Muscle Microdialysis as a Model Study to Relate the Drug Concentration in Tissue Interstitial Fluid and Dialysate

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The steady-state dialysis kinetics in buffer, erythrocyte suspension and muscle have been analyzed by clearance theory in the microdialysis study. "Tube" model has been demonstrated to be a useful model to relate the dialysis clearance, CL_D , the dialysis flow rate, F , and the permeability rate constant, PA , for microdialysis employing the transcranial type microdialysis probe. The effective dialysis coefficient (R_d), defined as the ratio of the *in vivo* PA and *in vitro* PA , was introduced to account for the differences between *in vivo* and *in vitro* microdialyses. The Arrhenius plot of the antipyrine permeability rate constant presented a single straight line in the range of 15—37 °C with an activation energy of 5.49 kcal/mol. A fairly good correlation was observed between the reciprocal of the permeability rate constant and the root of the molecular weight in the range of 18—1039. On the contrary, the molecular weight and the plasma membrane permeability were not determinant factors for R_d value determined in the erythrocyte suspension ($R_{d,erythrocyte}$), while the interstitial fluid space (100 – hematocrit)% of erythrocyte suspension plays a dominant factor to change $R_{d,erythrocyte}$. The *in vivo* permeability rate constant was determined in the muscle for [3H]water, [^{14}C]urea, antipyrine and [^{14}C]sucrose under the steady-state condition. No significant difference of R_d in muscle tissue was demonstrated for these four model substances. By using the R_d value, a hypothetical equation has been proposed to relate the concentration in the dialysate and the interstitial fluid at steady-state.

Keywords — antipyrine; clearance; dialysis; erythrocyte; effective dialysis coefficient; interstitial fluid; microdialysis; muscle; peptide

Introduction

Unbound drug concentration in the tissue interstitial fluid is well known to be a determinant for pharmacokinetics and pharmacological responses to drugs in the body. In the field of neurochemistry an innovative microdialysis technique has been developed to collect neurotransmitters in the brain interstitial fluid from the living animals directly.^{1–4)} Thus, an application of the microdialysis technique for pharmacokinetics and pharmacodynamics would provide more substantial insights into the kinetic analysis of drug distribution, elimination and pharmacological responses *in vivo*.

In the recent studies, we have employed the microdialysis technique to demonstrate a transcytosis of a dynorphin-like analgesic peptide through the blood-brain barrier (BBB) after the systemic administration⁵⁾ and also to confirm the differential transport of choline through the

BBB between normotensive and stroke-prone spontaneously hypertensive rats.⁶⁾ Although several investigators have studied the relationship between substrate concentrations in the tissue interstitial fluid and in the dialysate,^{7–11)} a more practical and reliable way would be necessary to extrapolate the "true" unbound concentration in the interstitial fluid from that in the dialysate for the tissue microdialysis.

Therefore, the purpose of the present study is to clarify the relationship of substrate concentration in the dialysate to the tissue interstitial fluid during the microdialysis. Muscle was used for a model tissue for the study since a permeation through the capillary of muscle is not restrictive for the substrate having a smaller molecular weight.

Materials and Methods

Animals — Male Wistar rats weighing

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250—350 g were purchased from Sankyo Laboratory Co. (Toyama, Japan) and had free access to food and water.

Chemicals — [^3H]Water (1 mCi/ml, MW 18.01) was purchased from Amersham International, Ltd., Buckinghamshire, U.K. [^{14}C]Urea (4.2 mCi/mmol, MW 60.1) and [^{14}C (U)]sucrose (5.0 mCi/mmol, MW 342.3) were purchased from New England Nuclear, Boston, U.S.A. [^{125}I]Ebiratide (H-Met(O_2)-Glu-[^{125}I]His-Phe(D)Lys-Phe-NH-(CH_2) $_8$ -NH $_2$ ·3CH $_3$ COOH, 1000 mCi/mg, MW 1091) and [^{125}I]E-2078 (CH $_3$ -[^{125}I]Tyr-Gly-Gly-Phe-Leu-Arg-CH $_3$ Arg-(D)Leu-NHC $_2\text{H}_5$, 0.82 to 21.4 mCi/mg, MW 1039) were kindly supplied from Hoechst Japan Ltd., Saitama, Japan and Eisai Co., Ltd., Tokyo, Japan, respectively. Clear-sol (Xylene based liquid scintillation cocktail) was purchased from Nacalai Tesque Inc., Kyoto, Japan. Antipyrine was obtained from Wako Pure Chemical Industries Ltd., Osaka, Japan. Other chemicals were of analytical grade and used without further purification.

