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Regular Articles

Effect of Application Volume and Area on the Absorption of Phenol Red as a Model Drug from the Liver Surface in Rats

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Shortened Title: EFFECT OF VOLUME AND AREA ON ABSORPTION FROM LIVER
SURFACE

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Abstract

We examined the importance of application volume and area in the absorption of phenol red as a model drug from the rat liver surface, for pharmaceutical formulation concerning administration form.

When 1 mg of phenol red was applied to the rat liver surface in-vivo using a cylindrical glass cell (i.d. 9 mm) in three volumes (0.1, 0.2 or 0.334 mL), the shape of the plasma concentration pattern differed greatly, particularly the maximum concentration. These patterns were well fitted by a two-compartment model with first-order absorption, and the obtained absorption rate constant K_a decreased inversely according to the application volume. The absorption ratio and biliary recovery of phenol red at 6 h was increased with glass cell area (i.d. 6, 9 or 14 mm; area 0.28, 0.64 or 1.54 cm²). Furthermore, the permeability coefficient P_{app} derived from K_a did not depend on application area, indicating no difference in absorption characteristics of liver surface. This also implies transport of a drug by a passive diffusion from the liver surface.

After intraperitoneal administration to the rat liver surface for clinical application, increase in application volume resulted in the delayed disappearance of phenol red from plasma. However, the difference was not as marked as that using a glass cell. The assumption that the effective area relating to the absorption changed with the application volume enabled us to estimate P_{app} .

Consequently, we speculate absorbability can be estimated precisely by considering application volume and area.

Introduction

Previously (Nishida et al 1994), we reported on drug absorption from the liver surface in rats, and demonstrated that direct application to the liver surface is useful for drug delivery to the target site in the liver. Furthermore, we examined the mechanism of drug absorption from the liver surface (Nishida et al 1995a,b; 1996), by using several organic anions and FITC-labeled dextrans of different molecular weights as model drugs.

An appropriate pharmaceutical modification for enhancement of drug release and adhesion with the liver surface should be considered to improve drug targeting to the liver. Therefore, application conditions such as volume and area are very important factors in the absorption from the liver surface.

In the present study, we selected phenol red as a model drug, the absorption mechanism of which has been already clarified (Nishida et al 1994; 1995a,b), and examined the influence of application volume and area of a glass cell on the absorption characteristics from the liver surface in rats. Furthermore, we also studied intraperitoneal administration of phenol red to the rat liver surface, for clinical application.

Materials and Methods

Chemicals

Phenol red was purchased from Nacalai Tesque, Inc. (Kyoto, Japan). All other chemicals were reagent grade products.

In-vivo experiment

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

Male Wistar rats (230-250 g) were anesthetized with sodium pentobarbitone (50 mg/kg, i.p.) and the left femoral artery was cannulated with a polyethylene tube (i.d. 0.5 mm, o.d. 0.8 mm; Dural Plastics, Dural, Australia). A 3 cm incision was made in the middle abdomen, and the common bile duct was cannulated with a polyethylene tube (i.d. 0.28 mm, o.d. 0.61 mm; Becton Dickinson & Co., Parsippany, NJ, U.S.A.). The phenol red solution was administered as follows.

Application to the rat liver surface: A cylindrical glass cell (i.d. 9 mm, effective area 0.64 cm²) was attached to the rat liver surface at the area of the left lobe with Aron Alpha (Sankyo Co. Ltd, Tokyo, Japan). The body temperature of the rats was kept at 37°C by a heat lamp throughout the experiment. The phenol red solution containing 1 mg was prepared in an isotonic phosphate buffer (pH 7.4) to yield a volume of 0.1, 0.2 or 0.334 mL, and added to the glass cell directly. The top of the glass cell was sealed with a piece of aluminum foil to prevent evaporation of the applied solution. In another experiment, phenol red was administered to the rat liver surface, by employing a cylindrical glass cell (i.d. 6 or 14 mm, effective area 0.28 or 1.54 cm²).

Intraperitoneal (i.p.) administration to the rat liver surface: Phenol red (1 mg) in an isotonic phosphate buffer (pH 7.4) at volumes of 0.2, 1 and 5 mL was administered

intraperitoneally to the liver surface. The injection point of i.p. administration was the division between the right and left lobe.

After administration, blood samples (200 μ l) were collected at selected times after dosing from the heparinized cannula inserted into the femoral artery over a 6-h period and centrifuged at 15000 rpm for 5 min. Bile samples were collected at appropriate time intervals for 6 h. At 6 h after the application, the urine remaining in the bladder was collected with a syringe. In addition, the solution remaining in the glass cell or peritoneal cavity was withdrawn.

