# Pharmacokinetics of Phenol Red in Rat Models of Liver Damage Prepared by Liver Targeting of Carbon Tetrachloride

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**Animal models prepared by treatment with carbon tetrachloride ($CCl_4$) have been used to examine drug disposition in hepatic disorder. However, previous studies demonstrated that systemic administration of $CCl_4$ impaired not only hepatic but also renal function. We recently reported that application of $CCl_4$ to the rat liver surface produced hepatic damage without impairing renal function. In the present study, we examined the pharmacokinetics of phenol red in our developed rat model. The rats treated with $CCl_4$ by liver surface application exhibited decreases in the biliary clearance of phenol red in comparison with normal rats from 0.54±0.03 to 0.31±0.06 ml/min, suggesting hepatic damage. In these rats, the renal clearance of phenol red did not decrease (0.50±0.16 ml/min *vs.* 0.46±0.07 ml/min in normal rats). On the other hand, oral and intraperitoneal treatments with $CCl_4$ reduced not only the biliary clearance of phenol red (0.34±0.03 ml/min in *p.o.* treated rats, 0.18±0.01 ml/min in i.p. treated rats) but also the renal clearance (0.26±0.05 ml/min in *p.o.* treated rats, 0.18±0.06 ml/min in i.p. treated rats) as compared with normal rats. These findings indicate that the rat model of liver damage prepared by liver surface application of $CCl_4$ is useful to investigate the effects of hepatic disorder on the pharmacokinetics of drugs.**

**Key words** animal model; liver damage; carbon tetrachloride; liver targeting; phenol red; pharmacokinetics

Since liver plays an important role in drug metabolism and excretion in the body, there is increasing interest in investigation of pharmacokinetics in liver diseases.[1,2] To predict drug disposition in patients with liver diseases, animal models prepared by treatment with toxic compounds have been used.[3—5] Carbon tetrachloride ($CCl_4$) has been extensively used for preparing experimental models of liver disease such as hepatic cirrhosis and drug-induced hepatopathy.[6—9] Previous reports demonstrated that oral and intraperitoneal administration of $CCl_4$ caused renal damage in rats in addition to damaging the liver.[10—13] These results indicated that the pharmacokinetic results evaluated with these animal models would be affected by not only hepatic but also renal disorders since kidney also plays a significant role in drug excretion. Recently, we attempted $CCl_4$ targeting to the liver in order to prepare rat models in which only the liver is significantly damaged.[12] By means of several biochemical and physiological parameters, it was shown that the liver was impaired without producing renal damage when $CCl_4$ was applied to the rat liver surface.

The purpose of the present study was to examine drug disposition in our developed rat model. For this study, we selected phenol red, a hydrophilic dye, as the model drug. This compound has been clinically used for renal function test in humans,[14] and it is excreted into bile and urine as a free form or conjugative metabolite (glucuronic acid conjugate) in rats.[15] After application of $CCl_4$ to the rat liver surface, phenol red was injected intravenously and its pharmacokinetics was evaluated. Effects of liver surface application of $CCl_4$ on the pharmacokinetics of phenol red were compared with those of oral and intraperitoneal treatments with $CCl_4$.

## MATERIALS AND METHODS

**Materials** $CCl_4$, olive oil and phenol red were obtained from Nacalai Tesque, Inc. (Kyoto, Japan). All other chemicals were commercial products of reagent grade.

**Animals** Male Wistar rats (250—290 g) were housed in cages in an air-conditioned room and maintained on a standard laboratory diet (MF, Oriental Yeast Co., Ltd., Tokyo, Japan) and water *ad libitum*. All experiments in the present study conformed to the Guidelines for Animal Experimentation in Nagasaki University.

**Liver Surface Application (LSA)** Rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and a midline incision about 3 cm in length was made in the abdomen. A cylindrical glass diffusion cell (i.d. 1.8 cm, area 2.54 $cm^2$) was attached to the liver surface with a thin film of surgical adhesive (Aron Alpha, Sankyo Co., Ltd., Tokyo).[12] One milliliter of $CCl_4$ (LSA group) was added directly to the diffusion cell, and treatment was continued for 2 h. The incision was sutured, and rats were housed in cages in an air-conditioned room with standard rat chow and water *ad libitum*.

***p.o.* and i.p. Treatments** Rats were anesthetized with sodium pentobarbital, and $CCl_4$ (100 $\mu$l) in olive oil was administered orally or intraperitoneally.

