TOXICOKINETICS OF PHENOBARBITAL IN RATS WITH DL-ETHIONINE-INDUCED LIVER INJURY

Michishige NOGUCHI, Koki TANAKA, Yuki KUWAMURA,
Tomiko FUKUSHIMA and Yoshiaki KAWAI

*Safety Research Laboratory, Tanabe Seiyaku Co., Ltd.,
16-89, 3-chome, Kashima Yodogawa-ku, Osaka 532, Japan*

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ABSTRACT—The toxicokinetic parameters of phenobarbital (PB) were assessed in a female rat model of liver disease. In a preliminary study to determine the optimum dose of DL-ethionine (ET) for creating liver damage, intraperitoneal injection of 250, 500, or 1,000 mg/kg of ET was done for 4 days. ET treatment caused an increase in serum GOT and GPT activity and a decrease in the serum glucose concentration. In the liver, triglycerides and free fatty acids were increased and glucose and S-adenosyl-methionine (SAM) were decreased. Histologic examination revealed diffuse fatty degeneration of the hepatocytes. These findings accorded with those already reported as characteristic of ET intoxication.

The toxicokinetic parameters for PB were determined after oral or intravenous administration of 100 mg/kg of PB to rats with ET (500 mg/kg, i. p.)-induced hepatotoxicity. After oral administration of PB, prolongation of the T_{max} , increased $AUC_{0-\infty}$, and decreased k_e and CL values were noted in ET-treated rats. When PB was given intravenously, the $AUC_{0-\infty}$ was increased while the values of α , β and CL were decreased. A high level of urinary excretion of PB persisted for 48 hr. Protein binding of PB was unchanged in ET-treated animals, but the extent of bioavailability of PB tended to increase. These results indicate that elimination of PB was impaired in the ET-treated rats.

KEY WORDS : Ethionine, Liver Injury, Phenobarbital, Toxicokinetics, Rat.

INTRODUCTION

The initial safety evaluation of drugs is mainly performed on the basis of animal toxicity tests. Recently, there has been increasing interest in pharmacokinetic research conducted from the viewpoint of toxicology (toxicokinetics) (Hawkins and Chasseaud, 1985 ; Yacobi et al., 1982). Toxicokinetic studies have been carried

out on healthy animals (Yuan et al., 1992), but many drugs are prescribed for patients with liver disease (Morgan et al., 1984 ; Williams, 1983). The drug metabolizing enzyme activity and detoxication mechanisms of patients with liver disease are likely to differ from those of healthy animals (Nakayama, 1979 ; Tsyrllov et al., 1976). Therefore, it would be desirable to assess toxicokinetics in a suitable animal model of liver disease.

Phenobarbital (PB) is a sedative and an antiepileptic drug that has long been used as a therapeutic agent. Alvin et al. (1975) have re-

Correspondence : Michishige NOGUCHI at the above address.

ported the effect of liver disease in man on the disposition of PB. Earlier studies (Breen et al., 1973; Iga et al., 1977) have also indicated that the clearance of PB, paraldehyde, or sulfobromophalein is disturbed in experimental liver disease induced by carbon tetrachloride. However, these studies did not elucidate the accumulation and elimination of these drugs in tissues, or characteristic and severity of liver diseases.

In the present study, we observed the time-courses of the PB concentrations in the serum, liver, kidney, or urine in rats with hepatic injury and comprehensively investigated toxicokinetic features of PB. Initially, the optimum dosage of DL-ethionine (ET) for producing the experimental hepatic injury model was determined because the toxic effect of this agent on the mice or rats liver has varied in previous reports (Berry and Friedman, 1977; Shull et al., 1966). Then we examined the toxicokinetics of PB in the rat model of ET induced hepatic injury.

MATERIALS AND METHODS

Experiment 1: Biochemistry and Pathology of DL-Ethionine-Treated Rats

Animals: Female Sprague-Dawley rats (Japan SLC) weighing 172–200 g were used at approximately 7 weeks of age. Rats were given rodent chow (CRF-1, Oriental Yeast Co.) and tap water *ad libitum*. The animal room was maintained at a temperature of $23 \pm 1^\circ\text{C}$, with a relative humidity of $55 \pm 5\%$ and a 12 hr light-dark cycle (lights on from 6:30 to 18:30).

