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In Vitro and *In Vivo* Evaluation of the Tissue-to-Blood Partition Coefficient for Physiological Pharmacokinetic Models

Jiunn Huei Lin,[1,4] Yuichi Sugiyama,[1,3] Shoji Awazu,[2] and Manabu Hanano[1]

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An important parameter used in physiologically based pharmacokinetic models is the partition coefficient (Kp), which is defined as the ratio of tissue drug concentration to the concentration of drug in the emergent venous blood of the tissue. Since Kp is governed by reversible binding to protein and other constituents in blood and tissue, an attempt was made here to estimate the Kp values for a model drug ethoxybenzamide (EB) by means of in vitro *binding studies and to compare these Kp values to those obtained from* in vivo *kinetic parameters observed following the administration of EB by two different routes, i.e., i.v. bolus injection and constant rate infusion. The Kp values obtained by using these three different methods were in reasonably good agreement suggesting that binding data obtained* in vitro *can successfully be used to estimate* in vivo *distribution.*

KEY WORDS: *in vitro* and *in vivo* correlation; tissue to plasma partition coefficient; tissue binding; physiological pharmacokinetics; ethoxybenzamide.

INTRODUCTION

Recently, the utility of physiologically based pharmacokinetic models has been demonstrated in describing quantitatively the distribution and elimination of drugs in various species (1–6). These models are different from the conventional compartmental models because all parameters involved have a specific physiological and/or physicochemical basis. One of these important parameters, which is required to solve the equations

[1]Faculty of Pharmaceutical Sciences, University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113, Japan.
[2]Department of Biopharmaceutics, Tokyo College of Pharmacy, Horinouchi, Hachioji, Tokyo 192-03, Japan.
[3]Author to whom all correspondence should be directed.
[4]Present address: Drug Metabolism Section, Merck Sharp Dohme Research Laboratory, West Point, Pennsylvania 19486.

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describing physiological models, is the partition coefficient (*Kp*) value, which is defined as the ratio of the drug concentration in the tissue and its emergent venous blood.

There is no general consensus on the most appropriate method for the determination of *Kp* values. However, the most common approach is to calculate *Kp* from blood and tissue concentrations after a drug is administered to animals. For example, Benowitz *et al.* (5) calculated the *Kp* values for lidocaine from steady-state blood and tissue concentration data following a constant rate infusion of the drug to monkeys. Harrison and Gibaldi (6) obtained *Kp* values for digoxin in rats in a similar manner. Harris and Gross (7), on the other hand, calculated the *Kp* values for adriamycin based on terminal (elimination) phase data following an i.v. bolus dose of the drug. Recently, Chen and Gross (8) have derived general analytic equations for calculation of *Kp* values when different routes of drug administration are used. However, no experimental data are available at present to validate their theoretical considerations.

Since the drug distribution ratio is governed by reversible binding to protein and other constituents in blood and tissue, it is possible to estimate the *Kp* values for each organ/tissue by *in vitro* tissue binding studies if it is assumed that the concentration of drug in a given organ is always in equilibrium with that in the emergent venous blood, i.e., the unbound drug concentration in blood is identical to that in the tissue. Indeed, Dedrick and Bischoff (1) calculated the *Kp* values for barbiturates from binding parameters obtained *in vitro* and predicted *in vivo* distribution of barbiturates in their pioneering work on physiological pharmacokinetics. The purpose of this study was to estimate the *Kp* values by means of *in vitro* binding studies using ethoxybenzamide (EB) as a model drug, and to compare these *Kp* values to those calculated from *in vivo* kinetic parameters observed after administering the drug either as an i.v. bolus or a constant rate infusion.

MATERIALS AND METHODS

Ethoxybenzamide (EB) was kindly supplied by Takeda Chemical Industries (Osaka, Japan) and was used without further purification. All other reagents were of analytical grade.

