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Absorption of Phenol Red and Bromphenol Blue as Model Drugs from the Peritoneal Cavity around the Liver Surface in Rats

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Abstract

The importance of the injection site on the pharmacokinetics of phenol red and bromphenol blue as model drugs after intraperitoneal administration into rat was examined.

Their absorption rate from the peritoneal cavity was faster after intraperitoneal administration to the liver surface than that after intraperitoneal administration to the distal small intestine, as shown by the increase in maximum concentration and decrease in mean residence time in plasma. A similar tendency was observed in the biliary excretion pattern. The enhanced absorption rate was supported by the significantly smaller amount of both drugs remaining in the peritoneal cavity at 15 min after liver surface administration than that after small intestine administration. The liver concentration of the model drugs at 15 min after liver surface administration was 1·5–2·0 times that after small intestine administration.

Accordingly, liver surface administration was shown to be effective with good absorption and efficient drug delivery to the liver.

In the chemotherapy of a localized tumour in the liver, direct drug injection to the liver has been suggested to enhance local drug distribution (Mattijssen et al 1992; Balemans et al 1993; Dubinett et al 1993). On the other hand, regional drug application to the organ surface via the intraperitoneal route may be advantageous for organ-specific drug delivery, particularly to the liver. However, the differences in the drug absorption from the peritoneal cavity between different injection sites have not been elucidated. In the present study, we examined the importance of the intraperitoneal injection site on the absorption of two organic anions (phenol red and bromphenol blue) as model drugs.

Materials and Methods

Chemicals

Phenol red and bromphenol blue were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). All other chemicals were of reagent grade.

In-vivo experiments

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

Male Wistar rats, 230–250 g, were anaesthetized with sodium pentobarbitone (50 mg kg^{-1}, i.m.), 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). An incision of approximately 2 cm 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, USA). Phenol red (2 or 6 mg) or bromphenol blue (2 mg) in an isotonic phosphate buffer (pH 7·4) (0·2 mL) was administered intraperitoneally to the area of either the liver surface or distal small intestine. The injection point for the liver surface was the division between the right and left lobe. The drug solution was administered to the small intestine 5 cm below the injection point for the liver surface.

After application of the drug solution, 200 μL blood was collected at selected times from the heparinized cannula inserted into the femoral artery over 4 h. Blood was centrifuged at 15 000 rev min^{-1} for 5 min. Bile samples were collected at appropriate time intervals for 4 h. In other experiments, the drug solution remaining in the peritoneal cavity was withdrawn by washing with saline at 15 or 60 min after dosing, and then the liver was excised.

Analytical methods

The concentrations of model drugs in plasma, bile and the remaining solution in the peritoneal cavity were determined.

The concentration of free phenol red was determined spectrophotometrically at 560 nm after dilution with 1 M NaOH. The total concentration of free phenol red and its metabolite 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 phenol red metabolite was estimated from the difference between these values.

The concentration of bromphenol blue was determined spectrophotometrically at 591 nm after dilution with isotonic phosphate buffer (pH 7·4) (Takada et al 1974).

The concentration of the model drug in the liver was determined as follows. The excised liver was homogenized in three times its weight of distilled water and of pH 6·5 phosphate buffer for analysis of phenol red and bromphenol blue, respectively. After 5 mL acetone was added to 5 mL of

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the liver homogenate, the mixture was shaken for 15 min, followed by centrifugation for 15 min at 3000 rev min^{-1}. The concentration of the drug in the resulting supernatant was determined as described above.

Calculation of moment parameters

The moment parameters for the plasma concentration profile of free phenol red and bromphenol blue (AUC_p, MRT_p) and those for biliary excretion rate–time curves of free phenol red and bromphenol blue ($AUC_{b,f}$, $MRT_{b,f}$), and of the phenol red metabolite ($AUC_{b,m}$, $MRT_{b,m}$) were calculated by numerical integration using a linear trapezoidal formula and extrapolation to infinite time based on a monoexponential equation (Yamaoka et al 1978).

Results and Discussion

Intraperitoneal administration has been extensively applied to treatment of the cancer 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 a drug to the target site.

The main purpose of this study was to examine the difference in pharmacokinetics after intraperitoneal administration of the drug at different injection sites. We selected phenol red and bromphenol blue as model drugs, because their in-vivo absorbability after application to rat liver surface employing a glass cell had already been determined (Nishida et al 1994, 1995).

Figs 1a and 1b show the plasma concentration profiles of phenol red (2 or 6 mg) and bromphenol blue (2 mg), respectively, after their intraperitoneal administration to the area around the liver surface or small intestine in rats. Intraperitoneally administered phenol red appeared in plasma with a peak concentration 30 min after dosing, followed by gradual disappearance. The plasma concentration of phenol red after liver surface administration was higher than that after small intestine administration up to 60 min, and its maximum concentration (C_{max}) was increased. On the other hand, the plasma concentration profile of bromphenol blue after liver surface administration reached a peak at 5 min. In this case, the C_{max} value after liver surface administration was 3·3 times that after small intestine administration. These findings suggest increased absorption from the peritoneal cavity around the liver surface in rats.