DL-ethionine treatment: DL-ethionine (ET) was purchased from Nacalai Tesque, Inc. and was dissolved in 0.25% NIKKOL HCO-60 of 0.1% carboxymethylcellulose sodium. ET was given intraperitoneally at doses of 250, 500, and 1,000 mg/kg for 4 consecutive days.

Biochemistry tests: After 4 days of ET treatment, the rats were killed under ether anesthesia. Blood samples were obtained from the abdominal aorta and were centrifuged at 3,000 rpm for 15 min at 4°C . The serum levels of GOT, GPT, LDH, ALP, protein (PR), ureanitrogen (UN), creatinine (CRN), glucose (GLU), total cholesterol (TC), triglycerides (TG), phospholipids (PL), and free fatty acids (FFA) were determined.

Tissue analysis: The liver was removed, weighed, and homogenized with 10 volumes of PBS (pH 7.4). The liver homogenates were centrifuged at 3,000 rpm for 30 min at 4°C and the supernatant was analyzed for PR, TG, GLU, and FFA. For determination of the S-adenosylmethionine (SAM) content, liver tissue was homogenized with 5 volumes of ice-cold 0.66 M HClO_4 . The liver homogenates were centrifuged at 3,000 rpm for 20 min at 4°C , and the supernatant was used for SAM determination by HPLC (Lieber et al., 1990).

Histopathology: Liver specimens were fixed in 10% neutral buffered formalin. Paraffin sections of the liver were prepared in the usual manner and were stained with hematoxylin-eosin (H·E). Formalin-fixed frozen sections of the liver were stained with Oil Red O, Nile blue, and Sudan III.

Small specimens of the liver from 8 rats (3 animals from the control group and 5 from the 1,000 mg/kg group) were fixed in 2% paraformaldehyde–2.5% glutaraldehyde in 0.1 N cacodylate buffer and then postfixed in 1% osmic acid in the same buffer. Ultrathin sections were cut and stained with uranyl acetate and lead citrate for examination with an electron microscope (JEM-100CX, Jeol).

Experiment 2: Toxicokinetics of Phenobarbital in DL-Ethionine-Treated Rats

Animals: Female Sprague-Dawley rats (Japan SLC) weighing 146–170 g were used at approximately 6 weeks of age. Other housing conditions were the same as “Experiment 1”.

Selection of PB dose and treatment: PB is used to treat epilepsy at doses of 2–8 mg/kg *via* the oral route (Svensmak and Buchthal, 1963; Buchthal and Lennox-Buchthal, 1972). Shenoy et al. (1982) reported that the TD50 for PB neurotoxicity was 61.1 mg/kg in rats (43.7–95.9 mg/kg; 95% confidence limits). Thus, 100 mg/kg of PB was selected as the toxic dose level. PB was purchased from Wako Pure Chemical Industries and was dissolved in physiological saline. A single dose of PB (100 mg/kg) was given intravenously or orally to rats treated with ET (500 mg/kg/day, *i.p.*) for 4 days and to control rats.

Blood, tissues, and urine samples: In a single experiment, small blood samples were sequential-

ly obtained by capillary tube from the tail veins of 5 rats in each of the ET-treated and control groups under light diethyl ether anesthesia at 0, 0.5, 1, 4, 7, 16, 24, and 48 hr after intravenous dosing of PB. Blood samples were centrifuged at 10,000 rpm for 5 min at 4°C and subjected to the PB assay. In another experiment, 5 rats each of the ET-treated and control groups were kept individually in metabolic cages after administration of PB. Urine samples were collected over 0–24 hr and 24–48 hr and the PB concentration was assayed. The 40 rats (5 animals at 8 time points) in each of the ET-treated and control groups were anesthetized with diethyl ether at 0, 0.5, 1, 4, 7, 16, 24, and 48 hr after administration of PB. Blood samples were withdrawn from the abdominal aorta, and centrifuged at 3,000 rpm for 20 min at 4°C. After blood collection, the animals were killed and their livers and kidneys were removed for PB assay. All samples were stored at –20°C until assayed.