Adult male Wistar strain rats weighing between 230 and 270 g were used in the steady-state studies. A polyethylene (PE 50) cannula was implanted into the right femoral artery and the left femoral vein of each animal under light ether anesthesia. All experiments were carried out 2 hr after completion of surgery. Food and water were withheld during the experiments. EB was administered as an i.v. bolus (20 mg/kg) via the femoral vein cannula followed immediately by a constant rate infusion of

7 mg/kg/hr for 60 min. Blood samples were collected at appropriate times during the infusion into heparinized microtubes, and plasma was separated by centrifugation. At the end of the infusion, the animals were exsanguinated by cutting the carotid artery. The abdomens of the animals were opened immediately, and the organs and tissues, including the liver, kidney, brain, heart, lung, small intestine, muscle, skin and adiopose tissue, respectively, were quickly excised. All tissues and organs were stored at −40°C pending analysis. The determination of EB concentrations in various tissues was performed as reported previously (9).

Data and kinetic parameters reported previously (9), from studies carried out in rats, were used for the calculation of *Kp* values following intravenous bolus (20 mg/kg) administration of EB.

In Vitro Plasma and Tissue Homogenate Binding

Nonmedicated rats of similar weight and strain as used in the steady-state studies were used for binding studies. After collecting blood from the carotid artery, the various organs and tissues were removed as described above. Diluted plasma (25 and 50% v/v) and tissue homogenate (either 25% or 25 and 50% w/v), with the exception of adipose tissue, were prepared in Tris-HCl buffer (pH 7.4) and dialyzed against buffer for 24 hr at 4°C prior to the binding studies. Adipose tissue was homogenized without the addition of buffer and was not dialyzed prior to the binding study since the use of buffer resulted in a separation of the homogenate into two layers. For the binding studies a dialysis cell (Kokugomu Co., Tokyo) having two chambers separated by Visking membrane (type 36/32; presoaked overnight in buffer) was used. EB solution in Tris-HCl buffer, pH 7.4 (seven initial concentrations in the range 0.05–0.6 mM) was added to one side of the membrane and tissue homogenates to the other side. The dialysis cells were shaken at 4°C for 48 hr for plasma and tissue homogenates with the exception of adipose tissue, which was shaken for 24 hr at 37°C. Preliminary studies showed that these conditions were optimal for equilibrium to be reached. The unbound fraction of EB in each tissue was determined by dividing the drug concentration in buffer by the concentration in the tissue homogenate. Binding to plasma was carried out using 25, 50, and 100% (undiluted) dilutions; to kidney and liver using 25 and 50% homogenates; and to other tissues using 25% homogenates. Binding to adipose tissue was determined using undiluted (100%) homogenate.

Theoretical Consideration

Since the elimination of EB proceeds almost exclusively through liver metabolism (9), the general equations for the calculation of *Kp* values

derived by Chen and Gross (8) based on the well-stirred model (10) can be used in their simplified form.

Constant-Rate Intravenous Infusion (Steady State)

When a drug is administered by a constant-rate intravenous infusion, the concentrations in each organ/tissue will eventually reach a steady state. At this steady state, the Kp for a non-eliminating tissue is given by

$$Kp = \frac{C_{i,ss}}{C_{B,ss}} \tag{1}$$

where C and Kp are concentration and partition coefficients, respectively, and the subscripts i, B, and ss denote tissue, blood pool, and steady state, respectively.

For the liver, being an eliminating organ, the Kp_H is given by

$$Kp_H = \left(\frac{Q_H + CLu_{int} \cdot fu_b}{Q_H}\right)\frac{C_{H,ss}}{C_{B,ss}} \tag{2}$$

where Q, CLu_{int}, and fu_b represent the blood flow rate, intrinsic clearance, and fraction of drug unbound in blood, respectively, and H denotes liver.

Intravenous Bolus Injection (Nonsteady-State)

When EB is administered as an i.v. bolus, a pseudodistribution equilibrium is attained in each organ/tissue following the initial distributive phase. During this terminal phase the Kp for a noneliminating tissue is given by

$$Kp_i = \frac{Q_i \cdot (C_{i,Z}/C_{B,Z})}{Q_i + (\lambda_Z \cdot V_{T,i})(C_{i,Z}/C_{B,Z})} \tag{3}$$

where λ_Z is the slope of the terminal disposition phase of the concentration-time curve, and V_T is the tissue volume. Thus, for liver, Kp_H is given by

$$Kp_H = \frac{(Q_H + CLu_{int} \cdot fu_b)(C_{H,Z}/C_{B,Z})}{Q_H + (\lambda_Z \cdot V_H)(C_{H,Z}/C_{B,Z})} \tag{4}$$

