Research paper

Absorption characteristics of compounds with different molecular weights after application to the unilateral kidney surface in rats

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Abstract

The aim of the present study is to clarify the absorption mechanism of a drug from the kidney surface membrane in rats. We studied the absorption characteristics of phenolsulfonphthalein (PSP) and other compounds with different molecular weights after their application to the rat kidney surface in vivo, employing a cylindrical diffusion cell (i.d. 6 mm, area 0.28 cm^2). The time course of free PSP amounts remaining in the diffusion cell obeyed first-order kinetics at a dose of 1 mg, and its rate constant k_a was calculated to be 0.0137 min^{-1}. Absorption ratios of PSP in 4 h were calculated from the amount recovered from the diffusion cell to be 91.4, 96.4 and 97.7 % at doses of 0.5, 1 and 1.5 mg, respectively. The area under the curve for the plasma concentration profile of free PSP was proportional to the application dose. It is thus suggested that the absorption process of PSP from the rat kidney surface does not approach saturation at a dose of 1.5 mg. Also, no significant difference was seen in the k_a values within the dose range of 0.5 - 1.5 mg, which were estimated by curve-fitting the plasma concentration profiles of free PSP in a two-compartment model with first-order absorption. Furthermore, we examined the importance of molecular weight on the absorption from the kidney surface using fluorescein isothiocyanate-dextrans (FDs) with molecular weights of 4,400 (FD-4), 11,000 (FD-10), 40,500 (FD-40) or 69,000 (FD-70), including the organic anions bromphenol blue and bromosulfonphthalein. The absorption ratios of FDs from the rat kidney surface in 6 h decreased with an increase in the molecular weight (76.1 % for FD-4, 54.4 % for FD-10, 11.5 % for FD-40 and 3.9 % for FD-70). A linear relationship was observed between the k_a and the reciprocal value with the square root of the molecular weight of these compounds. The limit of absorption from the rat kidney surface was extrapolated to be at a molecular weight of approximately 130,000.

Keywords: Absorption; Kidney surface; Rat; Molecular weight; Pharmacokinetics; Targeting

1. Introduction

The kidney plays such an important role in drug disposition in the body that there is an increasing interest to improve therapy for kidney diseases. It is desired that an administered drug should be distributed largely into the target site of the kidney, proposing to treat kidney diseases, e.g. a localized tumor. The renal arterial administration of poly (stylene-co-maleic acid)-conjugated neocarzinostatin (SMANCS)/lipiodol [1,2] or microcapsule chemoembolization [3-6] has been studied to target anticancer drugs at the unilateral kidney to enhance their efficacy and to reduce their toxicities.

In general, normal routes of drug administration have difficulties in reaching local sites of action in the unilateral kidney, because of high blood circulation rates and microvascular permeability in the kidney. Although the direct way is supposed to be a useful method, the direct injection route into the kidney is not suitable for targeted site-selective drug delivery. This is because directly injected drugs are rapidly cleared from the injected site with high blood flow, followed by drainage into the systemic circulation. In contrast, a kidney surface application does not stress the kidney with no injury, and continuous catheter administration may be capable of long-term application. In a previous study [7] we elucidated the kidney- and site-selective delivery of the anticancer drug 5-fluorouracil (5-FU), utilizing absorption from the kidney surface in the rats. At first, an experimental system employing a cylindrical diffusion cell attached to the unilateral kidney surface was established. After applying 5-FU to the rat kidney surface, the concentration of 5-FU in the applied kidney was significantly higher than concentrations in the non-applied kidney and other tissues. However, the absorption mechanism of a drug from the kidney surface remains to be examined.

The main purpose of the present study is to obtain basic information concerning the absorption mechanism from the kidney surface membrane. Kinetic analysis of drug

absorption from the kidney surface is of particular interest physiologically and important for clinical use of this administration method.   First, we analyzed absorption characteristics pharmacokinetically such as dose dependency in phenolsulfonphthalein (PSP) absorption from the kidney surface because its absorption characteristics from the rat liver surface had been fully investigated [8-11].

In addition, we selected four types of fluorescein isothiocyanate-dextrans (FDs) with different molecular weights as model macromolecules and the organic anions bromphenol blue (BPB) and bromosulfonphthalein (BSP), and studied their in vivo pharmacokinetics after application to the rat kidney surface.   There are some reports concerning the molecular weight dependence of peritoneal transport [12-17] and the accumulation of macromolecules in several organs after intraperitoneal (i.p.) administration [18,19].   However, the absorption characteristics of macromolecules across the kidney surface membrane is still unknown.

