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International Journal of Pharmaceutics 229 (2001) 45–55

international journal of pharmaceutics

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# Effects of cysteine on the pharmacokinetics of intravenous phenytoin in rats with protein–calorie malnutrition

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Received 27 March 2001; received in revised form 9 July 2001; accepted 11 July 2001

**Abstract**

The effects of cysteine on the pharmacokinetics of phenytoin and one of its metabolites, 5-(*p*-hydroxyphenyl)-5-phenylhydantoin (*p*HPPH) were investigated after intravenous administration of phenytoin, 25 mg/kg, to control rats (4-week fed on 23% casein diet) and rats with PCM (protein–calorie malnutrition, 4-week fed on 5% casein diet) and PCMC (PCM with oral cysteine supplementation, 250 mg/kg, twice daily starting from the fourth week). In rats with PCM and PCMC, the phenytoin hydroxylation (to form *p*HPPH) activities were significantly smaller (164, 103 and 95.3 pmol/min per mg protein for the control rats, and rats with PCM and PCMC, respectively) than that in control rats. In rats with PCMC, the intrinsic clearance of phenytoin, $CL_{int}$ was significantly slower than those in control rats and rats with PCM (0.175, 0.131 and 0.044 ml/min). The above data suggested that the formation of *p*HPPH could be reduced in rats with PCM and PCMC. This was supported by significantly smaller 24-h urinary excretion of *p*HPPH (54.7, 35.6 and 32.5% of intravenous dose of phenytoin) in rats with PCM and PCMC than that in control rats. In rats with PCM, the maximum velocity (0.344, 0.203 and 0.196 μg/min), apparent volume of distribution in central compartment (44.4, 65.4 and 72.2 ml/kg) of phenytoin, and total area under the plasma concentration–time curve from time zero to time infinity (609, 714 and 1210 μg min/ml), renal clearance (20.5, 13.4 and 4.67 ml/min per kg) and 24-h urinary excretion (54.7, 35.6 and 32.5% of intravenous dose of phenytoin) of *p*HPPH were not returned to control levels by cysteine supplementation (rats with PCMC). This could be mainly due to the fact that the phenytoin hydroxylation activity in rats with PCMC was not returned to control level. 

*Keywords:* Phenytoin and *p*HPPH; Pharmacokinetics; PCM; Cysteine; Rats

## 1. Introduction

Protein–calorie malnutrition (PCM) is considered to be a global problem, especially for chil-

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0378-5173/01/$ - see front matter 
PII: S0378-5173(01)00815-8

dren, infants and institutional elderly who are more susceptible to PCM (Denke and Wilson, 1998). A number of diseases including cancer, digestive disorders and AIDS are also associated with PCM (Wykes et al., 1996; Denke and Wilson, 1998). The rate of drug metabolism may be influenced by various physiological, genetic and environmental factors. Nevertheless, nutritional status was not usually investigated as a factor which may affect the pharmacokinetics of drugs, and hence, the pharmacodynamics of drugs. The changes in drug metabolism and pharmacokinetics in malnutrition were reviewed (Buchanan, 1978; Krishnaswamy, 1978).

The following results were obtained recently from our laboratories (Cho et al., 1999). Western blot analysis revealed that rats with protein restriction (5% casein diet, PCM) for 4 weeks exhibited 40–80% suppression in the hepatic CYP1A2, 2C11, 2E1 and 3A1/2 levels as compared with control (23% casein diet). However, CYP2B1/2 expression was not significantly affected by PCM. Northern blot analysis showed that hepatic CYP1A2, 2E1, 2C11 and 3A1/2 mRNAs also decreased in the state of PCM. Interestingly, the altered CYP450 expression by PCM completely or partially returned to the level of control by oral cysteine supplementation for one week (250 mg/kg, twice daily starting from the fourth week). Cysteine supplementation (rats with PCMC) also prevented decreases in CYP1A2, 2E1, 2C11 and 3A1/2 mRNA levels during PCM. The metabolic rate of ethoxyresorufin dealkylase monitored in the hepatic microsomes produced from rats with PCM was consistent with the changes in CYP1A2 apoprotein and mRNA expression.

The following results were also obtained from our laboratories. After intravenous administration of azosemide, a loop diuretic, primarily metabolized by cytochrome CYP1A in rats (Lee and Lee, 1997), the time-averaged nonrenal clearance ($CL_{NR}$) of azosemide decreased significantly in rats with PCM. Interestingly, cysteine supplementation (rats with PCMC) restored $CL_{NR}$ toward control (Cho et al., 1999; Kim et al., 2001). After intravenous administration of adriamycin, primarily metabolized by CYP3A in rats (Lee and Lee, 1999), the 24-h urinary excretion of M3, an aglycone metabolite of adriamycin, decreased in rats with PCM (Kim et al., in press). However, by cysteine supplementation (rats with PCMC), the 24-h urinary excretion of M3 increased significantly as compared with that in PCM rats (Kim et al., in press). Above data indicated that cysteine was efficient in restoring CYP450 expression and metabolic activities.

