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Effect of instillation method on the absorption of phenolsulphonphthalein as a model drug from the liver and small intestinal serosal surface in rats

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Abstract

We have examined the effect of the instillation method on the absorption of a drug from the liver and the small intestinal serosal surface in rats. We performed continuous microinstillation via an infusion pump and bolus instillation via a syringe, using phenolsulphonphthalein (phenol red) as the model drug. After continuous microinstillation of phenolsulphonphthalein 2.35 mg in 235 μL for 5 min on the liver and small intestinal serosal surface in rats, the AUC (area under the curve) of the plasma concentration profile up to 60 min was significantly higher compared with bolus instillation. A similar trend was observed after continuous microinstillation of phenolsulphonphthalein 2.35 mg in 117.5 μL for 2.5 min. The calculated absorption rate constants (K_a) after continuous microinstillation of phenolsulphonphthalein based on a two-compartment model with first-order absorption were higher than those after bolus instillation on the liver and small intestinal serosal surface at either instillation concentration. Moreover, K_a was increased after continuous microinstillation of 2.35 mg in 117.5 μL at either instillation site. Instillation of phenolsulphonphthalein on the liver surface resulted in a 1.2- to 2.3-fold higher K_a compared with the small intestinal serosal surface. This tendency was marked after continuous microinstillation of 2.35 mg in 117.5 μL. In conclusion, absorption could be enhanced by instilling a small amount of drug solution on the liver surface gradually and continuously, suggesting a promising approach for instillation site-selective drug delivery in the peritoneal cavity.

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Introduction

The intraperitoneal (i.p.) administration method has been used for application of anticancer drugs that are difficult for oral administration. For the intraperitoneal administration of liver cancer chemotherapy, the drug solution is expected to distribute preferentially around the diseased region in the liver. Although intravenous, hepatic arterial and portal administration have been attempted as targeted delivery methods (Anderson et al 1994), the administered drug tends to distribute through the entire liver. We have shown previously that direct injection into an organ is not suitable for site-selective drug delivery to the liver with a high blood flow, since directly injected drugs are rapidly cleared from the injection site and enter the systemic circulation (Nishida et al 1994). Therefore, it was suggested

that instillation of a small amount of drug solution continuously should be effective to keep a high concentration of drug around the liver surface. Recently, implantable infusion pumps have been developed for the treatment of several diseases (Hepp 1994), and endoscopic and laparoscopic operation techniques have been improved greatly (Stellato 1992). These advanced medical technologies should make possible the clinical use of continuous microinstillation of drugs on the liver surface and other regions in the peritoneal cavity.

After instillation of drug solution on the liver surface, drugs are believed to spread throughout the peritoneal cavity and to be diluted by serous fluid and ascites, leading to a reduction of drug accumulation around the instillation site. Therefore, we have examined the effect of the instillation method on the absorption characteristics from the liver surface and compared it with instillation on the small intestinal serosal surface in the peritoneal cavity. We have studied the influence of the instillation drug concentration and volume on the absorption rate. For this study, phenolsulphonphthalein (phenol red) was selected as the model drug because its absorption mechanism from the liver surface in rats has been clarified (Nishida et al 1995a). Phenolsulphonphthalein is a hydrophilic dye (organic anion), and it has been used clinically as a renal function test compound in man. It is excreted into bile and urine as a free form or conjugative metabolite in rats (Hart & Schanker 1966).

Materials and Methods

Animal study

The animal experiments conformed to the Guideline for Animal Experimentation in Nagasaki University. Male Wistar rats (230–270 g), which were not starved, were anaesthetized with sodium pentobarbitone (50 mg kg^{-1} body weight, intramuscular injection). An incision was made in the middle abdomen and the left femoral artery and common bile duct were cannulated with a polyethylene tube. Additional sodium pentobarbitone was administered as necessary during the experiment to maintain anaesthesia. Phenolsulphonphthalein (Nacalai Tesque, Inc., Kyoto, Japan) solution was prepared in isotonic phosphate buffer (pH 7.4) to yield a concentration of 10 or 20 mg mL^{-1}.

