PHARMACOKINETIC STUDY OF CARBAMAZEPINE AND ITS EPOXIDE METABOLITE IN HUMANS

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A major metabolite of carbamazepine (CBZ), CBZ-10, 11-epoxide (EPO), has been reported to possess anticonvulsant properties. Therefore, the present study was undertaken in order to develop a pharmacokinetic model to predict the behavior of EPO in the body after administration of CBZ. The serum concentration-time curves after oral administration of solution of CBZ (200 mg) or EPO (150 mg) in six healthy subjects showed the characteristic “nose”, suggesting that disposition of CBZ or EPO could be described by the two-compartment model. The kinetic parameters of disposition for CBZ and EPO were calculated by the method of Wagner, assuming the absolute bioavailabilities of CBZ and EPO to be 1.0 and 0.81, respectively. Total body clearance and elimination rate constant of EPO were very much larger than those of the parent drug but there was no statistically significant difference in the distribution volume between CBZ and EPO. The formation rate of EPO was calculated by a deconvolution method, and obeyed Michaelis-Menten kinetics.

Based on these findings, a pharmacokinetic model of the fate of CBZ and EPO in humans was developed and the time courses of CBZ and EPO in serum after oral administration of three tablet preparations and a solution containing 200 mg of CBZ were simultaneously fitted to this model by solving the differential equations by the Runge-Kutta-Gill method. There was good agreement between calculated and observed serum values, suggesting that the present model is appropriate to describe the formation and disposition of EPO from CBZ. The formation rate constant of EPO ($V_{\max}/K_m/V_1$) was approximately one-fifteenth of the elimination rate constant of EPO. This suggested a flip-flop model in which the formation of EPO was rate-limiting in humans. The observation that the serum concentrations of EPO after administration of CBZ were one-tenth to one-twentieth of those of the parent drug was well explained by the flip-flop kinetics of EPO, together with the large differences in total body clearance and elimination rate constant between CBZ and EPO.

Keywords — carbamazepine; carbamazepine-10, 11-epoxide; pharmacokinetic model; Michaelis-Menten kinetics; flip-flop model; deconvolution; human; bioavailability; two-compartment model

INTRODUCTION

When a metabolite of a drug has pharmacological activity, knowledge of the pharmacokinetics of the metabolite is important to insure the efficiency and safety of the drug. Carbamazepine (CBZ) is an effective drug for the treatment of patients with grand mal and psychomotor epilepsy¹⁾ and it is also the drug of choice for the treatment of trigeminal neuralgia.²⁻⁴⁾ A major biotransformation pathway of CBZ is epoxidation of the double bond at the 10, 11 position in the molecule, followed by hydration of the epoxide.^{5,6)} An intermediate metabolite of this pathway, carbamazepine-10, 11-epoxide (EPO), is the major metabolite in blood and has been detected in both cerebrospinal fluid (CSF)^{7,8)}

and brain tissue^{9,10)} of epileptic patients on CBZ therapy. In animal experiments, EPO has been found to possess anticonvulsant properties as well as the parent drug.^{11,12)} However, investigation of the disposition of EPO in humans has been limited to the report of Tomson *et al.*¹³⁾ Furthermore, there has been no *in vivo* study of the formation kinetics of EPO following CBZ administration in humans.

The present investigation was undertaken to determine the rate and extent of CBZ biotransformation to EPO and to develop a pharmacokinetic model of the fate of CBZ and EPO following CBZ administration in humans.

MATERIALS AND METHODS

Materials — CBZ was donated by Ciba-Geigy Japan Ltd. Since CBZ possesses a low aqueous solubility over a relatively wide pH range, a solution was prepared in a mixture of 50% propylene glycol, 20% ethanol and 30% water at a concentration of 10 mg/ml by the method of Levy *et al.*¹⁴⁾ A mixture of propylene glycol and water without ethanol did not maintain the CBZ in solution at room temperature. Three uncoated tablet preparation, Telesmin (Yoshitomi Pharmaceutical Co., Ltd., Lot No. Y016), Lexin (Fujinaga Pharmaceutical Co., Ltd., Lot No. BJ 291) and Tegretol (Ciba-Geigy Japan Ltd., Lot No. 00100), each containing 200 mg of CBZ, were purchased from commercial sources in Japan. The three tablet preparations showed different dissolution rates and absorption rates from each other, as reported previously.¹⁵⁾ EPO was synthesized chemically from CBZ according to the method of Frigerio *et al.*^{6,16)} and an EPO solution was prepared at a concentration of 7.5 mg/ml in propylene glycol. An antacid, Maalox (Yamanouchi Pharmaceutical Co., Ltd.), was also used; 10 ml of the solution contained 5.6 g of dried aluminum hydroxide gel and 0.4 g of magnesium hydroxide. All other chemicals and solvents used in this study were of analytical grade.

