Age-Related Changes in Pharmacokinetic Parameters of Phenytoin After Liver Resection in Rats

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INTRODUCTION

Therapeutic drug monitoring of phenytoin is made difficult by the saturable metabolic process in the liver, narrow therapeutic range, and large variations in individual metabolic capacity. Despite extensive information on the pharmacokinetics of phenytoin in humans (1) and rats (2,3), information on dosage regimens of phenytoin for critically ill patients is lacking.

After hepatic resection, changes in pharmacokinetic behavior may result from liver functional changes of drug metabolism (4). Moreover, phenytoin has been highly associated with hepatotoxicity and chronic liver enzyme abnormalities (5) attributed to enzyme induction. Regimens of antiepileptic drugs and side-effect profiles differ for infants, children, adults, and the elderly. However, the determinants of the changes in pharmacokinetic parameters that vary with age and disease state are still unknown. To predict the disposition of drugs, it is important to survey the factors relating to pharmacokinetic change. We tried to identify factors predictive of individual pharmacokinetic parameters of phenytoin after liver resection in rats.

MATERIALS AND METHODS

Materials

Sodium phenytoin was purchased from Tokyo Kasei Kogyo Co., and also as an injectable solution (Aleviatin, 250 mg/5 ml) from Dainippon Pharmaceutical Co., Ltd. All other chemicals were of reagent grade and were used without further purification.

Animal Experiments

Male Wistar rats (Japan SLC Inc., Shizuoka, Japan) at age of 8±1 and 50±1 weeks were used randomly in this study. All animal experiments complied with the standards set out in the Guidelines for the Care and Use of Laboratory Animals in Takara-machi Campus of Kanazawa University and adhered to the principles of Laboratory Animal Care of the NIH. The animals were divided into three experimental groups: normal, sham operated, and partially hepatectomized rats. The surgical removal of the two largest lobes of the liver was performed according to Higgins and Anderson (6), and was finished within 20 min. Sham operation was performed similarly without resection. Each rat was placed in an isolated cage for 10 days after the surgery before being used for the pharmacokinetic study. The pharmacokinetic experiments were carried out by essentially the same method as described previously (2, 7). Plasma samples were analyzed for albumin, total bilirubin, aspartate aminotransferase (AST), alanin aminotransferase (ALT), and nonesterified fatty acid (NEFA) which were determined using commercial kits (Wako Pure Chemical Co., Ltd., Japan). Microsome preparation and measurement of the activity of P-450 were the same as described by Omura and Sato (8).

Assay for Phenytoin

The drug concentration of phenytoin in plasma was determined by HPLC as described previously (2). The regression line was linear for phenytoin concentrations more than 0.2 μg/ml ($r>0.999$).

Data Analysis

The data were analyzed using a digital computer, FACOM-M760/20, at the Information Processing Center, Kanazawa University. All means are presented with their standard error (mean ± S.E.). Statistical analysis was performed using Student's t-test. All plasma concentration data were treated by nonlinear least squares regression analysis using the computer program (NONLIN)(9) fitting the observed data to Equations 1 and 2.

$$\frac{dC_1}{dt} = -k_{12}C_1 - \frac{V_{max}C_1f_p}{V_1(K_m + C_1f_p)} + k_{21}\frac{X_2}{V_1} \quad (1)$$

$$\frac{dX_2}{dt} = k_{12}C_1 V_1 - k_{21}X_2 \quad (2)$$

where K_m, V_{max}, V_1, k_{12}, and k_{21} are, respectively, the Michaelis Menten constant, maximum elimination rate, distribution volume of central compartment, apparent first order rate constant from compartment 1 to 2, and apparent first order rate constant from compartment 2 to 1. C_1 and X_2 are the plasma concentration and the amount of the drug in compartment 2, respectively. f_p is the plasma unbound fraction.

RESULTS AND DISCUSSION

In the present study, we investigated whether the V_{max} of phenytoin in rats varying with age correlates with hepatic function. The profiles of the mean plasma concentration of

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Abbreviations: aspartate aminotransferase (AST), alanin aminotransferase (ALT), nonesterified fatty acid (NEFA), maximum elimination rate (V_{max}).

