the general belief that the thermodynamic activities of water (reactant) and hydronium ion (catalyst) can be extremely low inside the micellar phase, one would expect the pH-profile of log k_{obs} shown in Fig. 1 (half-filled circles). Note that the overall rate-determining step in the hydrolysis of I is the protonation at C-5, which makes the Δ^5 bond polarized and susceptible to the subsequent water attack (Scheme I) (3). That is, if an intrinsic reactivity is assigned to the free acid present inside the micellar phase, regardless of the pH of the bulk phase, then the pH-profile of the observed rate constant should resemble that of the apparent partition coefficient. This analysis is consistent with what was observed.

In the presence of 1.0% hexadecyltrimethylammonium chloride, the apparent hydrolysis rate at pH below 3 is $\sim 1.5 \times 10^3$ fold slower than in the absence of the surfactant (Fig. 1). At neutral pH values, however, such a comparison can be made only after taking the general acid catalysis by buffer components into consideration. At pH 7.45, for instance, if the data shown in Fig. 1 are compared, only a 62-fold decrease in the rate is obtained. However, the hydrolysis rate shown in Fig. 1 in the absence of the surfactant is the rate extrapolated to zero buffer concentration, whereas that in the presence of the surfactant was obtained in a 0.165 *M* phosphate buffer of 0.50 *M* ionic strength. In an identical buffer system without the surfactant, it was found previously that the hydrolysis is ~4.56 faster than at zero buffer concentration (2). The net effect of 1.0% hexadecyltrimethylammonium chloride is, therefore, a reduction of the hydrolysis rate of ~280-fold; $t_{1/2}$ from 3.5 min to 16.5 hr.

Finally, one should not attempt to interpret the coincidental overlap of the rate constants for the hydrolysis of methyl ester (triangles in Fig. 1) and I in the presence of the surfactant at pH above 6. As discussed previously (4), the latter is a function of the concentration of both substrate and the surfactant present in a given system.

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Determination of Tissue to Blood Partition Coefficients in Physiologically-Based Pharmacokinetic Studies

GILBERT LAM *, MEI-LING CHEN, and WIN L. CHIOU ˣ

Received May 18, 1981, from the *Department of Pharmacy, College of Pharmacy, University of Illinois at the Medical Center, Chicago, IL 60680.* Accepted for publication August 10, 1981. * Present address: Stine Laboratory, E.I. du Pont Nemours and Co., Inc., Newark, DE 19711.

Abstract □ The partition coefficient between tissue and blood used in physiologically-based pharmacokinetic modeling analysis was investigated using the concept of clearance. New equations were derived and compared with previously reported equations in constant intravenous infusion and bolus injection methods. The importance of differentiating arterial from venous blood is discussed.

Keyphrases □ Partition coefficient—tissue to blood, physiologically-based pharmacokinetics □ Pharmacokinetics—determination of tissue to blood partition coefficients in physiologically-based pharmacokinetic studies □ Blood sampling—differences between arterial and venous blood, physiologically-based pharmacokinetic studies

The formulation of a physiologically-based pharmacokinetic model requires an accurate determination of physiological parameters such as blood flow, organ volume, partition coefficient, and clearance (1). The estimation of the tissue to blood partition coefficients for a compound is of special interest to pharmacokineticists because it can be directly measured in the laboratory. Recently, Chen and Gross (2) pointed out different methods by which tissue to plasma partition coefficients can be determined under specific experimental conditions. The following equations were used in constant infusion and bolus injection studies, respectively:

$$R = \left(1 + \frac{K}{Q}\right)\frac{C_t^\infty}{C_p^\infty} \qquad \text{(Eq. 1)}$$

$$R = \frac{(Q + K)C_t^0}{(QC_p^0 + \alpha V_t C_t^0)} \qquad \text{(Eq. 2)}$$

where R is the partition coefficient of drug between organ tissue and plasma, K was defined as the first-order elimination rate constant [but was used as organ clearance in their calculations (2)], Q is the flow rate of plasma in the organ, C_t^∞ and C_p^∞ are the concentrations of drug in tissue and plasma at steady state, C_t^0 and C_p^0 are the concentrations of drug in tissue and plasma at time zero extrapolated from the terminal phase, α is the terminal rate constant, and V_t is the volume of the organ or tissue. The present study examined Eqs. 1 and 2 from the concept of physiological clearance and derived new equations for the determination of R in constant infusion and bolus injection studies. Flow and concentration in terms of blood were dealt with instead of plasma.