It was published (Billings, 1984) that metabolism of phenytoin increased in rats pretreated with phenobarbital [a potent inducer of CYP2B1 (Guengerich, 1990) and CYP2B1/2 (Kawamura et al., 1999) in rats]. The main metabolite of phenytoin in rats, mice and humans is 5-(*p*-hydroxyphenyl)-5-phenylhydantion (*p*H-PPH) (Butler, 1957; Maynert, 1960). Although the CYP2B1/2 expression was not significantly affected in rats with PCM (Cho et al., 1999), the study on the phenytoin hydroxylation activity in hepatic microsomes in control rats and rats with PCM and PCMC seemed not to be investigated. The interaction of phenytoin with casein has been only studied for biopharmaceutical purpose (Rosen and Macheras, 1984, 1985). Phenytoin was chosen in the present study to find whether the phenytoin hydroxylation activity in hepatic microsomes changes in rats with PCM and the activity returns to control level in rats with PCMC. The purpose of this paper is to report the pharmacokinetic changes of phenytoin and *p*H-PPH after intravenous administration of phenytoin, 25 mg/kg, to control rats and rats with PCM and PCMC.

## 2. Materials and methods

### 2.1. Chemicals

Phenytoin (intravenous solution, 50 mg/ml ample) and *p*HPPH were obtained from Samjin Pharmaceutical Company (Seoul, South Korea) and Dr Ji Woo Lee (College of Pharmacy, Seoul National University, Seoul, South Korea), respectively. Cysteine, β-glucuronidase, β-nicotinamide dinucleotide phosphate (NADP), glucose-6-phosphate and glucose-6-phosphate dehydrogenase were purchased from Sigma Chemical Company

(St Louis, MO). Other chemicals were of reagent grade or high-performance liquid chromatographic (HPLC) grade, and, therefore, were used without further purification.

### 2.2. Rats and diets

Male Sprague–Dawley rats, weighing 145–195 g, were purchased from Charles River Company (Atsugi, Japan). Rats were assigned randomly to one of two diets containing either 23% (control rats) or 5% (rats with PCM) casein. Both diets were isocaloric and the compositions of the diets were listed (Cho et al., 1999). All rats were provided with food and water ad libitum and maintained on each diet for a 4-week period (Animal Center for Pharmaceutical Research, College of Pharmacy, Seoul National University, Seoul, South Korea). From the start of the fourth week, rats with PCM were divided randomly into two groups. One group was treated with oral cysteine, 250 mg/kg, twice daily (cysteine was dissolved in tap water to make 100 mg/ml, rats with PCMC) and other group (without cysteine) was treated with the same volume of tap water (rats with PCM). Food intake and body weight were recorded at least once a week to assess the influence of the low protein diet.

### 2.3. Pretreatment of rats

In the early morning after a 4-week fed on each diet, the jugular vein and the carotid artery of each rat were catheterized with polyethylene tubing (Clay Adams, Parsippany, NJ) under light ether anesthesia. Both cannulae were exteriorized to the dorsal side of the neck where each cannula terminated with a long Silastic tubing (Dow Corning, Midland, MI). Both Silastic tubings were covered with a wire to allow free movement of the rats. After the exposed areas were surgically sutured, each rat was housed individually in a rat metabolic cage (Daejong Scientific Company, Seoul, South Korea) and allowed to recover from anesthesia for 4–5 h before the study began. They were not restrained at any time during the whole experimental period.

### 2.4. Intravenous study

Phenytoin (phenytoin intravenous solution, 50 mg/ml, was diluted in 0.1 N NaOH), 25 mg/kg, was administered in 1-min via the jugular vein of control rats ($n = 7$) and rats with PCM ($n = 6$) and PCMC ($n = 7$). Total injection volume was approximately 1 ml. Approximately 0.25 ml of blood was collected via the carotid artery at 0 (to serve as a control), 1 (at the end of intusion), 5, 15, 30, 45, 60, 90, 120, 180, 240, 360 and 480 min after intravenous administration of phenytoin. At 60 min after intravenous administration, 1 ml of heparinized blood collected from each group of rats untreated with phenytoin was infused via the carotid artery to supply blood loss induced by blood sampling. Heparinized 0.9% NaCl-injectable solution, 0.3 ml, was flushed after each blood sampling to prevent blood clotting. After centrifugation, a 100-μl aliquot of each plasma sample was stored in a −70 °C freezer (Revco ULT 1490 D-N-S, Western Mednics, CA) until HPLC analysis of phenytoin and *p*HPPH (Tanaka et al., 1995). At the end of experiment (24 h), as much blood as possible was collected and plasma was collected for measuring the plasma protein binding. At the same time (24 h), the weight of each whole liver, kidney and stomach was recorded. Urine samples were collected between 0 and 24 h. Each metabolic cage was rinsed with 15 ml of distilled water and the rinsings were combined with each urine sample. After measuring the exact volume of combined urine sample, two l00-μl aliquots were stored in a −70 °C freezer until HPLC analysis of phenytoin and *p*HPPH (Tanaka et al., 1995). A 100-μl aliquot of urine sample was added to a 200-μl aliquot of 0.2 M sodium acetate buffer (pH 4.75) containing 1000 U of β-glucuronidase and the mixture was incubated for 3 h in a water-bath shaker kept at 37 °C and at a rate of 50 oscillations per min. After centrifugation, two 100-μl aliquots were stored in a −70 °C freezer until HPLC analysis of phenytoin and *p*HPPH (Tanaka et al., 1995).