Drug Administration — The data for serum concentrations of CBZ and EPO, after oral administration of the three tablet preparations and a solution containing 200 mg of CBZ to six of the subjects, were used from our previous report¹⁵⁾ to develop a pharmacokinetic model on the fate of CBZ and EPO. After an interval of 1 month, the same six subjects (four males and two females, listed in Table II) received EPO solution (150 mg/20 ml) orally with 80 ml of water at 9:00 a.m. after fasting overnight. No food or liquids were allowed until 3 h after drug administration. Blood samples were taken at 7.5, 15, 30, and 45 min, and at 1, 1.5, 2, 4, 7, 12, 24 and 32 h after administration. The serum samples were kept frozen at -20°C until assayed. This procedure was repeated with two of the same subjects at intervals of 1 month, the only difference being the addition of an antacid. Ten milliliters of the antacid were given immediately before and 15 and 30 min after ingestion of EPO.

Assay Method — The EPO concentration in serum was determined by the high-performance

liquid chromatography (HPLC) method reported previously.¹⁵⁾ The detection limit of EPO was 0.05 $\mu\text{g/ml}$.

Data Analysis — The area under the drug serum concentration-time curve (*AUC*) was calculated by the linear trapezoidal method and was extrapolated to infinity by dividing the last serum concentration by the slope (λ_2) of the terminal log-linear phase, since the disposition of both CBZ and EPO could be described by a two-compartment model. The half-life of the terminal phase ($t_{1/2(\lambda_2)}$) was calculated by the usual method. The maximum serum concentration (C_{max}) and the time of its occurrence (T_{max}) were obtained from the measured values. The serum concentration data of CBZ and EPO were analyzed by using a nonlinear regression method, with the weight function based on the reciprocal value of the square of the standard error for each datum. The computer program used was SALS¹⁷⁾ (statistical analysis with least-squares fitting, University of Tokyo) for nonlinear regression analysis and a deconvolution program¹⁸⁾ in which the anterior phenomenon was approximated by an appropriate exponential polynomial and the deconvolution operation was done from the posterior phenomenon by the point-area method. For data analysis, the Student's *t*-test for paired data was used. A *p*-value of 0.05 was considered significant.

RESULTS

Influence of Antacid on the Bioavailability of EPO

Because EPO has been found to decompose in gastric juice *in vitro*,¹³⁾ the effect of a magnesium-aluminum hydroxide antacid on the gastrointestinal absorption of EPO solution was determined in two subjects (Table I). When the EPO solution was given alone, the C_{max} and *AUC* values were smaller than those for EPO solution given with the antacid. The relative bioavailability values of EPO solution alone compared to the EPO solution plus antacid were 0.816 and 0.808 in subjects 1 and 2, respectively. However, there was no change in the elimination rate constant in the two subjects. These results suggested that EPO was decomposed in the gastrointestinal tract *in vivo* as well as *in vitro*¹³⁾ and that this decomposition was suppressed by the antacid. There was no difference between EPO alone and EPO plus antacid for T_{max} of subject 1, but the T_{max} of subject 2 was delayed by con-

TABLE I. *Bioavailability Parameters Following Oral Administration of EPO (150 mg) Solution without (I) or with (II) 30 ml of an Antacid*

Subject No.	$C_{\max}$ ($\mu\text{g/ml}$)	$T_{\max}$ (h)	AUC ($\mu\text{g} \cdot \text{ml}^{-1} \cdot \text{h}$)
1 (I)	2.38	0.75	22.66
(II)	2.85	0.75	27.76
2 (I)	2.17	0.75	20.38
(II)	2.45	2.00	25.22

comitantly administered antacid and the serum concentration-time curve of subject 2 had two peaks at 0.75 and 2.0 h after oral administration of the EPO solution with antacid. Therefore, the decomposition of EPO in the gastric juice was assumed to be 19% of the dose. Consequently, 81% of the dose was considered to be the available fraction for absorption of EPO solution, since absorption of EPO from the rat duodenum appears to be complete (to be published elsewhere).