THEORETICAL

The concept of clearance and its applications are well defined in the pharmacokinetic literature (3–7). In an eliminating organ or tissue, it describes the volume of incoming blood completely cleared of drug by the organ per unit time. Conventionally, it is expressed as the organ clearance, CL_s, and is defined as:

$$CL_s = \frac{r}{C_i} \qquad \text{(Eq. 3)}$$

where r is the rate at which the drug is removed from the organ and C_i is the drug concentration in the incoming blood. However, it has been observed that CL_s might be dependent on the blood flow through the organ and the use of intrinsic clearance, CL_i, was proposed to correct for the influence of blood flow (3). CL_i is defined as:

$$CL_i = \frac{r}{C_0} \qquad \text{(Eq. 4)}$$

where C_0 is the effluent venous blood concentration which is in equilibrium with the eliminating organ. It measures the maximum capacity of the organ to eliminate the drug. An important relation obtained from the above clearance equation is:

$$r = CL_s C_i = CL_i C_0 \qquad \text{(Eq. 5)}$$

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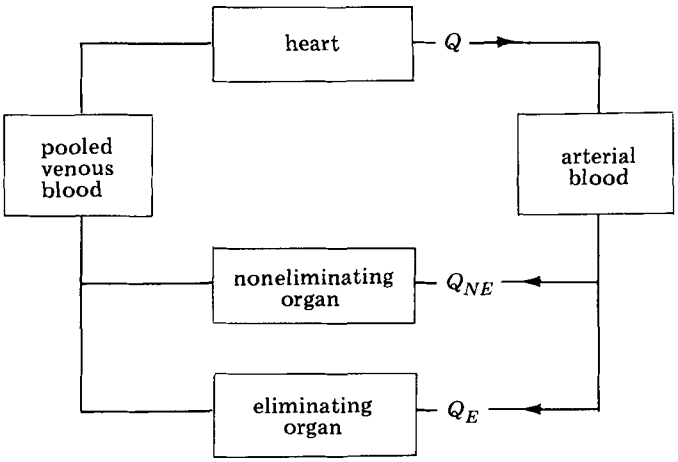

Scheme I—A simplified physiologically-based pharmacokinetic model in mammals.

This relationship describes the rate of removal of the drug from the eliminating organ in terms of either the influent arterial or effluent venous blood concentration. A simplified physiological model (Scheme I) is depicted for discussion purposes. Conventionally, the eliminating organs are the liver and kidney, while the noneliminating organs are the heart, muscle, adipose tissue, bone, etc. The lung is also considered as an eliminating organ for many drugs (8, 9), but due to its anatomical arrangement, it is treated separately. Nevertheless, the same reasoning could be applied to its treatment.

From the mass balance principle, the rate of drug accumulation in any organ can be described by the following equation:

$$V_t \frac{dC_t}{dt} = QC_i - QC_0 - CL_sC_i \qquad \text{(Eq. 6)}$$

In a well-stirred model, one generally assumes rapid equilibrium between organ tissue and its emerging venous blood (3, 10). The partition coefficient between tissue and blood is then defined as the ratio of the amount of drug per gram of the extravascular tissue (C_t) to the concentration of drug in the effluent venous blood:

$$R = \frac{C_t}{C_0} \qquad \text{(Eq. 7)}$$

Substituting into Eq. 6 yields:

$$V_t \frac{dC_t}{dt} = QC_i - \frac{QC_t}{R} - CL_sC_i \qquad \text{(Eq. 8)}$$

Partition coefficients can be estimated based on the conditions imposed by the method of administration, namely, constant infusion, intravenous bolus injection, and first-order absorption (2). Only the first two methods will be examined here.

Constant Infusion—It is apparent that at steady state, the differential term in Eq. 8 will become zero and:

$$R = \frac{QC_t^\infty}{(Q - CL_s)C_i^\infty} \qquad \text{(Eq. 9)}$$

Since $CL_s = QE$, and $C_i^\infty = C_0^\infty/(1 - E)$, where E is the extraction ratio of the organ, Eq. 9 will be reduced to:

$$R = \frac{C_t^\infty}{C_0^\infty} = \frac{C_t^\infty}{C_i^\infty(1 - E)} \qquad \text{(Eq. 10)}$$

In the case of a noneliminating organ, the concentration of the influent blood will be the same as the effluent blood at the steady state (7, 11) and the applicability of Eq. 10 is evident.