2. Materials and methods

2.1. Materials

PSP and BPB were purchased from Nacalai Tesque, Inc. (Kyoto, Japan).   BSP and FDs with average molecular weights of 4,400 (FD-4), 11,000 (FD-10), 40,500 (FD-40) or 69,000 (FD-70) were obtained from Sigma Chemical Co. (St. Louis, MO, USA).   All other chemicals were of reagent grade products.

2.2. Animal experiment

All animal procedures in the present study conformed to the Guideline for Animal Experimentation in Nagasaki University.

Male Wistar rats (220-290 g) were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and the left femoral artery was cannulated with a polyethylene tube (i.d. 0.5 mm, o.d. 0.8

mm, Dural Plastics, Dural, Australia).　　After the middle abdomen was cut open about 3 cm, the common bile duct was cannulated with a polyethylene tube (i.d. 0.28 mm, o.d. 0.61 mm, Becton Dickinson & Co., Parsippany, NJ, USA).　　The body temperature of rats was kept at 37 °C by a heat lamp during the experiment.　　The drug solution was prepared in an isotonic phosphate buffer (pH 7.4), and administered as follows.

Application to rat kidney surface: A cylindrical diffusion cell (i.d. 6 mm, area 0.28 cm^2) was attached to the rat left kidney surface with adhesive chemical Aron Alpha (Sankyo Co. Ltd., Tokyo, Japan).　　The drug solution (0.05 ml) was added to the diffusion cell directly. The top of the diffusion cell was sealed by a piece of aluminum foil to prevent evaporation of the applied solution.

I.v. administration: The drug solution (0.1 ml) was injected into the jugular vein.

After application of the drug solution a 200 µl blood sample was collected at selected times from the heparinized cannula inserted into the femoral artery over 4 or 6 h and centrifuged at 15,000 rpm for 5 min.　　Bile samples were collected at appropriate time intervals for 4 or 6 h.　　The urine was collected from the bladder directly by a syringe and the solution remaining in the diffusion cell was withdrawn at 4 or 6 h after the application.

2.3. Analytical methods

The concentrations of compounds in the plasma, bile, urine, and the solution remaining in the diffusion cell were determined as follows.

PSP: The concentration of free PSP was determined spectrophotometrically at 560 nm after dilution with 1 M NaOH solution.　　The total concentration of free PSP and its metabolite was measured in the same manner after they were subjected to acid hydrolysis (1 M HCl at 100 °C for 30 min) [20].　　The concentration of PSP metabolite was estimated from the difference between these values.

BPB: The concentration of BPB was determined spectrophotometrically at 600 nm after

dilution with an isotonic phosphate buffer (pH 7.4) [21].

BSP: The concentration of BSP was determined spectrophotometrically at 580 nm after dilution with 0.1 M NaOH solution [22].

FDs: The concentrations of FDs as fluorescence in the plasma, bile, urine and solution remaining in the diffusion cell were measured by a spectrophotofluorometer at excitation and emission wavelengths of 489 and 515 nm, respectively.

2.4. Calculation of pharmacokinetic parameters

The plasma concentration profile of free PSP was analyzed based on the statistical moment theory.   Moment parameters for the plasma concentration profile of free PSP (AUC_p, MRT_p) are calculated by numeral integration using a linear trapezoidal formula and extrapolation to infinite time based on a monoexponential equation [23].

2.5. Compartment model analysis

First, the plasma concentration (C_p) profiles of free PSP at doses of 0.5, 1 and 1.5 mg after i.v. administration into rats were fitted to the biexponential equation described as follows, by the nonlinear least-squares method [24].

$$C_p = \frac{D(\alpha - k_{21})}{V_c(\alpha - \beta)} e^{-\alpha \cdot t} + \frac{D(k_{21} - \beta)}{V_c(\alpha - \beta)} e^{-\beta \cdot t} \tag{1}$$

Hybrid parameters α and β are defined as $\alpha + \beta = k_{12} + k_{21} + k_{el}$ and $\alpha \cdot \beta = k_{21} \cdot k_{el}$. V_c is the volume of the central compartment. k_{el} is the first-order elimination rate constant from the central compartment. k_{12} and k_{21} are the first-order transfer rate constants between the central and peripheral compartment.   These parameters were substituted into the following equation for the plasma concentration after application of PSP to the rat kidney surface. Next, in the same way, the plasma concentration profile of free PSP after application to the rat kidney surface was fitted in a two-compartment model with first-order absorption by the nonlinear least-squares method [24].   In this model, the equation for plasma concentration

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of free PSP is given by the following equation.

$$C_p = \frac{F \cdot D \cdot k_a}{V_c}\left\{\frac{k_{21}-k_a}{(\beta-k_a)(\alpha-k_a)}e^{-k_a \cdot t} + \frac{k_{21}-\alpha}{(\beta-\alpha)(k_a-\alpha)}e^{-\alpha \cdot t} + \frac{k_{21}-\beta}{(\alpha-\beta)(k_a-\beta)}e^{-\beta \cdot t}\right\} \quad (2)$$

k_a is the first-order absorption rate constant for PSP absorption into the blood stream from the rat kidney surface.   F is the availability of PSP after application to the rat kidney surface.

