

Influence of Liver Disease on Phenolsulfonphthalein Absorption from Liver Surface to Examine Possibility of Direct Liver Surface Application for Drug Targeting

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We have examined the influence of liver disease on drug absorption from the liver surface membrane, regarded as the first barrier for drug targeting to the liver. The main purpose of this study is to examine the possibility of direct liver surface application as a drug targeting method. We employed rats intoxicated with carbon tetrachloride (CCl₄) or D-galactosamine (GAL) as the liver disease model, and examined drug absorption characteristics after application to the liver surface, by utilizing a cylindrical diffusion cell. In the liver-intoxicated rats, about 90% of a low molecular weight drug, phenolsulfonphthalein (PSP), as a model was absorbed from the liver surface in 6 h, similar to the normal rats (no treatment). Although the absorption rate was increased in the CCl₄ group, whereas slightly retarded absorption was observed in GAL group, there should be no serious problem for the clinical use of liver surface application. The PSP absorption from the liver surface in the CCl₄ group was indicated to obey first-order kinetics by elimination profile from the diffusion cell. The first-order absorption rate constant K_a values of PSP from the liver surface, obtained by a compartment model and elimination profile, were increased 1.3-fold in the CCl₄ group compared to the control. Moreover, we performed drug application to the liver surface in the peritoneal cavity to assume clinical use. The K_a of PSP in the CCl₄ group was about 4-fold larger than in the normal group, implying the importance of estimating changes in peritoneal drug absorption as a result of liver disease. Consequently, it is expected that there will be no marked decline in the absorption rate from the liver surface in a liver disease state, leading us to apply this administration method for liver targeting.

Key words drug targeting; liver disease; absorption; organ surface

Normal treatment for liver diseases *via* intravenous and oral administration routes has been frustrated by inadequate distribution into the desired site in the liver, as well as toxicity in other organs. To overcome this problem, we have developed a direct route by application to the liver surface and have clarified the absorption mechanism.¹⁻⁶ In our series of investigations, we found that application to the liver surface was useful for drug delivery to the liver, since the targeting efficacy of macromolecules to the liver was improved despite their slow absorption⁵ and the site-selective localization in the liver was enhanced by modification of application conditions of drug solution on the liver surface, such as infusion rate and pharmaceutical formulation.⁷ Furthermore, we demonstrated novel liver- and lobe-selective gene transfection with naked plasmid DNA by utilizing this administration method.⁸ Recently, implantable infusion pumps have been developed for the treatment of several diseases,⁹ and endoscopic and laparoscopic operation techniques have been markedly improved.¹⁰ These advanced medical technologies should make possible the clinical use of administration of drugs on the liver surface.

For applying this administration method to liver disease therapy based on drug targeting to the specific region, there is concern whether drug absorption might be influenced by changes in the physiological and pathological factors accompanying the liver disease. In addition, cancer patients may present additional problems because of the altered peritoneal anatomy with adhesions and post-surgical scarring. Accordingly, it is necessary to understand the changes in absorption caused by dilution of the drug solution with serous fluid, binding of the drug to components in the peritoneal fluid, or adhesion of the drug to the peritoneal surrounding organs.

There have been several physiological studies that examined the change in drug absorption from the peritoneal cavity in the diseased state.^{11,12} However, drug absorption across the specific peritoneal membrane is still unknown, although much has been learned about the advantages and limitations of intraperitoneal (i.p.) administration during the past decade.^{13,14}

In the present study, we prepared two different liver-intoxication models, carbon tetrachloride (CCl₄) or D-galactosamine-treated rats. There have been a few studies that reported elimination of drugs such as indocyanine green^{15,16} and glycyrrhizin^{17,18} from the plasma after i.v. administration in the experimental liver-intoxicated models with CCl₄ or D-galactosamine. We selected phenolsulfonphthalein (PSP), a low molecular weight organic anion, as a model drug to evaluate absorption in the diseased state, and then examined the changes in the absorption characteristics after application to the liver surface in liver-intoxicated rats. The absorption characteristics of PSP from the liver surface in normal rats have been well characterized.^{1-3,6} Since PSP is eliminated by biliary and urinary excretion and by conjugative metabolism,¹⁹⁻²¹ PSP should be appropriate as a marker compound.

