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Absorption of Organic Anions as Model Drugs Following Application to Rat Liver Surface In-vivo

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Abstract—Absorption of organic anions (phenol red, bromphenol blue and bromosulphonphthalein) has been studied after their application to rat liver surface in-vivo, employing a cylindrical glass cell (i.d. 9 mm, area 0·64 cm^2). Each drug appeared gradually in the blood with the peak level at about 1 h, after which its concentration declined slowly. Absorbed model drug was efficiently excreted into the bile. These observations appear to indicate the possibility of drug absorption from liver surface membrane. Absorption of model drugs was estimated to be more than 59% in 6 h. The biliary recovery and metabolism of phenol red did not change as compared with that after intravenous administration.

Liver plays an important role in drug disposition in the body, and there is an increasing interest in improving treatment of liver diseases. To treat liver disease, the administered drug should distribute largely into the liver target site. However, the usual routes of drug administration do not achieve a local site of action in the liver.

Although direct drug application to the liver surface should yield local drug distribution, drug absorption from the liver surface has not been reported in the literature.

In the present study, we have examined the absorption of organic anions (phenol red, bromphenol blue, bromosulphonphthalein) as model drugs, after application to the rat liver surface.

Materials and Methods

Chemicals

Phenol red and bromphenol blue were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Bromosulphonphthalein was obtained from Sigma Chemical Co. (St Louis, MO, USA). All other chemicals were of reagent grade.

In-vivo experiment

All animal experiments 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.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 cut of approximately 3 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). The body temperature of the rats was kept at 37°C by a heat lamp during the experiment. The test solution was prepared in an isotonic phosphate buffer (pH 7·4) to yield a concentration of 1 mg in 0·1 mL, and administered as follows.

Application to rat liver surface

A cylindrical glass cell (i.d. 9 mm, area 0·64 cm^2) was attached to the rat liver surface at the area of the left lobe with Aron Alpha (Sankyo Co. Ltd, Tokyo, Japan). The drug solution (0·1 mL) was added to the glass cell directly. The top of the glass cell was sealed by a piece of aluminium foil to prevent evaporation of the applied solution.

Intravenous administration

The drug solution (0·1 mL) was injected into the jugular vein.

Direct injection into rat liver

The test solution (0·1 mL) was directly injected over 20 s into the centre of the left lobe of the liver by using a syringe at a depth of 3 mm. The midpoint of the injection time was defined as time 0.

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 or 6 h. Blood was centrifuged at 15000 rev^{-1} min^{-1} for 5 min. Bile samples were collected at appropriate time intervals for 4 or 6 h. At 4 or 6 h after the application, urine was collected from the bladder directly by syringe. Following application to rat liver surface, the remaining dose solution in the glass cell was withdrawn at 6 h after dosing.

Analytical method

The concentrations of model drugs in plasma, bile, urine and remaining solution in the glass cell 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 600 nm after dilution with isotonic phosphate buffer (pH 7·4) (Takada et al 1974).

The concentration of bromosulphonphthalein was determined spectrophotometrically at 580 nm after dilution with 0·1 M NaOH (Frezza et al 1974).

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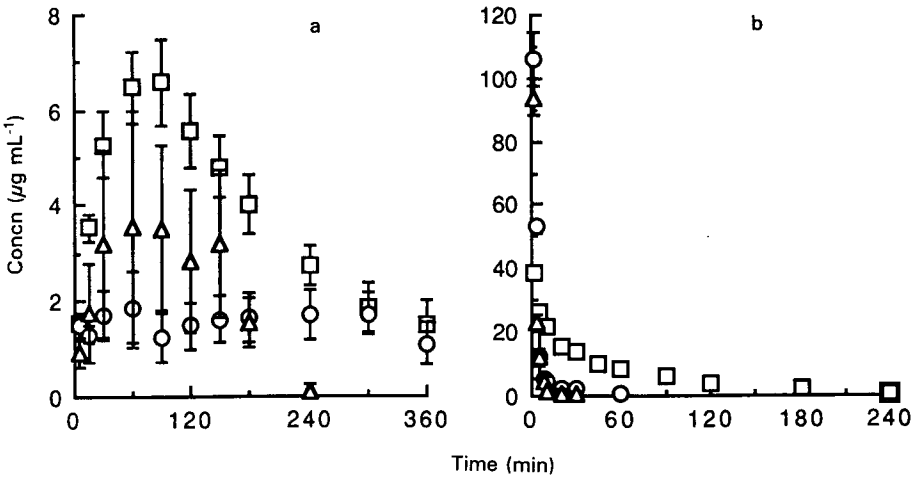