### 2.5. Tissue distribution study

Phenytoin (the same solution as used in the intravenous study), 25 mg/kg, was intravenously administered in 1-min via the jugular vein of control rats and rats with PCM and PCMC ($n=5$, each). Thirty min after intravenous administration, as much blood as possible was collected via the carotid artery in a heparinized tube and each rat was sacrificed by cervical dislocation. After centrifugation of blood samples, each plasma sample was added with four volumes of 0.9% NaCl-injectable solution, vortex-mixed and centrifuged. Approximately 1 g of each liver, lung, brain, heart, kidney, spleen, stomach, small intestine, large intestine, mesentery, fat and muscle was quickly removed, rinsed or perfused with cold 0.9% NaCl-injectable solution, blotted, minced, homogenized with four volumes of 0.9% NaCl-injectable solution in a tissue homogenizer (Ultra-Turrax T25, Janke & Kunkel, IKA Labortechnik, Staufen, Germany) and centrifuged immediately. Two 100-μl aliquots of plasma or the supernatant of each tissue homogenate were stored in a −70 °C freezer until HPLC analysis of phenytoin and *p*HPPH (Tanaka et al., 1995).

### 2.6. Measurement of plasma protein binding

The plasma protein binding of phenytoin in control rats and rats with PCM and PCMC after intravenous administration of phenytoin was measured using an equilibrium dialysis technique as reported earlier (Han et al., 1998). The concentration of phenytoin spiked into the plasma compartment was 10 μg/ml. After 24 h incubation, two 100-μl aliquots were removed from each compartment and stored in a −70 °C freezer until HPLC analysis of phenytoin (Tanaka et al., 1995).

### 2.7. Determination of phenytoin hydroxylation activity in rat liver microsomal fraction

Phenytoin hydroxylation activity was determined in the hepatic microsomal fraction. In the early morning after a 4-week fed on each diet, control rats and rats with PCM and PCMC ($n=4$, each) were sacrificed by cervical dislocation. Each liver was excised, chilled, blotted-dry with paper tissue and weighed. Hepatic microsomal fractions were prepared as described previously (Eriksson et al., 1978). Protein contents of liver microsomes were measured by the reported method (Lowry et al., 1951) and hepatic CYP450 contents were determined by the published method (Omura and Sato, 1964). Liver microsomes were stored in a −70 °C freezer until used.

The liver microsomes without substrate were preincubated at 37 °C for 3 min. Incubation with liver microsomes contained CYP450 (1 mg), NADP (1 mM), glucose-6-phosphate (10 mM), glucose-6-phosphate dehydrogenase (1 U/ml), magnesium chloride (5 mM), 100 mM potassium phosphate buffer (pH 7.4) and phenytoin (200 μM) in a total volume of 0.6 ml. The mixture was incubated for 15 min at 37 °C at a rate of 500 oscillations per min after the addition of the substrate. The reaction was terminated by the addition of 0.5 ml of 0.1 N HCl. Samples were stored in a −70 °C freezer until analysis of *p*H-PPH (Tanaka et al., 1995). Catalytic activities of all enzyme preparations were determined under conditions in which metabolism was proportional to CYP450 concentrations as well as the length of incubation.

### 2.8. HPLC analysis of phenytoin and pHPPH

The concentrations of phenytoin and/or *p*H-PPH in plasma, liver microsome and tissue homogenates were determined by reported method with slight modification (Tanaka et al., 1995). Briefly, to a l00-μl aliquot of sample in 1.5 ml eppendorf tube, a 50-μl aliquot of internal standard (fluorescin, 10 μg/ml in distilled water), a 100-μl aliquot of 0.1 N HCl and a 1-ml aliquot of methylene chloride were added. After vortexing for 5 min and centrifuging at 3000 rpm for 5 min, the organic phase was transferred into a clean eppendorf tube and evaporated under a gentle stream of nitrogen. The residue was reconstituted in a 100-μl aliquot of mobile phase and a 50-μl aliquot was injected directly onto the HPLC column. The mobile phase, 8 mM $KH_2PO_4$–acetonitril (70:30, v/v), was run at a flow rate of 1.2

ml/min and the column effluent was monitored by a UV detector set at 215 nm. Separation was achieved using a reversed-phase ($C_{18}$) column (250 mm, $l \times 4.6$ mm, i.d.; particle size, 5 μm; Supelcosil; Supelco, Bellefonte, PA). The detection limits for phenytoin and *p*HPPH in rat plasma, urine and tissue homogenates were all 100 ng/ml.

### 2.9. Pharmacokinetic analysis

In phenytoin pharmacokinetic analysis, all plasma concentration data were treated by nonlinear least squares regression analysis using the computer program (WINNONLIN) (Gabrielsson and Weiner, 1997) and the Eqs. (1) and (2) were fitted to the data (Nakashima et al., 1995).

