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Research Paper

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

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Purpose. The purpose of this study was to examine drug distribution in the liver after drug application to the rat liver surface.
Methods. Phenolsulfonphthalein (PSP) and fluorescein isothiocyanate dextran (MW 4400, FD-4) as model compounds or 5-fluorouracil (5-FU) was applied to the rat liver surface by employing a cylindrical diffusion cell (i.d. 9 mm, 0.64 cm^2). Then, blood and the remaining solution in the diffusion cell were collected at selected times, followed by excision of the liver. The excised liver was divided into three sites: the region under the diffusion cell attachment site (site 1), the applied lobe except for site 1 (site 2), and non-applied lobes (site 3).
Results. In the case of i.v. administration, there were no differences in PSP concentrations among the three sites of the rat liver, and the concentrations rapidly decreased. On the other hand, the PSP concentration in site 1 after application to the rat liver surface was considerably higher than in site 2 and site 3. In addition, the area under the curve (AUC) value (AUC_{site1}), calculated from the PSP concentration profile in site 1, was about 10 times larger than that in site 3. A similar trend of regional delivery advantage by liver surface application was observed in the case of the macromolecule model FD-4, with a marked AUC_{site1} of about 5 times larger than the other two sites. Moreover, we clarified that the anticancer drug 5-FU preferentially distributed in site 1 after application to the rat liver surface.
Conclusion. These results demonstrate the possibility of regional delivery of drugs to the liver by application to the liver surface.

KEY WORDS: absorption; 5-fluorouracil; liver surface; liver targeting; regional delivery.

INTRODUCTION

Regional drug delivery to the liver is of interest because the normal treatment of liver diseases with i.v. and oral administration routes have been complicated by inadequate drug delivery to specific region in the liver because of almost equal distribution by blood circulation. The direct injection route is supposed to be a useful method, but is not suitable for spatial controlled drug delivery to the target site in the liver because directly injected drugs are rapidly cleared from injected sites with high blood flow, followed by drainage into the systemic circulation (1).

Therefore, we have proposed the surfaces of organs as novel application and absorption sites of drugs, and studied the absorption mechanisms of model compounds after their application to the rat liver surface (1–4). In addition, we have reported that site-selective localization was enhanced continuously by instilling a small amount of the drug solution on the rat liver (5,6). Furthermore, we have demonstrated the liver lobe-selective gene transfection system utilizing the instillation of plasmid DNA to the liver surface in mice (7,8).

In the present study, we examined the distributions in the liver of phenolsulfonphthalein (PSP) and fluorescein isothiocyanate dextran (MW 4400, FD-4) as model compounds by dividing the liver into three different sites, and compared their regional delivery advantages with i.v. administration. We chose PSP and FD-4 as model compounds because their absorption characteristics from the liver surface have been investigated previously (1–4,9). Moreover, we have studied the liver- and region-selective delivery of the anticancer drug 5-fluorouracil (5-FU) by application to the liver surface in rats, as intraperitoneal (i.p.) therapy by 5-FU is a feasible means of administration to localized peritoneal cancers.

MATERIALS AND METHODS

Materials

PSP and 5-FU were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). FD-4 was obtained from Sigma Chemical Co.

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ABBREVIATIONS: AUC, area under the curve; FD-4, fluorescein isothiocyanate dextran (MW 4400); 5-FU, 5-fluorouracil; PSP, phenolsul-fonphthalein.

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(St. Louis, MO, USA). All other chemicals were of reagent grade.

Animal Experiments

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

PSP and FD-4 were dissolved in pH 7.4 isotonic phosphate buffer. 5-FU was dissolved in phosphate-buffered saline (pH 7.4).

