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Mechanism for Drug Absorption from Rat-liver Surface Membrane: Effect of Dose and Transport Inhibitors on the Pharmacokinetics of Phenol Red

KOYO NISHIDA, NORIHITO SATO, HITOSHI SASAKI AND JUNZO NAKAMURA

School of Pharmaceutical Sciences, Nagasaki University, 1–14 Bunkyo-machi, Nagasaki 852, Japan

Abstract

We examined the effect of dose and transport inhibitors on the pharmacokinetics of phenol red as a model drug after application to rat liver surface in-vivo, employing a cylindrical glass cell (i.d. 9 mm, area 0·64 cm^2), to elucidate the mechanism for drug absorption from liver surface membrane.

Absorption ratios of phenol red in 6 h were determined to be 91·1, 91·8 and 89·9% at a dose of 0·3, 1 and 3 mg, respectively. The AUC value for plasma concentration profile of phenol red was proportional to the dose. It is thus suggested that the absorption process of phenol red from rat liver surface does not approach saturability. The time course of the remaining amount of phenol red in the glass cell obeyed first-order kinetics at a dose of 0·3 mg, and its rate constant K_a was calculated to be 0·0069 min^{-1}. Moreover, no significant difference was seen in the K_a value within the dose range of 0·3–3 mg, which was estimated by curve fitting of the plasma concentration profile of phenol red after application to rat liver surface in the two-compartment model with first-order absorption. 2,4-Dinitrophenol (0·3 mg) and probenecid (0·5 and 1 mg), inhibitors of metabolic energy and anion transport, respectively, had no significant effect on the pharmacokinetics of phenol red after application to rat liver surface.

These data demonstrate that a specific transport mechanism such as active transport is not involved in phenol red absorption from rat liver surface membrane.

Liver site-specific drug delivery is of interest since normal treatment of liver diseases by intravenous and oral administration is frustrated by inadequate delivery into liver as well as toxicity in other organs. The direct way such as drug application to liver surface is considered to be a useful method for drug delivery to the target site in liver. In our previous paper (Nishida et al 1994), we selected organic anions (phenol red, bromphenol blue and bromosulphonphthalein) as model drugs and examined their in-vivo behaviour after application to rat liver surface. Absorption ratios in 6 h of model drugs were relatively large (>59%) and significant prolongation of blood concentration was observed. For therapeutic application to liver disease, it was suggested that further work was required to elucidate the mechanism for drug absorption from liver surface membrane.

The main purpose of the present study was to obtain information concerning the absorption mechanism from liver surface membrane. We selected phenol red as a model drug, as the absorption ratio was the largest of three organic anions.

Materials and Methods

Chemicals

Phenol red and 2,4-dinitrophenol were purchased from Nacalai Tesque, Inc., Kyoto, Japan. Probenecid was obtained from Wako Pure Chemical Industries, Ltd, Osaka, Japan. All other chemicals were of reagent grade.

Correspondence: K. Nishida, School of Pharmaceutical Sciences, Nagasaki University, 1–14 Bunkyo-machi, Nagasaki 852, Japan.

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). An incision 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. Phenol red dissolved in pH 7·4 phosphate buffer (0·1 mL) was 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 phenol red solution (0·3, 1 and 3 mg) was added to the glass cell directly. In another experiment, transport inhibitor 2,4-dinitrophenol (0·3 mg) or probenecid (0·5 or 1 mg) was added to the glass cell simultaneously.

Intravenous administration. The phenol red solution (0·3, 1 or 3 mg) was injected into the jugular vein.

Blood samples (200 μL) were collected at selected times after dosing from the heparinized cannula inserted into the femoral artery over a 6-h period and centrifuged at 15 000 rev min^{-1} for 5 min. Bile samples were collected at appropriate time intervals for 6 h. Six hours after the application, urine was collected from the bladder directly by syringe.

Following application to rat liver surface, phenol red solution remaining in the glass cell was withdrawn at 6 h after dosing. To study the time course of the remaining amount of phenol red in plasma and in the glass cell, and the cumulative amount of total phenol red excreted in bile and urine, certain experiments were carried out up to 0·5, 1, 2 and 4 h at a dose of 0·3 mg. The concentrations of free phenol red in plasma, bile, urine and remaining solution in the glass cell were 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 they 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.

