Regular Article

Influence of Liver Intoxication by Carbon Tetrachloride or D-Galactosamine on Absorption of Fluorescein Isothiocyanate-Dextran-10 and Other Marker Compounds with Different Molecular Weights from the Rat Liver Surface

Hirotaka Miyamoto, Kayoko Tsuda, Tominori Honda, Ayako Tokunaga, Shintaro Fumoto, and Koyo Nishida*

Department of Pharmaceutics, Graduate School of Biomedical Sciences, Nagasaki University; 1–7–1 Sakamoto-machi, Nagasaki 852–8501, Japan.
Received August 14, 2019; accepted November 24, 2019

We examined the influence of liver disease on the absorption from the liver surface of fluorescein isothiocyanate (FITC)-dextran 10 (FD-10, MW: 11000) and several marker compounds with different molecular weights. The purpose of this study was to determine the feasibility of liver surface application of macromolecular compounds in the disease state. We used male Wistar rats treated with carbon tetrachloride (CCl_4) or D-galactosamine (GAL). FD-10 and other marker compounds were applied to the liver surface using a cylindrical diffusion cell in liver-intoxicated rats. The blood, bile, urine, and the remaining solution in the diffusion cell were collected for assay. FD-10 was absorbed by first-order kinetics from the liver surface in the liver-intoxicated rat models. The calculated rate constant k_a values in the normal, CCl_4 and GAL groups were 0.000965, 0.00125 and 0.00104 min^{-1}, respectively. Increased absorption of FITC-dextrans in the liver-intoxicated rats was observed. In both CCl_4 and GAL groups, an inverse relationship was observed between the molecular weight and k_a from the rat liver surface of the marker compounds. The limits of the molecular weight absorbed from the liver surface were extrapolated to be 71200, 135000, and 105000 in the normal, CCl_4, and GAL groups, respectively. In conclusion, increased absorbability from the rat liver surface indicates that liver surface application for liver targeting of macromolecules in the diseased state is indeed feasible. Therefore, our findings can support further research on liver surface application of drugs under liver disease.

Key words liver disease; absorption; liver surface; dextran; carbon tetrachloride; D-galactosamine

INTRODUCTION

In previous studies, we have shown that application of low-molecular-weight compounds such as phenolsulfonphthalein and 5-fluorouracil to the liver surface enhanced drug delivery to desired sites in the liver.[1–5] The absorbability of macromolecules as well as their targeting efficacy were also improved.[2,5] The liver surface application might be useful for reduction of side effect and improve the effect of drugs because drugs administered to liver surface were selectivity accumulate into applied site.

In the previous studies, we employed normal rats as models of the normal liver state. However, we have to evaluate the effect of liver disease on absorption of drugs after liver surface application to use this method for liver disease because liver disease could alter the absorption of drugs after liver surface application.

In this study, we used carbon tetrachloride (CCl_4) or D-galactosamine (GAL) induced hepatitis model as a liver disease. In the CCl_4-intoxicated model, the membrane structure was changed due to super oxidation of membrane lipid and it is similar to drug induced hepatitis.[6,7] On the other hand, in the GAL-intoxicated model, there have been reported demonstrating inhibition of mRNA and protein synthesis in the cell and it is similar to virus induced hepatitis.[8–11] We have previously used rats intoxicated with either CCl_4 or D-GAL as basic liver disease models to examine the influence of the disease state on the absorption characteristics of a low molecular weight drug phenolsulfonphthalein (PSP) from the liver surface.[12] Although a few differences compared with the normal liver model were observed, there was no significant decline in the absorption characteristics of PSP after liver surface application to the liver disease model.[12] This suggests that the disease state does not significantly affect absorption of small-molecule drugs after liver surface application.