A cylindrical diffusion cell (i.d. 9 mm, 0.64 cm^2) (Fig. 1B) was attached to the liver surface (left lateral lobe, Fig. 1A) of male Wistar rats (250–310 g) with the biocompatible glue Aron Alpha (Sankyo Co. Ltd., Tokyo, Japan). As model compounds, PSP (10 mg/ml × 0.1 ml), FD-4 (50 mg/ml × 0.1 ml), or 5-FU solution (10 mg/ml × 0.5 ml) were added into the diffusion cell. The top of the diffusion cell was sealed with aluminum foil to prevent evaporation. Then, blood and the remaining solution in the diffusion cell were collected at selected times, followed by excision of the liver (PSP: 5, 15, 30, 60, 180, 360 min; FD-4: 30, 60, 180, 360 min, 5-FU: 30, 60, 120, 180, 360 min). As the schematic diagram of the rat liver shows in Fig. 1A, the excised liver was divided into the three sites: the region under the diffusion cell attachment site (site 1), the applied lobe except for site 1 (site 2), and the non-applied lobes (site 3).

Intravenous administration of the model compounds was performed for comparison. The model compound PSP (10 mg/ml × 0.1 ml), FD-4 (50 mg/ml × 0.1 ml), or 5-FU solution (10 mg/ml × 0.5 ml) was injected into the jugular veins of rats. Then, blood was collected at selected times, followed by excision of the liver (PSP: 5, 15, 30, 60, 180, 360 min; FD-4: 30, 60, 180, 360 min; 5-FU: 15, 30, 60, 120 min). The excised liver was similarly divided into the three sites.

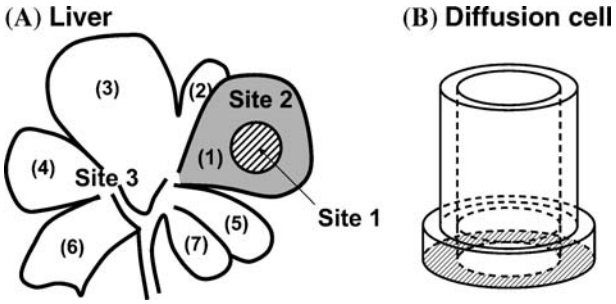

Fig. 1. Schematic diagram of the rat liver divided into the three different sites (sites 1, 2, and 3) (A) after drug application to the rat liver surface using a cylindrical diffusion cell (B). Name of the liver lobes: (1) left lateral lobe, (2) left medial lobe, (3) right medial lobe, (4) right lateral lobe, (5) caudate lobe, (6) quadrate lobe, and (7) papillary process lobe. The left lateral lobe was further separated into the region under the diffusion cell (site 1, slashed region) and the region not under the diffusion cell (site 2, hatched region). Other lobes (2–7) were pooled together into site 3 (open region). The diffusion cell (i.d. 9 mm, 0.64 cm^2) (B) was attached to site 1 of the rat liver using Aron Alpha biocompatible glue. All dimensions are approximate.

Analytical Methods

In the cases of PSP and FD-4, the excised liver was homogenized in ten- (site 1) or threefold (site 2 and site 3) of its weight of isotonic phosphate buffer (pH 7.4). Three milliliters of acetone was added to 1 ml (site 1) or 5 ml (site 2 and site 3) of the liver homogenate for extraction by modifying as previously described (10). Then, the mixture was shaken for 15 min, followed by centrifugation at 3000 rpm for 20 min at 4°C. The resulting supernatant was subjected to assay.

The concentrations of PSP in the plasma, liver, and the solution remaining in the diffusion cell were determined spectrophotometrically at 560 nm after dilution with 1 M NaOH. The concentrations of FD-4 as fluorescences in the plasma, liver, and the solution remaining in the diffusion cell were measured by a spectrophotofluorometer at excitation and emission wavelengths of 489 and 515 nm, respectively.