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

$$\frac{dX_2}{dt} = k_{12}C_1V_1 - k_{21}X_2 \quad (2)$$

where $K_m$, $V_{max}$, $V_1$, $k_{12}$ and $k_{21}$ are, respectively, the Michaelis–Menten constant, maximum elimination rate, apparent volume of distribution in central compartment, apparent first order rate constant from compartment 1 to 2 and apparent first order rate constant from compartment 2 to 1. The $C_1$ and $X_2$ are the plasma concentration and amount of the drug in compartment 2, respectively. The $f_p$ is the unbound fraction of phenytoin in plasma. The intrinsic clearance ($CL_{int}$) of phenytoin was calculated by dividing $V_{max}$ by $K_m$.

In *p*HPPH pharmacokinetic analysis, the total area under the plasma concentration–time curve from time zero to time infinity (AUC) was calculated by the trapezoidal rule-extrapolation method; this method utilized the logarithmic trapezoidal rule (Chiou, 1978) for the calculation of the area during the declining plasma-level phase, and the linear trapezoidal rule for the rising plasma-level phase. The area from the last data point to time infinity was estimated by dividing the last measured plasma concentration by the terminal rate constant. The time-averaged renal clearance ($CL_R$) was calculated by dividing the total amount of *p*HPPH excreted in 24-h urine by AUC of *p*HPPH (the contribution of $AUC_{24\ h-\infty}$ to total AUC of *p*HPPH was less than 0.98% in three groups of rats).

The mean values of $CL_R$ and $CL_{int}$ (Chiou, 1980), $V_1$ (Chiou, 1979) and terminal half-life (Eatman et al., 1977) were calculated by the harmonic mean method.

### 2.10. Statistical analysis

All results are expressed as mean ± S.D. A $P < 0.05$ was considered to be statistically significant using the Duncan's multiple range test of SPSS posteriori analysis of variance program among the three means for unpaired data.

## 3. Results

### 3.1. Effects of cysteine on body weight gain, food intake and organ weight in rats with PCM

The effects of cysteine on body weight gain, food, protein and calorie intakes and liver, kidney and stomach weights in the present intravenous study are listed in Table 1. Protein deprivation for 4 weeks (5% casein diet, PCM) caused a significant decrease in body weight gain (from 178 to 323 g in control rats vs. from 178 to 182 g in rats with PCM) and food consumption. For example, rats on 5% protein diet (rats with PCM) consumed approximately 50.3% less food than that on 23% protein diet (control rats), despite ad libitum supply of food. As a result, their protein and calorie intakes decreased significantly by 89.2 and 50.2%, respectively, in rats with PCM. Since the protein and calorie intakes decreased significantly in rats with PCM, it is important to realize that rats with PCM suffered from both protein and calorie deficiencies. Therefore, any changes in the pharmacokinetics of phenytoin in rats with PCM should be attributed to protein and calorie deficiencies and not solely to protein deficiency. The absolute liver (42.4% decrease) and kidney (41.0% decrease) weights decreased significantly in rats with PCM than those in control rats. Note that cysteine supplementation (rats with PCMC) did not restore the above mentioned parameters to control levels.

### 3.2. Pharmacokinetics after intravenous administration

The typical Michaelis–Menten type plasma concentration–time profiles of phenytoin after intravenous administration of the drug, 25 mg/kg, to control rats ($n = 7$) and rats with PCM ($n = 6$) and PCMC ($n = 7$) are shown in Fig. 1(A), and relevant pharmacokinetic parameters are listed in Table 2. The Eqs. (1) and (2) were well fitted to the plasma concentration–time profiles of phenytoin in three groups of rats with the Akaike

Table 1
Body weight, food, protein and calorie intakes, and liver, kidney and stomach weights after 1-min intravenous infusion of phenytoin, 25 mg/kg, to control rats and rats with PCM and PCMC

| | Control ($n = 7$) | PCM ($n = 6$) | PCMC ($n = 7$) |
|---|---|---|---|
| Initial body weight (g) | $178 \pm 13.0$ | $178 \pm 16.4$ | $174 \pm 10.9$ |
| Final body weight (g) | $323 \pm 42.6$[a] | $182 \pm 27.3$ | $172 \pm 14.6$ |
| Food intake (g/day per rat) | $16.8 \pm 2.82$[a] | $8.35 \pm 3.31$ | $7.64 \pm 2.39$ |
| Protein intake (g/day per rat) | $3.86 \pm 0.649$[a] | $0.418 \pm 0.166$ | $0.382 \pm 0.120$ |
| Calorie intake (kcal/day per rat) | $67.7 \pm 11.4$[a] | $33.7 \pm 13.4$ | $30.9 \pm 9.66$ |
| Liver weight (g) | $12.0 \pm 2.20$[a] | $6.91 \pm 1.24$ | $6.70 \pm 1.19$ |
| Liver weight (% of body weight) | $3.70 \pm 0.394$ | $3.81 \pm 0.444$ | $3.88 \pm 0.500$ |
| Kidney weight (g) | $2.66 \pm 0.327$[a] | $1.57 \pm 0.196$ | $1.57 \pm 0.108$ |
| Kidney weight (% of body weight) | $0.826 \pm 0.0566$[b] | $0.869 \pm 0.0737$ | $0.915 \pm 0.0908$ |
| Stomach weight (g) | $1.46 \pm 0.140$ | $1.00 \pm 0.160$ | $0.928 \pm 0.104$ |
| Stomach weight (% of body weight) | $0.457 \pm 0.0524$[b] | $0.555 \pm 0.0521$ | $0.539 \pm 0.0430$ |

Each value represents the mean $\pm$ S.D.
[a] Control group was significantly different ($P < 0.05$) from PCM and PCMC groups.
[b] Control group was significantly different ($P < 0.05$) from PCMC group.