Tissue homogenates: Liver and kidney homogenates (10% in PBS, pH 7.4) were centrifuged at 3,000 rpm for 20 min at 4°C and the supernatants thus obtained were used.

Assay of the PB concentration: For the PB assay, the sample (homogenate supernatants, serum, or urine), the internal standard (hexobarbital 20 µg/ml), saturated monobasic potassium phosphate solution, and chloroform were mixed (1:1:2:15), and the mixture was centrifuged for 15 min at 1,000 rpm. The chloroform fraction was evaporate to dryness under a nitrogen stream at 50°C and the residue was dissolved in mobile phase (1.0 ml). Then PB concentrations were determined by high performance liquid chromatography (Kabra et al., 1976).

Calculation of toxicokinetic parameters: The maximum serum or tissues concentration (C_{max}) and the time to reach C_{max} (T_{max}) were obtained in the oral administration study. The area under the serum or tissue concentration-time curve from time 0 to time ∞ ($AUC_{0-\infty}$) was calculated using the trapezoidal rule with extrapolation of the area to $t=\infty$ in the oral and intravenous studies. The serum concentration versus time data in the intravenous study was analyzed by the peeling algorithm on Multi Program software (Tanigawara et al., 1981) for estimation of the parameters A, B, α , and β for

the two-compartment model, and the serum, liver, and kidney concentration versus time data in the oral study by that for estimation of the parameter k_e for the one-compartment model. CL (clearance) in the oral and intravenous study was calculated using the following equation:

$$CL \text{ (ml/hr/kg)} = \frac{\text{dose}}{AUC_{0-\infty}}$$

Protein binding of PB: In the oral administration experiment, samples of the equilibrium dialysate (ultrafiltration system; Amicon) obtained 4 hr after dosing with PB at 100 mg/kg were assayed by HPLC. The protein binding ratio was calculated from the following equation:

$$\begin{aligned} &\text{Protein binding (\%)} \\ &= \frac{\text{Serum PB concentration} - \text{PB concentration in equilibrium dialysate}}{\text{Serum PB concentration}} \\ &\quad \times 100 \end{aligned}$$

Extent of bioavailability (EBA) and tissue/serum distribution ratio (T/S ratio): The EBA was calculated from $AUC_{0-\infty}$ in the oral and intravenous studies. The T/S ratio was calculated in the oral administration study using the following equation:

$$T/S \text{ ratio} = \frac{\text{Liver or kidney PB concentration}}{\text{Serum PB concentration}}$$

Statistical analysis: Results are represented as the mean $\pm$ S.D. Statistical processing was done by parametric or nonparametric analysis of variance using a Statistical Analysis package (Y.K.D. Co., Ltd.) and NEC PC-9801; MUSCOT computer program. Significant differences were defined at $p \leq 0.05$.

RESULTS

Experiment 1: Biochemical and Morphological Features of the DL-Ethionine-treated Rats

The body and liver weights of the ET-treated groups were significantly lower than those of the control group (Table 1).

Biochemistry tests revealed elevation of

Table 1. Body and liver weights and blood biochemistry in the DL-ethionine-treated rats.