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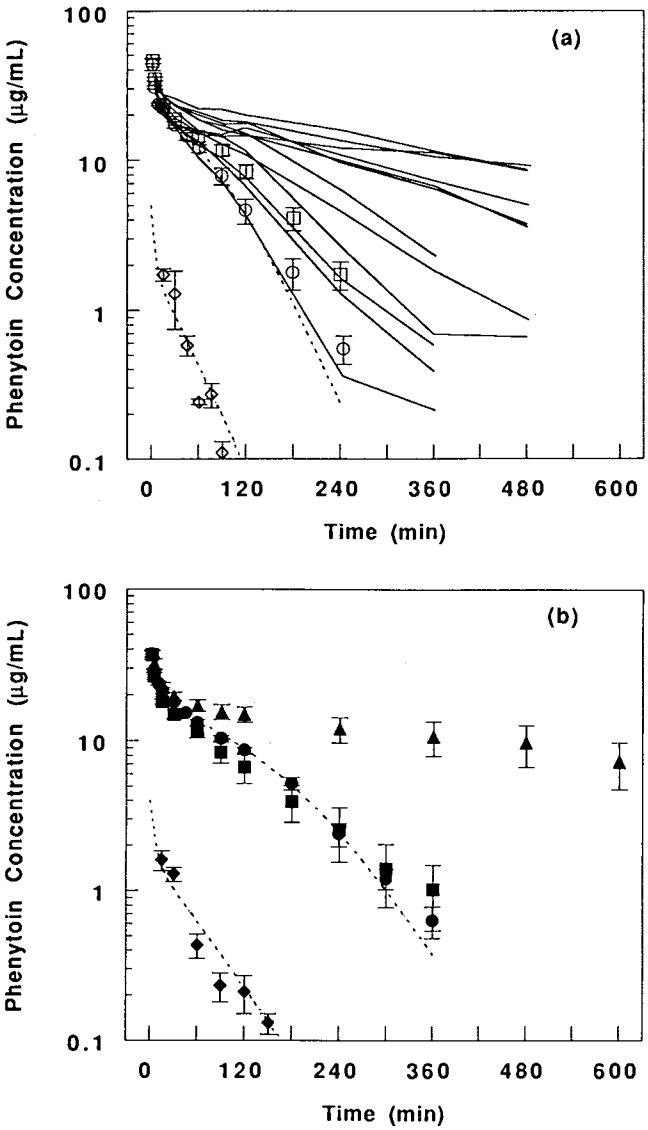

Fig. 1. Plasma phenytoin concentrations after 2-(◇) and 20-mg/kg (○) intravenous bolus dose to 8-week (a) and 50-week (b) old rats. Dotted lines were obtained by a nonlinear least squares regression analysis using the computer program NONLIN (9) fitting observed data in normal rats to Eqs. 1 and 2. In the case of partially hepatectomized 8-week-old rats, individual data were presented (lines). Each point represents the normal (○), sham operated (□), and the partially hepatectomized (△) rats for 8-week (open symbol) and 50-week (closed symbol) old rats. Mean±SE, n=3-7.

phenytoin *vs.* time after the intravenous bolus administration of 2 and 20 mg/kg were determined in the 8- and 50-week-old rats (Figure 1). The age of the rats had a marked influence on the distribution and elimination of phenytoin. Figure 1 also shows the plasma concentration vs. time profiles of phenytoin after the administration of 20 mg/kg in the 8- and 50-week-old rats at 10 days after the hepatic resection or sham operation. The partial hepatectomy markedly influenced the elimination of phenytoin in both groups of rats. As shown in Figure 1-a, the plasma levels of phenytoin at each time point in 8-week-old rats differed significantly among animals. Although there were no significant differences in plasma concentration at any time point after the administration between the 8-week-old sham operated and normal rats, there were big fluctuations in plasma levels at each joint in the partially hepatectomized rats. In contrast, relatively small individual differences were observed in the groups of 50-week-old rats. As shown in Figure 1-b, the plasma levels of phenytoin in the partially hepatectomized 50-week-old rats at each time point after the administration differed significantly from the sham operated and normal 50-week-old rats.