Figs 2a–c illustrate the biliary excretion rate-time curves of free phenol red and its metabolite, and bromphenol blue after administration of phenol red and bromphenol blue. During the first 60 min after liver surface administration, the biliary excretion of both drugs was more rapid than that after small intestine administration. This observation can be explained by the increased peritoneal absorption rate.

The overall absorption process can be evaluated with moment parameters. In particular, AUC_p and MRT_p are useful parameters for evaluating the drug absorbability from the peritoneal cavity. Table 1 summarizes the moment parameters for phenol red and bromphenol blue after liver surface or small intestine administration to rats.

The AUC_p values for both drugs after liver surface administration were larger than those after small intestine administration, although less markedly for phenol red. The difference in MRT_p values between intraperitoneal and intravenous administration corresponds to the mean time value for the absorption from the peritoneal cavity (MAT). MRT_p after intravenous administration of phenol red at a dose of 2 or 6 mg, and bromphenol blue were calculated to be 43·7, 43·6 and 9·5 min, respectively (unpublished data). As shown in Table 1, the MAT values for both drugs were shortened considerably by liver surface administration, the reduction being more marked for bromphenol blue.

With respect to biliary excretion, the $MRT_{b,f}$ for both drugs after liver surface administration was shorter than that after small intestine administration. The $AUC_{b,m}$ value for phenol red was increased in liver surface administration, suggesting that its metabolism is affected by the injection site.

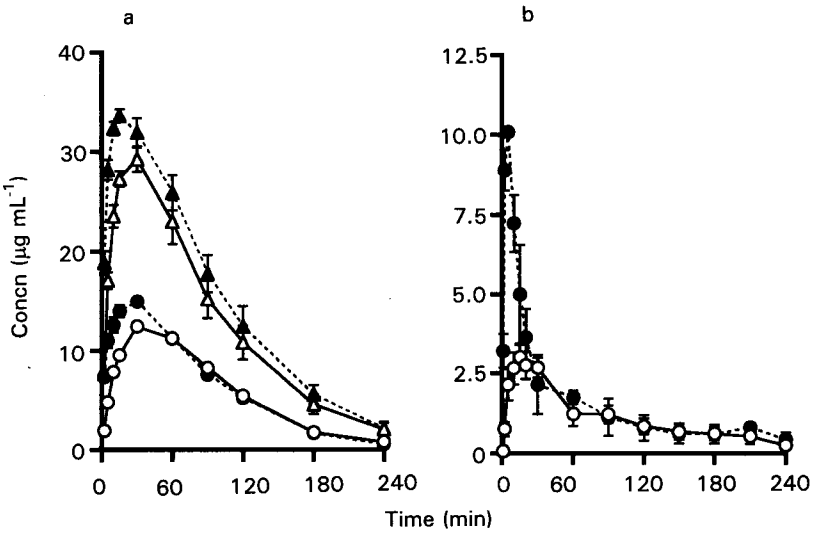

FIG. 1. Plasma concentration profiles of (a) phenol red at a dose of 2 (○, ●) or 6 mg (△, ▲), and (b) bromphenol blue (2 mg) (○, ●) after liver surface (●, ▲) or small intestine (○, △) administration. Values are means ± s.e. of at least three experiments.

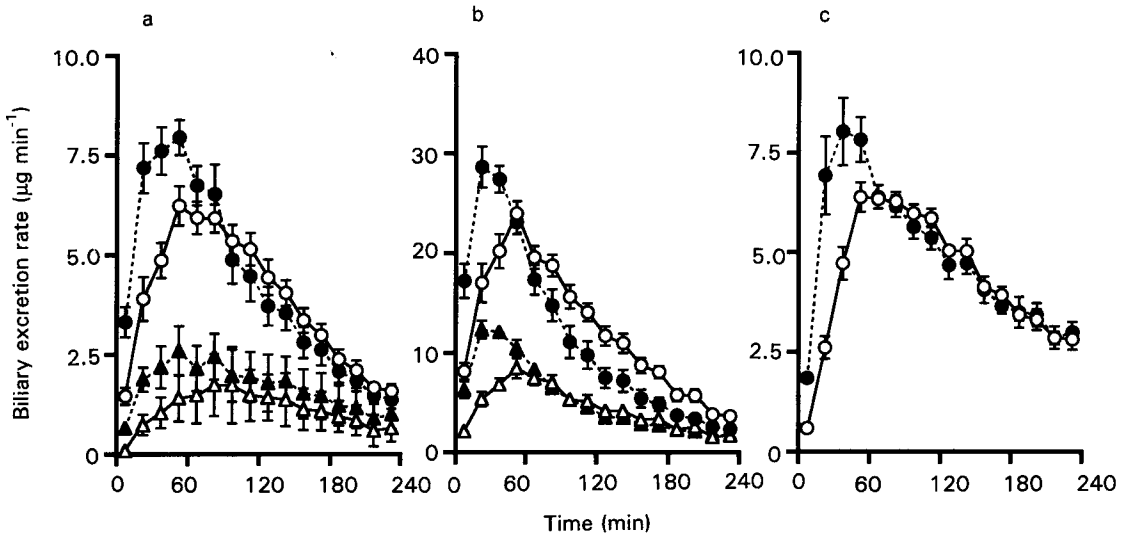

FIG. 2. Biliary excretion rate–time curves for free phenol red (○, ●) and its metabolite (△, ▲) at a dose of 2 (a) or 6 mg (b), and bromphenol blue (c) (2 mg) (○, ●) after liver surface (●, ▲) or small intestine (○, △) administration. Values are means ± s.e. of at least three experiments.