Analytical method

The concentrations of phenol red in the plasma, bile, urine and remaining solution in the glass cell or peritoneal cavity were determined as follows. The concentration of free phenol red was determined spectrophotometrically at 560 nm after dilution with a 1 M NaOH solution. The total concentration of free phenol red and its metabolite was similarly measured after subjection to acid hydrolysis (1 M HCl at 100°C for 30 min) (Hart & Schanker 1966).

Compartment model analysis

First, the plasma concentration (C_p) profile of phenol red after i.v. administration was fitted to the biexponential equation described as follows (eq. 1), by the nonlinear least-squares method (Yamaoka et al 1981).

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

Hybrid parameters α and β are defined as $\alpha + \beta = K_{12} + K_{21} + K_{el}$ and $\alpha \cdot \beta = K_{21} \cdot K_{el}$. V_c is the volume of the central compartment. K_{el} is the first-order elimination rate constant from the central compartment. K_{12} and K_{21} are the first-order transfer rate constants between the

central and peripheral compartment. These parameters were substituted into the following equation for the plasma concentration after application to the rat liver surface. The result for i.v. administration has been already reported (Nishida et al 1995a).

Next, in the same way, the plasma concentration profile of phenol red after application to the rat liver surface was fitted in the two-compartment model with first-order absorption, by the nonlinear least-squares method (Yamaoka et al 1981). In this model, the equation for plasma concentration is given by the following equation.

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

K_a is the first-order absorption rate constant for absorption into the blood stream from the rat liver surface or peritoneal cavity. F is the availability after application to the rat liver surface or i.p. administration to around the rat liver surface.

Statistical analysis

Statistical analysis was performed by applying unpaired Student's t -test. $p < 0.05$ was considered to be statistically significant. All results were expressed as the mean value \pm standard error of at least four experiments.

Results

Effect of application volume on absorption of phenol red from rat liver surface

First, the plasma concentrations of phenol red were determined for 6 h after application to the rat liver surface at a dose of 1 mg in three volumes (0.1, 0.2 or 0.334 mL) employing a glass cell (i.d. 9 mm), as illustrated in Fig. 1. We observed a marked decrease in maximum concentration and a prolongation of plasma concentration, as the application volume increased. The biliary excretion rate patterns of free phenol red and its metabolite showed a similar tendency (data not shown).

Table 1 lists the recovery of phenol red at 6 h in the bile, urine and glass cell after application to the rat liver surface at a dose of 1 mg in three volumes. The recovery ratio in the glass cell at 6 h was affected significantly by the application volume. The extent of absorption of phenol red in 6 h was calculated from the recovery ratio to be 91.8, 71.5 and 46.8 % of dose at volumes of 0.1, 0.2, and 0.334 mL, respectively. Also, the biliary recovery of phenol red in 6 h tended to decrease as the absorption ratio decreased according to the application volume.

Moreover, we tried to examine the effect of application volume, by applying a two-compartment model with first-order absorption (Nishida et al 1995a, 1996). As shown in Fig. 1, each curve fitted the experimental value, suggesting the validity of the pharmacokinetic analysis. The calculated K_a values listed in Table 1 were inversely proportional to the application volume.

Effect of application area on absorption of phenol red from rat liver surface

Next, we examined the effect of application area of the glass cell on the absorption of phenol red from the rat liver surface. Figures 2a and 2b illustrate the plasma concentration profiles of phenol red at a dose of 1 mg (10 mg/mL X 0.1 mL (a) or 3 mg/mL X 0.334 mL (b)) after

application to the rat liver surface with three different glass cells (i.d. 6, 9 or 14 mm, effective area 0.28, 0.64 or 1.54 cm²). At 10 mg/mL X 0.1 mL (Fig. 2a), the plasma concentration pattern at the large area (0.64 cm²) was sharp and the maximum concentration increased two-fold, compared to the small area (0.28 cm²). A similar relationship between the small and large glass cells was observed at 3 mg/mL X 0.334 mL, as shown in Fig. 2b. The biliary excretion rate profiles of free phenol red and its metabolite changed with the area of glass cell (data not shown).

Table 2 summarizes the recovery of phenol red at 6 h in the bile, urine and solution remaining in the glass cell under several experimental conditions. At application volumes of 0.1 and 0.334 mL, the absorption ratio from the rat liver surface at 6 h increased significantly 1.4 and 1.8 times, respectively, with increase in the application area. A similar trend was seen in the biliary recovery ratio of phenol red.