MATERIALS AND METHODS

Chemicals PSP and CCl₄ were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). D-Galactosamine was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All other chemicals were reagent grade products.

Animals Male Wistar rats were housed in cages in an air-conditioned room and maintained on standard rat food and water, freely available. All animal procedures in the pre-

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sent study conformed to the Guidelines for Animal Experimentation at Nagasaki University.

Intoxication of Rats with CCl₄ or D-Galactosamine
 We administered an olive oil solution of CCl₄ (0.4 ml/kg i.p. × 2 d) at 48 and 24 h prior to the *in vivo* experiment to the peritoneal cavity (CCl₄ group). For comparison, we administered D-galactosamine (300 mg/kg i.p. × 1 d) dissolved in saline to the peritoneal cavity (GAL group) at 24 h prior to the *in vivo* experiments.

We measured GOT and GPT levels in plasma by UV Test (Wako Pure Chemicals Industries, Ltd., Osaka, Japan) from plasma collected immediately before the *in vivo* experiment from each liver-intoxicated rat. The GOT level (Karmen unit) in the plasma of normal rats (66.3 ± 4.7, average ± standard error) was significantly increased in the CCl₄ (170.7 ± 15.6) and GAL (170.7 ± 21.2) groups. The same trend was observed in the plasma GPT levels (control, 24.9 ± 1.4; CCl₄ group, 56.0 ± 6.7; GAL group, 53.4 ± 7.8). We confirmed that liver injury was produced in the CCl₄ and GAL groups, judging from the significant increases in GOT and GPT levels compared with normal rats.

In Vivo Animal Experiment The Male Wistar rats (230–290 g) intoxicated with CCl₄ or D-galactosamine were anesthetized with sodium pentobarbital (50 mg/kg i.p.), and the left femoral artery and common bile duct were cannulated with a polyethylene tube. PSP was administered in different ways as follows.

Intravenous (i.v.) administration: The PSP solution (10 mg/ml × 0.1 ml) was injected into the jugular vein of the rats.

Application to the liver surface: A cylindrical diffusion cell (i.d. 9 mm, effective area 0.64 cm²) was attached to the rat liver surface of the left lobe with biocompatible glue (Aron Alpha, Sankyo Co. Ltd., Tokyo, Japan), and the PSP solution (3 or 10 mg/ml × 0.1 ml) was added to the diffusion cell directly, as illustrated in Fig. 1.

I.p. administration to the rat liver surface: PSP (1 mg) in 5 ml isotonic phosphate buffer (pH 7.4) was administered intraperitoneally to the liver surface in the rats. The injection point was the division between the right and left lobe.

Blood samples were collected from the femoral artery, followed by centrifugation. Bile was collected at selected times. At 4 or 6 h after the application, the urine remaining in the bladder was collected with a syringe, followed by withdrawing the solution remaining in the diffusion cell in the case of liver surface application.

Analytical Methods The concentrations of free PSP in the plasma, bile, urine and solution recovered from the diffusion cell were determined spectrophotometrically at 560 nm after dilution with 1 M NaOH solution. The total concentration of free PSP and its metabolite was similarly measured after subjection to acid hydrolysis.²²⁾

Calculation of Pharmacokinetic Parameters The moment parameters for the plasma concentration profile of free PSP (AUC_p , MRT_p), and biliary excretion of free PSP ($AUC_{b,f}$, $MRT_{b,f}$) and its metabolite ($AUC_{b,m}$, $MRT_{b,m}$), were calculated by numeral integration using a linear trapezoidal formula and extrapolated to infinite time based on a monoexponential equation.²³⁾

Compartment model analysis of the plasma concentration profile of PSP after application to the rat liver surface and i.p.

administration was performed as follows. First, the plasma concentration (C_p) profile of PSP after i.v. administration was fitted to the biexponential equation described in Eq. 1, 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 $\alpha \cdot \beta = K_{21} \cdot K_{el}$. D is the administration dose of PSP. 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 Eq. 2 in order to analyze the plasma concentration profile after application to the rat liver surface and i.p. administration. The result of i.v. administration of PSP was used under each disease state.