Microdialysis Cannula — The microdialysis probe was prepared as described previously⁶ using dialysis hollow fiber (i.d. 0.2 mm, MW cutoff 12500; Lento H.F., Organon Technica Corp., Oklahoma, U.S.A.) and a stainless steel tubing (o.d. 0.2 mm; MT Giken, Tokyo, Japan). The probe consists of a 22-mm long segment of dialysis hollow fiber with a length of fine stainless steel tubing inserted to a depth of 7 mm in each end and attached with a surgical adhesive (Aron Alpha A, Sankyo Co., Ltd., Tokyo, Japan). The distance of fiber where the dialysis takes place was 8 mm.

In Vitro Microdialysis Study — The probe was linked with a polyethylene tubing (SP10, Natsume Seisakusho Ltd., Tokyo, Japan) and was placed in a dish. The microdialysis study was carried out in 20 ml of Ringer-HEPES buffer (RHB: 141 mM NaCl, 4 mM KCl, 2.8 mM CaCl $_2$ ·2H $_2\text{O}$, 10 mM *N*-(2-hydroxyethyl)-piperazine-*N'*-2-ethanesulfonic acid (HEPES), pH 7.40) containing [^3H]water (0.15 $\mu\text{Ci/ml}$), [^{14}C]urea (0.3 $\mu\text{Ci/ml}$), antipyrine (100 $\mu\text{g/ml}$) or [^{14}C]sucrose (0.5 $\mu\text{Ci/ml}$) at 37 °C without any mixing. RHB was perfused throughout the probe at the various flow rate (1, 2, 2.5, 5, 10,

25 and 50 $\mu\text{l/min}$) and controlled with a syringe infusion pump (Model 22, Harvard Apparatus, South Natick, U.S.A.). The dialysate solution was collected three times during the dialysis periods. Taking account of the dead volume of the dialysis fiber, and also the attainment of the steady-state conditions for the substrate in the fluid of reservoir and the dialysis fiber, we had an appropriate interval to change the dialysis flow rate. Ten microliter of reservoir medium was sampled at the midpoint of the time intervals during each dialysate sample collection. For peptides, the following conditions were used; The concentrations of reservoir medium for [^{125}I]ebiratide and [^{125}I]E-2078 were 0.35 and 1 $\mu\text{Ci/ml}$, respectively, the perfusion flow rate was 10 and 2.5 $\mu\text{l/min}$, respectively. To prevent the adsorption of peptides to the fiber membrane surface of microdialysis fiber, 0.1% bovine serum albumin (BSA) was added to the perfusion and reservoir media.

Effect of Temperature on Microdialysis — In order to examine the effect of temperature on microdialysis, an *in vitro* dialysis study of antipyrine was performed in RHB at 15, 25 and 37 °C and at a constant flow rate of 50 $\mu\text{l/min}$ by the same procedure as described above.

Microdialysis Study in Erythrocyte Suspension — The bovine blood was centrifuged for 10 min at 3000 rpm at 4 °C, and the plasma was aspirated off. The erythrocytes were washed three times with cold saline and then with RHB at 4 °C. The erythrocyte suspension with the designed hematocrit (50—85%) was prepared by adding RHB.

The microdialysis probe was soaked in 30 ml of erythrocyte suspension mixed with a known amount of compounds. The final concentration was 0.15 $\mu\text{Ci/ml}$ for [^3H]water, 0.3 $\mu\text{Ci/ml}$ for [^{14}C]urea, 100 $\mu\text{g/ml}$ for antipyrine, 0.05 $\mu\text{Ci/ml}$ for [^{14}C]sucrose and 0.5 $\mu\text{Ci/ml}$ for [^{125}I]ebiratide. The dialysis was performed by perfusing RHB through the microdialysis cannula at a constant flow rate of 50 $\mu\text{l/min}$ except for [^{125}I]ebiratide. The dialysate samples were collected five times at 3 min interval. For [^{125}I]ebiratide, 20 mM ethylenediaminetetraacetic acid (EDTA) was added both in the erythrocyte suspension and the dialysate to prevent the

enzymatic metabolism. The dialysis was carried out at a constant flow rate of 10 $\mu\text{l}/\text{min}$ and the dialysate was collected three times for 20 min. The radioactivity or concentration in the dialysate and in the supernatants of erythrocyte suspension was determined. No remarkable sedimentation of erythrocyte was observed during the experimental period.