Calculation of moment parameters

The plasma concentration profiles and biliary excretion rate-time curves of free phenol red and its metabolite were analysed based on statistical moment theory. Moment parameters for the plasma concentration profile of free phenol red (AUC_p, MRT_p) and those for 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 using a linear trapezoidal formula and extrapolation to infinite time based on a monoexponential equation (Yamaoka et al 1978).

Compartment model analysis

First, the plasma concentration (C_p) profiles of phenol red at a dose of 0·3, 1 and 3 mg after intravenous administration into rat was fitted to the biexponential equation described as follows, by the nonlinear least-squares method (Yamaoka et al 1981):

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

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 plasma concentration in the application of phenol red to rat liver surface. Next, in the same way, the plasma concentration profile of phenol red after application to 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 of phenol red is given by the following equation:

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

K_a is the first-order absorption rate constant for phenol red absorption into the blood stream from rat liver surface. F is the availability of phenol red after application to rat liver surface.

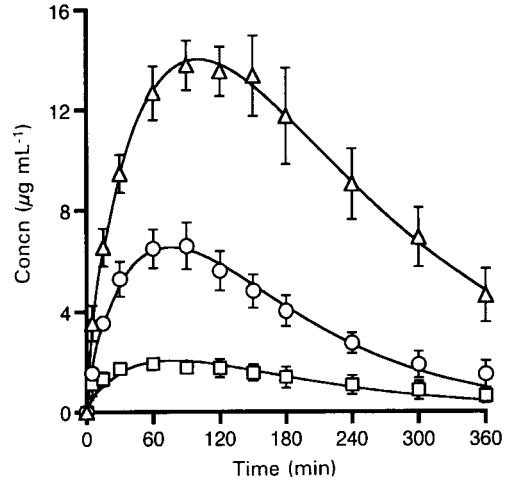

FIG. 1. Plasma concentration of phenol red after application to rat liver surface at a dose of 0·3 (□), 1 (○) and 3 mg (△). Each point represents mean ± s.e. of four experiments. The data for 1 mg was reported previously (Nishida et al 1994). Curves show simulated functions based on the pharmacokinetic parameters shown in Table 3.

Results and Discussion

Dose dependency

Fig. 1 shows the plasma concentration profiles of phenol red after application to rat liver surface at a dose of 0·3, 1 or 3 mg. At each dose, plasma concentration of phenol red reached a maximum at 1 or 1·5 h after dosing, followed by gradual disappearance.

Similarly, the shapes of biliary excretion rate-time curves of free phenol red and its metabolite after application of phenol red to rat liver surface were almost identical among every dose, as shown in Fig. 2.

Recovery (% of dose) of free phenol red and its metabolite in bile, urine and glass cell at doses of 0·3, 1 and 3 mg are given in Table 1. Absorption ratios of phenol red in 6 h calculated from the remaining amount of phenol red in the glass cell were 91·1, 91·8 and 89·9% at doses of 0·3, 1 and

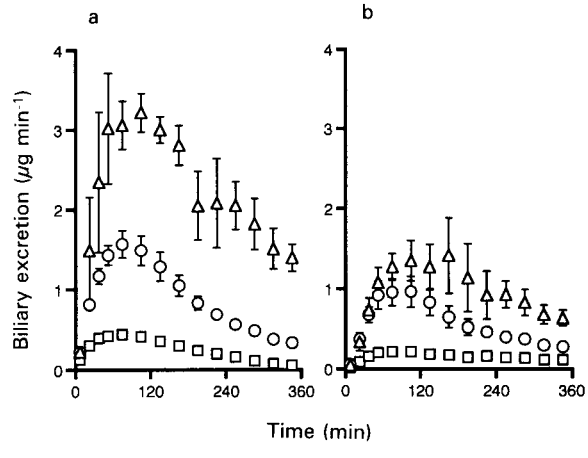

FIG. 2. Biliary excretion rate–time curves of free phenol red (a) and its metabolite (b) after application to rat liver surface at a dose of 0·3 (□), 1 (○) and 3 mg (△). Each point represents mean ± s.e. of four experiments. The data for 1 mg was reported previously (Nishida et al 1994).