Since EB is eliminated exclusively by the liver, and since the ratio of EB concentration in whole blood to that in plasma is equal to 1 (11), the term $CLu_{int} \cdot fu_b$ was calculated to be 2.02 ml/min using the relationship, body clearance $= (Q_H \cdot CLu_{int} \cdot fu_b)/(Q_H + CLu_{int} \cdot fu_b)$ assuming Q_H is 14.7 ml/min (11). Kp_H can now be calculated using these values for $CLu_{int} \cdot fu_b$, λ_Z, Q_H, and liver volume, respectively. The total body clearance and λ_Z were obtained from our previous study (9), while the volume of liver was obtained from the literature (11).

Estimation of the Kp Values from In Vitro Binding Studies

The binding of a drug to protein can be described by a Langmuir-type equation,

$$C_{\text{bound}} = \frac{B \cdot K_{\text{assoc}} \cdot Cu}{1 + K_{\text{assoc}} \cdot Cu} \tag{5}$$

where C_{bound} is bound drug concentration, Cu is unbound drug concentration, K_{assoc} is the association constant, and B is maximum binding capacity. When $K_{\text{assoc}} \cdot Cu$ is much smaller than 1, Eq. (5) can be simplified to

$$C_{\text{bound}} = B \cdot K_{\text{assoc}} \cdot Cu = \alpha \cdot Cu \tag{6}$$

where $\alpha = B \cdot K_{\text{assoc}}$.

Since it is difficult to perform binding studies with homogenized tissue without dilution, tissue homogenates are prepared in buffer, which necessarily results in reduction of protein concentration. If the drug binding characteristics are independent of protein concentration, then Eq. (6) can be expressed as

$$C'_{\text{bound}} = (B/d)K_{\text{assoc}} \cdot Cu = (\alpha/d)Cu \tag{7}$$

and

$$C_{\text{bound}} = d \cdot C'_{\text{bound}} \tag{8}$$

where d and C'_{bound} are the dilution factor and the observed bound drug concentration, respectively. Thus, Kp is given by

$$Kp = \frac{C_{\text{tissue}}}{C_{\text{blood}}} = \frac{(C_{\text{tissue bound}} + C_{\text{tissue unbound}})}{\gamma(C_{\text{plasma bound}} + C_{\text{plasma unbound}})} \tag{9}$$

where γ is the ratio of drug concentration in whole blood to that in plasma ($\gamma = C_{\text{blood}}/C_{\text{plasma}}$). Further, if Cu_{tissue} is assumed to be equal to Cu_{plasma}, then from Eqs. (6) and (9):

$$Kp = \frac{1 + \alpha_{\text{tissue}}}{\gamma(1 + \alpha_{\text{plasma}})} \tag{10}$$

where α (bound/free ratio) is given in Eq. (6) and γ is equal to 1 for EB (11).

RESULTS

EB plasma concentration reached a steady state approximately 15 min following the bolus dose and the initiation of the constant rate infusion (Fig. 1). At 60 min EB concentrations were determined in plasma and

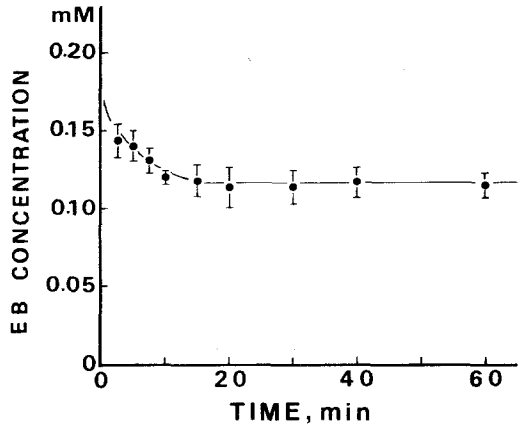

Fig. 1. Plasma concentrations of EB as a function of time after simultaneous intravenous bolus injection of 20 mg/kg of EB and a constant rate infusion at a rate of 7.0 mg/kg/hr. Each point represents the mean ± SD from four separate experiments.

various organs/tissues and were used to calculate *Kp* values, which are presented in Table I. Equations (3) and (4) were also used to calculate the *Kp* values for EB under nonsteady state conditions following the bolus dose of 20 mg/kg EB (Table I).