The concentration of 5-FU in the liver homogenate was determined by modifying the reported methods (11,12). The excised liver was homogenized in threefold of its weight of isotonic phosphate buffer (pH 7.4). Briefly stated, the liver homogenates (300 μl) were added to a solution of 5-bromouracil (20 μg/ml, 150 μl) dissolved in isotonic phosphate buffer (pH 7.4) as an internal standard, with 1 M sodium acetate buffer (pH 4.8, 100 μl), and 20% anhydrous sodium sulfate (500 μl). The mixtures were shaken with ethyl acetate (4 ml) for 10 min and centrifuged at 3000 rpm for 10 min. The organic layers (3 ml) were collected. Then ethyl acetate (4 ml) was added to the residue and the mixtures were shaken for 10 min, and thereafter centrifuged at 3000 rpm for 10 min. The organic layers (4 ml) were collected and the mixed organic layers (7 ml) were evaporated. The extraction residues were dissolved in 500 μl of distilled water and washed twice with 1 ml of hexane. Samples (100 μl) were injected onto the HPLC column (Cosmosil packed column 5C18-MS-II, 4.6 mm i.d. × 150 mm, Nacalai Tesque). An HPLC system (LC-6A, Shimadzu Co., Ltd., Kyoto, Japan) with a variable-wavelength UV detector (SPD-10A, Shimadzu) was used in reverse-phase mode. The detector wavelength, flow rate, and column temperature were set at 266 nm, 0.5 ml/min, and 25 °C, respectively. The mobile phase consisted of 10 mM sodium acetate buffer (pH 4.0).

Analytical Information

The limits of quantification of PSP, FD-4, and 5-FU were 0.010, 0.0027, and 0.20 μg/ml. The reproducibility of quantification was guaranteed by inter-day coefficient of variation (PSP, 1.1%; FD-4, 0.9%; 5-FU 0.9%) and intra-day coefficient of variation (PSP, 0.9%; FD-4, 0.4%; 5-FU, 0.5%). The accuracy of quantification was high (PSP, 98.6–103.3%; FD-4, 97.5–101.1%; 5-FU, 99.3–102.7%). The recoveries of PSP, FD-4, and 5-FU during extraction and precipitation of liver homogenate were 60.0%, 58.1%, and 76.7%, respectively.

Statistical Analysis

Statistical comparisons were performed by paired Student's *t*-test. A value of $p < 0.05$ was considered to be

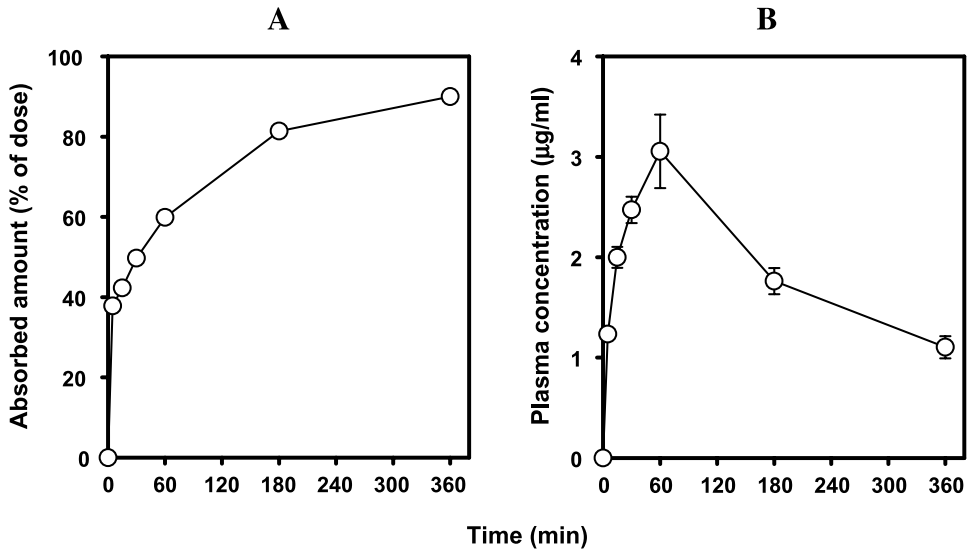

Fig. 2. Time courses of the PSP amounts absorbed from the liver surface (A) and the plasma concentration of PSP (B) after application to the liver surface in rats. Each point represents the mean ± SE of at least seven experiments.

significant. All values were expressed as the mean value ± standard error (SE) of at least four different independent experiments.

RESULTS AND DISCUSSION

We established an experimental system utilizing a cylindrical diffusion cell attached to the rat liver surface (1). This experimental system enabled us to examine drug absorption from the liver surface without interference by absorption from other sites, as a basic study for the development of pharmaceutical formulation. We first selected PSP as a low molecular weight model to examine the regional delivery advantage by liver surface application.