Next, in the same way, the plasma concentration profiles of PSP after application to the rat liver surface and i.p. administration were fitted based on a two-compartment model with first-order absorption, by the nonlinear least-squares method.²⁴⁾ In this model, the equation for the plasma concentration was as follows:

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

K_a is the first-order absorption rate constant for absorption into the blood stream from the rat liver surface. F is the availability after application to the rat liver surface.

Statistical Analysis Statistical analysis was performed by applying an unpaired Student's *t*-test after examination with analysis of variance (ANOVA). $p < 0.05$ was considered to be statistically significant. All results were expressed as mean values ± standard error of at least four experiments.

RESULTS AND DISCUSSION

The new administration route utilizing drug absorption from the liver surface should be effective for liver region specific targeting of drugs, such as bioactive peptide and genome medicine, if unchanged absorption rates of the drugs are guaranteed in the diseased state. Due to the decline in the metabolism and excretion of some compounds, several physiological changes in the body would occur in cases of liver disease; thus, there is a possibility of change in drug disposition. Therefore, we investigated whether drug absorption might be influenced by changes in the physiological and pathological factors accompanying liver disease.

Among the compounds causing liver injury, CCl₄ has often been employed to produce an acute liver injured animal model, because the symptom has been known to be similar to drug-induced liver injury and cirrhosis in humans.^{25,26)} On the other hand, the pathological organ image of the intoxicated liver by the D-galactosamine model attracts attention due to similarities to viral hepatitis in humans.²⁵⁾

The *in Vivo* Disposition of PSP after I.V. Administration in Liver-Intoxicated Model As a first step, we exam-

Table 1. Pharmacokinetic Parameters for Plasma Concentration Profiles of PSP and Cumulative Excretion (% of Dose) of PSP for 4 h after I.V. Administration to the Normal (None), CCl_4 - or D-Galactosamine (GAL)-Intoxicated Rats at a Dose of 1.0 mg

Treatment	K_{el} (min^{-1})	V_c (ml)	CL_{total} (ml/min)	Bile			Urine		
				Free	Metabolite	Total	Free	Metabolite	Total
None ^{a)}	0.033	20.4	0.63	39.1	19.8	58.9	20.2	6.8	26.9
	± 0.007	± 3.4	± 0.06	± 4.0	± 1.3	± 4.8	± 6.3	± 0.6	± 6.6
CCl_4	0.027	22.9	0.61	34.3	8.5*	42.8	25.9	6.2	32.1
	± 0.001	± 0.6	± 0.01	± 3.8	± 2.3	± 5.7	± 4.9	± 1.0	± 4.8
GAL	0.023	29.0	0.70	36.9	14.1	51.0	15.3	5.0	20.3
	± 0.004	± 2.0	± 0.14	± 4.0	± 2.6	± 5.2	± 4.9	± 1.2	± 5.9

Each value is the mean \pm S.E. of at least four experiments. Significantly different from the normal rat (* $p < 0.05$). a) Results were reported previously.²⁾

ined the change in PSP disposition in the rats after i.v. administration to each liver-intoxicated group. Figure 2A shows the plasma concentration profiles of PSP after i.v. administration on a semi-logarithmic scale, describing two phases in every profile. Retarded plasma disappearance was observed in the GAL group.

Table 1 summarizes the pharmacokinetic results of PSP after i.v. administration to each group using a non-linear least squares method program MULTI²⁴⁾ based on a two-compartment model. The distribution volume of the systemic circulation compartment (V_c) was larger in the GAL group than that in the normal group (no treatment). That is probably because the protein-unbound concentration of PSP in the plasma was increased according to the decrease in plasma total protein concentration in the GAL group.^{25,27,28)} However, the influence of liver disease was not recognized in the total body clearance CL_{total} . Table 1 also lists the cumulative excretion ratio for 4 h in the bile and urine of free PSP and its metabolite after i.v. administration. The biliary excretion ratio of the PSP metabolite in the CCl_4 group was significantly less than that in the normal group. This is likely responsible for the reduction in the conjugative metabolic process of PSP (glucuronic acid conjugate²²⁾) in the CCl_4 group.