FIG. 1. Plasma concentrations of phenol red (□), bromphenol blue (○) and bromosulphonphthalein (△) after application to rat liver surface (a) and intravenous administration (b) at a dose of 1 mg. Each point represents mean ± s.e. of four experiments.

Calculation of moment parameters

The plasma concentration profiles and biliary excretion rate-time curves of free phenol red and its metabolite, bromphenol blue and bromosulphonphthalein were analysed based on statistical moment theory. Moment parameters for the plasma concentration profile (AUC_p, MRT_p) and those for the biliary excretion rate-time curve (AUC_b, MRT_b) were calculated by numerical integration using a linear trapezoidal formula and extrapolation to infinite time based on a mono-exponential equation (Yamaoka et al 1978). For phenol red, moment parameters for the biliary excretion rate-time curves of free phenol red ($AUC_{b,f}$, $MRT_{b,f}$) and its metabolite ($AUC_{b,m}$, $MRT_{b,m}$) were calculated independently.

Results and Discussion

The main purpose of this study was to examine the possibility of drug absorption from the liver surface. Kinetic analysis of drug absorption from liver surface is of particular interest physiologically.

We selected three organic anions as model drugs because their disposition characteristics in liver had been investigated (Hart & Schanker 1966; Moller & Sheikh 1983; Klaassen & Watkins 1984; Tiribelli et al 1986, 1990; Kakutani et al 1992).

Fig. 1a shows the plasma concentration profiles of model drugs after application to the rat liver surface at a dose of 1 mg. Each model drug appeared in the blood at a low concentration ($< 6{\cdot}5\,\mu g\,mL^{-1}$). This observation suggests the occurrence of drug absorption from rat liver surface membrane which, seems to act as an effective barrier for several molecules to some extent. The plasma concentration of model drugs after application to rat liver surface reached a maximum at about 1 h after dosing and decreased gradually (Fig. 1a), representing the increased residence time in plasma as compared with intravenous administration (Fig. 1b). The prolongation of plasma concentration as compared with intravenous administration was marked for bromphenol blue and bromosulphonphthalein.