Disposition Kinetics of CBZ and EPO

Figure 1 shows the mean serum concentration-time curves obtained following oral administration of CBZ (200 mg) solution or EPO (150 mg) solution in six subjects. CBZ and EPO were each absorbed very rapidly from solution; the

FIG. 1. *Time Course of the Serum Concentration Following Oral Administration of CBZ (200 mg) Solution or EPO (150 mg) Solution in Six Subjects* ○, CBZ; ●, EPO. Solid lines is computer-fitted to the mean serum levels of CBZ or EPO. Each point represents the mean $\pm$ S.D.

peak time of CBZ or EPO was within 20–60 min (mean 48.3 min) or within 15–60 min (mean 40.0 min). These time courses showed the characteristic nose,¹⁹⁾ suggesting that disposition of CBZ or EPO can be described by the two-compartment model. This nose for CBZ or EPO was observed in all subjects. Therefore, a two-compartment model was adopted to describe the disposition of CBZ or EPO in humans and the pharmacokinetic parameters of CBZ and EPO based on the two-compartment model were estimated by the method of Wagner,²⁰⁾ assuming the absolute bioavailabilities of CBZ and EPO to be 1.0 and 0.81, respectively (Table II). The calculated pharmacokinetic parameters obtained from curve fitting to the mean serum concentrations of CBZ or EPO by using the initial values estimated by the Wagner method²⁰⁾ are also listed in Table II.

The epoxidation of the double bond at positions 10 and 11 in CBZ results in a marked increase in total body clearance (CL), the slope of the terminal log-linear phase (λ_2) and the elimination rate constant (k_{10}), a slight decrease in the distribution volume of the central compartment (V_1) and a marked decrease in the half-life of the terminal phase ($t_{1/2(\lambda_2)}$). There were statistically significant differences in CL and k_{10} between CBZ and EPO but there was no statistically significant difference in V_1 or V_{ss} between CBZ and EPO.

Four subjects among the six who were given EPO solution developed symptoms of drowsiness, malaise and slight dizziness over a period from 2–3 h to about 10 h after oral administration of drug. These symptoms were similar to the adverse reaction to CBZ,²¹⁾ indicating that EPO, as well as the parent drug, is potent in humans.

Formation Kinetics of EPO

TABLE II. Pharmacokinetic Parameters^{a)} Estimated from Serum Concentrations of CBZ and EPO Following Oral Administration

Subject No.	Sex	Age (yr)	Weight (kg)		C_1 ($\mu\text{g/ml}$)	λ_1 (h^{-1})	C_2 ($\mu\text{g/ml}$)	λ_2 (h^{-1})	V_1 (l/kg)	k_{12} (h^{-1})
1	M	26	78	CBZ	0.725	0.757	3.610	0.0173	0.592	0.120
				EPO	0.831	0.559	1.947	0.0903	0.561	0.110
2	F	32	53	CBZ	1.195	0.520	3.982	0.0181	0.729	0.111
				EPO	0.554	1.156	2.373	0.1135	0.783	0.174
3	M	23	59	CBZ	0.537	1.956	4.166	0.0153	0.721	0.220
				EPO	1.314	3.024	2.000	0.1039	0.622	1.093
4	F	27	44	CBZ	0.296	2.373	4.639	0.0186	0.921	0.140
				EPO	1.721	2.501	2.664	0.1106	0.630	0.872
5	M	23	55	CBZ	1.658	0.599	4.308	0.0154	0.610	0.157
				EPO	0.905	1.843	1.335	0.1135	0.986	0.629
6	M	23	48	CBZ	0.295	0.900	4.279	0.0204	0.911	0.045
				EPO	1.972	1.710	3.104	0.1198	0.499	0.550
Mean (S.D.)		25.7 (3.5)	56.2 (11.9)	CBZ	0.784 (0.543)	1.184 (0.782)	4.164 (0.347)	0.0175 (0.0020)	0.747 (0.142)	0.132 (0.057)
				EPO	1.216 (0.551)	1.799 (0.890)	2.237 (0.617)	0.1086 (0.0010)	0.680 (0.177)	0.571 (0.384)
				CBZ ^{e)}	1.342	1.037	4.246	0.0185	0.677	0.194
				EPO ^{e)}	1.479	1.118	2.191	0.1094	0.717	0.263

Subject No.	Sex	Age (yr)	Weight (kg)		k_{21} (h^{-1})	k_{10} (h^{-1})	CL ^{b)} (ml/h/kg)	V_{ss} ^{c)} (l/kg)	$t_{1/2}(\lambda_2)$ ^{d)} (h)
1	M	26	78	CBZ	0.633	0.0207	12.23	0.704	40.06
				EPO	0.419	0.1205	68.75	0.708	7.67
2	F	32	53	CBZ	0.404	0.0233	16.95	0.929	38.29
				EPO	0.959	0.1369	112.47	0.925	6.11
3	M	23	59	CBZ	1.735	0.0173	13.00	0.812	45.29
				EPO	1.867	0.1683	105.19	0.986	6.67
4	F	27	44	CBZ	2.232	0.0198	17.69	0.979	37.26
				EPO	1.563	0.1770	116.06	0.981	6.27
5	M	23	55	CBZ	0.437	0.0211	12.85	0.828	45.00
				EPO	1.144	0.1828	178.67	1.529	6.11
6	M	23	48	CBZ	0.853	0.0215	19.83	0.959	33.97
				EPO	1.092	0.1875	94.22	0.750	5.78
Mean (S.D.)		25.7 (3.5)	56.2 (11.9)	CBZ	1.049 (0.758)	0.0206 (0.0020)	15.43 (3.15)	0.869 (0.106)	39.98 (4.47)
				EPO	1.174 (0.501)	0.1622 (0.0272)	112.56 (36.59)	0.980 (0.294)	6.44 (0.67)
				CBZ ^{e)}	0.838	0.0229		0.834	
				EPO ^{e)}	0.815	0.1501		0.948	

a) Parameters estimated following oral administration of CBZ solution at a dose of 200 mg and EPO solution at a dose of 150 mg to 6 subjects.

