

TITLE PAGE

**Delivery Advantage to the Unilateral Kidney by Direct Drug
Application to the Kidney Surface in Rats and
Pharmacokinetic Verification based on a Physiological Model**

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ABSTRACT

The objective of this study was to evaluate the drug delivery advantage to the unilateral kidney by direct drug application to the rat kidney surface based on a physiological pharmacokinetic model. Under anesthesia, a cylindrical diffusion cell (i.d. 6 mm, area 0.28 cm²) was attached to the right kidney surface in rats. Phenolsulfonphthalein (PSP), an organic anion chosen as a model compound, was added into the diffusion cell. The free PSP concentration in the right (applied) kidney after application to the right kidney surface at a dose of 1 mg was significantly higher than that of the left (non-applied) kidney until 60 min after application. Similarly, the urinary excretion rate of free PSP from the applied kidney was much faster than that from the non-applied kidney, with a 2.6 times larger excreted amount in 240 min. These results imply the possibility that a considerable drug delivery advantage to the unilateral kidney could be obtained after direct absorption from the kidney surface. This tendency was also observed at the other application doses of 0.3 and 1.5 mg. On the other hand, fluorescein isothiocyanate dextran (Mw 4400, FD-4) was equally excreted into the urine from each kidney and the renal concentrations in the applied and non-applied kidneys were almost the same, possibly due to the involvement of passive

transport for the absorbed FD-4, i.e. glomerular filtration. The computer simulations of free PSP concentrations in the plasma and each kidney based on a physiological model after kidney surface application were consistent with the respective experimental data. Moreover, the delivery advantage of kidney surface application of PSP was verified by its comparison with other routes such as i.v. and intraarterial administrations.

Keywords: Kidney surface; Targeting; Unilateral delivery; Physiological model; Pharmacokinetics

INTRODUCTION

The kidney plays such an important role in maintaining homeostasis in the body that kidney diseases affect drug therapy in different ways. Therapeutic agents for kidney diseases have often been administered intravenously or orally. Following these administration methods, drugs tend to distribute in the whole body via the bloodstream, leading to inadequate delivery to local sites in the kidney and to toxicity in other organs.

Previously, we clarified the absorption mechanisms, such as the dose and molecular weight dependence of drug absorption from the kidney surface in rats, and found that

kidney surface application might be a useful method for drug delivery to the kidney (Nishida *et al.*, 2004). Furthermore, we demonstrated the kidney- and site-selective delivery of 5-fluorouracil utilizing absorption from the rat kidney surface (Kawakami *et al.*, 2002).

In the present study, we chose phenolsulfonphthalein (PSP) and fluorescein isothiocyanate dextran (Mw 4400, FD-4) as model compounds with different renal disposition characteristics (active and passive transport type compounds, respectively), and examined their delivery advantage to the unilateral kidney. Moreover, we constructed a physiological pharmacokinetic model after application to the rat unilateral kidney surface, in order to verify the delivery advantage of the model compound after kidney surface application, by its comparison with *i.v.* and intraarterial (*i.a.*) administrations.

MATERIALS AND METHODS

Materials

PSP was purchased from Nacalai Tesque, Inc. (Kyoto, Japan). FD-4 was obtained from the Sigma Chemical Co. (St Louis, MO, USA). All other chemicals were of reagent grade.

Animal Experiment

All animal procedures in the present study conformed to the Guide lines for Animal Experimentation in Nagasaki University. Under anesthesia, a cylindrical diffusion cell (i.d. 6 mm, area 0.28 cm²) was attached to the right kidney surface of male Wistar rats (250 - 280 g) with biocompatible glue (Aron Alpha, Sankyo Co. Ltd., Tokyo, Japan). The left femoral artery and both the right and left ureters were cannulated with polyethylene tubes. PSP at doses of 0.3, 1 and 1.5 mg or FD-4 (dose: 1 mg) were added directly into the diffusion cell. Then, blood samples and the urine from the right and left ureters were collected simultaneously at selected times. Also, the solution remaining in the diffusion cell was withdrawn, followed by excision of the kidneys and liver. The excised kidneys and liver were homogenized in three-fold of their weights of isotonic phosphate buffer (pH 7.4). After 1 ml of acetone was added to 1 ml of the homogenate, the mixture was shaken for 15 min, followed by centrifugation at 2,000 x g for 20 min at 4 °C. The resulting supernatant was subjected to assay.