3. Results and Discussion

3.1. Absorption of PSP after application to the rat kidney surface

To assess the absorption characteristics from the rat kidney surface membrane, we first studied the time course of the free PSP amount remaining in the diffusion cell after application to the rat kidney surface at a dose of 1 mg.   The extent of PSP absorption in 4 h was calculated as 96.4 % by the dose and the amount recovered from the diffusion cell.   As shown in Fig. 1, the semi-log plot of free PSP amount remaining in the diffusion cell made a straight line (correlation coefficient: $r^2 = 0.98$), indicating that PSP absorption from the rat kidney surface proceeds via first-order.   Its first-order absorption rate constant k_a was calculated to be 0.0137 min^{-1}.   Also, this result supports the employment of the two-compartment model incorporating first-order absorption to describe the plasma concentration profile of free PSP after application to the rat kidney surface.

3.2. Dose dependency on PSP absorption after application to the rat kidney surface

Fig. 2 shows the plasma concentration profiles of free PSP after application to the rat kidney surface at doses of 0.5, 1 and 1.5 mg.   At every dose, the plasma concentration of free PSP reached a maximum, followed by a gradual disappearance.   Table 1 lists the recovery of free PSP and its metabolite (% of dose) in the bile, urine and diffusion cell at 4 h

after i.v. administration or application to the kidney surface in rats at doses of 0.5, 1 and 1.5 mg.   Absorption ratios of PSP in 4 h calculated from the free PSP amount remaining in the diffusion cell were 91.4, 96.4 and 97.7 % at doses of 0.5, 1 and 1.5 mg, respectively, indicating no saturation of PSP absorption from the rat kidney surface membrane within the dose range used.

As shown in Table 1, free PSP and its metabolite were excreted into both bile and urine on each administration route.   The urinary and biliary recoveries of free PSP and its metabolite after application to the rat kidney surface were broadly similar to those after i.v. administration.   It is supposed that the drug metabolism and excretion processes of a drug are not affected by its application to the kidney surface.

Moment parameters are free from the complexities of a pharmacokinetic model, and thus AUC_p and MRT_p can be appropriate parameters to evaluate roughly the drug absorption rate and extent from the kidney surface.   Table 2 summarizes the moment parameters of free PSP after application to the rat kidney surface at doses of 0.5, 1 and 1.5 mg.   No significant difference was seen in the AUC_p/dose value among the three doses, indicating linearity of PSP absorption from the rat kidney surface membrane.   Also, MRT_p values were almost the same among the three doses.   The MRT_p values of free PSP after its application to the rat kidney surface were fairly larger than those after i.v. administration.   The prolonged MRT_p value is a reflection of the time required for absorption process across the kidney surface. The mean absorption time (MAT) of PSP across the rat kidney surface was calculated by subtracting the MRT_p (124 min) after i.v. administration from the MRT_p after application to the rat kidney surface, as listed in Table 2.

3.3. Pharmacokinetic analysis of PSP absorption after application to the rat kidney surface

We confirmed that the in vivo disposition after i.v. administration of PSP obeyed the two-

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compartment model, and the pharmacokinetic parameters were already obtained. The plasma concentration profile of free PSP after its application to the rat kidney surface was fitted into a two-compartment model with first-order absorption. The k_a values obtained by curve-fitting the plasma concentration profile of free PSP were calculated to be 0.0128, 0.0139 and 0.0141 min^{-1} at doses of 0.5, 1 and 1.5 mg, respectively. Fig. 2 also shows the fitted curves for the plasma concentration of free PSP after its application to the rat kidney surface at doses of 0.5, 1 and 1.5 mg, which had been reconstructed employing the estimated pharmacokinetic parameters. In general, agreement was observed between the fitted lines and experimentally observed data at every dose as shown in Fig. 2.