Dosage (mg/kg)	No. of Animals (Rats)	DL-ethionine-treated rats			
		0	250	500	1000
body weight (g)	6	194.8 ± 8.8	174.0 ± 8.1 ^{##}	170.2 ± 6.9 ^{##}	159.3 ± 6.2 ^{##}
absolute liver weight (g)	6	7.75 ± 0.83	6.57 ± 0.53	6.06 ± 0.52 [#]	5.42 ± 0.69 ^{##}
relative liver weight (%)	6	3.97 ± 0.27	3.77 ± 0.16 [#]	3.56 ± 0.22 ^{##}	3.39 ± 0.34 ^{##}
GOT (mU/ml)	6	51.0 ± 6.3	65.0 ± 10.4 [*]	80.2 ± 11.7 ^{***}	106.0 ± 41.9 ⁺
GPT (mU/ml)	6	23.4 ± 2.0	31.6 ± 7.5 ⁺	40.9 ± 10.4 ⁺⁺	44.3 ± 18.0 ⁺
LDH (mU/ml)	6	285 ± 177	133 ± 35	163 ± 26	213 ± 189
ALP (mU/ml)	6	490 ± 93	398 ± 93	462 ± 55	247 ± 94 ^{##}
PR (g/dl)	6	5.29 ± 0.16	5.31 ± 0.51	5.07 ± 0.39	3.85 ± 0.34 ^{##}
UN (mg/dl)	6	16.4 ± 1.3	19.2 ± 3.9	23.7 ± 8.4	18.5 ± 5.2
CRN (mg/dl)	6	0.61 ± 0.13	0.50 ± 0.08	0.73 ± 0.26	0.47 ± 0.13
GLU (mg/dl)	6	194 ± 16	159 ± 9 [#]	164 ± 18 [#]	141 ± 28 ^{##}
TC (mg/dl)	6	42.5 ± 4.6	27.7 ± 9.4 ^{**}	32.0 ± 23.8	28.9 ± 14.2
TG (mg/dl)	6	46.0 ± 12.2	43.2 ± 9.1	54.1 ± 23.8	39.3 ± 14.7
PL (mg/dl)	6	109 ± 11	81 ± 13	86 ± 23	99 ± 36
FFA (mEq/l)	6	0.46 ± 0.09	0.84 ± 0.19 ^{**}	0.78 ± 0.27 ⁺	0.76 ± 0.35

Values are the mean ± S.D.

* : Significant difference ($0.01 < P \leq 0.05$; Student's t-test) from the control.

** : Significant difference ($0.001 < P \leq 0.01$; Student's t-test) from the control.

*** : Significant difference ($P < 0.001$; Student's t-test) from the control.

: Significant difference ($0.01 < P \leq 0.05$; Dunnett's multiple comparison) from the control.

: Significant difference ($0.001 < P \leq 0.01$; Dunnett's multiple comparison) from the control.

+ : Significant difference ($0.01 < P \leq 0.05$; Aspin-Welch test) from the control.

++ : Significant difference ($0.001 < P \leq 0.01$; Aspin-Welch test) from the control.

GOT and GPT activity in the ET-treated animals, while the serum level of GLU was markedly reduced. The FFA concentration was increased in the 250 and 500 mg/kg groups, but no significant changes were noted in the serum levels of TG and PL (Table 1). The PR concentration and ALP activity were decreased in the 1,000 mg/kg group, which had the lowest body weight.

In the liver (Fig. 1), the SAM ($P < 0.001$) and GLU ($0.001 < P \leq 0.01$) contents were markedly significantly reduced. In contrast, the hepatic TG (250 and 1,000 mg/kg ; $0.001 < P \leq 0.01$, 500 mg/kg ; $P < 0.001$) and FFA (250 mg/kg ; $P < 0.001$, 500 and 1,000 mg/kg ; $0.001 < P \leq 0.01$) contents were significantly increased when compared with the control group. The hepatic PR content tended to decrease in the ET-treated animals.

ET-treated rats showed dose-dependent development of diffuse fatty degeneration of hepatocytes (Photo. 1). Electron microscopy re-

vealed an increase of fat droplets, mitochondria, and endoplasmic reticulum in the hepatocytes of the ET-treated rats, and a reduction of glycogen granules (Photo. 2).

Experiment 2 : Toxicokinetics of Phenobarbital in DL-Ethionine-Treated Rats

1. Toxicokinetic Parameters

1) Single intravenous dose

Figure 2 (upper panel) illustrates the serum PB concentration profile after intravenous administration. The serum PB level was higher in the ET-treated group than in the control group. Table 2 shows the toxicokinetic parameters calculated from the serum PB concentration profile. The $AUC_{0-\infty}$ was significantly about two times larger in the ET-treated group than in the control group, while the α (distribution phase) and β (elimination phase) values in the experimental parameter and CL value were smaller than in the control group (about half time the control value).

Fig. 1 Hepatic biochemical parameters in the DL-ethionine-treated rats. Column with vertical bars represent the mean and S.D.

* : Significant difference ($0.01 < P \leq 0.05$; Student's t-test) from the control.