After absorption from the rat liver surface, each model

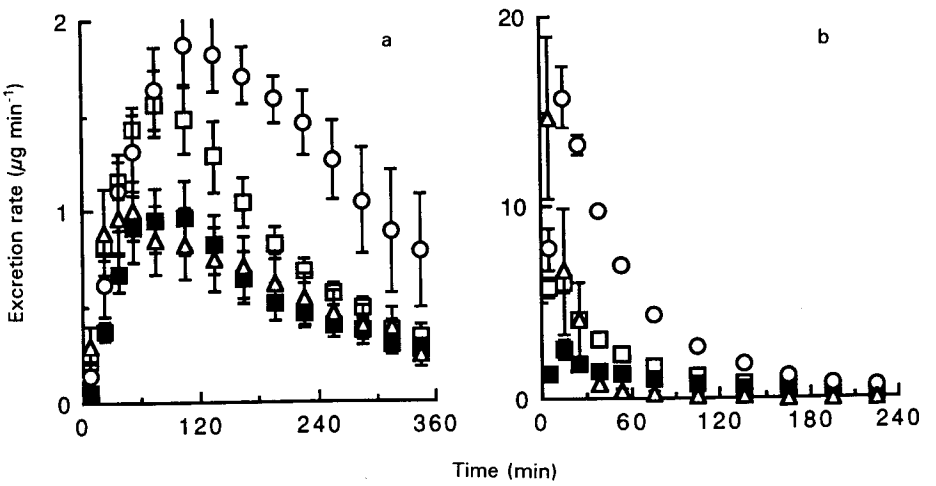

FIG. 2. Biliary excretion rate-time profiles of phenol red (□) and its glucuronic acid conjugate (■), bromphenol blue (○) and bromosulphonphthalein (△) after application to rat liver surface (a) and intravenous administration (b) at a dose of 1 mg. Each point represents mean ± s.e. of four experiments.

Table 1. Recovery of phenol red after application to rat liver surface, intravenous administration and direct injection into rat liver at a dose of 1 mg.

Route	Bile (%)			Urine (%)			Cell (%)
	Free	Metabolite	Total	Free	Metabolite	Total	Free
Liver surface	31·8 ± 2·5	18·8 ± 2·8	50·7 ± 5·0	17·1 ± 3·9	12·3 ± 3·1	29·3 ± 5·8	8·2 ± 2·0
Intravenous	39·1 ± 4·0	19·8 ± 1·3	58·9 ± 4·8	20·2 ± 6·3	6·8 ± 0·6	26·9 ± 6·6	–
Direct injection	23·9 ± 2·9	12·7 ± 2·5	36·6 ± 4·4	42·2 ± 5·3	9·9 ± 2·7	52·1 ± 5·5	–

Values are means ± s.e. of four experiments.

Table 2. Pharmacokinetic parameters for model drugs after application to rat liver surface, intravenous administration and direct injection into rat liver at a dose of 1 mg.

Drug	Route	Recovery (%)			AUC_p ($\mu g\,min\,mL^{-1}$)	MRT_p (min)	AUC_b ($\mu g\,min\,mL^{-1}$)	MRT_b (min)
		Bile	Urine	Cell				
Bromphenol blue	Liver surface	50·5 ± 8·6	1·1 ± 0·4	28·4 ± 4·2	604·2 ± 199·6	169·2 ± 12·3	686·1 ± 125·3	307·5 ± 74·8
	Intravenous	95·1 ± 2·2	0·9 ± 0·3	–	542·8 ± 76·8	2·1 ± 0·1	973·4 ± 23·9	66·9 ± 3·9
	Direct injection	77·6 ± 2·2	1·1 ± 0·4	–	398·4 ± 27·1	2·7 ± 0·3	853·9 ± 21·8	85·8 ± 3·8
Bromosulphonphthalein	Liver surface	22·5 ± 3·4	0·3 ± 0·1	41·4 ± 2·8	n.d.	n.d.	313·4 ± 57·6	284·5 ± 65·9
	Intravenous	86·3 ± 4·2	0·1 ± 0·1	–	366·5 ± 37·4	1·5 ± 0·2	903·4 ± 24·3	17·7 ± 2·4
	Direct injection	76·4 ± 1·9	0·3 ± 0·1	–	154·0 ± 6·9	1·9 ± 0·2	837·5 ± 21·4	25·4 ± 2·8

Values are means ± s.e. of four experiments. n.d., not determined.

Table 3. Moment parameters for phenol red after application to rat liver surface, intravenous administration and direct injection into rat liver at a dose of 1 mg.