As a comparison, the model compound solution was injected intravenously into the jugular vein of rats. Then, blood samples and the urine from the right and left ureters were collected simultaneously at selected times.

Also, the kidneys and liver were excised and homogenized by the same method described above.

Analytical Methods

The concentrations of free PSP in the plasma, kidney, liver, urine and the solution remaining in the diffusion cell were determined spectrophotometrically at 560 nm after dilution with 1 M NaOH. The total concentrations of free PSP and its metabolite were measured in the same manner after they were subjected to acid hydrolysis (2 M HCl at 100°C for 30 min) (Hart and Schanker, 1966). The concentration of the PSP metabolite (glucuronic acid conjugate) was estimated from the difference between these values. The PSP metabolite could not be detected in the plasma.

The concentrations of FD-4 as fluorescence in the urine, plasma, kidney and the solution remaining in the diffusion cell were measured by a spectrophotofluorometer at excitation and emission wavelengths of 489 and 515 nm, respectively.

Calculation of Organ Clearance

The areas under the curves of both the plasma (AUC_p) and renal concentration (AUC_r) profiles of free PSP were calculated using a linear trapezoidal formula (Yamaoka *et*

al., 1978). By utilizing urinary excretion values of free PSP from each kidney (X_u), the lateral renal clearance CL_r values (right: CL_{kidney1} , left: CL_{kidney2}) were calculated according to the following equation:

$$CL_r = \frac{X_u}{AUC_p} \quad (1)$$

Physiological Pharmacokinetic Analysis: Model Development and Verification

Figures 1A, 1B and 1C depict the physiological models employed in the present study for physiological pharmacokinetic analysis of PSP after i.v. administration (A), kidney surface application (B), and i.a. bolus administration or i.a. constant infusion (C), respectively. Each compartment is assumed to be under well-stirred conditions. In these models, elimination of PSP is considered to take place via both the liver and kidneys. The mass-balance equations of PSP in the cases of several administration methods are defined in the Appendix section. Each initial condition is also described in the Appendix section.

The model-fitting of the i.v. administration data of PSP was performed by the MULTI(RUNGE) program (Yamaoka and Nakagawa, 1983), written in Fortran 77. The differential equations 2-5 are numerically solved by the Runge-Kutta-

Gill method. The obtained PSP K_p values of the kidney and liver were used for the computer simulation of other administration methods. In the cases of kidney surface application and i.a. bolus or constant infusion, the concentrations of PSP in the plasma and each kidney were calculated using the Runge-Kutta-Gill method from the equations in the Appendix section.

RESULTS AND DISCUSSION

Plasma Concentrations and Urinary Excretion Rates of PSP from Each Kidney after Application to the Rat Right Kidney Surface

Figures 2A and 2B show the plasma concentration profiles of free PSP after i.v. administration or application to the right kidney surface in rats at a dose of 1 mg, respectively. The data of i.v. administration (Figure 2A) was employed for comparison of the delivery advantage and to provide basic information for computer simulations. The plasma concentration of free PSP reached a maximum 1 h after application to the right kidney surface and decreased thereafter. The absorption ratio of PSP from the right kidney surface in 4 h was calculated to be 85.6 % of dose.

Figures 3A and 3B show the urinary excretion rate

profiles of free PSP and its metabolite from each kidney after i.v. administration or application to the right kidney surface in rats at a dose of 1 mg, respectively. The urinary excretion rates of free PSP and its metabolite from each kidney were almost identical after i.v. administration (Figure 3A). On the other hand, the absorbed free PSP was excreted into the urine from the applied (right) kidney significantly until 120 min after application to the rat right kidney surface (Figure 3B), although the difference was not significant in the case of the PSP metabolite.

As listed in Table I, the urinary recovery of total PSP (free PSP and its metabolite) from the applied kidney in 4 h was twice that from the non-applied (left) kidney after application to the right kidney surface. There was also a significant difference in the urinary recovery of free PSP between the applied and non-applied kidneys, whereas urinary recovery of the PSP metabolite was not different, probably because the PSP metabolite distributed into both the applied and non-applied kidneys after metabolism by the liver. In addition, the lateral renal clearances (CL_r) of PSP from the applied (CL_{kidney1}) and non-applied kidney (CL_{kidney2}) were calculated to be 0.21 and 0.08 ml/min, respectively (Table I), suggesting a lateral delivery advantage by kidney surface application.