A good agreement was observed between experimental values and fitting lines (Figs. 2a and 2b), and the calculated K_a values are listed in Table 2. In general, the K_a value increased in proportion to the application area, indicating that application area determines the absorption rate from the rat liver surface.

Effect of application volume on pharmacokinetics of phenol red after i.p. administration to rat liver surface

For clinical application, we studied the peritoneal absorption of phenol red in different application volumes. Figure 3 shows the plasma concentration profiles of phenol red after i.p. administration to the rat liver surface at a dose of 1 mg in three volumes (0.2, 1 or 5 mL). The plasma concentration profiles after i.p. administration were delayed by increase in the application volume. However, there was no significant difference in the plasma concentration profiles among the three application volumes, irrespective of the large variation

(0.2 to 5 mL) relative to that obtained using a glass cell (0.1 to 0.334 mL).

Table 3 lists the biliary and urinary recovery of phenol red at 6 h after i.p. administration to the rat liver surface. For every application volume, more than 97 % of dose was absorbed from the peritoneal cavity in 6 h. Accordingly, no significant difference was seen for the biliary and urinary recovery of phenol red, by alteration in application volume (Table 3).

Moreover, we calculated the K_a value of phenol red by pharmacokinetic analysis of the plasma concentration profile, on the assumption of first-order absorption from the peritoneal cavity around the liver surface. In the pharmacokinetic analysis, best-fit curves in the three volumes were obtained as shown in Fig. 3, suggesting that this model can explain the pharmacokinetics after i.p. administration. The K_a after i.p. administration to the rat liver surface decreased with the increase in the application volume, as shown in Table 3.

Discussion

From the results obtained here, application volume and area appear to be significant factors in the application of drug to the liver surface. The absorption characteristics of drug from the liver surface can be precisely evaluated with the clearance concept. The elimination clearance of phenol red from the glass cell, which is assumed to be apparent absorption clearance $CL_{a,app}$ (mL/min), is expressed as follows,

$$CL_{a,app} = K_a \cdot V_a \quad (3)$$

where V_a is the application volume. Furthermore, the absorption clearance per application area is calculated by

$$P_{app} = \frac{K_a \cdot V_a}{Area} \quad (4)$$

where Area means effective application area of a glass cell (cm^2). This parameter (P_{app} , cm/min) is equivalent to the apparent diffusion coefficient, representing drug absorbability from the liver surface. The $CL_{a,app}$ and P_{app} of phenol red after application to the rat liver surface with a glass cell were calculated according to eqs 3 and 4, as summarized in Tables 1 and 2.

Although the K_a value decreased with the increase in application volume (Table 1), the $CL_{a,app}$ was not affected (about 1×10^{-3} mL/min). This suggests equal absorbability from the liver surface when identical glass cells are used. Accordingly, the application area should be a main determinant of absorption from the liver surface.

Furthermore, we studied the change in absorption from the liver surface using several glass cells with different application areas (0.28, 0.64 and 1.54 cm^2). In general, the absorbability of phenol red increased according to the application area judging from $CL_{a,app}$ (Table 2). On the other hand, no significant difference was seen in P_{app} among the three application areas (Table 2), ranging from 1.44×10^{-3} to 1.74×10^{-3} cm/min. This result

indicates that the liver surface membrane is broadly uniform with respect to absorption characteristics. Additionally, we confirmed the simple passive transport of drug across the liver surface (Nishida et al 1995a).

The application to the liver surface for clinical use is expected to be close to follow conventional administration into the abdominal cavity. Previously (Nishida et al 1995c), we clarified i.p. administration to the liver surface enhances drug delivery to the liver in rats, because the absorption rate of the model drugs from the peritoneal cavity around the liver surface was high, as compared with around the small intestine. I.p. administration has been extensively applied to the treatment of cancers restricted to the peritoneal cavity such as ovarian carcinoma, and is considered to be an effective method for organ-specific drug delivery, owing to ease of regional delivery of anticancer drugs to the target site.

Recently, several pharmaceutical preparations such as liposome (Allen et al 1992; Sharma et al 1996), microsphere (Cremers et al 1994; Hagiwara et al 1996) and carbon particle (Hagiwara et al 1988, 1992) have been used for controlled release of drug in peritoneal cavity. For i.p. administration, application volume influences the absorption rate of drug considerably (Nagy et al 1989; Barrett et al 1991; Bredberg et al 1994). However, changes in drug absorption from the peritoneal cavity with application volume have not been considered relative to alterations in effective absorption area.