: Significant difference ($0.01 < P \leq 0.05$; Dunnett's multiple comparison) from the control.

** : Significant difference ($0.001 < P \leq 0.01$; Dunnett's multiple comparison) from the control.

***: Significant difference ($P < 0.001$; Dunnett's multiple comparison) from the control.

Photo. 1 Fatty degeneration of liver cells in a female rat treated with DL-ethionine (H-E stain, $\times 300$).

Photo. 2 Numerous mitochondria and smooth-membraned concentric bodies are seen. An ET-treated female rat ($\times 7,800$).

Table 2. The toxicokinetic parameters after a single intravenous administration of phenobarbital at 100 mg/kg in the control and DL-ethionine-treated rats.

		control rats	DL-ethionine-treated rats
A	(μg/ml)	68.2	70.6
		±	±
		23.2	25.0
B	(μg/ml)	206	211
		±	±
		21	13
α	(hr)	0.234	0.155*
		±	±
		0.063	0.033
β	(hr)	0.043	0.018***
		±	±
		0.003	0.002
AUC _{0-∞}	(μg·h/ml)	4955	11843***
		±	±
		334	792
CL	(ml/h·kg)	20.2	8.4***
		±	±
		1.4	0.3

Serum concentrations were estimate by equation.

equation : $C_p = A \cdot \exp(-\alpha) + B \cdot \exp(-\beta)$

Values are the mean ± S.D.

* : Significant difference ($0.01 < P \leq 0.05$; Student's t-test) from the control.

*** : Significant difference ($P < 0.001$; Student's t-test) from the control.

Fig. 2 Mean serum concentrations of phenobarbital in DL-ethionine-treated rats after single oral or intravenous doses of 100 mg/kg (control rats ●, ET-treated rats ■).

Values represent the mean ± S.D. of 5 animals.

Fig. 3 Mean phenobarbital concentrations in the livers and kidneys of DL-ethionine-treated rats after a single oral dose of 100 mg/kg (control rats ●, ET-treated rats ■).

2) Single oral dose

Figure 2 (lower panel) and Figure 3 illustrate the serum, liver, and kidney PB concentrations in the ET-treated rats. The serum, liver, and kidney levels of PB were higher in the ET-treated group than in the control group. Toxicokinetic parameters are shown in Table 3. In the ET-treated rats, the serum C_{max} was significantly increased when compared with the control animals ($P < 0.001$). The CL values in serum, liver, and kidney in the rats treated with ET were about half time the control value. The T_{max} for serum, liver, and kidney in the ET-treated rats showed 7, 16, and 16 hr, respectively. The T_{max} for serum, liver, and kidney in the control rats showed 1, 4, and 0.5 hr, respectively. The $AUC_{0-\infty}$ for serum, liver, and kidney was in-

Table 3. The toxicokinetic parameters in the serum, liver, and kidney after a single oral administration of phenobarbital at 100 mg/kg in the control and DL-ethionine-treated rats.

		C_{max} ($\mu\text{g/ml}$ or g)	T_{max} (hr)	$AUC_{0-\infty}$ ($\mu\text{g}\cdot\text{h/ml}$)	k_e (hr^{-1})	CL (ml/h/kg)
serum	control rats	79.3	1	1997	0.042	43.9
		$\pm$				
	DL-ethionine-treated rats	103.9*	7	3598	0.028	23.1
		$\pm$				
liver	control rats	121.8	4	2734	0.051	32.9
		$\pm$				
	DL-ethionine-treated rats	128.4	16	4644	0.025	15.4
		$\pm$				
kidney	control rats	8.5	0.5	161	0.045	552.5
		$\pm$				
	DL-ethionine-treated rats	6.8	16	254	0.028	322.6
		$\pm$				

Each value was calculated from the mean concentrations of 5 rats.

The C_{max} data represents the mean $\pm$ S.D.

Serum, liver and kidney concentrations were estimate by equation.

equation : $C_p = A \cdot \exp(-\alpha) + B \cdot \exp(-\beta)$

* : Significant difference from the control ($0.01 < P \leq 0.05$; student's t-test).

creased in the ET-treated animals (about two times the control value), while the k_e in the ET-treated animals showed about half time the control value.