Table 1. Recovery of phenol red after application to rat liver surface at various doses in 6 h.

Dose (mg)	Bile (%)			Urine (%)			Cell (%)
	Free	Metabolite	Total	Free	Metabolite	Total	Free
0·3	29·4 ±4·2	18·1 ±2·6	47·4 ±4·4	15·4 ±4·8	23·4 ±4·8	38·7 ±6·5	8·9 ±2·1
1[a]	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
3	30·2 ±5·3	11·4 ±1·5	41·6 ±5·5	30·9 ±7·2	4·4 ±1·4	35·3 ±7·0	10·1 ±2·1

[a] Results were reported previously (Nishida et al 1994). Values are means ± s.e. of four experiments.

Table 2. Moment parameters for phenol red after application to rat liver surface at various doses.

Dose (mg)	AUC_p ($\mu g\,mL^{-1}\,min$)	AUC_p/dose (% · min mL^{-1})	MRT_p (min)	$AUC_{b,f}$ (μg)	$MRT_{b,f}$ (min)	$AUC_{b,m}$ (μg)	$MRT_{b,m}$ (min)
0·3	456·8 ±84·7	152·3 ±28·2	197·7 ±23·3	92·8 ±15·0	147·2 ±17·8	75·3 ±11·5	283·3 ±16·2
1[a]	1695·1 ±237·0	169·5 ±23·7	267·2 ±53·2	356·5 ±29·2	190·6 ±22·3	225·2 ±34·9	235·0 ±42·0
3	4534·3 ±354·6	151·1 ±11·8	238·8 ±15·1	1114·1 ±153·8	202·1 ±16·3	455·1 ±53·4	286·4 ±38·4

[a] Results were reported previously (Nishida et al 1994). Values are means ± s.e. of four experiments.

3 mg, respectively, indicating that the phenol red absorption from rat liver surface membrane shows no saturation within the dose range used. Biliary and urinary recovery ratio of phenol red metabolite decreased significantly at a dose of 3 mg, suggesting saturation of the metabolic process of phenol red at the highest dose.

A similar trend was seen after intravenous administration of phenol red into rats (data not shown).

Moment parameters for the plasma concentration profile of free phenol red and biliary excretion rate–time curves of free phenol red and its metabolite are listed in Table 2. No significant difference was seen in AUC_p/dose value among the three doses, suggesting the linearity of phenol red absorption from rat liver surface membrane. As expected from the patterns in Figs 1 and 2, MRT_p, $MRT_{b,f}$ and $MRT_{b,m}$ values were almost the same among the three doses.

Time course of phenol red distribution in plasma, bile, urine and glass cell

To assess the absorption characteristics from rat liver surface membrane, we studied the time course of phenol red distribution in plasma, bile, urine and glass cell after application to rat liver surface at a dose of 0·3 mg (Fig. 3). The remaining amount of phenol red in plasma determined as $C_p \times V_{plasma}$ (plasma volume in rat) was plotted against

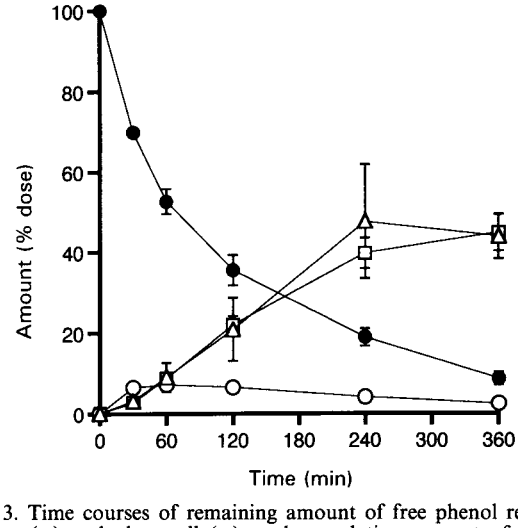

FIG. 3. Time courses of remaining amount of free phenol red in plasma (○) and glass cell (●), and cumulative amount of total phenol red excreted in bile (□) and urine (△) after application to rat liver surface at a dose of 0·3 mg. Each point represents mean ± s.e. of four experiments.