In Vivo Muscle Microdialysis Study — Male Wistar rats were intramuscularly anesthetized with ketamine (235 mg/kg) and xylazine (2.3 mg/kg). The left femoral artery was cannulated with polyethylene tube (SP 31) filled with heparin-saline solution (100 units/ml) for blood collection. Body temperature was maintained at 37 °C with heating lamps. The microdialysis probe was implanted in the right hind leg muscle or in the breast muscle followed by perfusing RHB into the microdialysis cannula at a constant flow rate of 10 $\mu\text{l}/\text{min}$. *In vitro* permeability rate constant of the dialysis probe was determined before the implantation. The same cannula as used in the corresponding *in vitro* experiments was used. After 15 min of the start of dialysis at a flow rate of 1 $\mu\text{l}/\text{min}$, each dose of [^3H]water (200 $\mu\text{Ci}/\text{kg}$), [^{14}C]urea (160 $\mu\text{Ci}/\text{kg}$), or [^{14}C]sucrose (200 $\mu\text{Ci}/\text{kg}$) was administered intravenously *via* a right jugular vein. For dialysis study of [^{14}C]urea and [^{14}C]sucrose, bilateral ligation of the renal artery and vein was performed to keep constant plasma levels of substances. For antipyrine, a bolus dose (75 mg/kg) was administered intravenously to rats *via* a jugular vein, immediately followed by the constant infusion of a maintenance dose (140 $\mu\text{g}/\text{min}/\text{kg}$) *via* a femoral vein cannula using an infusion pump (Model 22, Harvard Apparatus, South Natick, U.S.A.). From 35 min after a bolus injection of each substance, dialysate samples were collected at various flow rates in the order of 1, 10, 2, 50, 2.5, 25 and 5 $\mu\text{l}/\text{min}$ during the appropriate time intervals designed for [^3H]water and antipyrine after the appropriate time lapse before sampling to obtain the microdialysis steady-state condition. For [^{14}C]urea and [^{14}C]sucrose, the dialysates were collected at various flow rates in the order of 1, 10, 50 $\mu\text{l}/\text{min}$. Blood was withdrawn at the midpoint of each dialysate collection period.

Analytical Procedure — The radioactivities of the dialysate and reservoir medium were counted using a liquid scintillation counter (LSC 700, Aloka Ltd., Tokyo, Japan) for [^3H]water, [^{14}C]urea and [^{14}C]sucrose, a gamma-counter, ARC-605 (Aloka Co., Ltd., Tokyo, Japan) for [^{125}I]ebiratide and [^{125}I]E-2078. Antipyrine concentrations in plasma and dialysate were determined by a modification of the high-performance liquid chromatography (HPLC) method of Teunissen *et al.*¹²⁾ The HPLC system was constructed with a pump (TRI ROTAR-V, Japan Spectroscopic Co. (Jasco), Tokyo, Japan), UV spectrophotometer (UVIDEC-100-V, Jasco), automated sample processor (AS-L350, Jasco) and a data processor (C-R4A, Shimadzu Corporation, Kyoto, Japan). A 0.10 ml portion of the plasma sample was vigorously mixed for 15–30 s with 20 μl of the internal standard solution, 0.2 mg/ml phenacetin in ethanol, and 1 ml of dichlormethane-*n*-pentane (1:1). After centrifugation at 3000 rpm for 5 min, the organic layer (0.8 ml) was evaporated to dryness at room temperature. The residue was reconstituted with 0.1 ml of methanol and 0.15 ml of mobile phase (acetonitrile: 78 mM phosphate buffer = 1 : 3) and 30 μl aliquot was injected onto a stainless steel column (4.6 $\times$ 15 cm) filled with 5 μm of ODS (TSK gel ODS-80T_M, Toso Corporation, Tokyo, Japan). The dialysates were directly injected onto the HPLC column without any extraction and filtration. The HPLC conditions were: flow rate 1.0 ml/min; wavelength UV 254 nm. Peak area was used for quantification. The concentration was determined from the calibration curve prepared by the same procedure as that for the respective sample.