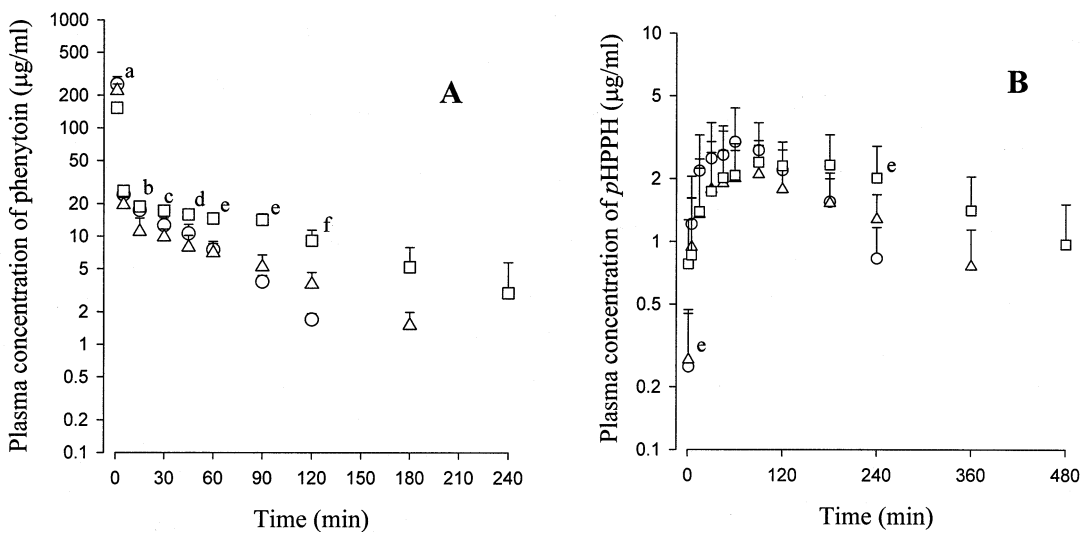


Fig. 1. Mean arterial plasma concentration-time profiles of phenytoin (A) and its metabolite, *p*HPPH (B), after 1-min intravenous infusion of phenytoin, 25 mg/kg, to control rats (○, $n = 7$) and rats with PCM (△, $n = 6$) and PCMC (□, $n = 7$). Vertical bars represent S.D. (a) PCMC group was significantly different ($P < 0.05$) from control group. (b) PCM group was significantly different ($P < 0.05$) from control groups. (c) PCM group was significantly different ($P < 0.05$) from control and PCMC groups. (d) PCM group was significantly different ($P < 0.05$) from PCMC group. (e) PCMC group was significantly different ($P < 0.05$) from control and PCM groups. (f) Each group (control, PCM and PCMC) was significantly different ($P < 0.05$).

Table 2
Pharmacokinetic parameters of phenytoin and its metabolites, *p*HPPH, after 1-min intravenous infusion of phenytoin, 25 mg/kg, to control rats and rats with PCM and PCMC

| | Control ($n=7$) | PCM ($n=6$) | PCMC ($n=7$) |
|---|---|---|---|
| *Phenytoin* | | | |
| $V_{max}$ (μg/min) | $0.334 \pm 0.101$[c] | $0.203 \pm 0.122$ | $0.196 \pm 0.060$ |
| $K_m$ (μg/ml) | $2.30 \pm 0.96$ | $1.32 \pm 0.55$ | $5.45 \pm 3.11$[d] |
| $CL_{int}$ (ml/min) | $0.175 \pm 0.050$ | $0.131 \pm 0.048$ | $0.044 \pm 0.019$[d] |
| $V_1$ (ml/kg) | $44.4 \pm 11.8$[c] | $65.4 \pm 21.8$ | $72.2 \pm 11.8$ |
| $k_{12}$ ( per min) | $0.871 \pm 0.101$[c] | $0.721 \pm 0.082$ | $0.744 \pm 0.047$ |
| $k_{21}$ (per min) | $0.045 \pm 0.017$ | $0.033 \pm 0.016$ | $0.042 \pm 0.012$ |
| $Ae_{Phe,0-24\ h}$[a] (% of dose)[b] | $0.067 \pm 0.093$[c] | $0.833 \pm 0.558$ | $0.982 \pm 0.803$ |
| Plasma protein binding (%) | $68.6 \pm 3.70$[c] | $55.0 \pm 10.1$ | $53.4 \pm 10.2$ |
| *pHPPH* | | | |
| AUC (μg min per ml) | $609 \pm 176$ | $714 \pm 259$ | $1210 \pm 515$[d] |
| Terminal half-life (min) | $83.8 \pm 49.1$ | $132 \pm 93.5$ | $232 \pm 42.4$[e] |
| $CL_R$ (ml/min per kg) | $20.5 \pm 7.70$ | $13.4 \pm 4.98$ | $4.67 \pm 4.40$[d] |
| $Ae_{pHPPH,\ 0-24\ h}$ (% of dose)[b] | $54.7 \pm 25.6$[c] | $35.6 \pm 14.2$ | $32.5 \pm 20.7$ |