Continuous microinstillation on the liver or small intestinal serosal surface

The phenolsulphonphthalein solution (20 mg $mL^{-1} \times$ 117.5 μL or 10 mg $mL^{-1} \times 235$ μL) was instilled using a polyethylene tube fixed by a clamp on the surface of the left lateral liver lobe or distal small intestine with an infusion pump (flow rate 0.047 mL min^{-1}) (Natsume, Tokyo, Japan) for 2.5 or 5 min, respectively.

Bolus instillation on the liver or small intestinal serosal surface

The phenolsulphonphthalein solution (20 mg $mL^{-1} \times$ 117.5 μL or 10 mg $mL^{-1} \times 235$ μL) was instilled momentarily onto the surface of the left lateral lobe or distal small intestine using a syringe.

After instillation of the phenolsulphonphthalein solution, 200 μL blood was collected at 2, 5, 10, 15, 30, 60, 90, 120, 150, 180, 210 and 240 min from the heparinized cannula inserted into the femoral artery. Blood was centrifuged at 15 000 rev min^{-1} for 5 min. Bile samples were collected at appropriate time intervals for 4 h. At 4 h after instillation, the solution remaining in the peritoneal cavity was withdrawn by washing with saline.

The concentration of free phenolsulphonphthalein was determined spectrophotometrically at 560 nm after dilution with 1 M NaOH. The total concentration of free phenolsulphonphthalein and its conjugative metabolite (glucuronic acid conjugate) was measured in the same manner after the samples were subjected to acid hydrolysis (1 M HCl at 100°C for 30 min) (Hart & Schanker 1966). The concentration of phenolsulphonphthalein metabolite was estimated from the difference between these values. The phenolsulphonphthalein metabolite could not be detected in the plasma.

Pharmacokinetic analysis with a compartment model

Compartment model analysis of the plasma concentration profile of phenolsulphonphthalein after bolus instillation or continuous microinstillation on the liver or small intestinal serosal surface in rats was performed based on a two-compartment model with first-order absorption as follows by the non-linear least-squares method (Yamaoka et al 1981). The pharmacokinetic models are illustrated in Figure 1.

Bolus instillation

The equation for the concentration of the central compartment (C_1) after bolus instillation was based on the model as shown in Figure 1 model B.

$$C_1 = \frac{F \cdot D \cdot K_a}{V_c}\left\{\frac{K_{21}-K_a}{(\beta-K_a)(\alpha-K_a)}e^{-K_a \cdot t} + \frac{K_{21}-\alpha}{(\beta-\alpha)(K_a-\alpha)}e^{-\alpha \cdot t} + \frac{K_{21}-\beta}{(\alpha-\beta)(K_a-\beta)}e^{-\beta \cdot t}\right\} \quad (1)$$