The obtained k_a of PSP (0.0139 min^{-1}) at a dose of 1 mg obtained by curve-fitting (Fig. 2) correlated closely with the value determined by an elimination profile of free PSP from the diffusion cell (0.0137 min^{-1}, Fig. 1), suggesting the validity of the pharmacokinetic model. In addition, no significant difference was seen in the k_a values at doses of 0.5, 1 and 1.5 mg, supporting the linearity of PSP absorption from the rat kidney surface membrane.

Accordingly, it is suggested that specialized transport processes such as active transport might not exist in the PSP absorption from the rat kidney surface membrane. Thus, a simple passive diffusion is considered to play an important role in the PSP absorption from the kidney surface. In the future, several factors influencing the drug absorption from the kidney surface should be clarified for practical clinical use.

3.4. Absorption of compounds with different molecular weights after application to the rat kidney surface

Since molecular weight seems to play an important role in drug absorption from the kidney surface membrane, the absorption mechanism of macromolecules itself needs to be carefully examined for clinical application of macromolecules such as biologically active

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peptides.　　We selected four different FDs, because dextrans are fairly resistant to metabolic degradation and their in vivo fate has been characterized fully in rats [25-28].

　　Fig. 3 illustrates the plasma concentration profiles of FDs with different molecular weights after application to the rat kidney surface at a dose of 1 mg.　　All FDs were absorbed from the kidney surface, followed by progressive appearances in the plasma. With regard to FD-40 and FD-70, their plasma levels either plateaued or continued to rise until 6 h after dosing, compared to FD-4 and FD-10.

　　Table 3 lists the recovery of FDs in 6 h together with three different organic anions (PSP, BPB, BSP) in the diffusion cell, bile and urine after application to the rat kidney surface at a dose of 1 mg.　　The absorption ratios of FDs from the rat kidney surface in 6 h were calculated from the amount recovered from the diffusion cell as 76.1 % for FD-4, 54.4 % for FD-10, 11.5 % for FD-40 and 3.9 % for FD-70.　　An increase in the molecular weight of FDs resulted in a decrease in the absorption rate from the rat kidney surface.

　　Fig. 4 summarizes the k_a of PSP, BPB, BSP and FDs (FD-4, FD-10, FD-40, FD-70) after application to the rat kidney surface.　　The calculated k_a of these compounds decreased with the increase in molecular weight as shown in Fig. 4, indicating that the drug absorption from the kidney surface was affected greatly by the molecular weight.

3.5. Relationship between molecular weights and absorption rate of compounds after application to the rat kidney surface

　　We compared the absorption rates of several compounds having different molecular weights to predict the absorption rate from the kidney surface by physicochemical characteristics such as molecular weight.　　The following equation has been proposed with respect to the drug absorption from the stomach and small intestine via passive diffusion [29,30] on the basis of the principle that the diffusibility of a substance in uniform solutions is

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approximately inversely proportional to the square root of the molecular weight of the drug ($\sqrt{Mw}$).

$$\frac{1}{\sqrt{Mw} \cdot k_a} = A + \frac{B}{P_a} \qquad (3)$$

Where P_a represents the partition coefficient and constants A and B are a correction factor to P_a and a constant for diffusion, respectively.

Because each model compound has high hydrophilicity, lipophilic index P_a of each model compound is very small.   This might enable us to assume that each P_a is approximately identical.   Then, the right side of Eq. (3) can be transformed as a fixed number.   As illustrated in Fig. 5, we examined the relationship between the k_a and the reciprocal value of the square root of the molecular weight ($1/\sqrt{Mw}$) of the compounds.   A linear relationship was observed between the k_a and $1/\sqrt{Mw}$ of these compounds (correlation coefficient: r^2 = 0.97), suggesting that these hydrophilic compounds with different molecular weights are absorbed from the rat kidney surface membrane via simple passive diffusion.

The molecular weight when the k_a value was 0 (x-intercept) was extrapolated to be 130,890 from the plot in Fig. 5.   We consider that the limit (so-called threshold value) of the molecular weight of the drug absorbed from the rat kidney surface is approximately 130,000, which is much larger than that restricting the movement of macromolecules from the peritoneum (Mw: ca. 50,000) reported by Flessner et al. [12] and Hirszel et al. [31]. Therefore, the pore size of the kidney surface membrane is likely to be larger compared to the general peritoneal membrane.   In addition, significant differences were seen in the absorption rate and molecular weight limit, compared to the liver surface membrane (approximately 70,000) [17], implying that organ surface membranes are not uniform with respect to absorption characteristics, probably because of differences in the intercellular gaps and pores among the peritoneal organ surfaces.   The basic information concerning

difference in absorption characteristics among the peritoneal organs might be useful to examine the pharmacokinetics of a drug in the peritoneal cavity. A simple pharmacokinetic model such as spatially distributed model proposed by Dedrick and Flessner [32] could be applied to perform pharmacokinetic calculations by utilizing permeability area product of each organ surface.