Similar to the application with a glass cell, the plasma concentration patterns of phenol red after i.p. administration to the rat liver surface were delayed and prolonged (Fig. 3), as the application volume increased. Obviously, the appearance of phenol red in plasma after i.p. administration was much faster than that using a glass cell. The absorption area is limited when the drug is applied with a glass cell, whereas the drug solution administered intraperitoneally distributes in the peritoneal cavity around the liver surface, resulting in increase of effective absorption area.

We calculated the apparent absorption clearance $CL_{a,app}$ on the assumption of first-order absorption from the peritoneal cavity, by regarding the volume of absorption compartment as the applied one. However, the derived $CL_{a,app}$ values differed considerably among the three volumes, as shown in Table 3. This result indicates that application area varies with application volume for i.p. administration.

The effective absorption area after i.p. administration is considered to depend on application volume, owing to drug diffusion in the peritoneal cavity. Therefore, we assumed that the administered solution exists in the peritoneal cavity as a sphere, in which the surface area is minimal. The surface area of the sphere of the administered volumes 0.2, 1, and 5 mL was estimated according to eq. 5 to be 1.7, 4.8 and 14.1 cm^2 , respectively. The corrected absorption clearance P_{app} was derived, as listed in Table 3. The derived P_{app} values were of the same magnitude among the three application volumes (0.45×10^{-2} - 0.61×10^{-2} cm/min), supporting the use of this assumption.

$$\text{Area} = 4\pi \cdot \left(\sqrt[3]{\frac{3 \cdot V_a}{4\pi}} \right)^2 \quad (5)$$

We do not think that the administered drug solution actually exists as a globular shape in the peritoneal cavity. Accordingly, it is necessary to understand the change in absorbability caused by dilution of drug solution with the serous fluid, binding of drug to the ingredient in peritoneal fluid or adhesion of drug to the peritoneal surrounding organ.

In conclusion, absorption rate from the liver surface appears to correlate with application volume and area. This should prove useful to pharmaceutical preparation of administration form.

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Figure titles and legends

FIG. 1. Plasma concentration profiles of phenol red at a dose of 1 mg in three volumes after application to rat liver surface. Curves show simulated functions based on the pharmacokinetic parameters shown in Table 1. Each point represents the mean \pm s.e. of four experiments.

FIG. 2. Plasma concentration profiles of phenol red at a dose of 1 mg (10 mg/mL X 0.1 mL (a) or 3 mg/mL X 0.334 mL (b)) at three application areas after application to rat liver surface. Curves show simulated functions based on the pharmacokinetic parameters shown in Table 2. Each point represents the mean \pm s.e. of four experiments.

FIG. 3. Plasma concentration profiles of phenol red at a dose of 1 mg in three volumes after intraperitoneal administration to rat liver surface. Curves show simulated functions based on the pharmacokinetic parameters shown in Table 3. Each point represents the mean \pm s.e. of four experiments.

Table

Table 1. Recovery in 6 h and pharmacokinetic parameters of phenol red at a dose of 1 mg in three volumes after application to rat liver surface.

Volume (mL)	Recovery (% of dose)			K _a	CL _{a,app}	P _{app}
	Glass cell	Bile	Urine			
0.1	8.2 ±2.0	50.7 ±5.0	29.3 ±5.8	9.20 ± 1.00	0.92 ± 0.10	1.44 ± 0.16
0.2	28.5** ±0.6	29.6* ±3.2	21.5 ±4.7	4.83** ±0.52	0.97 ±0.10	1.51 ±0.16
0.334	53.2** ±2.7	18.0** ±3.6	22.7 ±1.6	3.01** ±0.41	1.01 ±0.14	1.57 ±0.21

K_a: min⁻¹ X 10⁻³; CL_{a,app}: mL min⁻¹ X 10⁻³; P_{app}: cm min⁻¹ X 10⁻³. Each value is the mean ± s.e. of four experiments. Significantly different from the result at 0.1 mL (**p* < 0.05, ***p* < 0.01).

Table 2. Recovery in 6 h and pharmacokinetic parameters of phenol red at a dose of 1 mg after application to rat liver surface under various conditions.