Route	AUC_p ($\mu g\,min\,mL^{-1}$)	MRT_p (min)	$AUC_{b,f}$ ($\mu g\,min\,mL^{-1}$)	$MRT_{b,f}$ (min)	$AUC_{b,m}$ ($\mu g\,min\,mL^{-1}$)	$MRT_{b,m}$ (min)
Liver surface	1695·1 ± 237·0	267·2 ± 53·2	356·5 ± 29·2	190·6 ± 22·3	225·2 ± 34·9	235·0 ± 42·0
Intravenous	1618·9 ± 137·9	67·6 ± 10·7	396·4 ± 44·7	70·8 ± 8·8	215·7 ± 20·0	98·7 ± 19·0
Direct injection	1428·2 ± 139·3	58·9 ± 9·1	257·6 ± 35·4	59·3 ± 4·1	138·0 ± 23·2	85·6 ± 9·0

Values are means ± s.e. of four experiments.

drug was excreted into the bile, as shown in Fig. 2a. As for the plasma concentration profile, the biliary excretion rate after application to rat liver surface showed a delayed pattern as compared with that of intravenous administration (Fig. 2b). The biliary excretion rate patterns differed among the model drugs. The metabolite of phenol red (a glucuronic acid conjugate (Hart & Schanker 1966)) was also excreted into the bile.

Tables 1 and 2 list the recovery (% dose) of model drugs in the bile, urine and the glass cell on various administration routes. The extent of absorption was calculated, from the dose and the amount recovered in the glass cell after 6 h, as 91·8% for phenol red, 71·6% for bromphenol blue and 58·6% for bromosulphonphthalein.

Phenol red and its glucuronic acid conjugate were excreted into bile and urine after both routes of administration (Table 1), whereas bromphenol blue and bromosulphonphthalein were predominantly excreted in the bile (Table 2). The biliary recovery and metabolism ratios of phenol red after application to the rat liver surface were broadly similar to those after intravenous administration (Table 1). We suggest that the drug elimination and metabolism processes are not affected by application to liver surface.

Moment parameters are free from the complexities of a pharmacokinetic model, and thus AUC_p and MRT_p can be appropriate parameters for evaluating the drug absorbability from liver surface. Tables 2 and 3 summarize the moment parameters for model drugs after application to rat liver

surface. The AUC_p and MRT_p for bromosulphonphthalein after application to rat liver surfaces could not be calculated accurately because of its low plasma concentration.

The AUC_p values for phenol red and bromphenol blue after application to rat liver surface, which contain large errors, were roughly equal to those after intravenous administration (Tables 2, 3), supporting the good absorbability from rat liver surface. As expected, the MRT_p values for both model drugs after application to rat liver surface were larger than those after intravenous administration (Tables 2, 3).

The ratio of $AUC_{b,f}$ for phenol red and bromphenol blue following application to rat liver surface to those after intravenous administration were 0·899 and 0·705, respectively, which were in good agreement with recovery data estimates of absorption (Tables 2, 3). The $MRT_{b,f}$ and $MRT_{b,m}$ values for phenol red after application to rat liver surface were respectively 2·7 and 2·4 times larger than those after intravenous administration (Table 3). Similarly, a marked increase in MRT_b values was seen with the application of bromphenol blue and bromosulphonphthalein to rat liver surface (Table 2). From these results, the drug concentration in liver after application to rat liver surface would appear to be sustained as compared with that after intravenous administration, although its concentration was not determined directly.

We also examined the disposition characteristics of model drugs after direct injection into rat liver by use of a syringe. In this case, the plasma concentration profiles and biliary excretion rate patterns of each model drug (data not shown) were unaltered, as compared with those after intravenous administration (Figs 1, 2). The in-vivo behaviour of model drugs after direct injection into rat liver were considered to be basically similar to those after intravenous administration, judging from the pharmacokinetic parameters (Tables 1–3). It is suggested that the direct injection route is not suitable for target site-specific drug delivery to the liver with a high blood flow, because directly injected drug was rapidly cleared from the injection site, followed by drainage into the systemic circulation.

In conclusion, the present study provides evidence of drug absorption from rat liver surfaces. Such information should be useful in the development of a new administration route for drug delivery to the target site in liver. However, further work is required to elucidate the mechanism for drug absorption and to examine the drug absorption from the diseased liver surface for clinical application.

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