Data Analysis — The *in vitro* and *in vivo* relative recoveries (*R.R.*) were determined as follows:

$$R.R.(\%) \text{ (in vitro)} = \frac{C_d}{C_r} \times 100 \quad (1a)$$

$$R.R.(\%) \text{ (in vivo)} = \frac{C_d}{C_{\text{isf,u}}} \times 100 \quad (1b)$$

Fig. 1. (a) Dialysis Clearance (CL_D) and (b) Relative Recovery ($R.R.$) of $[^3H]$ Water in RHB (pH 7.40) at 37 °C as a Function of Dialysis Flow Rates

Each point and vertical bar represent the mean and S.E.M. of three experiments, respectively. When S.E.M. was small, it was included in the symbols. The data for CL_D were fitted to Eq. (4) by nonlinear least squares method. The solid line was generated from Eq. (4) using the PA value of 4.57 $\mu\text{l}/\text{min}$.

where C_d is the dialysate concentration of substances at the steady-state, C_r is the reservoir concentration in RHB, $C_{isf,u}$ is the unbound concentration in the interstitial fluid space at the steady state. $C_{isf,u}$ in muscle tissue would be the same as the unbound concentration in artery plasma at the steady-state, if the drug metabolism would be negligible in the interstitial fluid of the muscle. Thus, the alternative equation can be obtained.

$$R.R.(\%) \text{ (in vivo)} = \frac{C_d}{C_{p,u}} \times 100 \quad (2)$$

The dialysis clearance (CL_D) at the steady-state was defined as follows (see Appendix):

$$CL_D = F \times R.R. \quad (3)$$

where F is the dialysis flow rate through the microdialysis probe.

The data were analyzed by the clearance model to estimate the intrinsic permeability rate constant (PA_{vitro} or PA_{vivo}) at steady-state using a nonlinear least-squares regression method (MULTI program).¹³⁾ The derivation of the equation is described in Appendix and yields,

$$CL_D = F\{1 - \exp(-PA/F)\} \quad (4)$$

To compare the *in vivo* and *in vitro* microdialyses, the effective dialysis coefficient, R_d , was defined as follows:

$$R_d = \frac{PA_{\text{vivo}}}{PA_{\text{vitro}}} \quad (5)$$

Results

Effect of Flow Rate on the Dialysis Clearance

Fig. 2. Arrhenius Plots of the Permeability Rate Constant of Antipyrine (PA_{vitro})

The permeability rate constants were measured in RHB at 15, 25 and 37 °C at a constant flow rate of 50 $\mu\text{l}/\text{min}$. Each point represents the mean of three determinations.

Figure 1 shows a profile of the dialysis clearance (CL_D) and the relative recovery ($R.R.$) of $[^3H]$ water under the steady-state in RHB at 37 °C as a function of dialysis flow rate. CL_D increased with increase of dialysis flow rate and tended to attain plateau up to a flow rate of 10 $\mu\text{l}/\text{min}$. On the other hand, $R.R.$ was inversely related to the dialysis flow rate. The data for CL_D were fitted to Eq. (4) by nonlinear least squares method and the PA_{vitro} value of $[^3H]$ water was estimated to be $4.57 \pm 0.09 \mu\text{l}/\text{min}$ (mean $\pm$ S.D., $n=3$).

Temperature Dependency of Permeability Rate Constant of Antipyrine

The Arrhenius type plot for PA_{vitro} of antipyrine in RHB is shown in Fig. 2. From the slope of the line, the apparent activation energy for the microdialysis in RHB was estimated to be 5.49 kcal/mol.

Effect of Molecular Weight of Substrates on the Permeability Rate Constant

Figure 3 shows the relationship between root of the molecular weight and the reciprocal of PA_{vitro} (resistance) of $[^3H]$ water, $[^{14}C]$ urea, antipyrine, $[^{14}C]$ sucrose, $[^{125}I]$ ebiratide and $[^{125}I]$ E-2078 determined in RHB at 37 °C. A good linear relationship between two variables was obtained ($r=0.933$).