**Pharmacokinetics of Phenol Red** Twenty-four hours after administration of $CCl_4$, the femoral artery of rats was cannulated with a polyethylene tube (i.d. 0.5 mm, o.d. 0.8 mm; Dural Plastics, Dural, Australia) under anesthesia. Also, 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 body temperature of the rats was kept at 37 °C by a heat lamp during the experiment. Phenol red solution was prepared in isotonic phosphate buffer (pH 7.4) to yield a concentration of 10 mg/ml, and 0.1 ml of the solution was injected into the jugular vein. Blood samples 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

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(Type M-15-3, Sakuma, Tokyo). Bile samples were collected at appropriate time intervals for 6 h. At 6 h postinjection, urine was collected from the bladder directly by syringe. The concentrations of free phenol red in plasma, bile, and urine were determined spectrophotometrically at 560 nm after dilution with a 1 M NaOH solution. The total concentration of free phenol red and its metabolite was measured in the same manner after they were subjected to acid hydrolysis (2 M HCl at 100 °C for 30 min).[15] The concentration of phenol red metabolite was estimated from the difference between these values.

**Determination of Pharmacokinetic Parameters** The area under the plasma concentration–time curve ($AUC_p$) was calculated by numeral integration using a linear trapezoidal formula and extrapolation to infinite time based on a monoexponential equation.[16] In the same way, the area under the biliary excretion rate–time curves of free phenol red ($AUC_{b,f}$) and its metabolite ($AUC_{b,m}$) were determined independently. The biliary clearance ($CL_b$) and renal clearance ($CL_r$) were obtained by dividing the total amount of free phenol red excreted into the bile and urine within the collection period (6 h), respectively, by the area under the plasma concentration–time curve up to 6 h after dosing ($AUC_{p,6h}$). The metabolic clearance ($CL_m$) was also calculated by dividing the total amount of phenol red metabolite excreted into bile and urine by the $AUC_{p,6h}$.

**Statistical Analysis** Statistical analysis was performed by applying the unpaired Student's *t*-test, with $p<0.05$ considered statistically significant. All results are expressed as mean values±S.E. of six experiments.

## RESULTS

Figure 1 shows the plasma concentration profiles of free phenol red at a dose of 1 mg after intravenous administration to rats. Phenol red biphasically disappeared from the plasma in all groups. In $CCl_4$-treated rats (LSA, *p.o.* and i.p. groups), the plasma disappearances of phenol red were delayed compared with normal group.

Biliary excretion rate profiles of free phenol red and its metabolite after intravenous administration to rats are given in Fig. 2. At early time points, the biliary excretion rate of free phenol red in $CCl_4$-treated rats (LSA and i.p. groups) decreased as compared with those of normal group. Similar results were obtained in the biliary excretion of phenol red metabolite.

Table 1 summarizes the moment parameters for free phenol red and its metabolite after intravenous administration of phenol red. The $AUC_p$ values for *p.o.* and i.p. groups were larger than that for normal group. A similar tendency was observed in LSA group although the difference between LSA and normal group was not statistically significant owing to large fluctuations. In regard to the $AUC_{b,f}$ values, no significant difference was observed between $CCl_4$-treated and normal group. The $AUC_{b,m}$ values for $CCl_4$-treated groups were significantly lower than that for normal group.

To assess the *in vivo* behaviors of phenol red in $CCl_4$-treated rats, the clearance values were calculated (Table 2). Both $CL_b$ and $CL_r$ values for *p.o.* and i.p. groups were significantly lower than those for normal group. LSA group showed a decrease in $CL_b$ value as compared with normal group,

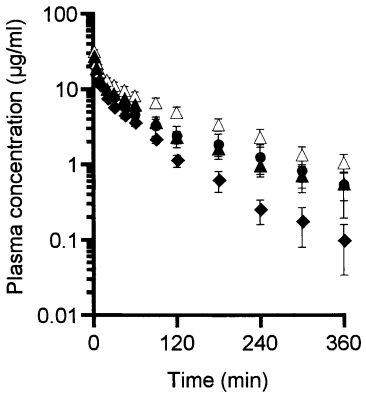


Fig. 1. Plasma Concentration *vs.* Time Profiles of Free Phenol Red after Intravenous Administration to Rats at a Dose of 1 mg/Rat under Several Conditions

◆, normal; ●, LSA; ▲, *p.o.*; △, i.p. Each point represents the mean±S.E. of six experiments.

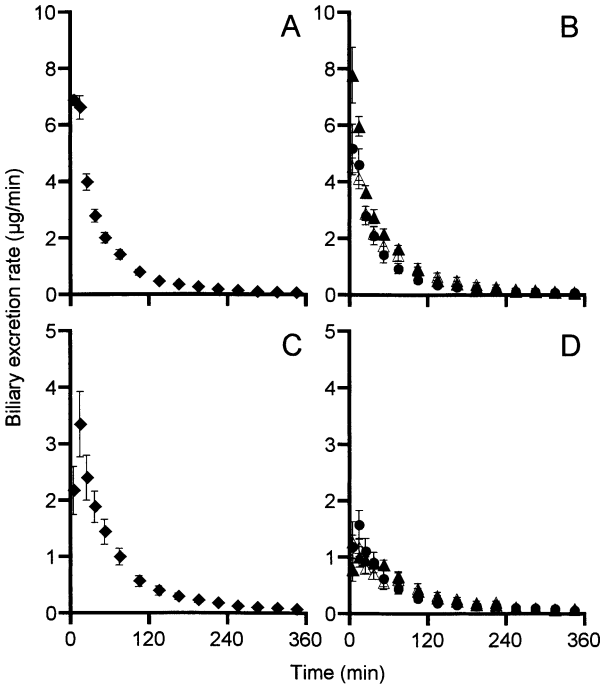


Fig. 2. Biliary Excretion Rate *vs.* Time Profiles of Free Phenol Red (A, B) and Its Metabolite (C, D) after Intravenous Administration to Rats at a Dose of 1 mg/Rat under Several Conditions