Absorption and Plasma Patterns of PSP After Application to the Rat Liver Surface

Figure 2 illustrates the time course of PSP absorption from the liver surface (A), and the plasma concentration profile of PSP (B) after its application to the liver surface in rats. Absorption of PSP from the rat liver surface was calculated to be about 90% of dose after 360 min. PSP appeared in the plasma at a peak level 60 min after dosing, and its concentration decreased gradually (Fig. 2B).

Distribution of PSP in the Different Sites After Application to the Rat Liver Surface

Figure 3 shows the liver concentrations of PSP in the three sites after application to the liver surface in rats at a dose of 1 mg. PSP concentration in site 1 (C_{site1}) reached a maximum 30 min after dosing, and was significantly higher than in site 2 (C_{site2}) and site 3 (C_{site3}) at 30 min. This trend was observed until 360 min after application (Fig. 3). The C_{site1} of PSP was not as high as that expected by the absorbed amount after the first 5 min (Fig. 2A), suggesting a slow distribution of absorbed PSP into the liver parenchymal cells.

Table I summarizes the ratios of the liver concentrations of PSP in the three sites and the plasma after application to the liver surface or i.v. administration in rats. The ratios at 30 min when we compare site 1 to site 2 (C_{site1}/C_{site2}) and site 3 (C_{site1}/C_{site3}) were as high as 5.9 and 12.8, respectively. In addition, the ratio of C_{site1} to the plasma concentration (C_{site1}/C_{plasma}) of PSP at 30 min after application was markedly higher than in the other two sites (C_{site2}/C_{plasma}, C_{site3}/C_{plasma}), as listed in Table I. This tendency after 60, 180, and 360 min was markedly higher (data not shown), implying the potent suppression of PSP drainage into the systemic circulation by liver surface application.

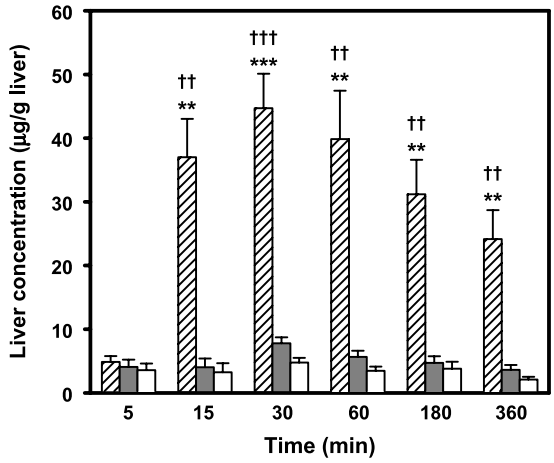

Fig. 3. Liver concentration of PSP in the three sites after application to the liver surface in rats at a dose of 1 mg. Key: site 1 (▨), site 2 (▬), site 3 (▭). Each bar represents the mean + SE of at least seven experiments. Significantly different from the result at site 2 (**$p < 0.01$, ***$p < 0.001$) or site 3 ($^{\dagger\dagger}p < 0.01$, $^{\dagger\dagger\dagger}p < 0.001$).

Table I. Ratios of Concentrations After 30 min and AUC Values Until 360 min of PSP After Application to the Liver Surface (LSA) or i.v. Administration in Rats at a Dose of 1 mg (LSA) or 0.5 mg (i.v.)

Concentration ratio	LSA	i.v.	AUC ratio	LSA	i.v.
C_{site1}/C_{site2}	5.9	1.2	AUC_{site1}/AUC_{site2}	6.6	1.0
C_{site1}/C_{site3}	12.8	1.2	AUC_{site1}/AUC_{site3}	9.6	1.1
C_{site1}/C_{plasma}	19.7	4.8	AUC_{site1}/AUC_{plasma}	16.5	4.5
C_{site2}/C_{plasma}	3.3	4.2	AUC_{site2}/AUC_{plasma}	2.5	4.3
C_{site3}/C_{plasma}	2.0	4.1	AUC_{site3}/AUC_{plasma}	1.8	3.9

Comparison of Regional PSP Delivery Advantage to the Applied Sites with i.v. Administration

Figure 4A shows the liver concentrations of PSP in the three sites 30 min after application to the liver surface (LSA) or i.v. administration in rats at a dose of 1 mg (LSA) or 0.5 mg (i.v.). After i.v. administration to rats, there were no differences in the PSP concentrations among the three sites (Fig. 4A) and PSP was rapidly eliminated thereafter (data not shown).