Effect of Erythrocyte on the Permeability Rate

Fig. 3. Relationship between the Reciprocal of the Permeability Rate Constant (PA_{vitro}) Determined in RHB (pH 7.40) at 37 °C and the Root of Molecular Weight of $[^3H]$ Water (○), $[^{14}C]$ Urea (◇), Antipyrine (□), $[^{14}C]$ Sucrose (△), $[^{125}I]$ Ebiratide (▽) and $[^{125}I]$ E-2078 (▲). Each point represents the mean of three experiments.

Constant

Figure 4 shows the relationship between the ratio of PA of $[^3H]$ water, antipyrine, $[^{14}C]$ sucrose, and $[^{125}I]$ ebiratide in erythrocyte suspension and that in RHB, which is the effective dialysis coefficient in erythrocyte suspension ($R_{d,\text{erythrocyte}}$), and % interstitial fluid volume ($100 - \text{hematocrit}$) of erythrocyte suspension. Despite the molecular weight of substances is 50 times different among the substrates, a statistically significant correlation was obtained ($r=0.921$).

Comparison of in Vitro and in Vivo Permeability Rate Constant

The *in vitro* and *in vivo* relative recoveries were determined for $[^3H]$ water, $[^{14}C]$ urea, antipyrine, and $[^{14}C]$ sucrose using Eq. (1a) and (2). Since no appreciable bindings to any plasma constituents for four substrates were observed, total plasma concentration at the steady state was assumed to be equal to $C_{p,u}$. The average concentrations in the plasma for $[^3H]$ water, $[^{14}C]$ urea, antipyrine and $[^{14}C]$ sucrose were $6.73 \times 10^5 \pm 0.01 \times 10^5 \text{ dpm}/\text{ml}$, $4.41 \times 10^5 \pm 0.12 \times 10^5 \text{ dpm}/\text{ml}$, $72.4 \pm 6.3 \mu\text{g}/\text{ml}$ and $1.96 \times 10^6 \pm 0.05 \times 10^6 \text{ dpm}/\text{ml}$, respectively. No significant time-dependencies of *in vitro* and *in vivo* $R.R.$ were observed. *In vitro* CL_D

Fig. 4. Relationship between $R_{d,\text{erythrocyte}}$ and % Interstitial Fluid Space ($100 - \text{Hematocrit}$)

Each point represents the mean of three to five determinations. The erythrocyte suspensions with the various hematocrit were prepared with bovine erythrocyte and RHB. Compound: $[^3H]$ water (○), antipyrine (□), $[^{14}C]$ sucrose (△), and $[^{125}I]$ ebiratide (▽).

Fig. 5. Comparison of *in Vivo* and *in Vitro* Dialysis Clearances (CL_D) of $[^3\text{H}]\text{Water}$, $[^{14}\text{C}]\text{Urea}$, Antipyrine and $[^{14}\text{C}]\text{Sucrose}$ at Different Flow Rates

In vitro CL_D (open circles) was measured at 37 °C in RHB (pH 7.40). *In vivo* CL_D (closed circles) was measured in the hind leg muscle of rats. Each point and vertical bar represent the mean and S.E.M. of three independent experiments, respectively. The solid lines were generated from Eq. (4), using parameters listed in Table I.

and *in vivo* CL_D values were calculated from Eq. (3) and were plotted in Fig. 5. *In vivo* CL_D value of all substrates was approximately one-third smaller than *in vitro* CL_D . The values of PA_{vivo} for all substrates were estimated by fitting to Eq. (4), and are listed in Table I together with PA_{vitro} values. Regarding PA_{vivo} values for

$[^3\text{H}]\text{water}$ and antipyrine, no significant differences were observed between hind leg muscle and breast muscle. The ratios of PA_{vivo} and PA_{vitro} , defined as the effective dialysis coefficient (R_d), are also listed in Table I. Almost the same values on R_d among substrates were obtained. The mean value and S.E.M. of R_d were 0.367 and