Since phenytoin is metabolized by a saturable process, simple description of clearance values is not applicable. Model dependent analysis is necessary, and a two compartment open model with saturable metabolic process was applied. The estimated pharmacokinetic parameters in 8- and 50-week-old rats are listed in Table I. The simulated curves were well fitted to the observed data in the distribution and elimination phases. The V_1 value changed about 2-fold in the 50-week old rats versus the 8-week old rats, with this difference significant. However, there were no significant differences in K_m or V_{max} between the 8- and 50-week-old rats. When parameters were normalized for body weight, significant decreases were noted in V_{max}/BW values of the 50-week-old rats versus the 8-week-old rats, whereas the significant difference in the V_1/BW values between the 8- and 50-week-old rats disappeared. The mean V_{max} value of phenytoin in 50-week-old rats was almost the same as that of 8-week-old rats (Table I), whereas the mean V_{max}/BW value in 8-week-old rats was higher than that of 50-week-old rats suggesting that the V_{max} values may be related to liver weight rather than body weight in normal rats.

In the present model of partial hepatectomy, it is known that the liver was less active cytologically from the fourth to the seventh days after the operation, and the preoperative ratio of weight of the liver to weight of the body was essentially restored from the tenth to the fourteenth day (6). The physiological and biochemical data for the rats used in this study are summarized in Table II. The AST of the 50-week-old rats was elevated 1.4-fold, as compared with the 8-week-old rats, whereas the values of ALT, serum free fatty acid, total bilirubin, albumin, and hepatic cytochrome P-450 did

Table I. Estimated Pharmacokinetic Parameters of Phenytoin in 8- and 50-week-old Rats[a]

Pharmacokinetic parameters	8-week-old	50-week-old
K_m[b] (μg/mL)	2.36 ± 0.34	1.81 ± 0.25
V_{max}[c] (μg/min)	67.5 ± 4.8	64.9 ± 4.0
V_1[d] (mL)	111 ± 7	257 ± 14
k_{12}[e] (min^{-1})	0.114 ± 0.021	0.0788 ± 0.0129
k_{21}[e] (min^{-1})	0.108 ± 0.010	0.0749 ± 0.0068
V_{max}/BW[f] (μg/min/kg)	244 ± 26	126 ± 18
V_1/BW (mL/kg)	401 ± 41	499 ± 69

[a] mean ± SD; each value was obtained by a nonlinear least squares regression using the computer program (NONLIN) (9) fitting the observed data to Eqs. 1 and 2.
[b] Michaelis Menten constant.
[c] Maximum elimination rate.
[d] Distribution volume of central compartment.
[e] Apparent 1st order elimination rate constant.
[f] BW (Body Weight).

not differ. A significant increase in the plasma unbound fraction of phenytoin was observed in the 50-week-old rats as compared to the 8-week-old rats. A 10-days post-surgery, the fraction of unbound phenytoin was 0.338 in partially hepatectomized animals and 0.242 in sham-operated 50-week-old rats.

The AST values of the partially hepatectomized rats were elevated 2- and 4-fold as compared with the 8-week- and 50-week-old sham operated rats, respectively. The serum free fatty acid and total bilirubin of the partially hepatectomized rats were elevated significantly as compared with the 8-week-old sham operated rats. Decreased values of hepatic cytochrome P-450 were observed in the 50-week-old rats, whereas the values of serum free fatty acid and total bilirubin did not change. Individual differences in the values of AST were found in the partially hepatectomized rats of both ages.

We determined the pharmacokinetic parameters on the tenth day after the operation when there was only little influence by the sham operation on the pharmacokinetics. Regeneration of the liver is activated sharply in the early postoperative period (Table II) (6); however, delay in the elimination of phenytoin was observed in all of the older rats studied. The plasma concentration data in each animal of the partially hepatectomized rats were treated by nonlinear least squares regression analysis to determine each V_{max} value