To expand the findings of the previous study, here, we examined the influence of liver disease on the absorption of macromolecular compounds from the rat liver surface using fluorescein isothiocyanate-labeled dextran (FITC-dextran) with a molecular weight of 11 kDa (FD-10), since the absorption characteristics of FD-10 from the normal liver surface has been well characterized.[2] Next, we examined the effect of liver disease on the correlation between absorbability and molecular weight of compounds, using FITC-dextrans with different molecular weights as well as several low molecular weight drugs.

MATERIALS AND METHODS

Chemicals FITC-dextrans of different molecular weights: 4 kDa (FD-4), 10 kDa (FD-10), 40 kDa (FD-40), and 70 kDa (FD-70); bromosulfophthalein (BSP); CCl_4; and D-GAL were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Bromophenol blue (BPB) was obtained from Nacalai Tesque,

* To whom correspondence should be addressed. e-mail: koyo-n@nagasaki-u.ac.jp

Inc. (Kyoto, Japan). All other chemicals were reagent-grade products.

Animal Experiment Animal care and experimental procedures were performed in accordance with the Guidelines for Animal Experimentation of Nagasaki University with approval of the Institutional Animal Care and Use Committee.

Intoxication of Rats with CCl_4 or D-GAL Male Wistar rats (230–290 g) were used in this study. The liver intoxicated model rat was prepared according to previous report.[12] In briefly, the CCl_4 in olive oil solution (20% (v/v)) was intraperitoneally (i.p.) injected (0.4 mL/kg) 2 times every 24 h. The GAL dissolved in saline was i.p. injected (300 mg/kg) only 1 time. Twenty-four hours after final injection of CCl_4 or GAL, the pharmacokinetics of drug was evaluated.

The aspartate transaminase (AST) and alanine transaminase (ALT) levels in plasma were determined immediately before the *in vivo* experiment through the Wako Transaminase CII test (Wako Pure Chemical Corporation, Ltd., Osaka, Japan). The plasma was obtained before pharmacokinetic evaluation (24 h after final administration of CCl_4 or GAL). The plasma AST level (IU/L) of normal rats (49.5 ± 3.5, average ± standard error (S.E.)) was significantly increased in the CCl_4 (127.4 ± 11.6) and GAL (127.4 ± 15.8) groups. A similar trend was observed in the plasma ALT levels (control, 15.8 ± 0.9; CCl_4 group, 35.4 ± 4.2; GAL group, 33.8 ± 4.9). Liver injury was thus confirmed to have been induced in the CCl_4 and GAL groups.

Administration of Marker Compounds to the Intoxicated Rats *in Vivo* The rats intoxicated with CCl_4 or GAL were anesthetized with sodium pentobarbital (50 mg/kg, i.p.), and the left femoral artery and common bile duct were cannulated using a polyethylene tube. The marker compounds were administered as follows.

For intravenous (i.v.) administration, the FD-10 solution (10 mg/mL × 0.1 mL) was injected into the jugular vein of the rats.

Marker compounds were administered to the liver surface as previously reported.[5] Briefly, a cylindrical diffusion cell (i.d. 9 mm, effective area 0.64 cm^2) was attached to the liver surface of the left lobe with biocompatible glue (Aron Alpha, Sankyo Co., Ltd., Tokyo, Japan) in the rats. FITC-dextrans (FD-10, FD-40, or FD-70) solution (50 mg/mL × 0.1 mL) or other marker compounds (10 mg/mL × 1 mL) were directly added to the diffusion cell.

Blood samples were collected from the femoral artery, followed by centrifugation. Bile was collected at the selected times. At 4 or 6 h after the administration, the urine in the bladder was collected with a syringe. In case of liver surface application, the solution remaining in the diffusion cell was withdrawn at appropriate times. In some cases, for FD-10, the solution in the diffusion cell was collected at appropriate time intervals.