PSP Concentration in Each Kidney after Application to the Rat Right Kidney Surface

Figures 4A and 4B show the concentration of free PSP in the right and left kidneys after i.v. administration (A) or application to the right kidney surface (B) in rats, respectively. The free PSP concentration after i.v. administration in each kidney declined rapidly, and the concentration ratio in the right and left kidneys was about 1 at any time point (Figure 4A). On the contrary, the free PSP concentration in the applied kidney (App) 5 min after application was 2.4 times higher than in the non-applied kidney (Non-app) (Figure 4B). Thereafter, the App/Non-app concentration ratio of free PSP had a relatively high range between 1.2 and 1.5. Then, distribution of free PSP in the non-applied kidney could be due to re-distribution from the systemic circulation (Figure 4B).

Moreover, the free PSP concentration in the applied kidney was considerably higher than the plasma concentration (data not shown). The concentration ratio (App/plasma) increased with time to 5.1 at 120 min, implying a gradual accumulation of PSP in the applied kidney. The lateral availability, expressed by the AUC_r

value of free PSP concentration in the applied kidney (1881.3 $\mu\text{g}\cdot\text{min}/\text{g}$ kidney), was 1.3-fold larger than that of the non-applied kidney (1452.7 $\mu\text{g}\cdot\text{min}/\text{g}$ kidney). These results suggest the usefulness of the kidney surface administration method for drug delivery to the unilateral kidney.

Urinary Excretion and Renal Concentration of FD-4 from Each Kidney after Application to the Rat Right Kidney Surface

For the purposes of examining different types of compounds, FD-4 (Mw 4400) was selected as a different renal disposition type excreted by glomerular filtration. The absorption ratio of FD-4 from the right kidney surface in 4 h was calculated from the amount remaining in the diffusion cell to be 21.2 % of dose. Table II lists the renal concentration and urinary recovery in 4 h of FD-4 after application to the right kidney surface. There were no marked differences between the applied and non-applied kidneys concerning their renal concentrations and urinary recoveries of FD-4 (Table II). In addition, the renal clearances (CL_r) of FD-4 from the applied and non-applied kidneys were calculated to be 0.62 and 0.56 ml/min, respectively. This was probably because the first-pass extraction ratio of FD-4 by the applied kidney was so low

that most of the absorbed FD-4 tended to distribute in the plasma (1.4 µg/ml at 4 h) and other organs, including the non-applied kidney.

Accordingly, there was a marked difference in the delivery advantage to the applied kidney between the different excretion types of active tubular secretion (PSP) and glomerular filtration (FD-4), i.e., active or passive renal uptake, respectively.

Dose Dependency of PSP Absorption and the Lateral Delivery Advantage from Each Kidney

The effects of different administration conditions, such as the effect of application dose on absorption and renal concentration, were examined. The recoveries (% of dose) of free PSP and its metabolite in the bile, urine and diffusion cell at doses of 0.3, 1 and 1.5 mg are summarized in Table III. The absorption ratios of PSP in 4 h, calculated from the amount remaining in the diffusion cell, were 83.3, 85.6 and 85.0 % of dose at doses of 0.3, 1 and 1.5 mg, respectively, indicating that PSP absorption from the rat kidney surface shows no saturation within the dose range used.

There was no dose-dependency for the lateral delivery advantage of PSP, judging from the App/Non-app

concentration ratios at 60 and 120 min, with the ratio between 1.2 and 1.4 (Table IV). In addition, the App/Non-app ratios of urinary excretion of free PSP in 4 h did not change significantly among the three doses (ratio: 1.7-2.2). The linear dose proportionality of PSP suggests that local renal concentrations are a direct result of tissue uptake and distribution of PSP after absorption from the applied kidney surface.

Pharmacokinetic Verification of Preferential PSP Distribution in the Applied and Non-applied Kidneys Based on Physiological Model Analysis

Since a good physiological pharmacokinetic model could form theoretical considerations of the advantages of kidney surface application of PSP, we constructed a physiological model for PSP as illustrated in Figure 1B, and simulated the PSP distribution in each kidney and the plasma after kidney surface application. The physiological and pharmacokinetic parameters necessary for the computer simulation are listed in Table V.

An identical PSP distribution for each kidney after i.v. administration to the rat (Figure 5A) was confirmed by curve-fitting based on the physiological model (Figure 1A). Figure 5B illustrates the computer simulations of free PSP

concentration in each kidney and the plasma after application to the right kidney surface in rats at a dose of 1 mg, together with experimental data. In general, the simulations were consistent with the respective experimental values (Figure 5B), supporting the validity of this pharmacokinetic analysis. From the simulation curve (Figure 5B), PSP applied to the lateral kidney surface is considered to distribute preferentially in the administered kidney for a long period of time.