Hybrid parameters α and β are defined as $\alpha+\beta =$

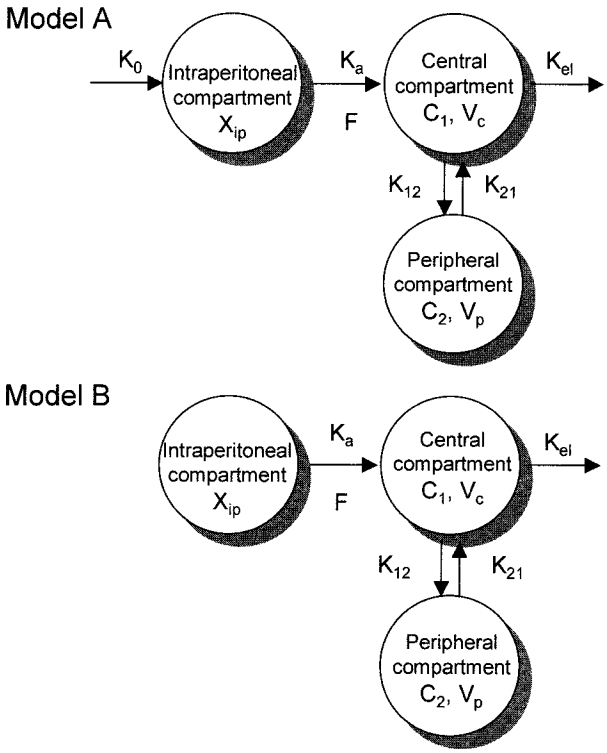

Figure 1 Pharmacokinetic model for phenolsulphonphthalein after continuous microinstillation or bolus instillation. Model A, $t < t_0$, continuous microinstillation during drug instillation; model B, $t \geqq t_0$, continuous microinstillation or bolus instillation. X_{ip}, amount in intraperitoneal compartment; C_1, concentration of central compartment; C_2, concentration of peripheral compartment; K_0, instillation rate; K_a, first-order absorption rate constant; K_{el}, first-order elimination rate constant; K_{12}, K_{21}, first-order transfer rate constants; V_c, volume of central compartment; V_p, volume of peripheral compartment.

$K_{12}+K_{21}+K_{el}$ and $\alpha\cdot\beta = K_{21}\cdot K_{el}\cdot V_c$ 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 compartments. K_a is the first-order absorption rate constant for absorption into the blood stream from the liver or small intestinal serosal surface. D is the instilled dose, and F is the availability after instillation. The result for intravenous administration of phenolsulphonphthalein was reported by Nishida et al (1995a).

Continuous microinstillation

We first analysed the plasma concentration profile after the end of instillation (t_0) with a two-compartment model as shown in Figure 1 model B according to equation 2 by the non-linear least-squares method (Yamaoka et al 1981). In equation 2, $X_{ip}(0)$ is the drug amount in the intraperitoneal compartment at the end of instillation (t_0). $C_1(0)$ and $C_2(0)$ are concentrations of the central and peripheral compartment at the end of instillation (t_0), respectively. After obtaining the pharmacokinetic parameters (K_a and F), the plasma concentration profile before the end of instillation ($t < t_0$) was simulated according to equation 3. In equation 3, K_0 is the drug instillation rate.

(i) $t \geqq t_0$:

$$C_1 = \frac{F\cdot K_a\cdot X_{ip}(0)}{V_c}\left\{\frac{K_{21}-K_a}{(\beta-K_a)(\alpha-K_a)}e^{-K_a(t-t_0)} + \frac{K_{21}-\alpha}{(\beta-\alpha)(K_a-\alpha)}e^{-\alpha(t-t_0)} + \frac{K_{21}-\beta}{(\alpha-\beta)(K_a-\beta)}e^{-\beta(t-t_0)}\right\} + C_1(0)\left\{\frac{K_{21}-\alpha}{\beta-\alpha}e^{-\alpha(t-t_0)} + \frac{K_{21}-\beta}{\alpha-\beta}e^{-\beta(t-t_0)}\right\} + \frac{C_2(0)\cdot K_{21}}{\beta-\alpha}\{e^{-\alpha(t-t_0)} - e^{-\beta(t-t_0)}\} \quad (2)$$

(ii) $t < t_0$:

$$C_1 = \frac{F\cdot K_0\cdot K_a}{V_c}\left\{\frac{K_{21}}{K_a\cdot\alpha\cdot\beta} + \frac{K_a-K_{21}}{K_a(\beta-K_a)(\alpha-K_a)}e^{-K_a\cdot t} + \frac{\alpha-K_{21}}{\alpha(\beta-\alpha)(K_a-\alpha)}e^{-\alpha\cdot t} + \frac{\beta-K_{21}}{\beta(\alpha-\beta)(K_a-\beta)}e^{-\beta\cdot t}\right\} \quad (3)$$

Statistical analysis

Statistical analysis was performed by applying the unpaired Student's *t*-test. $P < 0.05$ was considered to be statistically significant. All values were expressed as the mean ± standard error of at least four experiments.