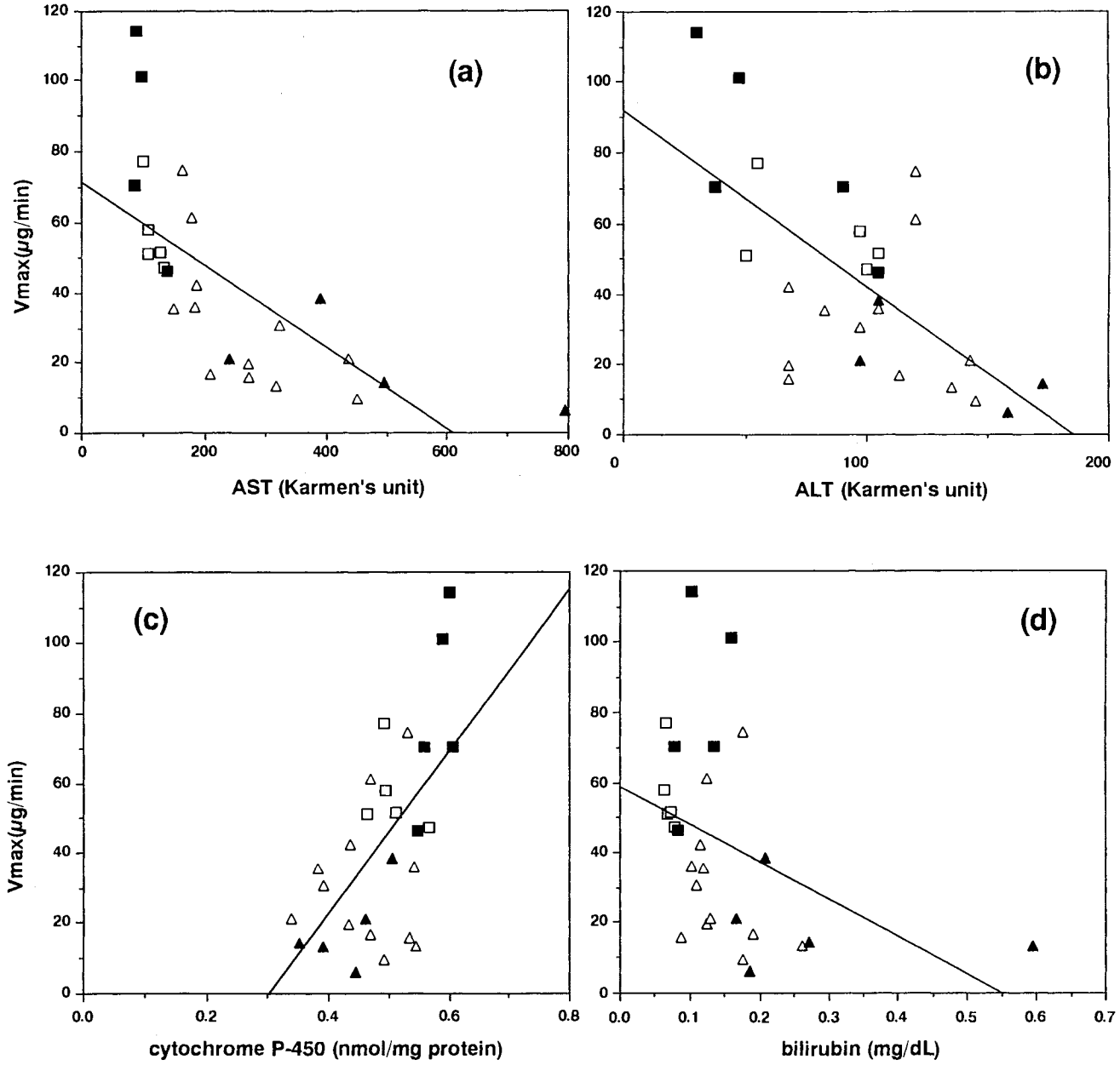

Fig. 2. Comparison of maximum elimination rate (V_{max}) with biochemical parameters, AST (a), ALT (b), cytochrome P-450 (c), bilirubin (d) in partially hepatectomized and sham operated 8- and 50-week-old rats at 10-day interval after each operation. Each point represents the sham operated (□) and the partially hepatectomized (△) rats for 8-week (open symbol) and 50-week (closed symbol) old rats. Solid line represents the simple linear regression line.