2. Tissue/Serum Phenobarbital Distribution Ratio

Figure 4 shows the time course of the T/S ratios (tissue/serum distribution ratios) after oral administration of PB. The T/S ratio of the liver/serum showed no change in the ET-treated rats at 0.5–7 hr, but the T/S ratio at 24–48 hr was tended to increase in the ET-treated rats when compared with the control group. The T/S ratio of the kidney/serum at early stage was significant-

Fig. 4 Serum concentration to tissue (liver, kidney) ratios of phenobarbital at each time point after a single oral dose of 100 mg/kg in the DL-ethionine treated rats (control rats ●, the ET-treated rats ■). Values represent the mean $\pm$ S.D. of 5 animals. *: Significant difference from the control ($0.01 < P \leq 0.05$; Student's t-test).

ly decreased the ET-treated rats when compared with the control group ($0.01 < P \leq 0.05$).

3. Protein Binding

The serum protein binding ratio after oral administration of PB was similar in the ET-treated rats and control animals (control vs. ET-treated: 46.6 ± 10.0 vs. $41.5 \pm 7.9\%$; mean $\pm$ S.D.).

4. Urinary Excretion

Table 4 shows the cumulative urinary excretion of PB after oral administration at 100 mg/kg. In the ET-treated group, urinary excretion PB from 0 to 48 hr was either unchanged or falls slightly.

5. Extent of Bioavailability

Table 5 shows the extent of bioavailability (EBA) of PB after oral dosing. The EBA of PB was slightly higher in the ET-treated rats when compared with the control group.

DISCUSSION

The purpose of the present study was to clarify the toxicokinetic parameters of PB in an experimental model of liver disease. Increased toxicity or persistence of the pharmacological effects of PB has been observed in patients with liver diseases due to impairment of the drug metabolizing enzymes or other detoxication mechanisms (Conney, 1967). For example, reduced clearance of PB (Alvin et al., 1975), antipyrine (Branch et al., 1973, 1976), and indocyanine green (Branch et al., 1976) has been reported in patients with liver disease. Howev-

Table 4. The cumulative urinary excretion of phenobarbital after an oral administration of phenobarbital at 100 mg/kg in the control and DL-ethionine-treated rats.

	hours after PB treatment (hr)	urine volume (ml)	urinary excretion of PB (μ g/day)
control rats	0~24	12.5 ± 3.8	4318 ± 1160
	24~48	13.0 ± 10.2	1740 ± 249
	0~48	25.5	6058
DL-ethionine-treated rats	0~24	6.5 ± 4.4	3349 ± 992
	24~48	12.0 ± 7.3	2973 ± 1759
	0~48	18.5	6322

Each value represents the mean $\pm$ S.D. (n=5).

Table 5. Extent of bioavailability of phenobarbital of 100 mg/kg in the control and DL-ethionine-treated rats.

	AUC _{0-∞} in serum ($\mu\text{g}\cdot\text{h}/\text{ml}$)		EBA (%)
	i.v.	p.o.	
control rats	4955	2275	45.9
DL-ethionine-treated rats	11843	4336	56.8

$$\text{EBA (\%)} = \frac{\text{AUC (p.o.)} \cdot \text{D (i.v.)} \cdot \text{ke (p.o.)}}{\text{AUC (i.v.)} \cdot \text{D (p.o.)} \cdot \beta \text{ (i.v.)}} \times 100 (\%)$$

EBA : Extent of bioavailability (%)

D : Dose

er, detailed reports on drug kinetics using experimental models of liver disease are rare. Breen et al. (1973) found impaired clearance of PB in rats with CCl_4 -induced hepatic injury, but the kinetics of PB in experimental liver disease is still unclear.

In the present study, we initially confirmed the features of ET toxicity. After 4 days of treatment with ET, the hepatic lipid content was significantly increased while the GLU and SAM contents were significantly decreased. In addition, histologic examination revealed diffuse fatty degeneration of the liver cells. Van Phi and Soling (1982) have reported that a rapid reduction of hepatic SAM was possibly important in the mechanism of ET intoxication. In addition, Feo et al. (1986) have reported that a reduction of the SAM content was closely related to fat accumulation in hepatocytes. The present biochemical (SAM and lipid levels) and morphological changes of the liver in ET injury largely agree with those reported previously (Van Phi and Soling, 1982; Feo et al., 1986; Berry and Friedman, 1977; Shull and Kisilevsky, 1971; Shull et al., 1966).