Each value represents the mean ± S.D.
[a] Total amount excreted in 24-h.
[b] Expressed in terms of phenytoin.
[c] Control group was significantly different ($P<0.05$) from PCM and PCMC groups.
[d] PCMC group was significantly different ($P<0.05$) from control and PCM groups.
[e] PCMC group was significantly different ($P<0.05$) from control group.

information criteria (AIC) values ranging from $-22.3$ to $+70.3$.

In rats with PCM, the $V_1$ of phenytoin was significantly larger (42.3% increase) than that in control rats and this could be due to increased unbound fraction of phenytoin in plasma (43.3% increase) in the rats (Table 2). The $V_{max}$ of phenytoin was significantly slower (39.2% decrease) and the amount of unchanged phenytoin excreted in 24-h urine ($Ae_{Phe,\ 0-24\ h}$) was significantly greater (1140% increase) in rats with PCM than those in control rats (Table 2). However, the $K_m$ and $CL_{int}$ of phenytoin were not significantly different between control rats and rats with PCM (Table 2).

In rats with PCMC, the $V_{max}$ and $V_1$ of phenytoin and $Ae_{Phe,\ 0-24\ h}$ were not significantly different compared with those in rats with PCM, however, they were significantly slower (41.3% decrease), larger (62.6% increase) and greater (1370% increase), respectively, than those in control rats (Table 2). In rats with PCMC, the $K_m$ of phenytoin was significantly higher than those in control rats and rats with PCM (139 and 313% increase, respectively), and the $CL_{int}$ of phenytoin was significantly slower than those in control rats and rats with PCM (74.9 and 66.4% decrease, respectively) (Table 2).

The formation of *p*HPPH was fast; the *p*HPPH was detected in plasma from the first blood sampling time (1 min) and reach its peak within 60 min for all three groups of rats (Fig. 1(B)). In rats with PCM, the percentages of intravenous dose of phenytoin excreted in 24-h urine as *p*HPPH ($Ae_{pHPPH,\ 0-24\ h}$) was significantly smaller (34.9% decrease) than that in control rats, however, the AUC, terminal half-life and $CL_R$ of *p*HPPH were not significantly different between two groups of rats (Table 2). In rats with PCMC, the AUC of *p*HPPH was significantly greater than those in control rats (98.7% increase) and rats with PCM (69.5% increase), and the $CL_R$ of *p*HPPH was significantly slower than those in control rats (65.1% decrease) and rats with PCM (77.2% decrease) (Table 2). In rats with PCMC, the terminal half-life of *p*HPPH and $Ae_{pHPPH,\ 0-24\ h}$ were significantly longer (177% increase) and smaller (40.6% decrease), respectively, than those in control rats, however, they were not significantly different compared with those in rats with PCM (Table 2).

### 3.3. Tissue distribution study

The tissue distribution of phenytoin and *p*HPPH 30 min after intravenous administration of phenytoin, 25 mg/kg, to control rats, and rats with PCM and PCMC ($n=4$, each) are shown in Figs. 2 and 3, respectively. In control rats, rat tissues studied had a poor affinity for phenytoin; the tissue-to-plasma ($T/P$) ratios were less-than-unity in all the tissues studied (Fig. 2) and this could be supported by considerably small value of

$V_1$, 44 ml/kg (Table 2). In rats with PCM, the $T/P$ ratios of phenytoin in kidney, stomach, large intestine and brain were significantly greater than those in control rats (Fig. 2) and this could be due to an increase in unbound fraction of phenytoin in plasma of rats with PCM as mentioned earlier. Cysteine supplementation (rats with PCMC) had no effect on tissue distribution of phenytoin in rats with PCM; the $T/P$ ratios of phenytoin were not significantly different between rats with PCM and PCMC in all rat tissues studied (Fig. 2).

In control rats, *p*HPPH had a good affinity to rat tissues studied; the $T/P$ ratios of *p*HPPH were greater-than-unity in liver, kidney, small intestine, heart and stomach (Fig. 3). In rats with PCM, the $T/P$ ratios of *p*HPPH in liver, small intestine and stomach were significantly smaller and that in kidney were significantly greater than those in control rats (Fig. 3). Cysteine supplementation (rats with PCMC) had no effect on tissue distribution of *p*HPPH in rats with PCM; the $T/P$ ratios of *p*HPPH were not significantly different between rats with PCM and PCMC in all rat tissues studied except in muscle (Fig. 3).