Change in Absorption Characteristics of PSP after Application to the Rat Liver Surface As a next step, we examined the change in the absorption characteristics of PSP from the rat liver surface in the liver-intoxicated group. The absorption site was limited and restricted to the liver surface by attaching a cylindrical diffusion cell to the liver surface in rats, as illustrated in Fig. 1, without interference by absorption from the other sites. Figure 2B shows the plasma concentration profile of PSP after application to the rat liver surface under different conditions. Although the plasma concentration of PSP reached a maximum at 1 or 1.5 h after application in each group, the elimination of PSP from the plasma in the GAL group was delayed compared with the normal group, similar to i.v. administration (Fig. 2A).

Table 2 summarizes the recovery ratio of free PSP and its conjugate in the diffusion cell, bile and urine at 6 h after application to the rat liver surface. The absorption ratios of PSP from the liver surface at 6 h in the normal, CCl_4 and GAL group were calculated, respectively, to be 91.8, 93.3 and 89.0% of the dose from the recovery ratio in the diffusion cell. Moment parameters of PSP after application to the rat liver surface are listed in Table 3. There was a trend towards a longer MRT in the GAL group compared with the normal group. That could be mainly due to the prolongation of PSP

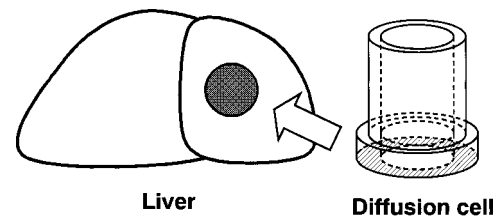


Fig. 1. A Cylindrical Diffusion Cell Made by Glass Employed to Selectively Study the Absorption of a Drug from the Rat Liver Surface

The diffusion cell was attached to the left lateral lobe of the rat liver, using Aron Alpha biocompatible glue in the hatched region. The limited and restricted absorption region is indicated by the closed mark. All dimensions are approximate.

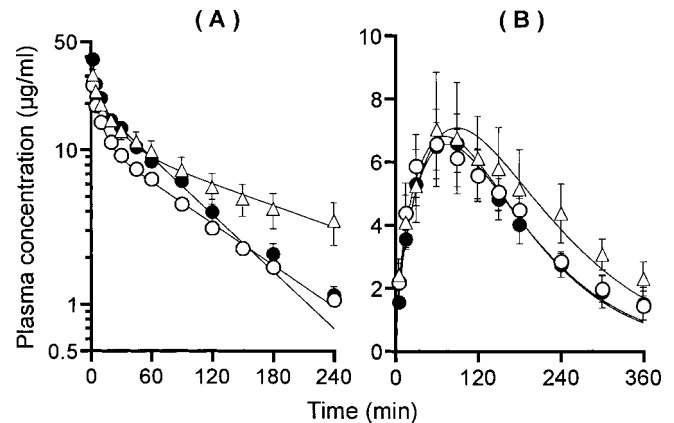


Fig. 2. Plasma Concentration Profiles of Free PSP after I.V. Administration (A) or Application to the Liver Surface (B) in the Normal (●), CCl_4 - (○) or D-Galactosamine-Intoxicated Rats (△) at a Dose of 1.0 mg

Curves show the simulated function based on the pharmacokinetic parameters shown in Tables 1 and 4. Each point represents the mean \pm S.E. of at least four experiments. Results in the normal group were reported previously.²⁾

residence time after absorption from the liver surface, judging from decreased K_{el} in the GAL group (Table 1).