Analytical Methods The marker compounds in plasma, bile, urine, and the solution remaining in the diffusion cell were assayed as follows. The concentrations of FITC-dextrans as fluorescence were measured by a spectrophotofluorometer at excitation and emission wavelengths of 489 and 515 nm, respectively.[13] The concentration of BPB was determined spectrophotometrically at 591 nm.[14] The total concentration of free BSP and its metabolite was determined spectrophotometrically at 580 nm after dilution with 0.1 M NaOH solution.[15]

Calculation of Pharmacokinetic Parameters The plasma concentration (C_p) profile of FD-10 after i.v. administration was fitted to the biexponential eq. (1), by the nonlinear least-squares method.[16]

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

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 FD-10. 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. Area under the curve (AUC_p) and total body clearance (CL_{tot}) were calculated by $A/\alpha + B/\beta$ and $k_{el} \cdot V_c$, respectively.

Statistical Analysis All results are expressed as the mean value ± S.E. of at least three experiments. Statistical comparisons were performed by Dunnett's test after examining with ANOVA. $p < 0.05$ was considered to be statistically significant, compared to normal rats. Statistical analysis of correlation was performed by Pearson's correlation.

RESULTS

Disposition of FD-10 after i.v. Administration to the Liver-Intoxicated Rats We first evaluated the change in the disposition of FD-10 after its i.v. administration in the liver disease group. Figure 1A illustrates the plasma concentration-time profiles of FD-10 after i.v. administration to the normal and liver-intoxicated rats. The FD-10 plasma concentration at elimination phase was considerably higher in the GAL group than in the normal and the CCl_4 groups.

Table 1 summarizes the pharmacokinetic parameters of FD-10 analyzed based on a two-compartment model to the plasma concentration-time profiles after i.v. administration. k_{el} and CL_{tot} were lower in the liver-intoxicated group, whereas there was no change in V_c. CL_{tot} of FD-10 in the GAL group significantly decreased to about 60% of the control. Table 1 also lists the urinary and biliary excretion ratio 4 h after i.v. administration of FD-10. Significant decline in urinary and biliary excretion of FD-10 was observed in the GAL group.

Change in the Plasma Profile and Recovery Ratio of FD-10 after Application to the Rat Liver Surface in the Liver-Intoxicated Rats Figure 1B shows the plasma concentration-time profiles of FD-10 after application of FD-10 to the rat liver surface in the normal and liver-intoxicated models. Similar to normal rats, FD-10 appeared in the plasma after absorption from the liver surface in the liver diseased groups. While the plasma concentration of FD-10 was not changed in the CCl_4 group, plasma concentration of FD-10 in the GAL group was significantly increased compared to control.

Table 2 summarizes the recovery ratios of FD-10 in the diffusion cell, bile and urine 6 h after application to the rat liver surface. The absorption ratios calculated from the recovery ratio in the diffusion cell of FD-10 from the liver surface at 6 h in the normal, CCl_4 and GAL group were 20.0, 23.8 and 23.5% of the applied dose, respectively. No significant change in urinary and biliary excretion of FD-10 was observed in the CCl_4 and GAL groups.

Time Course of the Recovery Ratio in the Diffusion Cell after Application of FD-10 to the Rat Liver Surface in