TABLE I. Comparison of Permeability Rate Constant of $[^3\text{H}]\text{Water}$, $[^{14}\text{C}]\text{Urea}$, Antipyrine and $[^{14}\text{C}]\text{Sucrose}$ in Ringer-HEPES Buffer (pH 7.40) and in the Rat Muscle

Compound	PA_{vitro} ($\mu\text{l}/\text{min}$)	n	Region of muscle	PA_{vivo} ($\mu\text{l}/\text{min}$)	n	$PA_{\text{vivo}}/PA_{\text{vitro}}$ ($= R_d$)
$[^3\text{H}]\text{Water}$	3.82 ± 0.04	(3)	Hind leg	1.45 ± 0.04	(3)	0.380
	4.20 ± 0.13	(3)	Breast	1.62 ± 0.05	(3)	0.387
$[^{14}\text{C}]\text{Urea}$	2.23 ± 0.04	(3)	Hind leg	0.792 ± 0.020	(3)	0.355
	1.10 ± 0.03	(3)	Hind leg	0.447 ± 0.015	(3)	0.406
Antipyrine	1.10 ± 0.03	(3)	Breast	0.439 ± 0.017	(3)	0.399
$[^{14}\text{C}]\text{Sucrose}$	0.454 ± 0.009	(3)	Hind leg	0.148 ± 0.014	(3)	0.326
Mean $\pm$ S.E.M.						0.367 ± 0.034

0.034, respectively (ranged from 0.33—0.40).

Discussion

According to the conventional calculation procedure for estimating the tissue interstitial fluid concentration by calibrating the microdialysis probe in a saline solution containing a known concentration of the substrate,¹⁾ the estimated unbound concentration is known to result in the underestimation from the "true" interstitial fluid concentration.⁴⁾ This would be ascribed, in part, to a reduction of the permeability rate through the dialysis probe in the tissue. Therefore, we estimated the permeability rate constants of substrates through the microdialysis probe in the *in vitro* and the *in vivo* tissue employing a clearance concept.^{14,15)} Muscle was used as a model tissue since the transcapillary transport of low molecular weight substrates in the muscle tissue is not restricted.¹⁶⁾

The apparent activation energy estimated in the *in vitro* microdialysis (5.49 kcal/mol) corresponded to the diffusion energy of the solutes in aqueous solution.¹⁷⁾ This suggests that the steps controlling the transport of solute are the diffusion through the membrane and the aqueous barrier (diffusion layer) adjacent to both sides of the membrane. Thus, the permeability rate constant of microdialysis would be proportional to the diffusion coefficient of the model substrates in aqueous solution. Moreover, since the product of the diffusion coefficients and root of the molecular weight is known to be approximately constant,¹⁸⁾ the reciprocal of the permeability rate constant would be correlated with the root of the molecular weight of the model substrates. The significant positive correlation observed in Fig. 3 demonstrates this relationship.

The permeability rate constant of substrates estimated in the erythrocyte suspension and/or the muscle tissue resulted in the decrease in comparison with that in the buffer. Thus, it is very important to examine the determinant factors influencing the difference between them. The reciprocal of the steady-state *in vitro* and the *in vivo* permeability rate constants (resistance) estimated in the present study would be expressed from the series resistant equation as the sum of

the reciprocal of permeability rate constants of the membrane (PA_m), of the unstirred water layer at the inner side (PA_{in}) and at the outer side of the probe ($PA_{vitro, out}$ or $PA_{vivo, out}$).

$$\frac{1}{PA_{vitro}} = \frac{1}{PA_{in}} + \frac{1}{PA_m} + \frac{1}{PA_{vitro, out}} \quad (6)$$

$$\frac{1}{PA_{vivo}} = \frac{1}{PA_{in}} + \frac{1}{PA_m} + \frac{1}{PA_{vivo, out}} \quad (7)$$

According to the recent findings,^{8,9,11)} the resistance of the outer side of the probe is suggested to be dominant for the substrate penetration of the microdialysis membrane (*i.e.*, PA_{in} , $PA_m \gg PA_{vitro, out}$, $PA_{vivo, out}$). Therefore, Eq. (6) and (7) could be reduced to Eq. (8) and (9), respectively.

$$\frac{1}{PA_{vitro}} = \frac{1}{PA_{vitro, out}} \quad (8)$$

$$\frac{1}{PA_{vivo}} = \frac{1}{PA_{vivo, out}} \quad (9)$$

As a significant mixing for the reservoir solution would affect the thickness of the unstirred water layer of the outer side of the dialysis fiber and would result in the increase of the $PA_{vitro, out}$ value, one may not be able to approximate the relation of Eq. (6) to (8). Thus, in the present study, the reservoir solution was not stirred to obtain the relation of Eq. (8).