Comparison of the Lateral Delivery Advantage by Kidney Surface Application with i.a. Administration

We compared the lateral delivery advantage by kidney surface application, based on this physiological model analysis, with i.a. administration methods as useful routes for renal carcinoma therapy. We used PSP as a model and performed physiological simulations in the rat under normal administration conditions using several parameters after i.a. bolus or constant infusion. Figure 6 shows the simulations of free PSP concentrations in the plasma and each kidney based on the physiological organ model (Figure 1C), by i.a. bolus administration (A) or constant infusion at a rate of 4.2 $\mu\text{g}/\text{min}$ for 240 min (dose: 1 mg) (B) into the rat renal artery.

In the case of i.a. administration with a bolus (Figure 6A), the concentration of free PSP in the applied kidney declined to the same level as the non-applied kidney approximately 5 min after administration, indicating the rapid elimination of the lateral delivery advantage. On the other hand, the free PSP concentration in the applied kidney was sustained at a higher level compared with the non-applied kidney in the case of i.a. constant infusion, as shown in Figure 6B. However, the rapid disappearance of the lateral delivery advantage was recognized soon after the removal of constant infusion (240 min). Because i.a. constant infusion has the disadvantage of rapid elimination after the cessation of infusion, the continuation of constant infusion is necessary to retain an effective drug concentration in the kidney.

From the physiological pharmacokinetic analysis, the kidney surface application route could provide an advantage similar to renal artery infusion, since the absorbed drug would be taken up by the kidney before reaching the systemic circulation. For the enhancement of renal uptake and reduction of toxicity in the normal organs outside the kidney, including the other kidney, the specific bioactive recognition system in the kidney needs to be appropriately added to the molecules, such as a receptor for specific peptides (Elfarra *et al.*, 1995; Hwang and Elfarra, 1991;

Lash *et al.*, 1997) and/or non-specific association by electric cationic charges (Takakura *et al.*, 1990). Furthermore, the delivery advantage and drug concentration in the applied lateral kidney could be retained by pharmaceutical formulation modifications such as viscous and bioadhesive additives, by increasing contact time with the membrane around the site of absorption. In the case of administration routes via the blood stream, such as i.v. and i.a. administration, modification of the pharmaceutical formulation is difficult due to washout by fast renal plasma flow. Therefore, the kidney surface application is considered to be advantageous to improve the control of drug concentration and limit distribution around the applied area.

In conclusion, direct drug application of PSP as a model to the unilateral kidney surface could effectively provide an efficient drug delivery system to the unilateral kidney. Its lateral delivery advantage has been verified based on physiological pharmacokinetic model simulation.

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NOMENCLATURE

C_{kidney1} : right (applied) kidney concentration ($\mu\text{g/ml}$)

C_{kidney2} : left (non-applied) kidney concentration ($\mu\text{g/ml}$)

C_{liver} : liver concentration ($\mu\text{g/ml}$)

C_{plasma} : plasma concentration ($\mu\text{g/ml}$)

C_{cell} : drug concentration in diffusion cell ($\mu\text{g/ml}$)

V_{kidney1} : volume of right (applied) kidney (ml)

V_{kidney2} : volume of left (non-applied) kidney (ml)

V_{liver} : volume of liver (ml)

V_{plasma} : volume of plasma (ml)

V_{cell} : volume of applied drug solution in diffusion cell (ml)

Q_{kidney1} : plasma flow rate of right (applied) kidney (ml/min)

Q_{kidney2} : plasma flow rate of left (non-applied) kidney (ml/min)

Q_{liver} : liver plasma flow rate (ml/min)

Q_{plasma} : total plasma flow rate (ml/min)

k_a : first-order absorption rate constant from kidney surface (min^{-1})

R_{infusion} : constant renal artery infusion rate ($\mu\text{g}/\text{min}$)

CL_a : absorption clearance from kidney surface (ml/min)

$K_{p,\text{kidney1}}$: right (applied) kidney to plasma partition coefficient

$K_{p,\text{kidney2}}$: left (non-applied) kidney to plasma partition coefficient

$K_{p,\text{liver}}$: liver to plasma partition coefficient

$CL_{\text{int},\text{kidney1}}$: intrinsic clearance of right (applied) kidney (ml/min)