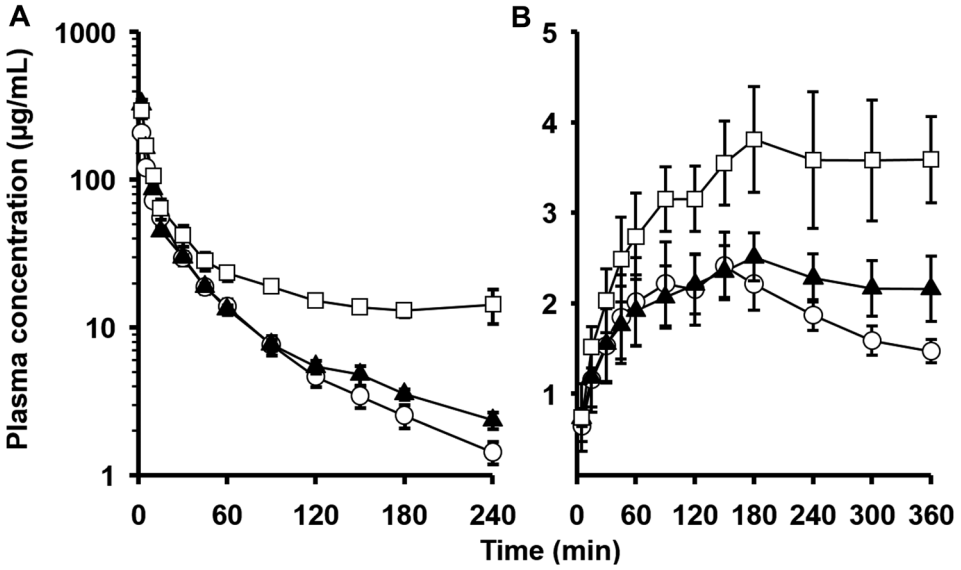

Fig. 1. Plasma Concentration Profiles of FD-10 after i.v. Administration on a Semi-log Scale (A) or Liver Surface Application (B) to the Normal (○), CCl_4- (▲) or GAL-Treated Rats (□) at a Dose of 5.0 mg

Each point represents the mean ± S.E. of at least three experiments.

Table 1. Pharmacokinetic Parameters Based on a Two-Compartment Model for the Plasma Concentration Profiles and Urinary and Biliary Excretion of FD-10 4 h after Its i.v. Administration to the Normal, CCl_4- or GAL-Treated Rats at a Dose of 5.0 mg

	Pharmacokinetic parameters				Recovery (% of dose)	
	k_{el} (min^{-1})	V_c (mL)	CL_{tot} (mL/min)	AUC_p ($\mu g \cdot min/mL$)	Bile	Urine
Normal	0.146 ± 0.014	10.8 ± 1.0	1.551 ± 0.118	3192 ± 167	0.9 ± 0.1	69.9 ± 1.6
CCl_4	0.131 ± 0.004	9.6 ± 0.4	1.267 ± 0.088	4131* ± 291	0.9 ± 0.1	64.8 ± 2.0
GAL	0.095 ± 0.019	10.4 ± 1.9	0.899** ± 0.056	5835* ± 371	0.4* ± 0.1	39.4** ± 3.0

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

Table 2. Recovery (% of Dose) of FD-10 in the Diffusion Cell, Bile, and Urine at 6 h after Application to the Normal, CCl_4- or GAL-Treated Rat Liver Surface

	Diffusion cell	Bile	Urine
Normal	80.0 ± 1.5	0.2 ± 0.1	10.7 ± 2.8
CCl_4	76.2 ± 2.1	0.2 ± 0.1	9.5 ± 3.1
GAL	76.5 ± 2.4	0.3 ± 0.1	8.9 ± 1.5

Each value represents the mean ± S.E. of at least four experiments.

Liver-Intoxicated Rats Figure 2 illustrates the time courses of the amount of FD-10 remaining in the diffusion cell after liver surface application of FD-10 in the liver-intoxicated rats. The amount of FD-10 in the diffusion cell decreased linearly on a semi-logarithmic scale under every condition. The first-order absorption rate constant k_a values in the normal, CCl_4 and GAL group were calculated, respectively, to be 0.000965, 0.00125 and 0.00104 min^{-1} from the regression straight-line slope shown in Fig. 2.