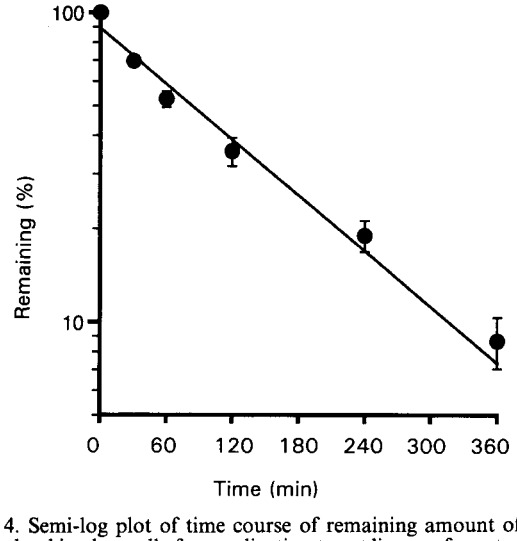

FIG. 4. Semi-log plot of time course of remaining amount of free phenol red in glass cell after application to rat liver surface at a dose of 0·3 mg. Each point represents mean ± s.e. of four experiments.

Table 3. Pharmacokinetic parameters for plasma concentration profiles of phenol red after application to rat liver surface at various doses.

Dose (mg)	K_a (min^{-1})	F	V_c[a] (mL)	k_{12}[a] (min^{-1})	k_{21}[a] (min^{-1})	k_{el}[a] (min^{-1})
0·3	0·0079 ±0·0003	0·96 ±0·03	19 ±1	0·11 ±0·02	0·16 ±0·03	0·034 ±0·006
1	0·0092 ±0·0010	0·97 ±0·02	20 ±3	0·13 ±0·04	0·13 ±0·02	0·033 ±0·007
3	0·0081 ±0·0011	0·97 ±0·03	23 ±2	0·15 ±0·04	0·12 ±0·01	0·033 ±0·003

[a] Parameters were obtained from the data of intravenous administration of phenol red into rat, using a two-compartment model. Values are means ± s.e. of four experiments.

time in Fig. 3, where V_{plasma} was estimated from Bischoff et al (1971). A relatively small amount of phenol red (<7·2%) was detected in plasma. Cumulative total phenol red (free phenol red and its metabolite) excreted in bile and urine reached a plateau at 4 h after dosing and exhibited a similar pattern. Therefore, we conclude there is no significant difference in urinary and biliary excretion of phenol red with respect to rate.

On the other hand, the remaining amount of phenol red in the glass cell declined rapidly. A semi-log plot of the remaining amount of phenol red in the glass cell gave a straight line (correlation coefficient, 0·99) as shown in Fig. 4, indicating that the phenol red absorption from rat liver surface proceeds via a first-order process. The rate constant K_a was calculated to be 0·0069 min^{-1}; this result supports the use of a two-compartment model incorporating first-order absorption to describe the plasma concentration profile of phenol red after application to the rat liver surface.

Compartment model analysis

Pharmacokinetic parameters for phenol red after intravenous administration into rats are listed in Table 3, and were used in the following analysis. The plasma concentration profile of phenol red after application to rat liver surface was fitted using a two-compartment model with first-order absorption. K_a and F obtained by curve fitting are given in Table 3. Fig. 1 shows the simulation curves for plasma concentration of phenol red after application to rat liver surface at a dose of 0·3, 1 and 3 mg, which were reconstructed employing the estimated parameters shown in Table 3. In general, a good agreement was observed between fitted lines and experimentally observed data at each dose. The obtained K_a value correlated closely with the value determined according to the elimination profile of phenol red from the glass cell (Fig. 4), suggesting the validity of this pharmacokinetic model. No significant difference was seen in the K_a value at the doses of 0·3, 1 and 3 mg (Table 3). Accordingly, linearity of phenol red absorption from rat liver surface membrane was confirmed.