$CL_{\text{int},\text{kidney2}}$: intrinsic clearance of left (non-applied) kidney (ml/min)

$CL_{\text{int},\text{liver}}$: intrinsic clearance of liver (ml/min)

CL_a : absorption clearance from diffusion cell (ml/min)

f_u : unbound fraction in plasma

CL_{liver} : hepatic clearance (ml/min)

CL_r : lateral renal clearance (ml/min)

CL_{kidney1} : lateral renal clearance (CL_r) of right (applied) kidney

CL_{kidney2} : lateral renal clearance (CL_r) of left (non-applied) kidney

APPENDIX

Mass-balance equations for the physiological model by different administration routes

(i) i.v. administration

$$V_{\text{plasma}} \cdot \frac{dC_{\text{plasma}}}{dt} = Q_{\text{kidney1}} \cdot \frac{C_{\text{kidney1}}}{K_{p,\text{kidney1}}} + Q_{\text{kidney2}} \cdot \frac{C_{\text{kidney2}}}{K_{p,\text{kidney2}}} + Q_{\text{liver}} \cdot \frac{C_{\text{liver}}}{K_{p,\text{liver}}} - Q_{\text{plasma}} \cdot C_{\text{plasma}} \quad (2)$$

$$V_{\text{kidney1}} \cdot \frac{dC_{\text{kidney1}}}{dt} = Q_{\text{kidney1}} \cdot C_{\text{plasma}} - Q_{\text{kidney1}} \cdot \frac{C_{\text{kidney1}}}{K_{p,\text{kidney1}}} - f_u \cdot \text{CL}_{\text{int,kidney1}} \cdot \frac{C_{\text{kidney1}}}{K_{p,\text{kidney1}}} \quad (3)$$

$$V_{\text{kidney2}} \cdot \frac{dC_{\text{kidney2}}}{dt} = Q_{\text{kidney2}} \cdot C_{\text{plasma}} - Q_{\text{kidney2}} \cdot \frac{C_{\text{kidney2}}}{K_{p,\text{kidney2}}} - f_u \cdot \text{CL}_{\text{int,kidney2}} \cdot \frac{C_{\text{kidney2}}}{K_{p,\text{kidney2}}} \quad (4)$$

$$V_{\text{liver}} \cdot \frac{dC_{\text{liver}}}{dt} = Q_{\text{liver}} \cdot C_{\text{plasma}} - Q_{\text{liver}} \cdot \frac{C_{\text{liver}}}{K_{p,\text{liver}}} - f_u \cdot \text{CL}_{\text{int,liver}} \cdot \frac{C_{\text{liver}}}{K_{p,\text{liver}}} \quad (5)$$

Initial condition ($t = 0$) is $V_{\text{plasma}} \cdot C_{\text{plasma}} = \text{dose}$, $C_{\text{kidney1}} = C_{\text{kidney2}} = C_{\text{liver}} = 0$.

(ii) Kidney surface application

$$V_{\text{cell}} \cdot \frac{dC_{\text{cell}}}{dt} = -V_{\text{cell}} \cdot k_a \cdot C_{\text{cell}} = -\text{CL}_a \cdot C_{\text{cell}} \quad (6)$$

$$V_{\text{plasma}} \cdot \frac{dC_{\text{plasma}}}{dt} = Q_{\text{kidney1}} \cdot \frac{C_{\text{kidney1}}}{K_{p,\text{kidney1}}} + Q_{\text{kidney2}} \cdot \frac{C_{\text{kidney2}}}{K_{p,\text{kidney2}}} + Q_{\text{liver}} \cdot \frac{C_{\text{liver}}}{K_{p,\text{liver}}} - Q_{\text{plasma}} \cdot C_{\text{plasma}} \quad (7)$$

$$V_{\text{kidney1}} \cdot \frac{dC_{\text{kidney1}}}{dt} = V_{\text{cell}} \cdot k_a \cdot C_{\text{cell}} + Q_{\text{kidney1}} \cdot C_{\text{plasma}} - Q_{\text{kidney1}} \cdot \frac{C_{\text{kidney1}}}{K_{p,\text{kidney1}}} - f_u \cdot \text{CL}_{\text{int,kidney1}} \cdot \frac{C_{\text{kidney1}}}{K_{p,\text{kidney1}}} \quad (8)$$