As an index of regional availability expressed by the AUC of the liver concentration profile, Fig. 4B shows the AUC of each site until 360 min after the administration of PSP, corrected by the absorbed amount after application to the liver surface or i.v. administration in rats. The AUC of the C_{site1} profile (AUC_{site1}) of PSP after application to the liver surface was markedly higher than in site 2 (AUC_{site2}) and site 3 (AUC_{site3}), by 6.6 and 9.6 times, respectively, while values were identical in each site after i.v. administration, as illustrated in Fig. 4B. These results indicate that the liver surface application of the small molecule model compound PSP results in a high regional availability around the applied site.

Absorption and Plasma Patterns of FD-4 After Application to the Rat Liver Surface

Since there was a significant relationship between the molecular weight and absorption rate constant after the application of model compounds to the liver (9), molecular weight appeared to play an important role in drug accumulation after absorption from the liver surface. We selected FD-4 as a model macromolecule, and examined the molecular weight dependence of drug distribution after absorption from the liver surface in rats, for the clinical application of biologically active macromolecules such as epidermal growth factor.

Figure 5 illustrates the time course of the amount of FD-4 absorbed from the liver surface (A) and the plasma concentration profile of FD-4 after application to the liver surface in rats (B) at a dose of 5 mg. The amount of FD-4 absorbed from the diffusion cell increased to about 30% of dose after 360 min, as shown in Fig. 5A. The plasma concentration of FD-4 reached a maximum after 60 min, followed by a slow decline (Fig. 5B).

Distribution of FD-4 in the Different Sites After Application to the Rat Liver Surface

Figure 6 shows the liver concentrations in the three sites of FD-4 after application to the liver surface in rats at a dose of 5 mg. At 30 min after application, FD-4 was not detected in any sites, probably because of the low absorption rate of FD-4 from the liver surface (Fig. 5A) and the slow diffusion of absorbed FD-4 into the liver parenchymal cells. Thereafter, C_{site1} of FD-4 showed a maximum at 60 min after application, and was significantly higher than in the other two sites at 60, 180, and 360 min after application.

Table II summarizes the ratio of the liver concentration after 60 min and the AUC of the liver concentration profile until 360 min after FD-4 application to the liver surface or i.v. administration in rats at a dose of 5 mg. The C_{site1}/C_{site2} and

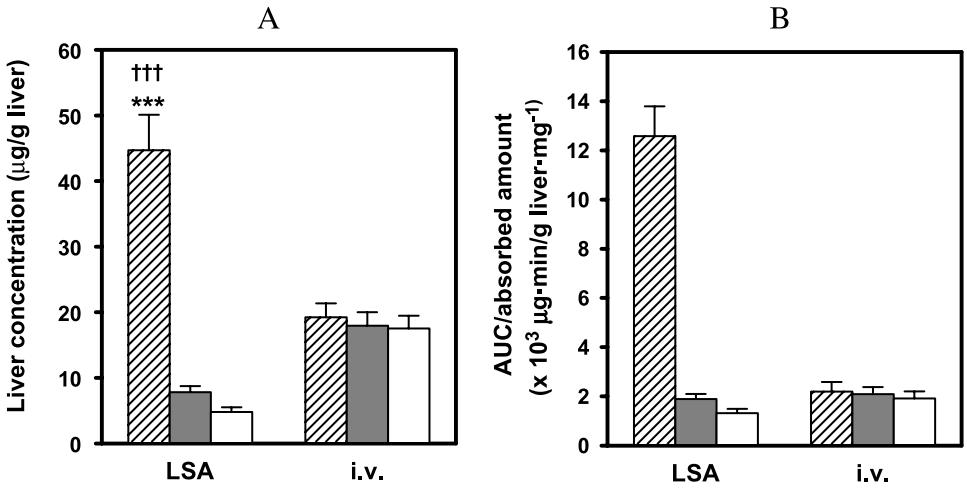

Fig. 4. Liver concentration of PSP after 30 min (A) and the AUC of the PSP concentration profile until 360 min in each site, corrected by the absorbed amount (B) after application to the liver surface or i.v. administration in rats at a dose of 1 mg (LSA) or 0.5 mg (i.v.). Key: site 1 (▨), site 2 (▬), site 3 (▭). Each bar represents the mean + SE of at least seven experiments. Significantly different from the result at site 2 (*** $p < 0.001$) or site 3 ($^{\dagger\dagger\dagger}p < 0.001$) (A).