Absorption Ratio of Other Marker Compounds with Different Molecular Weights 6 h after Application to the Rat Liver Surface in Liver-Intoxicated Model We examined the absorption from the liver surface of the other marker compounds with different molecular weights in the CCl_4 and GAL groups. Figure 3 shows the absorption ratios calculated from the amount remaining in the diffusion cell of

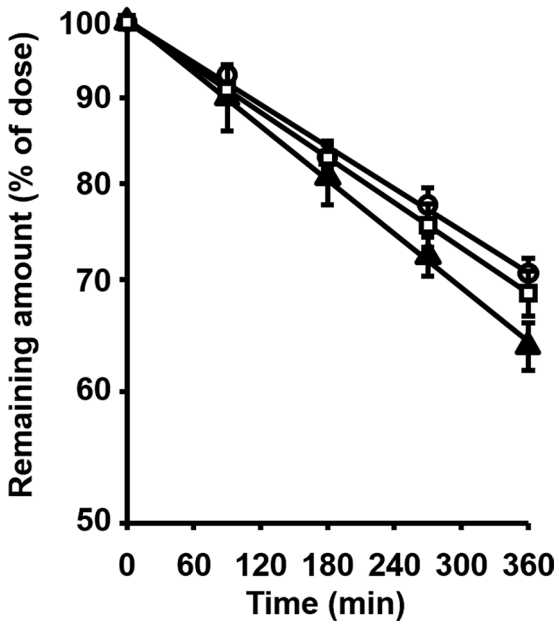

Fig. 2. Semi-log Plot of Time Courses of FD-10 Amount Remaining in the Diffusion Cell after Application to the Normal (○), CCl_4- (▲) or GAL-Treated Rats (□) Rat Liver Surface at a Dose of 5.0 mg

Each point represents the mean ± S.E. of at least three experiments.

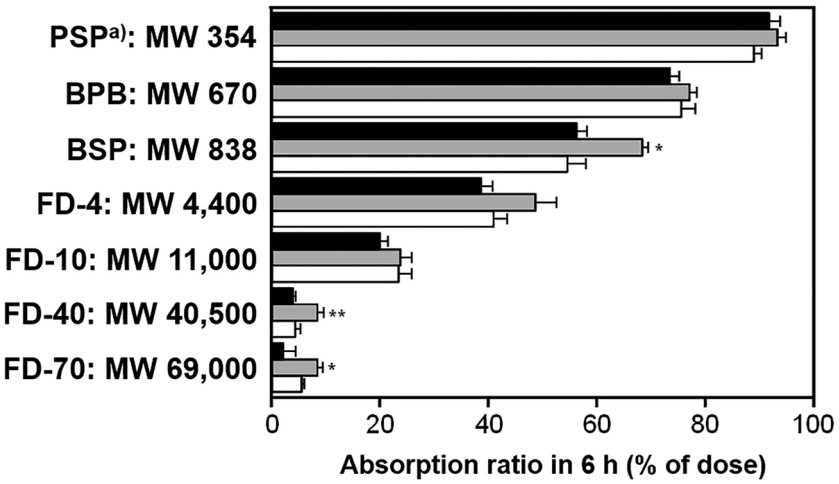

Fig. 3. Absorption Ratio 6 h (% of Dose) after Application of the Marker Compounds with Different Molecular Weights to the Normal (Closed Bar), CCl_4-Treated (Gray Bar) or GAL-Treated (Open Bar) Rat Liver Surface Each Point Represents the Mean + S.E. of at Least Four Experiments

Significantly different from the normal rat (*$p < 0.05$, **$p < 0.01$). [a] Results of phenolsulfonphthalein (PSP) were reported previously.[2,12]

these marker compounds as well as that of PSP 6 h after liver surface application in the normal, CCl_4 or GAL group.[12] The absorption from the liver surface of marker compounds with different molecular weights in the CCl_4 group increased by more than 4% compared with the normal group. In case of the GAL group, this trend was not evident with PSP and BSP but was observed for FITC-dextrans.