PA_{vitro} and PA_{vivo} would be also expressed in terms of the diffusion parameters as follows.^{11,19)}

$$PA_{vitro} = \frac{S_{vitro}D_{vitro}}{L_{vitro}} \quad (10)$$

$$PA_{vivo} = \frac{\alpha S_{vivo}D_{vivo}}{\lambda^2 L_{vivo}} \quad (11)$$

where D_{vitro} and D_{vivo} are the diffusion coefficients of the substrates in buffer solution and the interstitial fluid, respectively, S_{vitro} and S_{vivo} are the effective surface areas of the fiber membrane at the condition of *in vitro* and *in vivo*, respec-

tively. L_{vitro} is the diffusion distance in buffer solution. λ^2 is the tortuosity factory to account for the distance as the solute moves around the probe, that is, $\lambda^2 L_{\text{vivo}}$ is the diffusion distance in tissue. α is the interstitial volume fraction. As the diffusion coefficient is related to the reciprocal of the root of molecular weight, *i.e.*,

$$\begin{aligned} D_{\text{vitro}} &= \beta (\text{MW})^{-1/2} \\ D_{\text{vivo}} &= \gamma (\text{MW})^{-1/2} \end{aligned}$$

where β and γ are the coefficient relating $(\text{MW})^{-1/2}$ and D_{vitro} and D_{vivo} , respectively, the ratio of D_{vivo} and D_{vitro} can be expressed by the constant value, *i.e.*, γ/β . Therefore, when R_d is defined as the ratio of PA_{vivo} and PA_{vitro} , the following equation can be obtained.

$$R_d = \alpha \frac{S_{\text{vivo}}}{S_{\text{vitro}}} \frac{L_{\text{vitro}}}{\lambda^2 L_{\text{vivo}}} \frac{\gamma}{\beta} \quad (12)$$

This formula suggests that the effective dialysis coefficient (R_d) would be ascribed to the difference of the diffusion distance of the outer surface of the fiber, the difference of the effective surface area between *in vivo* and *in vitro*, and the interstitial fluid space. Moreover, Eq. (12) suggests that R_d value would be independent of the molecular weight of the substrate.

The positive correlation in the erythrocyte suspension (Fig. 4), which was used as an *in vitro* model of tissue, suggests that R_d mainly depends on the interstitial fluid volume fraction and is independent of the molecular weight, as indicated from Eq. (12). Moreover, it is of great interest that no difference of $R_{d,\text{erythrocyte}}$ was observed among sucrose, antipyrine and water, while sucrose does not permeate through the plasma membrane but water and antipyrine do. This suggests that the diffusion distance of the outer surface of the fiber membrane and/or the effective surface area of the outer side of the fiber would not be influenced by the characteristic of the plasma membrane permeability of the substrate. Moreover, as evident from Table I, almost similar R_d values (0.33–0.40) were obtained among $[^3\text{H}]\text{water}$, $[^{14}\text{C}]\text{urea}$, antipyrine and $[^{14}\text{C}]\text{sucrose}$ in hind leg muscle and breast

muscle, demonstrating that the findings obtained in the erythrocyte suspension were reproduced in muscle tissue.

In the present study, we have adopted the clearance concept to analyze the relationship between the dialysis flow rate and the dialysate concentration. As in the case of the analysis of hepatic elimination,¹⁴⁾ one of the advantages for the present analysis is that the rate limiting step can be easily known by comparing the dialysis clearance and the dialysis flow rate. To find the appropriate dialysis flow rate for the determination of the permeability rate constant, it would be helpful to know the rate limiting step for the tissue microdialysis. The other advantage for use of the dialysis clearance is that we could know whether the tissue microdialysis will change the averaged substrate concentration in the tissue interstitial fluid or not. Accordingly, by comparing the dialysis clearance and the influx rate of the substrate from the vascular fluid to the tissue interstitial fluid, we could find the appropriate condition for the microdialysis to minimize the changes of the substrate concentration in the tissue interstitial fluid.