Time Course of the Recovery Ratio of PSP in the Diffusion Cell To examine the absorption mechanism of PSP from the liver surface in the CCl_4 group, we studied the time course of the PSP recovery ratio from the diffusion cell at a dose of 0.3 mg (Fig. 3). We selected the CCl_4 group because its absorption rate from the liver surface was increased (Table 2), giving us important information on intraperitoneal drug delivery systems such as the existence of active transport. In addition, we compared the result of the normal rats at a dose of 0.3 mg which had been obtained previously.²⁾

The recovery ratio of PSP in the diffusion cell in the CCl_4

Table 2. Recovery (% of Dose) of PSP at 6 h after Application to the Normal, CCl₄- or D-Galactosamine-Intoxicated Rat Liver Surface at a Dose of 1.0 mg

Treatment	Diffusion cell		Bile		Urine		
	Free	Free	Metabolite	Total	Free	Metabolite	Total
None ^{a)}	8.2 ±2.0	31.8 ±2.5	18.8 ±2.8	50.7 ±5.0	17.1 ±3.9	12.3 ±3.1	29.3 ±5.8
CCl ₄	6.7 ±1.6	28.1 ±2.6	7.8* ±2.3	35.9 ±4.8	26.5 ±7.0	12.8 ±1.9	39.3 ±6.0
GAL	11.0 ±1.4	28.6 ±5.7	14.8 ±2.8	43.4 ±8.3	20.4 ±7.6	7.2 ±2.7	27.6 ±10.2

Each value is the mean ± S.E. of at least four experiments. Significantly different from the normal rat (**p* < 0.05). a) Results were reported previously.²⁾

Table 3. Moment Parameters of PSP after Application to the Normal, CCl₄- or D-Galactosamine-Intoxicated Rat Liver Surface at a Dose of 1.0 mg

Treatment	AUC _p (μg · min/ml)	MRT _p (min)	AUC _{b,f} (μg)	MRT _{b,f} (min)	AUC _{b,m} (μg)	MRT _{b,m} (min)
None ^{a)}	1695.1 ±237.0	267.2 ±53.2	356.5 ±29.2	190.6 ±22.3	225.2 ±34.9	235.0 ±42.0
CCl ₄	1856.3 ±124.7	255.1 ±64.5	320.8 ±42.4	183.2 ±26.7	131.8 ±46.1	359.6 ±58.1
GAL	1981.2 ±378.1	347.8 ±81.1	347.8 ±73.2	205.9 ±22.4	274.1 ±53.3	445.4 ±143.7

Each value is the mean ± S.E. of at least four experiments. a) Results were reported previously.²⁾

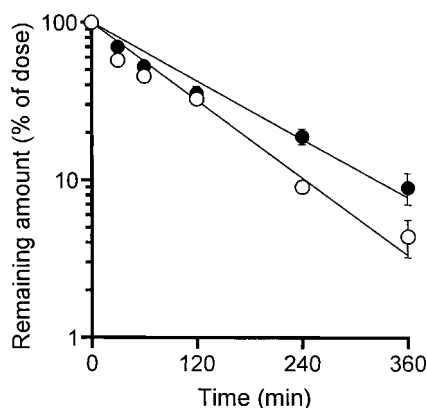


Fig. 3. Semi-Log Plot of the Free PSP Amount Remaining in the Diffusion Cell after Application to the Normal (●) or CCl₄-Intoxicated Rat Liver Surface (○) at a Dose of 0.3 mg (3 mg/ml × 0.1 ml)

Each point represents the mean ± S.E. of at least four experiments. Results in the normal group were reported previously.²⁾

group decreased linearly on a semi-logarithmic scale, indicating that the absorption process of PSP from the liver surface followed first-order kinetics in the CCl₄ group, similar to normal rats.²⁾ The *K_a* values in the normal and CCl₄ groups were calculated to be 6.9 × 10⁻³ and 9.2 × 10⁻³ min⁻¹, as listed in Table 4, respectively, from the regression straight lines in Fig. 3.