$$V_{\text{kidney2}} \cdot \frac{dC_{\text{kidney2}}}{dt} = Q_{\text{kidney2}} \cdot C_{\text{plasma}} - Q_{\text{kidney2}} \cdot \frac{C_{\text{kidney2}}}{K_{p,\text{kidney2}}} - f_u \cdot \text{CL}_{\text{int,kidney2}} \cdot \frac{C_{\text{kidney2}}}{K_{p,\text{kidney2}}} \quad (9)$$

$$V_{\text{liver}} \cdot \frac{dC_{\text{liver}}}{dt} = Q_{\text{liver}} \cdot C_{\text{plasma}} - Q_{\text{liver}} \cdot \frac{C_{\text{liver}}}{K_{p,\text{liver}}} - f_u \cdot \text{CL}_{\text{int,liver}} \cdot \frac{C_{\text{liver}}}{K_{p,\text{liver}}} \quad (10)$$

Initial condition ($t = 0$) is $V_{\text{cell}} \cdot C_{\text{cell}} = \text{dose}$, $C_{\text{kidney1}} = C_{\text{kidney2}} = C_{\text{liver}} = 0$.

(iii) i.a. bolus administration to unilateral kidney

$$V_{\text{plasma}} \cdot \frac{dC_{\text{plasma}}}{dt} = Q_{\text{kidney1}} \cdot \frac{C_{\text{kidney1}}}{K_{p,\text{kidney1}}} + Q_{\text{kidney2}} \cdot \frac{C_{\text{kidney2}}}{K_{p,\text{kidney2}}} + Q_{\text{liver}} \cdot \frac{C_{\text{liver}}}{K_{p,\text{liver}}} - Q_{\text{plasma}} \cdot C_{\text{plasma}} \quad (11)$$

$$V_{\text{kidney1}} \cdot \frac{dC_{\text{kidney1}}}{dt} = Q_{\text{kidney1}} \cdot C_{\text{plasma}} - Q_{\text{kidney1}} \cdot \frac{C_{\text{kidney1}}}{K_{p,\text{kidney1}}} - f_u \cdot \text{CL}_{\text{int,kidney1}} \cdot \frac{C_{\text{kidney1}}}{K_{p,\text{kidney1}}} \quad (12)$$

$$V_{\text{kidney2}} \cdot \frac{dC_{\text{kidney2}}}{dt} = Q_{\text{kidney2}} \cdot C_{\text{plasma}} - Q_{\text{kidney2}} \cdot \frac{C_{\text{kidney2}}}{K_{p,\text{kidney2}}} - f_u \cdot \text{CL}_{\text{int,kidney2}} \cdot \frac{C_{\text{kidney2}}}{K_{p,\text{kidney2}}} \quad (13)$$

$$V_{\text{liver}} \cdot \frac{dC_{\text{liver}}}{dt} = Q_{\text{liver}} \cdot C_{\text{plasma}} - Q_{\text{liver}} \cdot \frac{C_{\text{liver}}}{K_{p,\text{liver}}} - f_u \cdot \text{CL}_{\text{int,liver}} \cdot \frac{C_{\text{liver}}}{K_{p,\text{liver}}} \quad (14)$$

Initial condition ($t = 0$) is $V_{\text{kidney1}} \cdot C_{\text{kidney1}} = \text{dose}$, $C_{\text{plasma}} = C_{\text{kidney2}} = C_{\text{liver}} = 0$.

(iv) i.a. constant infusion to unilateral kidney

$$V_{\text{plasma}} \cdot \frac{dC_{\text{plasma}}}{dt} = Q_{\text{kidney1}} \cdot \frac{C_{\text{kidney1}}}{K_{p,\text{kidney1}}} + Q_{\text{kidney2}} \cdot \frac{C_{\text{kidney2}}}{K_{p,\text{kidney2}}} + Q_{\text{liver}} \cdot \frac{C_{\text{liver}}}{K_{p,\text{liver}}} - Q_{\text{plasma}} \cdot C_{\text{plasma}} \quad (15)$$

$$V_{\text{kidney1}} \cdot \frac{dC_{\text{kidney1}}}{dt} = R_{\text{infusion}} + Q_{\text{kidney1}} \cdot C_{\text{plasma}} - Q_{\text{kidney1}} \cdot \frac{C_{\text{kidney1}}}{K_{p,\text{kidney1}}} - f_u \cdot \text{CL}_{\text{int,kidney1}} \cdot \frac{C_{\text{kidney1}}}{K_{p,\text{kidney1}}} \quad (16)$$