Table II. Pathophysiological Characteristics of Normal, Sham Operated, and Partially Hepatectomized 8- and 50-Week-Old Rats

	8-week-old					
	Normal		Sham Operated		Hepatectomized	
	mean	(SEM, n)	mean	(SEM, n)	mean	(SEM, n)[a]
Body Weight (g)	277	(4, 33)	309	(4, 5)	295	(5, 12)
Liver Weight (g)	10.7	(0.3, 33)	9.56	(0.5, 5)	8.71	(0.12, 12)[d]
Serum transaminase activity						
AST (Karmen's Unit)	143	(12, 13)	117	(7, 5)	262	(29, 12)[c]
ALT (Karmen's Unit)	49	(5, 13)	82	(12, 5)	105	(8, 12)
Serum free fatty acid (mEq/L)	1.01	(0.09, 13)	0.78	(0.04, 5)	1.01	(0.06, 12)[d]
Serum total bilirubin (mEq/L)	0.11	(0.03, 13)	0.07	(0.00, 5)	0.14	(0.01, 12)[c]
Serum albumin (g/dL)	3.66	(0.12, 13)	2.99	(0.33, 5)	3.21	(0.19, 12)
Hepatic cytochrome P-450						
(nmol/mg protein)	0.555	(0.019, 11)	0.51	(0.02, 5)	0.46	(0.02, 12)
Plasma unbound fraction	0.249	(0.007, 29)	0.23	(0.01, 9)	0.27	(0.01, 14)
	50-week-old					
	Normal		Sham Operated		Hepatectomized	
	mean	(SEM, n)[b]	mean	(SEM, n)	mean	(SEM, n)[a]
Body Weight (g)	515	(15, 20)[c]	495	(16, 5)	508	(21, 5)
Liver Weight (g)	13.6	(0.6, 20)[c]	12.5	(0.8, 5)	9.48	(0.61, 5)[d]
Serum transaminase activity						
AST (Karmen's Unit)	204	(17, 10)[c]	104	(12, 4)	480	(117, 4)[d]
ALT (Karmen's Unit)	50	(4, 10)	62	(15, 5)	133	(19, 4)[d]
Serum free fatty acid (mEq/L)	1.16	(0.11, 12)	0.95	(0.06, 5)	1.53	(0.26, 5)
Serum total bilirubin (mEq/L)	0.147	(0.021, 12)	0.11	(0.02, 5)	0.29	(0.08, 5)
Serum albumin (g/dL)	3.37	(0.10, 12)	3.28	(0.20, 4)	3.14	(0.10, 3)
Hepatic cytochrome P-450						
(nmol/mg protein)	0.61	(0.03, 10)	0.58	(0.01, 5)	0.43	(0.03, 5)[c]
Plasma unbound fraction	0.299	(0.011, 25)[c]	0.24	(0.01, 7)	0.34	(0.04, 5)[d]

[a] *t*-test between sham operated and partially hepatectomized rats.
[b] *t*-test between 8- and 50-week old rats.
[c] Values are significantly different ($p < 0.01$).
[d] Values are significantly different ($p < 0.05$).

using Equations 1 and 2 by fixing the parameters of K_m, V_1, k_{12}, and k_{21} to the values of age matched-control rats. The terminal half-life was significantly increased from 120 min. to 360 min. by the partial hepatectomy in younger rats. We selected the V_{max} value as an index to interpret the individual capacity of metabolism. Because the values of k_{12} and k_{21} are useful to explain only the early distribution phase, moreover, the changes in the values of K_m and V_1 will be included in the observed V_{max} value. The individual values of V_{max} ranged from 10 to 75 μg/min among the sham and partially hepatectomized 8-week-old rats. In contrast, the partially hepatectomized 50-week-old rats showed a 5-fold increase with relatively small intraindividual difference in the plasma concentration compared with the age-matched sham-operated controls. Figure 2 shows the relationships between V_{max} values and each biochemical parameter. The correlation coefficients of V_{max} with AST, ALT, and P-450 were −0.668, −0.650, and 0.604 respectively. The correlation coefficients are high and significant ($p < 0.05$). There were no significant correlations between V_{max} values and bilirubin, albumin, or free fatty acid. Factors related to the rate of regeneration after liver resection, include coagulation, fibrinolysis (10), insulin, glucagon, estradiol and androgen (11). Changes in all these indices may cause the age-related differences in V_{max} of phenytoin. A best correlation between the values of AST and V_{max} was obtained suggesting that there is a close relation between metabolic capacity and liver function during hepatic regeneration after partial hepatectomy.

Since there are considerable fluctuations during regeneration and recovery from hepatic damage after liver resection, estimation of the clearance of drugs in patients undergoing hepatic resection is difficult. Our results indicate that the dosage regimen in the aged should not be identical to that in young people. We conclude that AST is a good index of metabolic function of phenytoin, and that an altered AST could relate to hepatic regeneration.

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