Compartment Model Analysis The *in vivo* disposition of PSP after application to the liver surface in the liver-intoxicated group could be explained using a compartment model incorporating a first-order absorption process similar to the normal group. We performed model-fitting of the plasma concentration profile after application to the rat liver surface using the non-linear least squares method program MULTI.²⁴⁾ As shown in Fig. 2B, the fitting curves and experimental values agreed in general, showing the validity of this

Table 4. First-Order Absorption Rate Constant (*K_a*) for PSP after Application to the Normal, CCl₄- or D-Galactosamine-Intoxicated Rat Liver Surface

Treatment	Dose (mg)	Method	<i>K_a</i> (min ⁻¹ × 10 ⁻³)
None ^{a)}	0.3	Elimination profile ^{b)}	6.9
	1.0	Curve-fitting ^{c)}	9.2 ± 1.0
CCl ₄	0.3	Elimination profile ^{b)}	9.2
	1.0	Curve-fitting ^{c)}	12.0 ± 2.5
GAL	1.0	Curve-fitting ^{c)}	8.9

The *K_a* value obtained by curve-fitting is the mean ± S.E. of at least four experiments. a) Results were reported previously.²⁾ b) *K_a* was calculated from the elimination profile of PSP from the diffusion cell (Fig. 3). c) *K_a* was obtained by the curve-fitting of the plasma concentration profile of PSP (Fig. 2B).

compartment model analysis, suggesting that this pharmacokinetic model can be applied in the liver-intoxicated model. In addition, both pharmacokinetic methods provide similar pharmacokinetic parameter estimates in every experimental group, as listed in Table 4. Obviously, the obtained *K_a* of PSP by curve-fitting was increased 1.3-fold in the CCl₄ group, similar to the trend by the elimination profile from the diffusion cell (Table 4).

Although the absorption rate of PSP was increased in the CCl₄ group and decreased slightly in the GAL group, there was no drastic difference between the normal and liver-intoxicated groups, implying feasibility for drug targeting utilizing the unchanged absorption rate from the liver surface, even in the diseased state. The liver is covered by a serous membrane containing monolayer squamous epithelial cells, and a space between the serous membrane and hepatic parenchymal cell is supported by connective tissue, in which capillaries of the portal vein and hepatic artery circulate. The absorption of PSP from the liver surface is considered to occur through the intercellular gap and pore in the serous membrane and connective tissue, rather than *via* the transcellular lipid route, since PSP is highly ionized and has a small partition coefficient.

cient at physiological pH.²⁹⁾ We speculate that the size or number of the intercellular gap and pore in the serous membrane and connective tissue could be altered by a physiological change in the liver disease state. However, further work needs to be performed to examine this point.

Influence of Liver Disease on the Absorption of PSP after Application in the Peritoneal Cavity Application of a drug to the liver surface for clinical use would closely follow conventional administration into the peritoneal cavity. The i.p. route of drugs has attracted attention because the peritoneal cavity is a potential space for peritoneal dialysis as a long-term renal replacement therapy and/or i.p. chemotherapy of cancer restricted to the peritoneal cavity, such as peritoneal carcinomatosis and ovarian cancer. The drug absorption from the peritoneal cavity, including the liver surface membrane, could be affected by various pathological and physiological factors such as adhesion among the internal organs or an increase in ascites. Therefore, we examined the absorption from the liver surface in the CCl₄ group, in which the absorption mechanism was investigated after i.p. administration to the liver surface.

Figure 4 shows the plasma concentration profiles of PSP after i.p. administration to the liver surface in the normal and CCl₄ groups. The maximum plasma concentration in the CCl₄ group was increased significantly, from 8.6 to 11.1 µg/ml, compared with the normal group, and the reduction in time to reach the maximum concentration was observed, indicating faster absorption in the CCl₄ group. Table 5 lists the cumulative excretion ratios of PSP in the bile and urine at 6 h after i.p. administration to the rat liver surface.

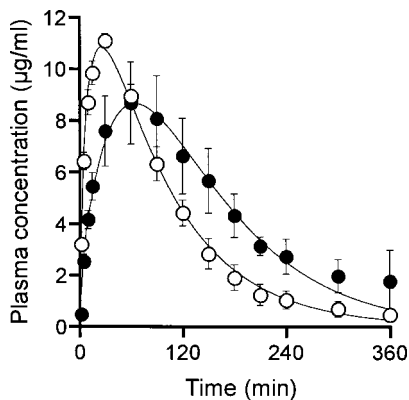


Fig. 4. Plasma Concentration Profiles of Free PSP after I.P. Administration to the Liver Surface in the Normal (●) or CCl₄-Intoxicated Rats (○) at a Dose of 1.0 mg (0.2 mg/ml × 5.0 ml)

Curves show the simulated function based on the pharmacokinetic parameters shown in Tables 1 and 6. Each point represents the mean ± S.E. of at least four experiments.