$$V_{\text{kidney2}} \cdot \frac{dC_{\text{kidney2}}}{dt} = Q_{\text{kidney2}} \cdot C_{\text{plasma}} - Q_{\text{kidney2}} \cdot \frac{C_{\text{kidney2}}}{K_{p,\text{kidney2}}} - f_u \cdot \text{CL}_{\text{int,kidney2}} \cdot \frac{C_{\text{kidney2}}}{K_{p,\text{kidney2}}} \quad (17)$$

$$V_{\text{liver}} \cdot \frac{dC_{\text{liver}}}{dt} = Q_{\text{liver}} \cdot C_{\text{plasma}} - Q_{\text{liver}} \cdot \frac{C_{\text{liver}}}{K_{p,\text{liver}}} - f_u \cdot \text{CL}_{\text{int,liver}} \cdot \frac{C_{\text{liver}}}{K_{p,\text{liver}}} \quad (18)$$

Initial condition ($t = 0$) is $C_{\text{plasma}} = C_{\text{kidney1}} = C_{\text{kidney2}} = C_{\text{liver}} = 0$.

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TABLE I Urinary recoveries of free PSP and its metabolite at 4 h and lateral renal clearance values (CL_r) after i.v. administration or application to the rat right kidney surface at a dose of 1 mg

Excretion site	Urinary recovery (% of dose)			CL_r (ml/min)
	Free	Metabolite	Total	
i.v. administration				
Right kidney	14.3 ±2.4	5.7 ±0.7	20.0 ±2.5	0.18 ±0.02
Left kidney	13.2 ±2.1	4.9 ±0.8	18.2 ±1.9	0.16 ±0.02
(Right/Left)	(1.1)	(1.2)	(1.1)	(1.1)
Kidney surface application (KSA)				
Applied kidney (App)	14.1* ±2.6	5.4 ±1.2	19.5 ±9.8	0.21 ±0.04
Non-applied kidney (Non-app)	6.1 ±1.5	3.7 ±0.5	9.8 ±1.5	0.08 ±0.02
(App/Non-app)	(2.3)	(1.5)	(2.0)	(2.6)

Each value is the mean ± S.E. of four (i.v.) and five (KSA) experiments.

Significantly different from the non-applied kidney with the use of the paired Student's *t*-test (**P* < 0.05).

Ratios (Right/Left or App/Non-app) are shown in parenthesis.

TABLE II Renal concentration and urinary recovery in 4 h of FD-4 after application to the rat right kidney surface at a dose of 1 mg

Excretion site	Concentration ($\mu\text{g/g}$ kidney)				Urine
	15 min	30 min	60 min	120 min (% of dose)	
App	1.9 ± 0.5	2.9 ± 0.8	5.6 ± 1.1	5.3 ± 0.8	5.25 ± 1.40
Non-app	1.8 ± 0.7	3.2 ± 0.9	6.2 ± 1.1	4.6 ± 0.9	5.12 ± 1.26
(App/Non-app)	(1.1)	(0.9)	(0.9)	(1.2)	(1.0)

Each value is the mean \pm S.E. of at least four experiments. Ratio (App/Non-app) are shown in parenthesis.

TABLE III Recoveries (% of dose) of free PSP and its metabolite in 4 h after application to the rat right kidney surface at doses of 0.3, 1 and 1.5 mg

Dose (mg)	Diffusion cell (%) Free	Urinary recovery (% of dose)					
		Applied kidney			Non-applied kidney		
		Free	Metabolite	Total	Free	Metabolite	Total
		-----	-----	-----	-----	-----	-----
0.3	16.7 ±2.7	14.1* ±3.1	N.D.	14.1 ±3.1	6.3 ±1.5	N.D.	6.3 ±1.5
1	14.4 ±0.8	14.1* ±2.6	5.4 ±1.2	19.5 ±3.3	6.1 ±1.5	3.7 ±0.5	9.8 ±1.5
1.5	15.0 ±3.8	14.2* ±2.0	4.4 ±0.7	18.6 ±2.3	8.5 ±1.0	3.4 ±0.8	11.9 ±1.2

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

N.D.: not detected.

Significantly different from the non-applied kidney with the use of the paired Student's *t*-test (**P* < 0.05).