Area (cm ²)	Volume (mL)	Recovery (% of dose)			K _a	CL _{a,app}	P _{app}
		Glass cell	Bile	Urine			
0.28	0.1	32.6** ±2.0	23.9** ±3.4	35.8 ±2.3	4.91** ±0.57	0.49** ±0.06	1.74 ±0.20
0.64	0.1	8.2 ±2.0	50.7 ±5.0	29.3 ±5.8	9.20 ±1.00	0.92 ±0.10	1.44 ±0.16
0.64	0.334	53.2 ±2.7	18.0 ±3.6	22.7 ±1.6	3.01 ±0.41	0.97 ±0.10	1.57 ±0.21
1.54	0.334	17.6** ±1.1	33.0 ±5.6	33.4 ±6.3	7.88** ±0.77	2.63** ±0.26	1.71 ±0.17

K_a: min⁻¹ X 10⁻³; CL_{a,app}: mL min⁻¹ X 10⁻³; P_{app}: cm min⁻¹ X 10⁻³. Each value is the mean ± s.e. of four experiments. Significantly different from the result at 0.64 cm² (**p < 0.01).

Table 3. Recovery in 6 h and pharmacokinetic parameters of phenol red at a dose of 1 mg in three volumes after intraperitoneal administration to rat liver surface.

Volume (mL)	Recovery (% of dose)		K_a	$CL_{a,app}$	P_{app}
	Bile	Urine			
0.2	51.3 ±9.6	26.0 ±7.9	3.71 ±0.57	0.74 ±0.11	0.45 ±0.07
1	43.6 ±8.0	36.2 ±9.8	2.96 ±0.72	2.96* ±0.72	0.61 ±0.15
5	57.7 ±2.3	20.9 ±7.1	1.34 ** ±0.25	6.75** ±1.25	0.48 ±0.09

K_a : $\text{min}^{-1} \times 10^{-2}$; $CL_{a,app}$: $\text{mL min}^{-1} \times 10^{-2}$; P_{app} : $\text{cm min}^{-1} \times 10^{-2}$. Each value is the mean \pm S.E. of four experiments. Significantly different from the result at 0.2 mL (* $p < 0.05$, ** $p < 0.01$).