◆, normal; ●, LSA; ▲, *p.o.*; △, i.p. Each point represents the mean±S.E. of six experiments.

Table 1. *AUC* Values for the Plasma Concentration *vs.* Time Profiles and Biliary Excretion Rate *vs.* Time Profiles of Free Phenol Red and Its Metabolite after Intravenous Administration to Rats at a Dose of 1 mg/Rat under Several Conditions

| Group | $AUC_p$ (μg/ml·min) | $AUC_{b,f}$ (μg) | $AUC_{b,m}$ (μg) |
|---|---|---|---|
| Normal | 664.5±42.5 | 342.8±26.1 | 218.7±29.8 |
| LSA | 1188.6±331.9 | 259.4±31.3 | 122.3±20.6* |
| *p.o.* | 1243.5±160.8* | 366.0±28.9 | 124.0±9.3* |
| i.p. | 1908.5±278.4** | 311.3±42.2 | 123.7±14.0* |

Each value represents the mean±S.E. of six experiments. Significant differences from the normal group (*$p<0.05$, **$p<0.01$).

Table 2. Clearance Values of Phenol Red after Intravenous Administration to Rats at a Dose of 1 mg/Rat under Several Conditions

| Group | $CL_b$ (ml/min) | $CL_r$ (ml/min) | $CL_m$ (ml/min) |
|---|---|---|---|
| Normal | 0.54±0.03 | 0.46±0.07 | 0.38±0.06 |
| LSA | 0.31±0.06** | 0.50±0.16 | 0.24±0.05 |
| *p.o.* | 0.34±0.03*** | 0.26±0.05* | 0.16±0.02** |
| i.p. | 0.18±0.01*** | 0.18±0.06* | 0.13±0.03** |

Each value represents the mean±S.E. of six experiments. Significant differences from the normal group (* $p<0.05$, ** $p<0.01$, *** $p<0.001$).

while the $CL_r$ value did not decrease as the result of the compensatory increase in the amount excreted into urine. The $CL_m$ values were reduced by systemic treatments of $CCl_4$.

## DISCUSSION

Excretion of various amphiphilic compounds occurs in both liver and kidney; their excretion by one route increases when the other is impaired.[17] In regard to phenol red, it was shown that the renal excretion increased to compensate for reduced biliary excretion in the bile duct-ligated rats.[18] Similarly, the compensatory effect on phenol red elimination was observed in the rats treated with $CCl_4$ by liver surface application in the present study.

On the other hand, oral and intraperitoneal treatments with $CCl_4$ showed reductions of both biliary and renal clearances of phenol red. This confirms the previous observations that systemic administration of $CCl_4$ impairs not only hepatic but also renal function.[10—13] Numerous studies demonstrated that $CCl_4$ toxicity is dependent on the reduction to trichloromethyl radicals catalyzed by cytochrome P-450 (CYP) such as CYP2E1 and CYP2B.[19—21] When $CCl_4$ is administered systemically, the liver is commonly assumed to be the major target organ due to its high content of CYP. However, it was shown that $CCl_4$ administered systemically in rats was distributed in the kidney at higher concentrations than in the liver.[22] Furthermore, CYP2E1 and CYP2B were reported to exist in the rat tubular cells.[23] Therefore, marked decreases in renal clearance of phenol red are certainly responsible for the impaired tubular secretion by renal distribution of $CCl_4$.

The metabolic clearance of phenol red was reduced by systemic administration of $CCl_4$. Glucuronidation enzymes, UDP-glucuronosyltransferases are present in many tissues as well as liver in rats.[24] Thus, the metabolism of phenol red in the rats administered systemically with $CCl_4$ would be affected by the change in not only hepatic but also extrahepatic enzymes.

In conclusion, we examined the pharmacokinetics of phenol red in rats with liver damage prepared by liver surface application of $CCl_4$. In these rats, the biliary clearance of phenol red decreased while the renal clearance did not. On the other hand, the rats treated systemically with $CCl_4$ showed decreases not only in biliary clearance of phenol red but also in the renal clearance. These findings indicate that the model of liver damage prepared by liver surface application of $CCl_4$ is useful to investigate the effects of hepatic disorder on the pharmacokinetics of drugs.

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