Table I. *Kp* Values (Partition Coefficient of Drug in Tissue to Drug in its Emergent Blood) Determined by Different Methods[a]

	Kp (constant infusion)	*Kp* (bolus injection)	*Kp*[b] (*in vitro*)	α[c]
Plasma				0.493 ± 0.09
Liver	1.59 ± 0.20	1.59[d] ± 0.20	1.45 ± 0.17	1.16 ± 0.22
Kidney	1.26 ± 0.05	1.30[d] ± 0.13	1.48 ± 0.15	1.21 ± 0.21
Brain	0.95 ± 0.11	0.90[d] ± 0.06	1.06 ± 0.08	0.584 ± 0.065
Lung	1.00 ± 0.18	0.84[d] ± 0.03	1.12 ± 0.07	0.673 ± 0.091
Heart	1.12 ± 0.12	0.92[d] ± 0.08	1.12 ± 0.07	0.678 ± 0.047
Muscle	0.81 ± 0.06	0.66[d] ± 0.03	0.95 ± 0.07	0.419 ± 0.055
Skin	1.07 ± 0.12	0.95[e] ± 0.28	1.15 ± 0.07	0.718 ± 0.067
Adipose tissue	0.80 ± 0.05	0.68[e] ± 0.11	0.87 ± 0.08	0.299 ± 0.017
Small intestine	0.62 ± 0.09	0.52[e] ± 0.07	0.68 ± 0.04	0.016 ± 0.005

[a] Data are expressed by average ± SD ($n = 3$–6).
[b] *Kp* values were calculated using Eq. (10) in the text for a set of α values, of plasma and tissue taken from the same rat, which are listed in the next column as average ± SD ($n = 3$–4).
[c] Bound to unbound ratio given by Eq. (6) in the text.
[d] *Kp* values were calculated by Eqs. (3) or (4) in the text, using the tissue/plasma data taken from Table II in ref. (9).
[e] For these tissues, during the early period, the levels of EB did not parallel those in plasma. Therefore, the tissue/plasma ratios were calculated from the data (Fig. 2 in ref. 9) at 15, 30, 60, and 90 min for skin, adipose, and small intestine.

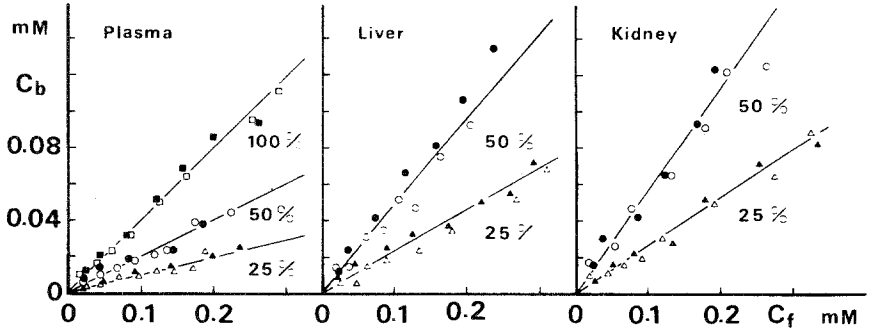

Fig. 2. Binding of EB to rat plasma, liver, and kidney homogenates. Plasma (v/v): 25% (▲, △); 50%(●, ○); and 100% (■, □). Liver homogenates (w/v): 25% (▲, △) and 50% (●, ○). Kidney homogenates (w/v): 25% (▲, △) and 50% (●, ○). Equilibrium dialysis was performed at 4°C for 48 hr. Open and closed symbols represent separate experiments, respectively.

The effect of dilution of plasma or tissue homogenates was examined with 25, 50, and 100% plasma and 25 and 50% liver and kidney homogenates. As shown in Fig. 2, EB binding was directly proportional to protein concentration in plasma or tissue homogenates. Further, the unbound fraction of EB in plasma and various organs/tissues was independent of initial EB concentration (Fig. 3). The bound/free ratios(α) of EB in plasma and various tissues are presented in Table I as are the *Kp* values of EB calculated on the basis of *in vitro* binding studies employing Eq. (10).