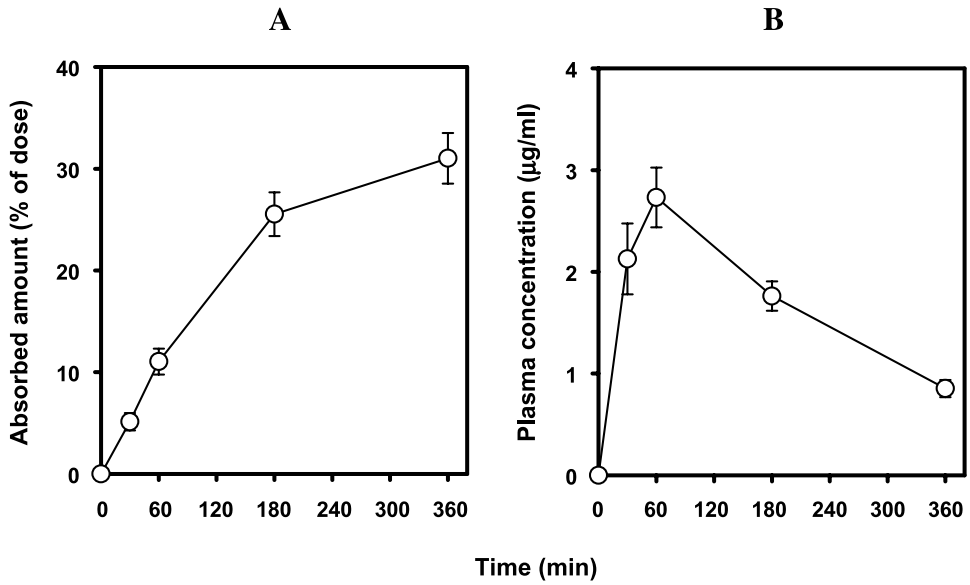

Fig. 5. Time courses of the FD-4 amounts absorbed from the liver surface (A) and the plasma concentrations of FD-4 (B) after application to the liver surface in rats. Each point represents the mean ± SE of at least seven experiments.

C_{site1}/C_{site3} values 60 min after FD-4 administration were 7.9 and 6.0, respectively. The C_{site1}/C_{plasma} of FD-4 after 60 min was extremely high, with a ratio of 26.8, compared to C_{site2}/C_{plasma} (0.2) and C_{site3}/C_{plasma} (0.5), indicating a higher regional availability of FD-4 in the liver compared to systemic drainage.

Comparison of the Regional FD-4 Delivery Advantage to the Applied Sites with i.v. Administration

Figure 7A shows the liver concentrations of FD-4 after 60 min in the three sites after application to the liver surface or i.v. administration in rats at a dose of 5 mg. In the case of i.v. administration, the FD-4 concentration of every site was very low (<1.3 μg/g liver) and there were no differences among the three sites (Fig. 7A). On the other hand, the C_{site1}/C_{site2} and C_{site1}/C_{site3} values of FD-4 after 60 min were considerably higher after liver surface application compared with i.v. administration (values are listed in Table II).

Figure 7B shows the AUC until 360 min of the FD-4 concentration in each site, corrected by the absorbed amount after application to the liver surface or i.v. administration in rats at a dose of 5 mg. The AUC_{site1} of FD-4 after application to the liver surface was markedly higher than in the other two sites (AUC_{site2}, AUC_{site3}), with the ratios listed in Table II, whereas the AUC values of the FD-4 concentration profile in each site after i.v. administration were extremely low and identical (Fig. 7B). These results imply that liver surface application could enable the efficient accumulation of macromolecule drugs around MW 5000 in local sites of the liver.