Results and Discussion

Effect of the instillation method on the absorption of phenolsulphonphthalein from the liver and small intestinal serosal surface

We performed continuous microinstillation via an infusion pump and bolus instillation via a syringe of phenolsulphonphthalein solution 2.35 mg in 235 or 117.5 μL on the liver and small intestinal serosal surface in rats. The plasma concentration profiles of phenolsulphonphthalein are shown in Figure 2 in a solution of 2.35 mg in 235 μL. The plasma concentrations of phenolsulphonphthalein after continuous microinstillation of 2.35 mg in 235 μL for 5 min on the liver and small intestinal serosal surface were higher in the first 60 min compared with those after bolus instillation. The maxi-

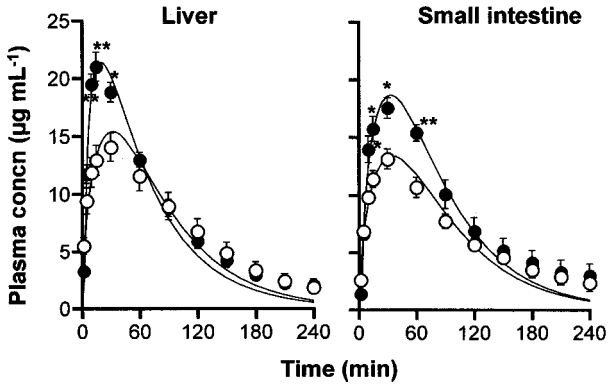

Figure 2 Plasma concentration profiles of phenolsulphonphthalein after continuous microinstillation for 5 min at a flow rate of 0.047 mL min^{-1} (●) or bolus instillation (○) of 2.35 mg in 235 μL on the liver or small intestinal serosal surface in rats. Curves show the simulated function based on the pharmacokinetic parameters listed in Table 2. Each point represents the mean ± s.e. of at least four experiments. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the result after bolus instillation.

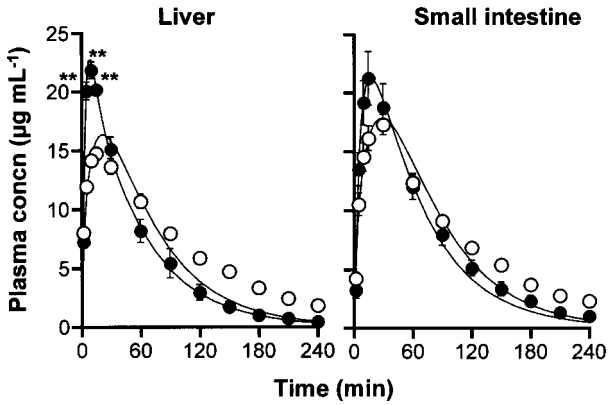

Figure 3 Plasma concentration profiles of phenolsulphonphthalein after continuous microinstillation for 2.5 min at a flow rate of 0.047 mL min^{-1} (●) or bolus instillation (○) of 2.35 mg in 117.5 μL on the liver or small intestinal serosal surface in rats. Curves show the simulated function based on the pharmacokinetic parameters listed in Table 2. Each point represents the mean ± s.e. of at least four experiments. $^{**}P < 0.01$ compared with the result after bolus instillation.

mum plasma concentration was increased by 1.5- and 1.3-fold after continuous microinstillation on the liver and small intestinal serosal surface, respectively, compared with bolus instillation (Figure 2).

Figure 3 shows similar findings for instillation of phenolsulphonphthalein solution of 2.35 mg in 117.5 μL. A similar tendency of higher maximum concentration was observed in the plasma concentration profiles of phenolsulphonphthalein after continuous microinstillation of 2.35 mg in 117.5 μL for 2.5 min, compared with bolus instillation (Figure 3). Accordingly, an increase in the absorption of phenolsulphonphthalein by continuous microinstillation was suggested.

Table 1 summarizes the recovery ratio in the peritoneal cavity, and biliary and urinary excretion ratios of phenolsulphonphthalein at 4 h after instillation on the liver or small intestinal serosal surface under different instillation conditions. Since the absorption from the peritoneal cavity was almost completed in 4 h, the recovery ratios of phenolsulphonphthalein from the peritoneal cavity were less than 3 % of the dose. The total biliary excretion ratio after 4 h of phenolsulphonphthalein bolus instillation was higher than that after continuous microinstillation. The urinary excretion ratio after continuous microinstillation was higher than after bolus instillation, although the difference was not significant. The difference in the biliary and urinary excretion ratios of phenolsulphonphthalein might be considered to be alterations in the absorption route after continuous microinstillation and bolus instillation.