DISCUSSION

Linearity in all processes, which is a necessary assumption in the derivation of the equations describing *Kp* values, is roughly verified in the present study since EB has a relatively high K_m value as compared to the unbound plasma concentrations obtained in the bolus (9) and steady-state studies (Fig. 1). Further, the drug binding ratio exhibits drug concentration independence over the range of plasma (or tissue) concentrations studied (Fig. 3).

Although Shen and Gibaldi (12) reported that drug binding to serum (bovine) albumin depends on albumin concentration in a complex manner, the binding of EB to rat plasma and to tissue (liver and kidney) homogenates was found to be linearly related to protein concentration (Fig. 2). Many other investigators have also reported that drug binding is independent of homogenate protein concentration (13–15). Further, it has been shown with a series of eight drugs that homogenation does not alter the binding properties at least as far as binding to rat diaphragm is concerned (16).

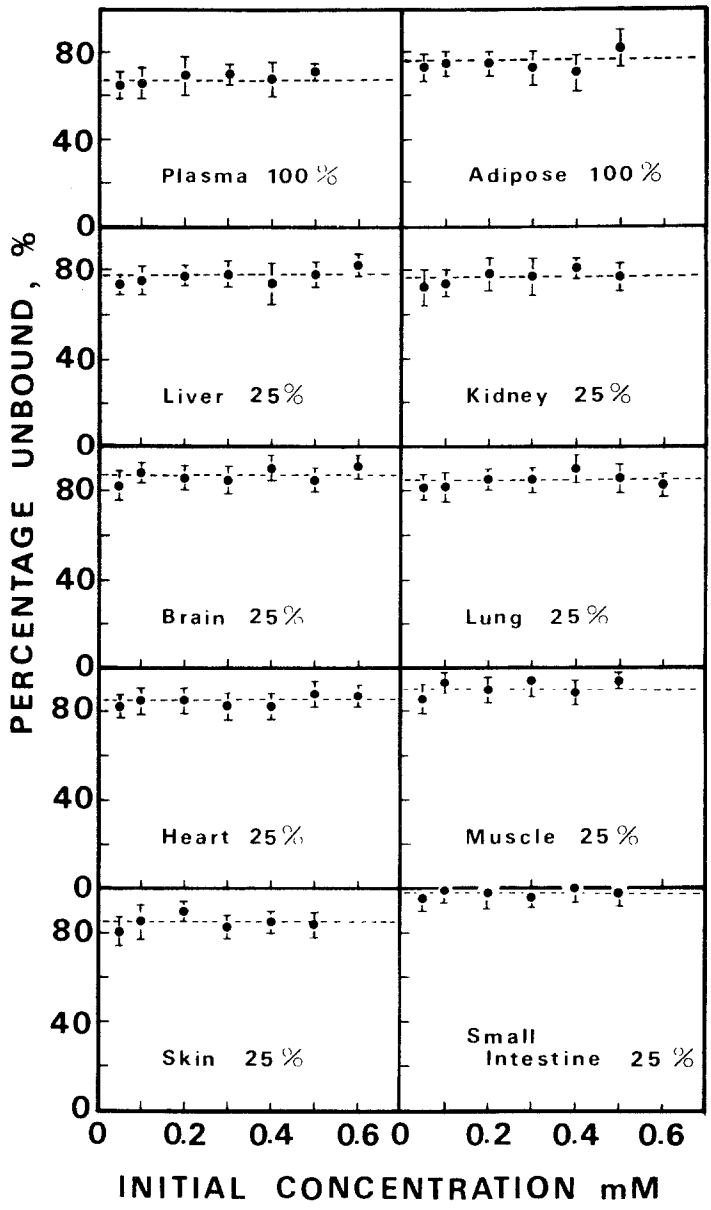

Fig. 3. Binding of EB to plasma and various organ/tissue homogenates. Equilibrium dialysis was performed at 4°C for 48 hr with plasma and all tissues with the exception of adipose tissue, which was dialyzed for 24 hr at 37°C. Each point represents the mean ± SD from 3–4 separate experiments.