When PB was intravenously administered to the ET-treated rats, α , β and CL values were significantly decreased (about half time the control value), and the AUC_{0-∞} was increased (about two times the control value). These results indicate that the elimination of PB was impaired by ET-induced liver injury.

A single oral administration of PB to ET-treated rats resulted in prolongation of the T_{max} (control vs. ET-treated : 1 vs. 7 hr; serum, 4 vs. 16 hr; liver, 0.5 vs. 16 hr; kidney) and a decrease

of ke and CL values (about half time the control value), and an increase of AUC_{0-∞} (about two times the control value) in the serum, liver, and kidneys. These are also supported by the persistent urinary excretion of PB in the ET-treated animals. When PB was administered to ET-treated rats, the CL value was lower in intravenous administration than in oral administration. This was attributed to reduced blood flow in the tail vein resulting from a combination of anesthetic action of these drugs (PB and ET) at sampling of the intravenous test.

The T/S ratio of the liver/serum distribution of PB over the period from 16 to 24 hr after PB dosing was similar in the ET-treated rats and the control animals. This result is in agree with the finding that the K_p value of PB was not changed in rats with CCl_4 -induced hepatic injury (Breen et al., 1973). However, the T/S ratio of the kidney/serum distribution was significantly lower in the ET-treated rats at an early stage (0.5–4.0 hr), indicating that transport of PB into the tissues was impaired at an early stage. The T/S ratio of the liver/serum distribution was slightly higher in the ET-treated rats at 24–48 hr, indicating that elimination of PB into the liver was slightly hastened at 24–48 hr. The T/S ratio in the kidney was lower than that in the liver, this evidence suggests that a main excretion of PB is a key process the liver rather than the kidney, and an excretion to urine from the kidney is steeply hastened.

It has been reported that the serum protein binding of drugs such as warfarin and sulfadiazine (Boobis and Chignell, 1979) was increased by liver disease. In the present study, however, the

protein-binding ratio of PB was unchanged in the ET-treated rats. Thus, it seems possible that the transport of PB across the cell membrane was not disturbed in the ET-treated rats.

As mentioned above, ET-treated rats showed slightly delayed urinary excretion of PB. The exact cause of this phenomenon is still unknown, but because renal function of the ET (500 mg/kg)-treated rats was not impaired (Exp. 1, Table 1). It seems possible that hepatocyte injury was related to impaired elimination of PB.

The EBA of PB showed a tendency to increase in the ET-treated rats. This change in the EBA of the ET-treated rats might have been associated with enhanced concentration of PB in blood.

Clinical studies have shown that some drugs are more likely to accumulate than others when given to patients with liver disease (Williams, 1983). Prolongation of the half-life ($T_{1/2}$) of antipyrine and indocyanine green has been reported in patients with chronic liver disease (Branch et al., 1973, 1976), and prolongation of the $T_{1/2}$ of propranolol, morphine, and lidocaine has been reported in liver cirrhosis (Williams, 1983). Animals with CCl_4 -induced liver injury show prolongation of the $T_{1/2}$ of PB and antipyrine (Breen et al., 1973) and a decrease in sulfobromophthalein transport (Iga et al., 1977). The results of the present study are in agree with these earlier observations.

In conclusion, the present study with an experimental rat model of liver disease demonstrated the changes in toxicokinetic parameters of serum and tissues such as increased $AUC_{0-\infty}$, prolonged T_{max} , and decreased k_e and CL (the oral test) or α , β and CL values (the intravenous test). The persistent urinary excretion of PB in the ET-treated animals was also seen. These evidences suggest accumulation of PB in the ET-treated rats, which probably resulted in the impairment of the drug metabolizing enzyme and other detoxication mechanisms. From the results of the present study we can conclude that the toxicokinetic study in animals with experimentally impaired liver is useful for safety assessment of hepatotoxic agents.

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