Table 2 shows the level of phenol red and bromphenol blue in the peritoneal cavity and liver at 15 and 60 min after administration.

The amount remaining in the peritoneal cavity after liver surface administration was smaller than that after small intestine administration for both drugs, although the difference was not significant for bromphenol blue at 60 min. This supports the high absorption of these model drugs from the peritoneal cavity around the liver surface.

The liver concentrations of phenol red and bromphenol blue at 15 min after liver surface administration were 1·5–2·0 times those after small intestine administration, which suggests that the former route of administration enhances drug delivery to the liver.

The effects of molecular weight (Dedrick et al 1978; Litterst et al 1982), charge (Lukas et al 1971), and injection volume (Barrett et al 1991; Bredberg et al 1994) on the drug absorption after intraperitoneal administration have been reported. In contrast, the present study provides evidence of a difference in pharmacokinetics after intraperitoneal administration according to the injection site. However, the mechanism underlying this phenomenon is not clear.

Previously (Nishida et al 1994, 1995), we examined the in-vivo behaviour of model drugs after application to liver surface in rats, using a cylindrical glass cell (i.d. 9 mm; area 0·64 cm^2), to clarify drug absorption from the liver-surface membrane. The absorption rates of phenol red and bromphenol blue at 6 h were relatively high (91·8 and 71·6% of dose, respectively). The difference in the peritoneal absorption of model drugs with the injection site might be closely related to the good absorbability from the liver-surface membrane.

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Table 1. Moment parameters for plasma concentration and biliary excretion rate-time curves for free phenol red and its metabolite, and bromphenol blue after liver surface or small intestine administration in rats.

	Phenol red (2 mg)		Phenol red (6 mg)		Bromphenol blue (2 mg)	
Parameter	Intestine	Liver	Intestine	Liver	Intestine	Liver
AUC_p (μg min mL^{-1})	1443·9 ± 72·8	1533·6 ± 53·4	3255·5 ± 232·9	3663·7 ± 306·1	224·5 ± 8·3	433·9* ± 61·6
MRT_p (min)	79·3 ± 0·8	67·2** ± 2·2	70·1 ± 5·5	57·9* ± 1·8	61·3 ± 3·5	25·9** ± 1·7
MAT[a] (min)	35·6	23·5	26·5	14·3	51·8	16·4
$AUC_{b,f}$ (μg)	1037·8 ± 58·9	1115·1 ± 108·0	3283·3 ± 99·9	3009·6 ± 173·4	1566·1 ± 74·9	1621·2 ± 71·1
$AUC_{b,m}$ (μg)	347·3 ± 160·5	534·8 ± 179·0	1143·1 ± 134·1	1461·0 ± 72·7	–	–
$MRT_{b,f}$ (min)	131·8 ± 5·5	112·6* ± 5·9	109·5 ± 4·9	88·6* ± 4·1	214·9 ± 8·0	192·5 ± 20·1
$MRT_{b,m}$ (min)	175·7 ± 16·7	182·7 ± 29·9	113·8 ± 5·5	118·9 ± 8·4	–	–

[a]MAT (mean absorption time) is the difference in MRT_p values between intravenous and intraperitoneal administration. *$P < 0.05$, **$P < 0.01$ Student's t-test compared with small intestine administration. Each value is the mean ± s.e. of at least three experiments.

Table 2. Recovery (% of dose) in peritoneal cavity and concentration in liver of phenol red and bromphenol blue after liver surface or small intestine administration in rats.

Drug	Site	Peritoneal cavity (%)		Liver μg (g tissue)$^{-1}$	
		15 min	60 min	15 min	60 min
Phenol red (2 mg)	Intestine	57·9 ± 2·6	27·2 ± 2·1	4·1 ± 0·4	4·7 ± 0·2
	Liver	49·9* ± 2·4	16·3* ± 2·5	8·3** ± 0·9	10·6** ± 0·8
Phenol red (6 mg)	Intestine	58·0 ± 2·4	27·1 ± 1·1	15·1 ± 0·8	12·5 ± 2·2
	Liver	43·6** ± 2·4	16·9** ± 1·1	26·5** ± 0·2	15·8 ± 0·8
Bromphenol blue (2 mg)	Intestine	63·0 ± 2·4	36·7 ± 2·0	22·0 ± 2·3	31·2 ± 1·8
	Liver	47·0** ± 2·3	35·4 ± 1·9	33·1** ± 1·6	31·1 ± 1·8

*$P < 0.05$, **$P < 0.01$ Student's t-test compared with small intestine administration. Each value is the mean ± s. e. of at least three experiments.

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