TABLE IV Renal concentrations of free PSP at 60 and 120 min after application to the rat right kidney surface at doses of 0.3, 1 and 1.5 mg

Dose (mg)	60 min			120 min		
	App	Non-app	(App/Non-app)	App	Non-app	(App/Non-app)
0.3	5.8* ±0.9	4.6 ±0.8	(1.3)	4.9*** ±0.7	3.4 ±0.6	(1.4)
1	20.8** ±1.0	17.1 ±1.1	(1.2)	15.6 ±2.1	12.5 ±0.8	(1.2)
1.5	26.2* ±4.0	20.2 ±3.8	(1.3)	20.8** ±2.7	15.1 ±1.6	(1.4)

Each value is the mean ± S.E. of at least four experiments. Significantly different from non-applied kidney with the use of the paired Student's *t*-test (* *P* < 0.05, ** *P* < 0.01, *** *P* < 0.001). Ratios (App/Non-app) are shown in parenthesis.

TABLE V Physiological and pharmacokinetic parameters of a 250 g rat used for computer simulation based on a physiological model

Parameter	Plasma	Lateral kidney	Liver
Compartment size, V (ml or g) ^a	7.8	0.5	10.0
Flow rate, Q (ml/min) ^b	13.4	1.4	8.0
$f_u \cdot CL_{int}$ (ml/min) ^c	-	0.10	0.77
Tissue to plasma partition coefficient K_p ^d	-	3.5	3.3

f_u : unbound fraction in plasma. CL_{int} : intrinsic clearance.

Parameters were from the literature ^a (Gerlowski and Jain, 1983), ^b (Lin *et al.*, 1982). ^c Results were obtained previously (Nishida *et al.*, 1995).

^d K_p values for the kidney and liver were obtained by simultaneous model-fitting the concentration profiles in the plasma, kidney and liver after i.v. administration to rats (Fig. 4A).

FIGURE CAPTIONS

FIGURE 1 Physiological pharmacokinetic models for the in vivo disposition of PSP after i.v. administration (A), application to the right (applied) kidney surface (B), i.a. administration with a bolus or a constant mode (C) in the rat.

Q_{plasma} : total plasma flow rate Q_{liver} : liver plasma flow rate Q_{kidney1} , Q_{kidney2} : lateral kidney plasma flow rate CL_a : absorption clearance CL_{liver} : hepatic clearance CL_{kidney1} , CL_{kidney2} : lateral renal clearance

FIGURE 2 Plasma concentration profiles of free PSP after i.v. administration (A) or application to the right kidney surface (B) in rats at a dose of 1 mg. Each point represents the mean \pm S.E. of four (A) or five (B) experiments.

FIGURE 3 Urinary excretion rate profiles of free PSP and its metabolite from each kidney after i.v. administration (A) or application to the right kidney surface (B) in rats at a dose of 1 mg.

Key: (A) Right kidney; ●: free PSP, ▲: PSP metabolite. Left kidney; ○: free PSP, △: PSP metabolite. (B) Applied kidney; ●: free PSP, ▲: PSP metabolite. Non-applied

kidney; ○: free PSP, △: PSP metabolite. Each point represents the mean ± S.E. of four (A) or five (B) experiments. Significantly different from the non-applied kidney by the paired Student's *t*-test (**P* < 0.05).

FIGURE 4 Renal concentration of free PSP after i.v. administration (A) or application to the right kidney surface (B) in rats.

(A) Right kidney (■), left kidney (□). (B) Applied kidney (■), non-applied kidney (□). Each column represents the mean + S.E. of at least four experiments. Significantly different from non-applied kidney with the use of the paired Student's *t*-test (** *P* < 0.01 *** *P* < 0.001).

FIGURE 5 Best-fitting curves after i.v. administration (A) and computer simulations of the free PSP concentrations in each kidney and the plasma after application to the right kidney surface (B) in rats at a dose of 1 mg.

Key: Right kidney (applied) (●); left kidney (non-applied) (○); plasma (△). The best-fitting and simulation curves were obtained based on the physiological and pharmacokinetic parameters listed in Table V. Line; broken line ---: plasma; solid line —: right (applied)

kidney; dotted line ···: left (non-applied) kidney.

FIGURE 6 Computer simulations of free PSP concentrations in the plasma, applied and non-applied kidneys based on a physiological organ model by bolus administration at a dose of 1 mg (A) or the constant infusion mode at a rate of 4.2 µg/min for 240 min (1 mg) (B) into the rat renal artery.

Simulation curves were obtained based on the physiological and pharmacokinetic parameters listed in Table V. Line: broken line ---: plasma; solid line —: applied kidney; dotted line ···: non-applied kidney.