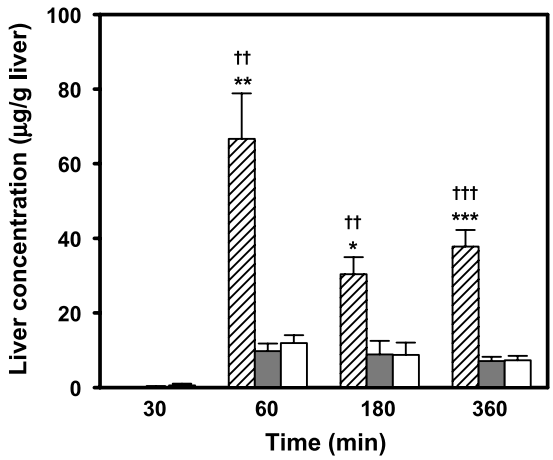

Fig. 6. Liver concentrations of FD-4 in the three sites after application to the liver surface in rats at a dose of 5 mg. Key: site 1 (▨), site 2 (▬), site 3 (▭). Each bar represents the mean + SE of at least seven experiments. Significantly different from the result at site 2 (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$) or site 3 ($^{\dagger\dagger}p < 0.01$, $^{\dagger\dagger\dagger}p < 0.001$).

Distribution of 5-FU in the Different Sites After Application to the Rat Liver Surface

The time-dependent anticancer drug 5-FU is commonly used in clinical oncology practices (13). Although the oral administration of 5-FU has been used for convenience, this administration route has the disadvantage that the oral bioavailability of 5-FU is low and erratic (14,15). Therefore,

Table II. Ratios of Concentrations After 60 min and AUC Values Until 360 min of FD-4 After Application to the Liver Surface (LSA) or i.v. Administration in Rats at a Dose of 5 mg

Concentration ratio	LSA	i.v.	AUC ratio	LSA	i.v.
C_{site1}/C_{site2}	7.9	1.1	AUC_{site1}/AUC_{site2}	4.8	1.0
C_{site1}/C_{site3}	6.0	1.1	AUC_{site1}/AUC_{site3}	4.5	1.0
C_{site1}/C_{plasma}	26.8	1.8	AUC_{site1}/AUC_{plasma}	21.1	1.5
C_{site2}/C_{plasma}	3.9	1.7	AUC_{site2}/AUC_{plasma}	4.4	1.5
C_{site3}/C_{plasma}	4.8	1.6	AUC_{site3}/AUC_{plasma}	4.7	1.5

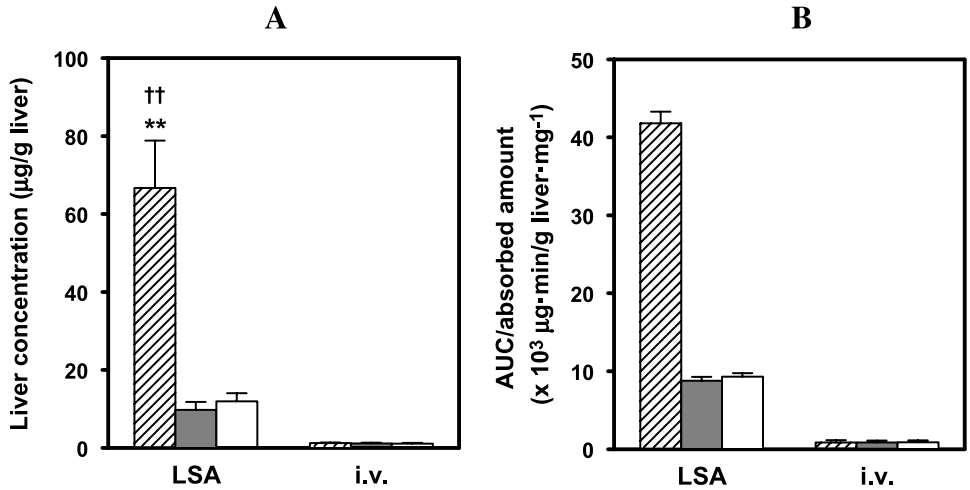

Fig. 7. Liver concentration of FD-4 after 60 min (A) and the AUC of the FD-4 concentration profile until 360 min in each site, corrected by the absorbed amount (B) after application to the liver surface or i.v. administration in rats at a dose of 5 mg. Key: site 1 (▨), site 2 (▬), site 3 (▭). The i.v. data were corrected by the absorbed amount of FD-4 at 60 min after application to the liver surface. Each bar represents the mean + SE of at least seven experiments. Significantly different from the result at site 2 (**$p < 0.01$) or site 3 ($^{\dagger\dagger}p < 0.01$) (A).