Pharmacokinetic analysis of the plasma concentration profiles of phenolsulphonphthalein

To examine the time course of the absorption rate of phenolsulphonphthalein, the area under the curve (AUC) of the plasma concentration of phenolsulphonphthalein (AUC_p) was calculated at 10, 15, 30, 60 and 240 min (Table 2). Continuous microinstillation on the liver and small intestinal serosal surface exhibited higher AUC_p values of phenolsulphonphthalein up to 60 min after instillation compared with those after bolus instillation at either concentration, even though no difference was observed in AUC_p values up to 240 min. This indicated increased absorption from the liver and small intestinal serosal surface by continuous microinstillation. The trend was marked in continuous microinstillation on the liver compared with the small intestinal serosal surface.

The plasma concentration profiles of phenolsulphonphthalein were analysed based on a compartment model (illustrated in Figure 1), assuming that the absorption process after continuous microinstillation and bolus instillation followed first-order kinetics. For continuous microinstillation, the plasma concentration profile during drug instillation ($t < t_0$) was simulated based on model A (Figure 1) using the K_a value obtained with the model fitting of the plasma concentration profile after drug instillation ($t \geqq t_0$) based on the pharmacokinetic

Table 1 Recovery (% of dose) after 4 h of phenolsulphonphthalein continuous microinstillation or bolus instillation on the liver or small intestinal serosal surface in rats.

Site	Method	Peritoneal cavity	Bile			Urine		
			Free	Metabolite	Total	Free	Metabolite	Total
2.35 mg in 235 μL								
Liver	Continuous (5 min)	1.9 ± 0.6	25.2 ± 4.7	13.8 ± 1.4	39.0 ± 5.6	30.8 ± 4.3	9.0 ± 1.7	39.8 ± 5.8
	Bolus	1.7 ± 0.3	33.6 ± 2.3	10.1 ± 2.9	43.7 ± 4.5	20.7 ± 3.7	4.8 ± 1.3	25.5 ± 3.1
Small intestine	Continuous (5 min)	1.4 ± 0.5	26.4 ± 4.9	16.6 ± 2.4	43.0 ± 7.2	34.2 ± 5.7	5.7 ± 1.2	39.9 ± 5.5
	Bolus	2.7 ± 0.8	32.5 ± 5.3	16.0 ± 3.2	48.5 ± 7.9	20.3 ± 3.0	6.1 ± 1.8	26.4 ± 4.5
2.35 mg in 117.5 μL								
Liver	Continuous (2.5 min)	1.4 ± 0.6	24.4 ± 5.0	12.0 ± 3.0	36.4 ± 6.9	35.4 ± 4.6	5.2 ± 0.7	40.6 ± 4.7
	Bolus	2.0 ± 0.2	35.8 ± 4.4	12.1 ± 3.1	47.9 ± 4.1	24.9 ± 2.0	4.5 ± 0.9	29.4 ± 1.7
Small intestine	Continuous (2.5 min)	1.5 ± 0.3	32.6 ± 3.7	15.2 ± 2.2	47.8 ± 4.6	37.8 ± 3.2	4.3 ± 1.2	42.1 ± 3.8
	Bolus	2.2 ± 0.2	35.6 ± 1.0	16.0 ± 1.8	51.6 ± 2.0	28.0 ± 3.0	6.1 ± 1.8	34.1 ± 4.5

Each value is the mean ± s.e. of at least four experiments.

Table 2 AUC_p and K_a of phenolsulphonphthalein after continuous microinstillation or bolus instillation on the liver or small intestinal serosal surface in rats.