Effect of transport inhibitors

From these results, it is suggested that phenol red absorption from rat liver surface membrane is explained mostly by passive diffusion. The saturable specialized absorption

Table 4. Recovery of phenol red after application to rat liver surface at a dose of 0·3 mg in 6 h with or without transport inhibitors.

Condition	Bile (%)			Urine (%)			Cell (%)
	Free	Metabolite	Total	Free	Metabolite	Total	Free
Control	29·4 ±4·2	18·1 ±2·6	47·4 ±4·4	15·4 ±4·8	23·4 ±4·8	38·7 ±6·5	8·9 ±2·1
+2,4-Dinitrophenol (0·3 mg)	30·6 ±5·1	18·2 ±4·2	48·8 ±5·4	19·3 ±5·4	28·8 ±2·8	48·1 ±6·8	5·5 ±0·8
+Probenecid (0·5 mg)	25·7 ±3·8	19·9 ±1·8	45·7 ±5·4	21·8 ±3·3	26·1 ±2·7	47·9 ±5·8	7·8 ±0·8
+Probenecid (1 mg)	21·1 ±2·9	13·9 ±2·5	35·0 ±5·0	27·1 ±4·2	30·4 ±1·9	57·5 ±5·2	10·8 ±1·5

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

Table 5. Moment parameters of phenol red after application to rat liver surface at a dose of 0·3 mg with or without transport inhibitor.

Condition	AUC_p ($\mu g\,mL^{-1}\,min$)	MRT_p (min)	$AUC_{b,f}$ (μg)	$MRT_{b,f}$ (min)	$AUC_{b,m}$ (μg)	$MRT_{b,m}$ (min)
Control	456·8 ±84·7	197·7 ±23·3	92·8 ±15·0	147·2 ±17·8	75·3 ±11·5	283·3 ±16·2
+2,4-Dinitrophenol (0·3 mg)	325·0 ±71·9	168·9 ±17·5	95·9 ±16·4	124·3 ±8·8	65·2 ±13·4	221·9 ±32·7
+Probenecid (0·5 mg)	300·0 ±41·8	250·0 ±73·2	87·4 ±16·3	171·0 ±29·8	75·3 ±5·6	243·9 ±6·5
+Probenecid (1 mg)	268·0 ±43·4	243·6 ±33·8	68·0 ±9·4	146·5 ±13·6	51·8 ±7·6	248·7 ±24·6

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

process of phenol red in rat lung is known to be inhibited by metabolic inhibitors and structurally related organic anions (Enna & Schanker 1973; Gardiner & Schanker 1976). Thus, we examined the effect of the metabolic inhibitor 2,4-dinitrophenol, which blocks oxidative phosphorylation, on the phenol red absorption to determine whether phenol red absorption depends on metabolic energy. We also studied the influence of a structurally-related organic anion, probenecid, on the phenol red absorption, to determine the structural specificity of the absorption system of phenol red.

Biliary and urinary recovery (% of dose) of free phenol red and its metabolite at a dose of 0·3 mg in the presence of 2,4-dinitrophenol (0·3 mg) and probenecid (0·5 and 1 mg) did not change compared with control, as shown in Table 4. Absorption ratios of phenol red, 6 h after application to rat liver surface, ranged from 89·2 to 94·5% in the presence of transport inhibitors. 2,4-Dinitrophenol and probenecid as transport inhibitors did not cause a reduction of the phenol red absorption from rat liver surface.

The plasma concentration profiles of phenol red after application to rat liver surface in the presence of transport inhibitors was almost the same as that of control (data not shown). A similar trend was seen in the biliary excretion rate-time curves of free phenol red and its metabolite after application to rat liver surface (data not shown).

Moment parameters for phenol red after application to rat liver surface in the presence of transport inhibitors are given in Table 5, which were of the same magnitude as that of control. Accordingly, it is concluded that transport inhibitors 2,4-dinitrophenol and probenecid, had no significant effect on the phenol red absorption from rat liver surface membrane.

Consequently, it is suggested that a specialized transport process might not exist for the absorption of phenol red from rat liver surface membrane, and a simple passive diffusion system is considered to play an important role.

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