Correlation between Drug Absorbability and Molecular Weight after Application to the Liver Surface in Liver-Intoxicated Rats In the liver-intoxicated groups, the absorption ratio of the marker compounds from the liver surface tended to decrease with increasing molecular weight (Fig. 3). We examined the relationship between k_a and the reciprocal value of the square root of the molecular weight ($1/\sqrt{Mw}$) of the marker compounds, as illustrated in Figs. 4A and 4B, for CCl_4 and D-GAL treatment, respectively. A significant linear relationship was observed between the k_a and $1/\sqrt{Mw}$ of the marker compounds (correlation coefficient R: CCl_4 group 0.984; GAL group 0.965) in the liver-intoxicated rats, similar to the trend observed in previously examined normal rats.[2]

The molecular weights in the normal, CCl_4 and GAL groups when k_a is 0 were extrapolated to be 71195,[2] 105020 and 135237, respectively. The limits of the molecular weight of the compound absorbed from the liver surface were considered to be different between the disease states.

DISCUSSION

In order to demonstrate the possibility of liver surface application for macromolecules in liver disease, we first examined the absorption characteristics of FD-10 after application to the liver surface in liver-intoxicated rat models. The plasma concentration of FD-10 after application to the liver surface was high in the GAL group but remained unchanged in the CCl_4 group. This could be due to the glomerular filtration process of FD-10 that was restrained owing to the decline in blood flow rate in the GAL group compared to normal,[17] as indicated by a decline in CL_{tot} (Table 1). The excretion of FD-10 into the urine also decreased in the GAL group after i.v. administration (Fig. 1A).

We studied the time course of the recovery ratio of FD-10 in the diffusion cell (Fig. 2) in order to analyze pharmacokinetically the absorption mechanism of FD-10 after rat liver surface application in the liver-intoxicated state. The results suggest that the absorption of FD-10 from the liver surface in the CCl_4 and GAL groups followed first-order kinetics similar to that of the control group. The k_a values in CCl_4 and GAL groups increased by 1.3 and 1.1 times, respectively, compared to control. This confirmed that the absorption rate of FD-10 from the liver surface in the liver-intoxicated groups increased compared with the normal group. This trend was particularly evident in the CCl_4 group.

Similar to the absorption ratio of low molecular weight compound PSP in a previous report, significant decline was not observed in the absorption ratio of FD-10 from the liver surface in the liver disease model.[12] The absorption ratio from the liver surface of other FITC-dextrans with different molecular weights was higher in the liver-intoxicated groups compared to the normal group (Fig. 3). Accordingly, there should be no serious problem for the liver surface application of macromolecules.

We also examined the influence of molecular weight on drug absorbability in the CCl_4 and GAL groups. We chose BPB and BSP as other low molecular weight compounds and FITC-dextrans with different molecular weights. As shown in Fig. 3, the absorbability from the liver surface declined with increasing molecular weight in both the CCl_4 and GAL groups. In our previous study on normal rats, there was also a reverse relationship between molecular weight and permeability of the peritoneal liver membrane surface.[2] This trend is typically characteristic of simple passive diffusion. Even in the CCl_4 and GAL groups, significant correlation between molecular weight and absorption rate constant was observed (Fig. 4). This suggests that the marker compounds with different molecular weights are absorbed from the liver surface membrane *via* simple passive diffusion even in the disease state. However, the liver disease model used in this study seemed mild because the AST and ALT was slightly elevated. Further study is needed used severe liver disease or liver fibrosis model to evaluate the effect of liver disease on drug absorption from liver surface.