Thus, the results of Fig. 2 and observations from many reports in the literature permitted the calculation of the partition coefficient for EB using *in vitro* binding data and Eqs. (6) and (10).

As can be seen from Table I and Fig. 4, there is very good agreement between the *Kp* values obtained from the constant rate infusion (steady state) study and the bolus study. In no case did the *Kp* values determined by different methods differ by more than 20% from each other. These data therefore confirm the validity of the general equations derived by Chen and Gross for calculation of the *Kp* values.

In order to prevent drug metabolism during equilibrium dialysis in the 25 and 50% tissue homogenates, particularly liver, the binding studies were performed at 4°C. On the other hand, binding of EB to adipose tissue was carried out at 37°C for technical reasons. It therefore becomes necessary

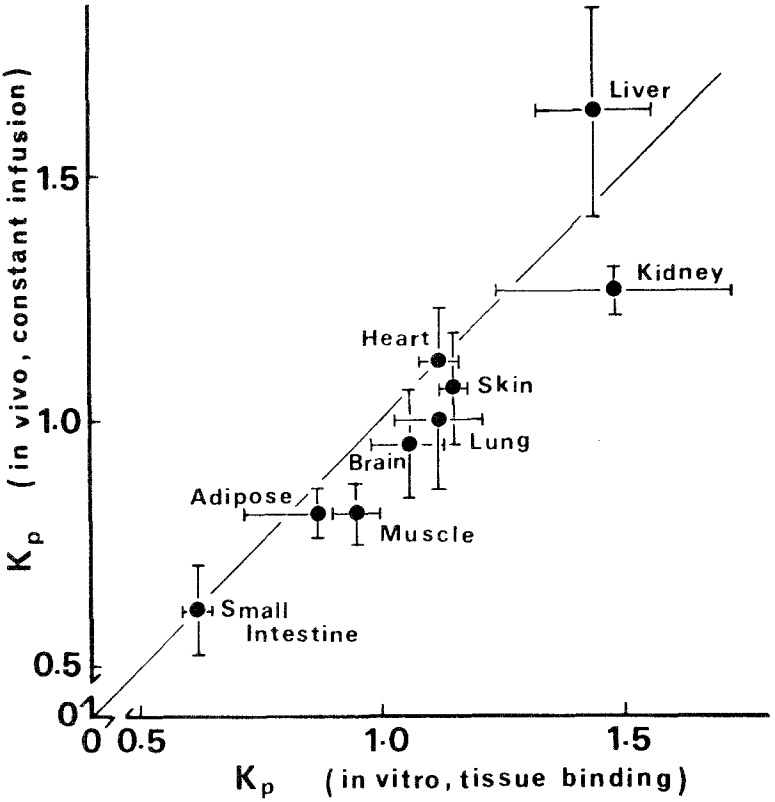

Fig. 4. Relationship between the *Kp* values obtained from *in vivo* constant rate infusion (steady state) and *in vitro* binding data, as presented in Table I. Each point represents the mean ± SD from four separate experiments (*in vivo*) and 3–4 separate experiments.

to consider the effect of temperature on EB binding. Although the effect of temperature on EB binding was not examined in our study, it is generally noted that the fraction of drug bound to proteins tends to decrease with an increase in temperature for a number of drugs (17). However, temperature does not affect the binding of all drugs, since Igari *et al.* (18) have recently reported that a change in temperature does not affect the binding of thiopental to bovine serum albumin. In any case, the binding to plasma and all tissues with the exception of adipose tissue was carried out at the same (4°C) temperature, minimizing any temperature effect on the *in vitro* binding parameters of EB.

In conclusion, then, the *Kp* values for EB determined from *in vitro* binding data and calculated from kinetic parameters obtained after administering the drug by two different routes are in good agreement. This observation suggests that the binding data obtained *in vitro* can successfully be used to predict *in vivo* distribution. Our observations, however, do in no way imply that binding of drugs to plasma and/or tissue is the sole determinant of *in vivo* distribution. Hemodynamic factors and active processes may also be involved.

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