### 3.4. Phenytoin hydroxylation activity in rat liver microsome

Formation of *p*HPPH from phenytoin in rat liver microsome increased linearly with increased incubation time and was dependent on the concentrations of microsomal protein for up to 5 mg/ml in the presence of NADPH generating system. The phenytoin hydroxylation activities in control rats and rats with PCM and PCMC ($n = 4$, each) are listed in Table 3. In rats with PCM and PCMC, the microsomal protein contents (34.8 and 31.1% decrease, respectively), cytochrome P450 contents (32.5 and 37.7% decrease, respectively) and phenytoin hydroxylation activi-

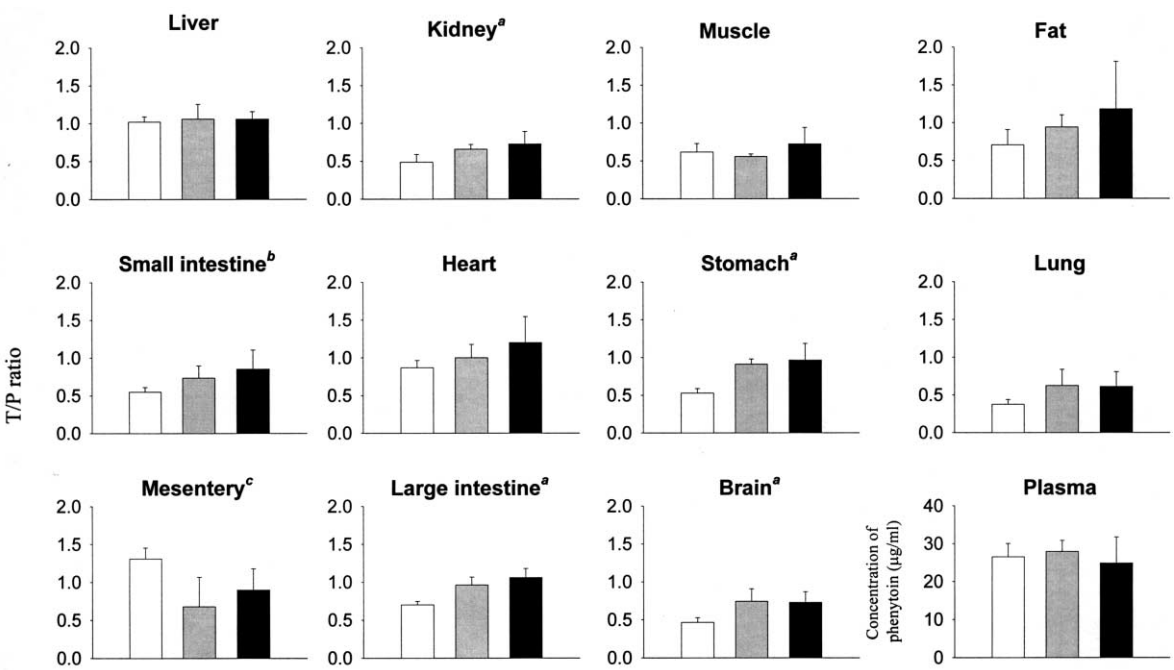


Fig. 2. Mean tissue-to-plasma ($T/P$) ratios and plasma concentrations (μg/ml) of phenytoin (last figure) 30 min after 1-min intravenous infusion of phenytoin, 25 mg/kg, to control rats (white, $n = 5$) and rats with PCM (gray, $n = 5$) and PCMC (black, $n = 5$). Vertical bars represent S.D. (a) Control group was significantly different ($P < 0.05$) from PCM and PCMC groups. (b) Control group was significantly different ($P < 0.05$) from PCMC group. (c) Control group was significantly different ($P < 0.05$) from PCM group.