The peritoneum that covers the liver is composed of a

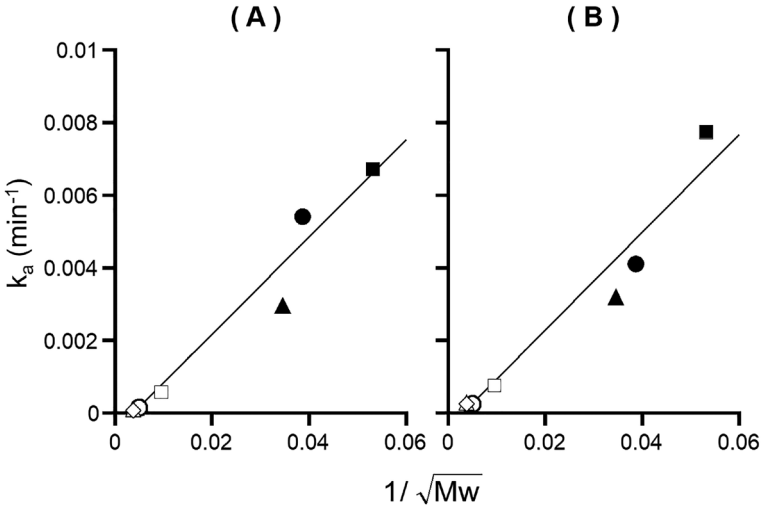

Fig. 4. Relationship between the Molecular Weight and First-Order Absorption Rate Constant (k_a) of the Marker Compounds after Application to the CCl_4- (A) or GAL-Treated (B) Rat Liver Surface

k_a was calculated from the amount remaining in the diffusion cell 6 h after application to the rat liver surface Key: ■, PSP [a]; ●, BPB; ▲, BSP; □, FD-4; ○, FD-10; △, FD-40; ◇, FD-70. [a] Results of PSP were reported previously.[12]

serous membrane containing monolayer squamous epithelial cells. Absorption of a drug from the liver surface is considered to occur through the intercellular route, since marker compounds have small partition coefficients at physiological pH. Therefore, changes in absorbability of the marker compounds must have occurred due to changes in the gap and pore characteristics in the serous membrane in the liver disease state. From the study of the correlation between molecular weight and absorption rate constant, the molecular weight limit (normal: 71195) was observed to have increased by more than 1.5 times in the liver-intoxicated rats.[2] This suggests an increase in the size of the pores or cell gaps on the liver surface membrane epithelium following CCl_4 or GAL-treatment.

We speculate that the size or number of the intercellular gaps and pores in the serous membrane and/or connective tissue could be altered by physiological changes in the liver disease state. Similar observations have been noted in previous studies on models intoxicated with either GAL or CCl_4. However, the absorption ratio of drug more than MW838 was increased in CCl_4 compared to control or GAL. In the GAL-intoxicated model, there have been reports demonstrating inhibition of mRNA and protein synthesis in the cell.[10,11] On the other hand, the membrane structure was changed due to super oxidation of membrane lipid in CCl_4 treatment.[6,7] This difference might cause the alteration of drug absorption from liver surface in CCl_4 treated rat. Characterizations of the physiological changes accompanying the liver disease state may then be performed to further understand the mechanism of liver surface absorption of macromolecules in the disease state.

CONCLUSION

The absorption of marker compounds from the liver surface obeyed first-order kinetics, and the absorption rate constant of the disease state was 1.5 folds of the control. The increased absorbability of FITC-dextrans from the liver surface of liver-intoxicated rats suggests that macromolecular compounds can be applied to the liver surface for liver-targeting of macromolecules in the disease state. A good correlation was observed based on the relationship between k_a and molecular weight liver disease model, similar to that of the control. The molecular weight limit of absorption was also shown to have increased in the liver-intoxicated models. Taken together, liver surface application of molecules in the disease state is possible and therefore has potential for treatment of liver disease.

Acknowledgments We wish to thank Yuki Nakakoga, Sayaka Sakamoto, Mari Nagano and Kurumi Ota for skilled technical assistance. This study was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Conflict of Interest The authors declare no conflict of interest.