we studied the distribution in the liver of 5-FU after application to the liver surface in rats.

Figure 8A and B shows the liver concentrations in the three sites of 5-FU after application to the liver surface or i.v. administration, respectively, in rats at a dose of 5 mg. After the i.v. administration of 5-FU, the concentrations of 5-FU in the three sites were almost the same after 15 min, and 5-FU was not detected thereafter, as shown in Fig. 8B. After the liver surface application of 5-FU, on the other hand, 5-FU was preferentially distributed in site 1 after 30, 60, 120, 180, and 360 min (Fig. 8A). The regional delivery of 5-FU implies that liver surface application would reduce the systemic side effects, because of the low 5-FU concentrations in the other sites.

The introduction of special affinity toward the liver to the drug molecules could enhance the regional delivery advantage in the liver and reduce toxic effects in the normal liver regions and other organs. In addition, there is the advantage of flexible formulation modifications, such as viscous and bioadhesive additives, compared to the other administration routes such as i.v. and intraarterial administrations. Recently, implantable infusion pumps have been developed for treatment of several diseases, and endoscopic and laparoscopic operation techniques have made remarkable progress. Therefore, a combination of these advanced medical technologies and pharmaceutical modifications could make possible the clinical application for a drug to the liver surface in the peritoneal cavity.

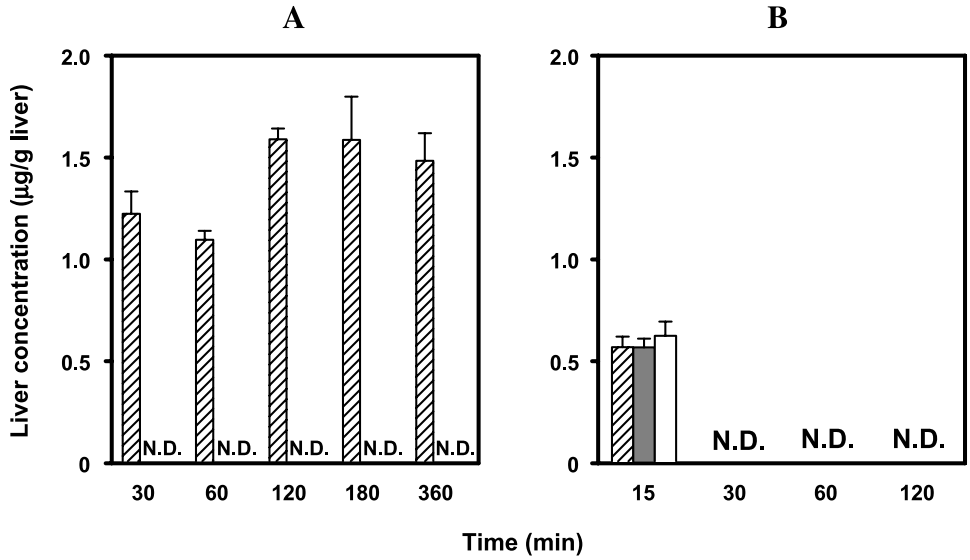

Fig. 8. Liver concentration of 5-FU in each site after application to the liver surface (A) or i.v. administration (B) in rats at a dose of 5 mg. Key: site 1 (▨), site 2 (▬), site 3 (▭). Each bar represents the mean + SE of at least four experiments. N.D.: not detected.

In conclusion, these results demonstrated that liver surface application could achieve the regional delivery of drugs to the liver. This administration system, utilizing direct absorption from the liver surface, is expected to be a safe and effective treatment for liver diseases and cancers.

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