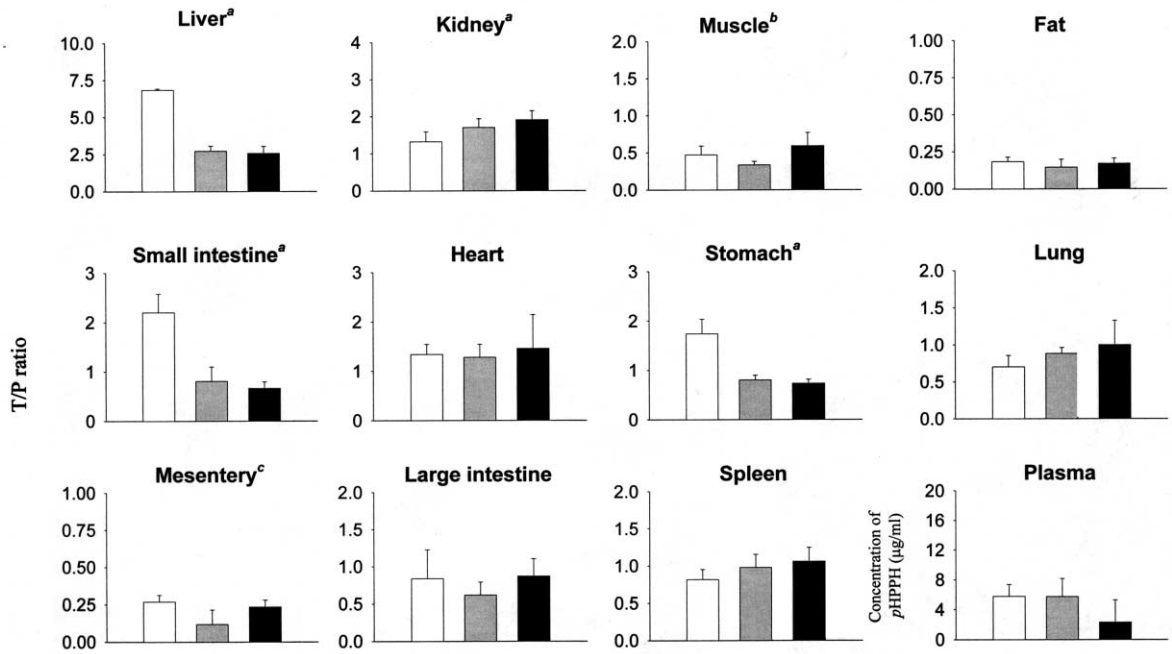


Fig. 3. Mean tissue-to-plasma ($T/P$) ratio and plasma concentrations (μg/ml) of *p*HPPH (last figure) 30 min after 1-min intravenous infusion of phenytoin, 25 mg/kg, to control rats (white, $n = 5$) and rats with PCM (gray, $n = 5$) and PCMC (black, $n = 5$). Vertical bars represent S.D. (a) Control group was significantly different ($P < 0.05$) from PCM and PCMC groups. (b) PCM group was significantly different ($P < 0.05$) from PCMC group. (c) Control group was significantly different ($P < 0.05$) from PCM group.

Table 3
Hepatic microsomal protein and cytochrome P450 and phenytoin hydroxylation activity in control rats and rats with PCM and PCMC

| | Control ($n = 4$) | PCM ($n = 4$) | PCMC ($n = 4$) |
|---|---|---|---|
| Protein (mg/g liver) | $14.0 \pm 0.717$[a] | $8.62 \pm 0.183$ | $9.64 \pm 1.35$ |
| Cytochrome P450 (nmol/mg protein) | $1.02 \pm 0.0270$[a] | $0.689 \pm 0.0527$ | $0.635 \pm 0.121$ |
| Phenytoin hydroxylation activity (pmol/min per mg protein) | $164 \pm 21.8$[a] | $103 \pm 29.9$ | $95.3 \pm 36.8$ |

Each value represents the mean ± S.D.
[a] Control group was significantly different ($P < 0.05$) from PCM and PCMC groups.

ties (37.2 and 41.9% decrease, respectively) decreased significantly than those in control rats.

## 4. Discussion

It was reported that the rate of phenytoin metabolism (Billings, 1984) and in vitro liver microsomal covalent binding of [$^{14}$C]-phenytoin (Roy and Snodgrass, 1988) increased in rats pretreated with phenobarbital [an inducer of CYP2B1 (Guengerich, 1990) and CYP2B1/2 (Kawamura et al., 1999) in rats], and the CYP2B1/2 expression was not significantly affected in rats with PCM (Cho et al., 1999). Therefore, it was expected that the pharmacokinetic parameters of phenytoin and *p*HPPH between control rats and rats with PCM could not be significantly different. However, some pharmacokinetic parameters of phenytoin and *p*HPPH

were significantly different between two groups of rats (Table 2) mainly due to significantly lower phenytoin hydroxylation activity in rats with PCM than that in control rats (Table 3). Hence, it could be expected that the formation of *p*HPPH from phenytoin could be smaller in rats with PCM. This could be supported by 24-h urinary excretion of *p*HPPH; the $Ae_{pHPPH,\ 0-24\ h}$ was significantly smaller in rats with PCM than those in control rats (Table 2). In rats with PCMC, the $CL_{int}$ of phenytoin was significantly smaller than those in control rats and rats with PCM (Table 2). Therefore, it could be expected that the formation of *p*HPPH could be the smallest in rats with PCMC among three groups of rats. This could be proved by the significantly smaller percentages of intravenous dose of phenytoin excreted in 24-h urine as *p*HPPH than those in control rats and rats with PCM (Table 2). Note that in rats with PCMC, the AUC of *p*HPPH was significantly greater than those in control rats and rats with PCM, and this could be due to significantly longer terminal half-life (could be due to significantly slower CL of *p*HPPH) and significantly slower $CL_R$ of *p*HPPH (Table 2). It was reported that some pharmacokinetic parameters of azosemide (Cho et al., 1999; Kim et al., 2001) and adriamycin (Kim et al., in press) in rats with PCM were partially or completely returned to control levels by cysteine supplementation (rats with PCMC). However, in the present study, all the pharmacokinetic parameters of both phenytoin and *p*HPPH in rat with PCM were not restored to control levels by cysteine supplementation. It was mainly due to the fact that phenytoin hydroxylation activity and $CL_{int}$ of phenytoin were not restored to control levels in rats with PCM by cysteine supplementation. The above data suggested that the phenytoin hydroxylation activities were not directly related to CYP2B1 in rats. It was reported (Correia, 1995) that phenobarbital is also an inducer of CYP2C6, 2C7 and 3A1/2. It was also reported (Bachmann et al., 1988) that phenytoin may not effectively discriminate between inductive or inhibitory effects of xenobiotics when used as a single sample probe of hepatic drug-metabolizing activity in rats.

## Acknowledgements

This study was supported in part by Korea Science and Engineering Foundation, 2000-2-21700-002-5.

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