Table 5. Recovery (% of Dose) of PSP at 6 h after I.P. Administration to the Liver Surface in the Normal or CCl₄-Intoxicated Rats at a Dose of 1.0 mg (0.2 mg/ml × 5.0 ml)

Treatment	Bile			Urine		
	Free	Metabolite	Total	Free	Metabolite	Total
None	40.7 ±4.2	16.9 ±3.3	57.7 ±2.3	15.5 ±5.6	5.4 ±2.0	20.9 ±7.1
CCl ₄	25.5* ±3.3	5.6* ±1.4	31.1** ±3.7	27.5 ±2.6	23.2* ±5.6	50.7** ±4.1

Each value is the mean ± S.E. of at least four experiments. Significantly different from the normal rat (**p* < 0.05, ***p* < 0.01).

Each recovery ratio of PSP varied considerably between the normal and CCl₄ groups, although no significant change was recognized in the total biliary and urinary excretion ratios of free PSP and its metabolite. This is probably because the absorption of PSP from the peritoneal cavity in the CCl₄ group was rapid, and the hepatic uptake amount of PSP during the early phase after i.p. administration was decreased due to the decline in liver function by the CCl₄ treatment.

Pharmacokinetic Parameters after Application of PSP in the Peritoneal Cavity We calculated the *K_a* of PSP from the liver surface in the peritoneal cavity, as listed in Table 6, by using the curve-fitting program MULTI,²⁴⁾ assuming a two-compartment model with first-order absorption after i.p. administration to the liver surface. The *K_a* of PSP in the CCl₄ group was about 4-fold larger than that of the normal group, indicating a different trend of the result on the liver surface application using the diffusion cell (Table 4). Moment parameters of PSP after i.p. administration in the normal and CCl₄ groups are also summarized in Table 6. The difference in *MRT_p* values between i.p. and i.v. administration corresponds to the mean time value for the absorption from the peritoneal cavity (MAT). As listed in Table 6, the MAT after i.p. administration of PSP was shortened considerably by the CCl₄ treatment.

The reason for the marked change in *K_a* and MAT in the CCl₄ group after i.p. administration to the liver surface is suggested to be because the drug was distributed into the peritoneal cavity over a wide area according to the probable increase in the ascites amount accompanied by the CCl₄ treatment, since i.p. administration cannot limit the area for drug absorption. In addition, another factor of the change in the binding of a drug with components such as protein in the ascites, and the membrane drug diffusivity of the peritoneal organs, should be considered in the case of actual liver surface application in the peritoneal cavity.

In conclusion, there should be no marked influence of liver

Table 6. Moment Parameters and *K_a* of PSP after I.P. Administration to the Liver Surface in the Normal or CCl₄-Intoxicated Rats at a Dose of 1.0 mg (0.2 mg/ml × 5.0 ml)

Treatment	<i>AUC_p</i> (µg · min/ml)	<i>MRT_p</i> (min)	MAT (min)	<i>K_a</i> (min ⁻¹ × 10 ⁻³)
None	1596.2 ±149.1	136.1 ±9.6	75.5 ±9.6	13.6 ±2.4
CCl ₄	1498.1 ±131.5	118.9 ±14.8	44.8 ±14.8	52.9** ±4.8

Each value is the mean ± S.E. of at least four experiments. Significantly different from the normal rat (***p* < 0.01).

disease on drug absorption characteristics after liver surface application, although a few differences were recognized in the liver-intoxicated model. We expect that the liver surface application method could be applied for clinical use as a novel targeting method. In addition, these physiologically interesting results obtained in this study will be useful for optimizing peritoneal drug targeting.

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