Site	Method	AUC_p (μg mL^{-1} min)					K_a (min$^{-1} \times 10^{-2}$)
		0–10	0–15	0–30	0–60	0–240	
2.35 mg in 235 μL							
Liver	Continuous (5 min)	103.5 ± 4.5*	204.6 ± 8.9**	502.8 ± 23.9**	978.4 ± 45.5*	1920.9 ± 135.7	7.41 ± 0.45**
	Bolus	80.8 ± 9.4	142.7 ± 15.7	344.8 ± 34.1	728.2 ± 69.2	1714.7 ± 215.3	4.00 ± 0.60
Small intestine	Continuous (5 min)	64.6 ± 4.6	138.7 ± 10.1	387.8 ± 23.6*	880.8 ± 44.1*	2038.7 ± 146.8	3.93 ± 0.48
	Bolus	58.3 ± 4.3	111.2 ± 7.8	294.8 ± 20.3	651.4 ± 49.0	1573.3 ± 168.7	3.24 ± 0.23
2.35 mg in 117.5 μL							
Liver	Continuous (2.5 min)	152.7 ± 4.8**	257.6 ± 6.5**	521.6 ± 13.7**	870.0 ± 43.0	1436.0 ± 145.8	19.35 ± 2.35***
	Bolus	103.0 ± 2.2	175.2 ± 4.2	387.6 ± 12.3	751.4 ± 30.4	1669.0 ± 78.8	6.28 ± 0.59
Small intestine	Continuous (2.5 min)	109.7 ± 11.2	210.6 ± 21.5	510.4 ± 51.3	971.0 ± 91.2	1766.4 ± 156.0	8.38 ± 1.10*
	Bolus	88.9 ± 8.0	165.5 ± 13.6	415.8 ± 26.0	860.3 ± 47.1	1909.2 ± 110.6	4.44 ± 0.44

Each value is the mean ± s.e. of at least four experiments. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with the result after bolus instillation.

model B shown in Figure 1. Figures 2 and 3 show the fitting curves under different instillation conditions. In general, they agreed well with the experimental values and, therefore, the validity of this compartment model analysis was confirmed. The deviation between the experimental and fitted values was observed in some data series (Figures 2 and 3) due to the curve fitting procedure (non-linear least-square regression).

The K_a values of phenolsulphonphthalein under different conditions are listed in Table 2. In the comparison of identical instillation sites, the K_a values of phenolsulphonphthalein after continuous microinstillation were larger compared with the values after bolus instillation. This supported the idea of the improvement of absorbability by continuous microinstillation. The K_a of phenolsulphonphthalein was increased by 1.4 to 2.6-fold by reducing the instillation volume from 235 to 117.5 μL, as shown in Table 2. This finding was consistent with our observation that the K_a of phenolsulphonphthalein after intraperitoneal administration to the rat liver surface increased with decreasing application volume (Nishida et al 1997).

Influence of the instillation site on the absorption rate of phenolsulphonphthalein

The pharmacokinetic analysis suggested that continuous microinstillation of phenolsulphonphthalein resul-

ted in faster absorption from the peritoneal cavity. This tendency was significant after continuous microinstillation of 2.35 mg in 117.5 μL for 2.5 min on the liver surface, when the K_a was approximately 3-fold higher compared with bolus instillation (Table 2). When comparing the K_a values at identical instillation volumes, the instillation of phenolsulphonphthalein on the liver surface increased the K_a value by 1.2 to 2.3-fold compared with instillation on the small intestinal serosal surface. We reported previously (Nishida et al 1995b) that the absorption rate from the peritoneal cavity was faster after intraperitoneal administration on the liver surface than after intraperitoneal administration on the distal small intestinal serosal surface. This was shown by the increase in the maximum concentration and the decrease in the mean residence time of phenolsulphonphthalein in plasma.

When drugs are instilled continuously, it appears that the drug concentration at the instillation site can be maintained because of the restricted distribution compared with bolus instillation. In a previous study, the concentration of phenolsulphonphthalein at the instillation site in the liver (left lateral lobe) was significantly higher than at the other lobes of the liver after continuous microinstillation (Nakamura et al 1999). However, when the same volume of drug solution was instilled on the small intestine or injected intravenously, no significant difference in drug concentration between the left lateral and other lobes was found, suggesting a uniform distribution of phenolsulphonphthalein in the liver (Nakamura et al 1999).

In conclusion, a good absorption of phenolsulphonphthalein from the instilled site of the liver was attained by continuous microinstillation, possibly leading to restricted drug distribution in the liver. Further information should be clarified for clinical relevance in the future.

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