume of distribution and capillary clearance cancel each other [Eq. (10)]. This contrasts with the effect of plasma protein binding on the steady-state elimination rate for an eliminating organ (e.g., liver), where binding reduces the elimination rate (6).

The fit of Eq. (6) to the previously published myocardial accumulation and washout data for isradipine (7) and the myocardial accumulation data for propafenone (8) was excellent. The fitted value for permeability was extremely large for isradipine, which indicates that the isradipine diffuses very readily into the myocardium. Although permeability was much lower for propafenone (246 mL/min) compared with isradipine, it was still relatively large compared with the perfusion flow rate of 19 mL/min. The fitted values of mean V_u (79.3 and 96.2 mL/g for accumulation and washout of isradipine, respectively) were comparable to the value determined directly by measurement of myocardial isradipine content [mean, 104 ± 23 mL/g (7)]. This comparison could not be made for propafenone because heart weights were not

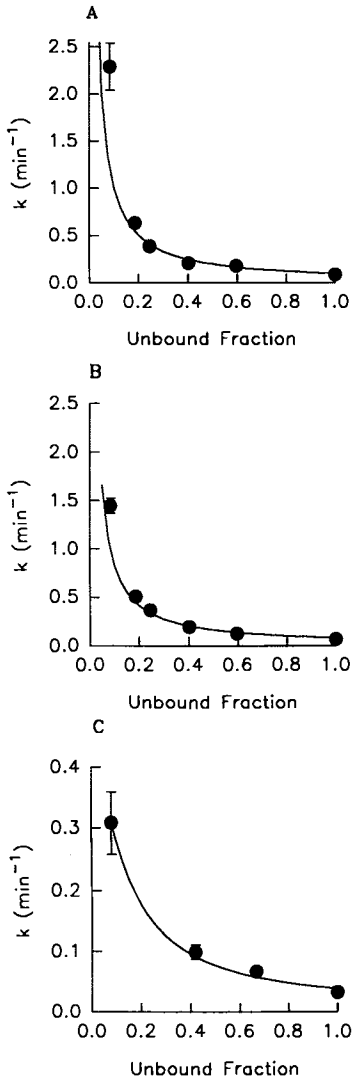

Fig. 4. Relationship between k and f_u for isradipine for single-pass perfusion of the isolated perfused heart during accumulation (A) and washout (B) of isradipine and during accumulation of propafenone (C). Experimentally observed data (•) published previously (7,8), and fit of Eq. (6) to the data (——).

reported. Moreover, the effect of plasma protein binding on perfusate drug outflow versus time profiles during both accumulation (isradipine, propafenone) and washout (isradipine) were as predicted in Figs. 2 and 3, respectively. Therefore, Eq. (6) appears to be an appropriate equation for describing the influence of plasma protein binding on the accumulation and washout rate constant.

There are several assumptions inherent in the model we have used to analyze the myocardial accumulation and washout data. These assumptions include single-compartment kinetic behavior for the myocardium, equal blood flow rate in individual capillaries with absence of A–V shunting, individual capillaries of equal permeability, and equal capillary permeability in both directions. During uptake, drug concentration is assumed to decline exponentially along the length of the capillary and the uptake rate is assumed proportional to the unbound drug concentration. Furthermore, binding is assumed to be at equilibrium along the length of the capillary, i.e., ligand dissociation is not rate-limiting. It is also assumed that the uptake process does not deplete the unbound drug concentration at the cell surface below the unbound concentration in the bulk due to diffusional barriers presented by unstirred water layers adjacent to the cell. Goresky *et al.* (16) have incorporated the effect of unequal flows in the capillaries into Eq. (1) and Weisiger *et al.* (17) have incorporated the effect of slow ligand dissociation from protein and of diffusional barriers due to unstirred layers into Eq. (2). More complex models incorporating these effects might be necessary to account for the kinetics of uptake of certain substances, such as the extremely highly protein-bound fatty acid palmitic acid (18–20). However, the simple model described by Eq. (6) appears adequate for accounting for the effects of protein binding on myocardial accumulation and washout of isradipine and propafenone.

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