In conclusion, we clarified the absorption mechanism such as dose and molecular weight dependence of drug absorption from the kidney surface in rats. Such information would help this new administration route to improve specific delivery of therapeutic agents to the diseased region in the unilateral kidney.

Acknowledgements

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Table

Table 1

Recovery (% of dose) of free PSP and its metabolite 4 h after application to the kidney surface or i.v. administration in rats

Dose (mg)	Diffusion cell (Free)	Bile			Urine		
		Free	Metabolite	Total	Free	Metabolite	Total
Kidney surface application							
0.5	8.6 ± 1.0	30.7 ± 3.2	18.6 ± 3.7	49.3 ± 1.8	16.8 ± 2.0	13.8 ± 1.6	30.7 ± 2.8
1	3.6 ± 0.4	34.1 ± 2.7	16.0 ± 2.3	50.1 ± 3.1	13.6 ± 3.7	11.9 ± 2.9	25.5 ± 4.1
1.5	2.3 ± 0.6	33.1 ± 3.9	20.2 ± 3.1	53.3 ± 6.3	16.8 ± 6.4	8.6 ± 1.5	25.4 ± 7.6
I.v. administration							
1	-	28.8 ± 3.5	14.4 ± 1.5	43.2 ± 2.1	23.4 ± 2.8	4.1 ± 0.8	27.5 ± 3.5

Each value is the mean ± S.E. of four experiments.

Table 2

Moment parameters of free PSP after application to the kidney surface or i.v. administration in rats

Dose (mg)	AUC_p (µg·min/ml)	MRT_p (min)	MAT (min)
Kidney surface application			
0.5	535 ± 119	170 ± 27	46.2 ± 27.1
1	1097 ± 166	176 ± 44	52.4 ± 43.9
1.5	1781 ± 198	198 ± 21	74.6 ± 20.5
I.v. administration			
1	955 ± 3	124 ± 4	-

Each value is the mean ± S.E. of four experiments.

Table 3

Recovery (% of dose) of the compounds with different molecular weights 4 or 6 h after application to the rat kidney surface at a dose of 1 mg

Compound	Mw	Time (h)	Diffusion cell	Bile	Urine
PSP	354	4	3.6 ± 0.4	50.1[a] ± 3.1	25.5[a] ± 4.1
BPB	670	4	10.5 ± 0.8	55.4 ± 2.3	2.4 ± 1.2
BSP	838	4	19.0 ± 4.2	61.9 ± 3.7	2.5 ± 0.7
FD-4	4,400	6	23.9 ± 1.2	N.D.	67.3 ± 3.5
FD-10	11,000	6	45.6 ± 1.6	N.D.	48.5 ± 2.5
FD-40	40,500	6	88.5 ± 0.7	N.D.	5.5 ± 0.6
FD-70	69,000	6	96.1 ± 1.0	N.D.	1.6 ± 0.1

Each value is the mean ± S.E. of four experiments.

N.D.: not detected.

[a] Total recovery of free PSP and its metabolite.

Figure captions

Fig. 1.　　Semi-log plot of time course of free PSP amount remaining in the diffusion cell after application to the rat kidney surface at a dose of 1 mg.　　Each point represents the mean ± S.E. of at least four experiments.

Fig. 2.　　Plasma concentration profiles of free PSP after application to the rat kidney surface at doses of 0.5 (▲), 1 (○) and 1.5 mg (●).　　Each point represents the mean ± S.E. of four experiments.　　Curves show the simulated function based on the pharmacokinetic parameters by two-compartment model.

Fig. 3.　　Plasma concentration profiles of FD-4 (●), FD-10 (○), FD-40 (▲) and FD-70 (△) after application to the rat kidney surface at a dose of 1 mg.　　Each point represents the mean ± S.E. of four experiments.

Fig. 4.　　First-order absorption rate constant k_a of PSP, BPB, BSP and FDs (FD-4, FD-10, FD-40, FD-70) after application to the rat kidney surface at a dose of 1 mg.　　Each column represents the mean + S.E. of four experiments.　　k_a was calculated from the amount remaining in the diffusion cell at 4 or 6 h.

Fig. 5.　　Relationship between the molecular weight of the compounds and first-order absorption rate constant k_a after application to the rat kidney surface.　　Each point represents the mean ± S.E. of four experiments.　　1: PSP, 2: BPB, 3: BSP, 4: FD-4, 5: FD-10, 6: FD-40, 7: FD-70.