Bolus Intravenous Injection—For a linear multicompartmental system, the concentration in each compartment will decay at the same rate after the attainment of pseudo-distribution equilibrium. Both the arterial and the venous blood concentration will be of the form:

$$C = C^0e^{-\alpha t} \qquad \text{(Eq. 11)}$$

at the terminal phase; C^0 and α are the same as defined previously. Substitution of Eq. 11 and its derivative into Eq. 8 yields:

$$-\alpha V_tC_t^0e^{-\alpha t} = QC_i^0e^{-\alpha t} - \frac{QC_t^0}{R}e^{-\alpha t} - CL_sC_i^0e^{-\alpha t} \qquad \text{(Eq. 12)}$$

so that:

$$R = \frac{QC_t^0}{(Q - CL_s)C_t^0 + \alpha V_tC_t^0} \qquad \text{(Eq. 13)}$$

RESULTS AND DISCUSSION

The derived equations provide the theoretical basis for estimating the partition coefficient of drugs between tissue and blood. It is obvious that Eqs. 9 and 13 are different from Eqs. 1 and 2. If CL_iC_0 is in the place of CL_sC_i in Eq. 6, and knowing that $CL_s = CL_iQ/(CL_i + Q)$, the same conclusion can be drawn. The discrepancy between the newly derived and the reported equations lies in the definition of the clearance in the mass balance equation. The effluent concentration, C_0 or C_t/R, in the elimination rate in Eq. 6 should only be used with CL_i.

For constant infusion studies, R can be calculated by simply taking the ratio of the drug concentration in the tissue to the effluent venous blood as indicated in Eq. 10. The arterial blood concentration can also be used if the extraction ratio of the compound by the organ is known. Either arterial or venous blood concentration can be used interchangeably for the R determination of a noneliminating organ.

Equation 13, on the other hand, offers the tool to estimate R from bolus injection data. Its limitation, however, is quite severe because all of the physiological and clearance parameters must be known before applying the equation. It also requires the exclusive use of arterial blood concentration in its application. In the case of a noneliminating organ, Eq. 13 could be reduced to:

$$R = \frac{C_t^0}{C_0^o} = \frac{C_t}{C_0} \qquad \text{(Eq. 14)}$$

By recognizing the fact that $QC_i + \alpha V_tC_t = QC_0$ and $CL_s = 0$. The use of effluent venous blood concentration would then be most appropriate. It is of interest to note that for such an organ, venous blood sampling avoids the cumbersome restrictions dictated by Eq. 13. It also avoids the use of literature physiological parameter values, since errors in them may be compounded in the determination of R.

The previous discussions suggest the importance of the source of blood sampling. As indicated in Eqs. 10 and 14, venous blood concentration can be used for determining R; however, venous blood must be obtained from the outflow of the organ of interest. Since the venous concentrations can vary among organs (11) many venous samples might have to be taken from different organs to satisfy the conditions prescribed by the equations. In reality, venous blood is often obtained from one peripheral vein or the pooled venous blood (such as studies in mice or rats); the validity of such practices appears questionable in light of the arguments presented. In addition, many tissues and blood (plasma) flow parameters are often pooled and scaled accordingly, making the possibility of obtaining venous blood for these tissues almost impossible.

Systemic arterial blood, on the other hand, is generally regarded as homogeneous in the body (7, 11). Sampling it from any site could closely reflect the influent arterial blood concentration in all other organs except for the lung (7, 8). Because of its association with CL_s in Eq. 6, literature clearance data can be used readily without any modifications, since organ clearance is estimated routinely by the quotient of the total amount of drug eliminated from a particular route and the total area under the blood (plasma) level–time curve. In addition, sampling of arterial blood is relatively easy in experimental animals and the advantage of this approach is obvious.