REFERENCES

1) Kodama Y, Fumoto S, Nishi J, Nakashima M, Sasaki H, Nakamura J, Nishida K. Absorption and distribution characteristics of 5-fluorouracil (5-FU) after an application to the liver surface in rats in order to reduce systemic side effects. *Biol. Pharm. Bull.*, **31**, 1049–1052 (2008).
2) Nishida K, Sato N, Sasaki H, Nakamura J. Absorption characteristics of dextrans with different molecular weights from the liver surface membrane in rats: implications for targeting to the liver. *J. Drug Target.*, **4**, 141–150 (1996).
3) Kodama Y, Horishita M, Fumoto S, Mine T, Miyamoto H, Yoshikawa N, Hirata H, Sasaki H, Nakamura J, Nishida K. Effect of viscous additives on the absorption and hepatic disposition of 5-fluorouracil (5-FU) after application to liver surface in rats. *J. Pharm. Pharmacol.*, **64**, 1438–1444 (2012).
4) Nishida K, Sato N, Sasaki H, Nakamura J. Absorption of organic anions as model drugs following application to rat liver surface in-vivo. *J. Pharm. Pharmacol.*, **46**, 867–870 (1994).
5) Nishida K, Fujiwara R, Kodama Y, Fumoto S, Mukai T, Nakashima M, Sasaki H, Nakamura J. Regional delivery of model compounds and 5-fluorouracil to the liver by their application to the liver surface in rats: its implication for clinical use. *Pharm. Res.*, **22**, 1331–1337 (2005).
6) Weber LW, Boll M, Stampfl A. Hepatotoxicity and mechanism of

action of haloalkanes: carbon tetrachloride as a toxicological model. *Crit. Rev. Toxicol.*, **33**, 105–136 (2003).
7) Boll M, Weber LW, Becker E, Stampfl A. Mechanism of carbon tetrachloride-induced hepatotoxicity. Hepatocellular damage by reactive carbon tetrachloride metabolites. *Z. Naturforsch. C*, **56**, 649–659 (2001).
8) Keppler DO, Rudigier JF, Bischoff E, Decker KF. The trapping of uridine phosphates by D-galactosamine. D-glucosamine, and 2-deoxy-D-galactose. A study on the mechanism of galactosamine hepatitis. *Eur. J. Biochem.*, **17**, 246–253 (1970).
9) Keppler D, Lesch R, Reutter W, Decker K. Experimental hepatitis induced by D-galactosamine. *Exp. Mol. Pathol.*, **9**, 279–290 (1968).
10) Decker K, Keppler D. Galactosamine induced liver injury. *Prog. Liver Dis.*, **4**, 183–199 (1972).
11) Gujral JS, Farhood A, Jaeschke H. Oncotic necrosis and caspase-dependent apoptosis during galactosamine-induced liver injury in rats. *Toxicol. Appl. Pharmacol.*, **190**, 37–46 (2003).
12) Nishida K, Honda T, Nakashima M, Sasaki H, Nakamura J. Influence of liver disease on phenolsulfonphthalein absorption from liver surface to examine possibility of direct liver surface application for drug targeting. *Biol. Pharm. Bull.*, **26**, 988–993 (2003).
13) Stock RJ, Cilento EV, McCuskey RS. A quantitative study of fluorescein isothiocyanate–dextran transport in the microcirculation of the isolated perfused rat liver. *Hepatology*, **9**, 75–82 (1989).
14) Takada K, Narumiya O, Muranishi S. Biopharmaceutical study of the hepato-biliary transport of drugs. III. Binding characteristics of bromphenol blue and amaranth to the liver cytoplasmic Y and Z binding proteins *in vitro*. *Chem. Pharm. Bull.*, **23**, 729–735 (1975).
15) Baker KJ, Bradley SE. Binding of sulfobromophthalein (BSP) sodium by plasma albumin. Its role in hepatic BSP extraction. *J. Clin. Invest.*, **45**, 281–287 (1966).
16) Yamaoka K, Nakagawa T. A nonlinear least squares program based on differential equations, MULTI (RUNGE), for microcomputers. *J. Pharmacobiodyn.*, **6**, 595–606 (1983).
17) Kimura T, Nakayama S, Yamao T, Kurosaki Y, Nakayama T. Pharmacokinetics of indocyanine green in rats with experimentally induced hepatic diseases. *Biol. Pharm. Bull.*, **16**, 1140–1145 (1993).