The ultimate purpose of our study is to find a precise and practical way to extrapolate the “true” concentration in interstitial fluid from the dialysate concentration at the steady-state. Rearrangement of Eq. (1b) and Eqs. (3)–(5) yields the following equation to extrapolate the “true” unbound concentration in the tissue interstitial fluid, $C_{\text{isf}, u}$, from the *in vitro* permeability rate constant, PA_{vitro} , and the dialysate concentration, C_d .

$$C_{\text{isf}, u} = \frac{C_d}{1 - \exp(-R_d PA_{\text{vitro}}/F)} \quad (13)$$

As the effective dialysis coefficient, R_d , is demonstrated to be insensitive to the molecular weight and the plasma membrane permeability, the advantage of this conventional method using the above equation would be that R_d value of antipyrine or $[^3\text{H}]\text{water}$ could be used as reference compound to extrapolate the $C_{\text{isf}, u}$ from C_d .²⁰⁾

In conclusion, the effective dialysis coefficient (R_d) to relate the *in vitro* microdialysis to the *in*

vivo microdialysis has been demonstrated to be independent of molecular weight and the plasma membrane permeability of four model substrates. By using the R_d value, a useful equation at the steady-state has been proposed to extrapolate the "true" unbound concentration in the tissue interstitial fluid from the dialysate concentration. The method for estimating "true" unbound concentration in the interstitial fluid will contribute to clarify the microscopic distribution mechanisms of drugs in various tissues and the quantitative relationship between the interstitial fluid concentration of drugs and the related pharmacological activity.

Appendix

In order to describe the microdialysis phenomena, we introduced the dialysis clearance (CL_D) with the following assumptions.

1. The dialysis tube is composed of the homogeneous cylindrical semipermeable membrane which has the length of L .
2. Dialysate solution enters and leaves the dialysis tube at a constant flow rate (F). No appreciable convection is produced.
3. Substrate permeates with a constant influx permeability rate constant (PA_{inf}) from the outer medium into the dialysate solution, along with a concentration gradient. Substrate also permeates from the dialysate solution to the outer medium with a constant efflux rate constant (PA_{eff}) equal to PA_{inf} , that is $PA_{inf} = PA_{eff} = PA$.

The dialysis clearance can be defined as follows:

$$CL_D = \frac{dX_d/dt}{C_r} \quad (A1)$$

where dX_d/dt is the amount of substrate cleared in the dialysate solution from the outer medium per unit time. C_r is the substrate concentration in the outer medium.

Eq. (A1) is also expressed as follows at steady-state:

$$CL_D = \frac{F \cdot C_d}{C_r} \quad (A2)$$

where C_d is the concentration of substrate in the dialysate. Since C_d/C_r is the relative recovery ($R.R.$):

$$CL_D = F \times R.R. \quad (A3)$$

can be obtained.

Based on the above assumptions, the mass balance equation of the substrate amount in a dialysis tube at steady-state is given by the following equation:

$$F \frac{dC_d(x)}{dx} + \frac{PA_{eff}}{L} C_d(x) - \frac{PA_{inf}}{L} C_r = 0 \quad (A4)$$

By adopting assumption (3) described above, Eq. (A4) leads to:

$$\frac{dC_d(x)}{dx} = -\frac{PA}{F \cdot L} \{C_d(x) - C_r\} \quad (A5)$$

where $C_d(x)$ is the substrate concentration in the dialysis tube at point x . Integration of Eq. (A5) from point zero to L for x gives

$$\ln \left\{ \frac{C_d(L) - C_r}{C_d(0) - C_r} \right\} = \frac{PA}{F \cdot L} (L - 0) \quad (A6)$$

As $C_d(0) = 0$ and $C_d(L) = C_d$, we obtain the substrate concentration in dialysate during tissue microdialysis as follows:

$$\frac{C_d}{C_r} = 1 - \exp(-PA/F) \quad (A7)$$

Substituting Eq. (A7) into Eq. (A2) gives

$$CL_D = F \{1 - \exp(-PA/F)\} \quad (A8)$$

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