Figure

FIG. 1

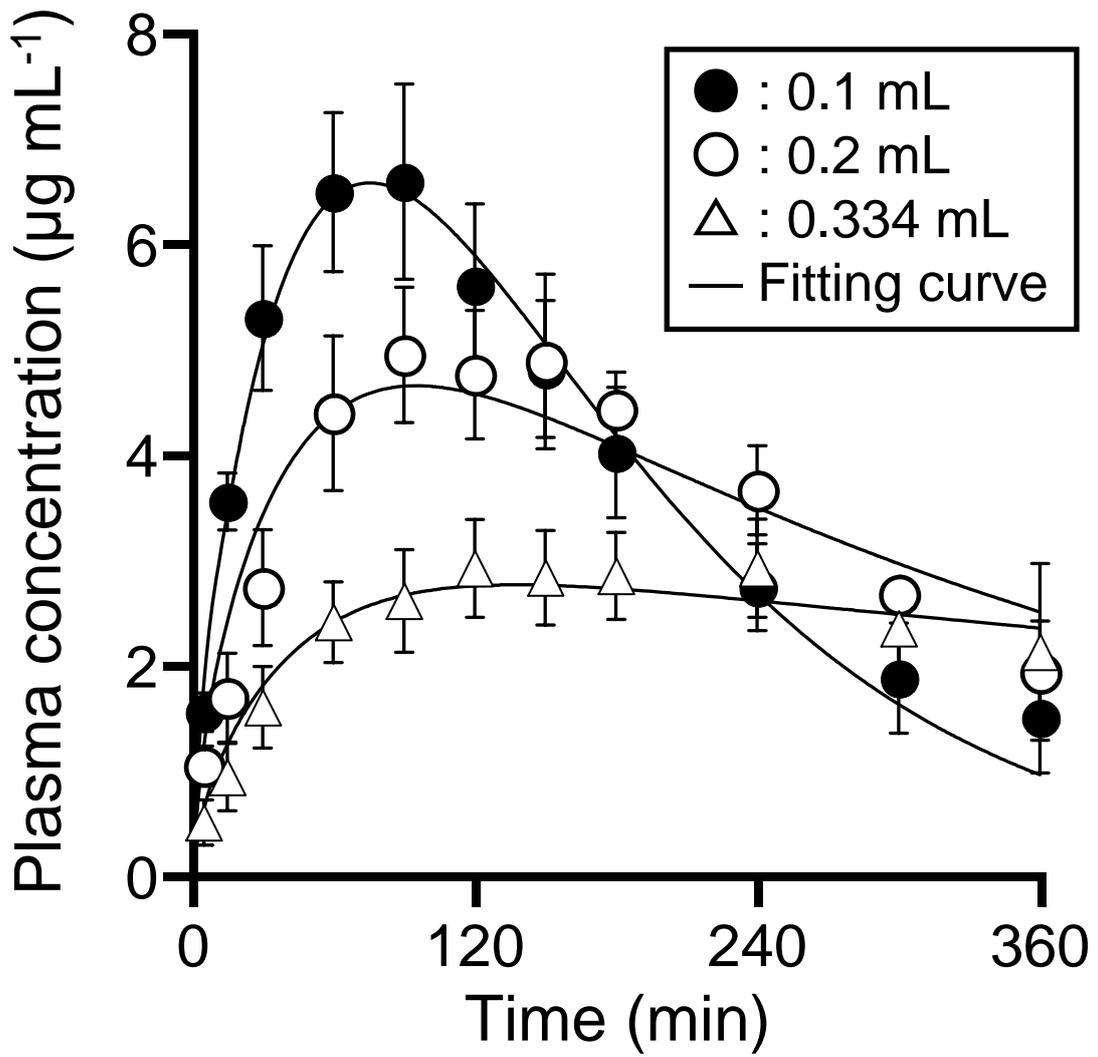


FIG. 2

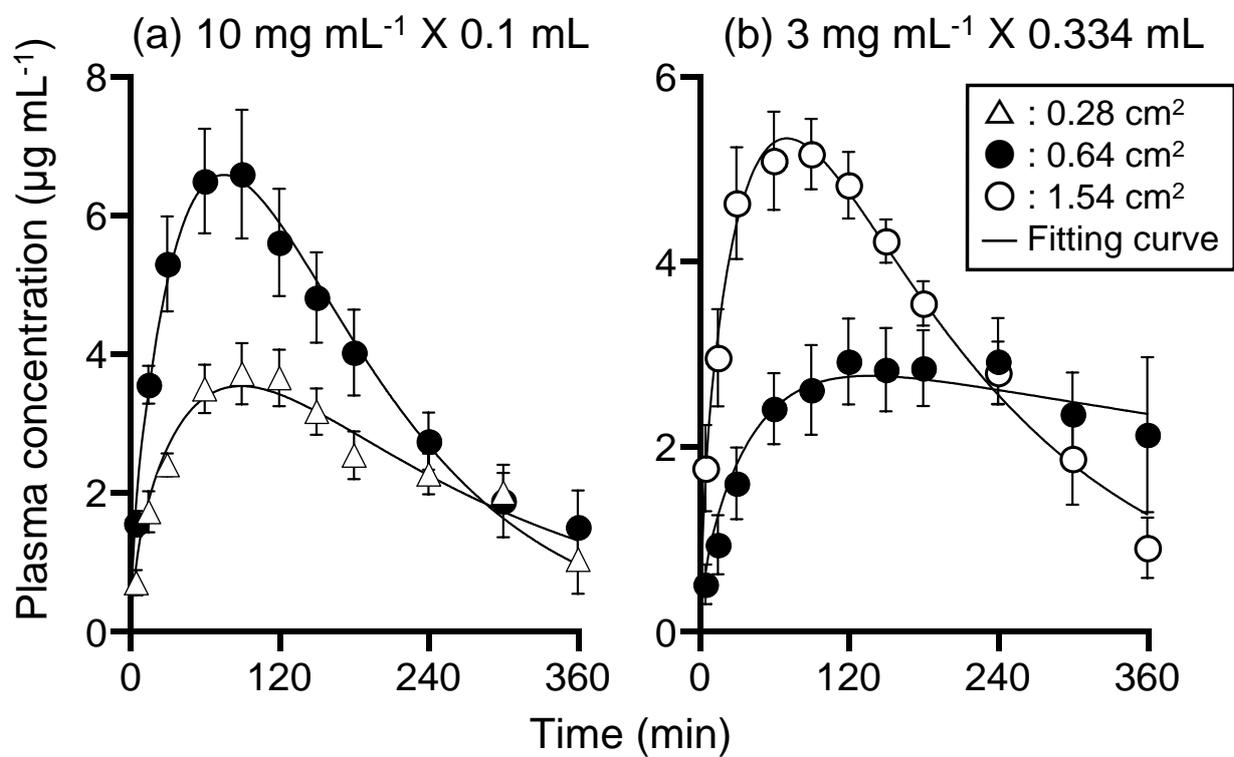


FIG. 3