The source of blood sampling is not only important in the determination of R, but may also be significant in the overall successfulness of a physiologically-based pharmacokinetic model. From a mathematical standpoint, the predicted blood (plasma) levels from the system of first-order differential equations are, in fact, those of the arterial blood (12–17). Therefore, it seems only logical to sample arterial blood for comparison with the predicted levels. A brief review of the literature, however, revealed that the source of blood sample could be any one of the following: arterial[lidocaine (12) and procaine (13)], jugular venous [sulfobromophthalein (14) and digoxin (15)], peripheral venous [adriamycin (16)], and total pooled plasma [methotrexate (17)]. It is apparent that no general rule has been adopted in this area. Arterial sampling certainly is justifiable physiologically and should be used. This is particularly true in view of a recent study which showed significant arteriovenous plasma concentration difference for six compounds after intravenous administration to dogs and rabbits (11).

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Conductivity Studies of Suspension Systems in Different States of Aggregation

BERNARD ECANOW ˣ, JUDY M. WEBSTER, and MARTIN I. BLAKE

Received February 8, 1980, from the *College of Pharmacy, Department of Pharmacy, University of Illinois at the Medical Center, Chicago, IL, 60612.* Accepted for publication July 29, 1981.

Abstract □ The electrical conductivity effects of dispersed, coagulated, and flocculated systems were investigated using sulfamerazine powder, an insoluble, hydrophobic drug to prepare the suspension systems. For the dispersed systems, a peak in conductivity was observed at a drug concentration between 5 and 15%. The critical coagulating concentration was defined as the concentration of drug at which a maximum in specific conductance was observed. At this concentration, a maximum number of charged particles were in the system. Coagulated suspensions showed higher conductance values than the dispersed systems at equivalent concentrations; however, the critical coagulating concentration value appeared to be the same. For flocculated suspensions there was an increase in conductance with drug concentration with no perceptible peak conductance value.

Keyphrases □ Conductivity—use in studies of suspension systems in different states of aggregation □ Aggregation states—electrical conductivity effects on dispersed, coagulated, and flocculated systems □ Suspension systems—electrical conductivity effects on dispersed, coagulated, and flocculated systems

As defined in the United States Pharmacopeia (1), suspensions are preparations of finely divided, undissolved drugs in liquid vehicles. Insoluble particles dispersed in a liquid medium have large specific surface areas which render the suspension system thermodynamically unstable. The particles tend to settle and form aggregates which have a reduced surface area and, thus, a decreased surface free energy. This results in a system of greater thermodynamic stability. Two types of aggregation are identified: coagulation and flocculation. Unfortunately, these terms are used frequently in the literature in a way that confuses the nature of the systems being described (2).

Here, a dispersed system in water is described as consisting of primary particles acting as independent entities in the bulk water polar medium. The settling process, in general, is relatively slow with each particle settling separately.

In a coagulated system the aggregated particles, including adsorbed surface films, are in surface contact with each other and each aggregate of particles (coagula) acts as a unit. The particles are held together by film–film bonds. The interstitial water is structured and exhibits nonpolar behavior. Coagulated suspensions tend to form caked systems which can be difficult, if not impossible, to redisperse.

In a flocculated system the aggregated particles are held together by one of several mechanisms: adsorption bridging, chemical bridging, or long-range Van der Waals forces (secondary minimums). The particles settle out as a "floc," a loosely packed aggregate having a network-like structure. A hard cake does not form and the sediment is readily redispersed to the original suspension form. The water medium is bulk polar water.

The classification of these systems was first reported by Ecanow *et al.* (3) and has since been referred to by others (4). The properties of dispersed, coagulated, and flocculated systems have been compared in terms of caking (5), sedimentation rate (6), rheology (3), gas adsorption (7), and filtration rates (8). In the present study, the electrical conductivity effects of these systems were investigated. Sulfamerazine powder, a hydrophobic drug, was used to prepare the suspensions, and docusate sodium (I), an anionic surfactant, rendered the drug particles hydrophilic in the formation of the dispersed and the coagulated systems. Compound I and aluminum chloride were used to form the flocculated systems (9) of sulfamerazine particles.

EXPERIMENTAL

Materials—Sulfamerazine[1] was USP grade and ranged in particle size from 5 to 20 μm. Docusate sodium[2] USP was employed as the surfactant, and aluminum chloride[3] NF served as the flocculating agent. All other chemicals were reagent grade and were used without further treatment.

[1] Sigma Chemical Co., Lot 103C 2660.
[2] Aerosol OT, Fisher Scientific Co., Lot 732561.
[3] Mallinckrodt Chemical Works, Lot WLJD.

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