Parameters for the model after intravenous administration

b) $CL = FD/AUC$, with the F values of CBZ and EPO assumed to be 1.0 and 0.81, respectively.

c) $V_{ss} = (1 + k_{12}/k_{21})V_1$

d) $t_{1/2(\lambda_2)} = 0.693/\lambda_2$

e) Parameters obtained by curve fitting to the mean serum levels of CBZ or EPO after oral administration, with the first-order absorption rate constants for CBZ and EPO of 4.40 and 2.89 h^{-1} , respectively.

FIG. 2. Calculated Serum Concentration-Time Curves (Solid Lines) and Observed Concentrations of CBZ (○) and EPO (●) after Oral Administration of CBZ Preparations

Solid lines are calculated values based on Table III and dotted lines are calculated values by assuming that the epoxidation of CBZ is first-order. See text for details. Each point represents the mean $\pm$ S.D.

The mean serum concentration-time courses of CBZ and EPO observed following oral administration of the three tablet preparations (A, B, C) and a solution containing 200 mg of CBZ in six subjects are shown in Fig. 2. EPO levels in the serum after administration of CBZ were very low, being one-tenth to one-twentieth of the serum CBZ levels. The time to reach peak concentration (T_{max}) of CBZ was 1–4 h after oral administration of the three tablets and the solution of CBZ, while T_{max} of EPO was observed within 24 h after administration of CBZ in all cases.

The disposition of EPO in humans was suggested to be apparently linear,¹³⁾ and it was assumed that the absolute bioavailability of EPO from propylene glycol solution was 0.81. Based on this assumption, the following equation is applicable for calculation of the formation rate of EPO:

$$B(t) = \int_0^t G(\theta) A(t-\theta) d\theta \quad (1)$$

where $A(t)$ is the serum EPO level after an intravenous injection of EPO, estimated by using

the kinetic parameters which were calculated from the serum levels after oral administration of EPO solution, $B(t)$ is the serum level of EPO

FIG. 3. Time Courses of the Weight Function Values, $G(t)$, Calculated from the Deconvolution between the Serum Levels of Each EPO after Intravenous Injection of EPO and Oral Administration of Preparations

○ — ○, solution; ● — — — ●, tablet A; ● — — — ●, tablet B; X — — — X, tablet C.

FIG. 4. Lineweaver-Burk Plot for Epoxidation of CBZ in Humans

○, solution; ●, tablet A; ◐, tablet B; X, tablet C.

See text details.

at time t after oral administration of the three tablet preparations and the solution of CBZ and $G(t)$ is the weight function, *i.e.*, the formation rate of EPO. $G(t)$ values were calculated from the deconvolution between $A(t)$ values and $B(t)$ values. Figure 3 shows the time courses of calculated $G(t)$ values after oral administration of the three tablet preparations and the solution of CBZ. Because the $G(t)$ values were calculated from serum EPO levels at time t after oral administration of CBZ, there is a possibility that the $G(t)$ values immediately after administration reflect the absorption rate of CBZ. In the previous report,¹⁵⁾ the three tablet preparations (A, B, C) and the solution of CBZ were mostly absorbed during the 4 h after administration. Therefore, based on Michaelis-Menten kinetics for EPO formation, a Lineweaver-Burk plot was prepared between the product of $G(t)$ value after 4 h and unit impulse dose of EPO, and the midpoint concentration of CBZ obtained by extrapolation from each sampling point (Fig. 4). The values of maximum velocity of epoxidation ($V_{\max}$) and Michaelis constant (K_m) were 0.156 mg/h/kg and 18.57 $\mu\text{g/ml}$, respectively.

Pharmacokinetic Model of the Fate of CBZ and Its Epoxide Metabolite

A pharmacokinetic model of the fate of CBZ

FIG. 5. Pharmacokinetic Model of the Fate of CBZ and EPO in Humans

k , rate constant; $V_{\max}$, maximum velocity of epoxidation; k_m , Michaelis constant of epoxidation. Concentration C_1 , CBZ in GI tract; C_2 , CBZ in central compartment; C_3 , CBZ in peripheral compartment; C_4 , EPO in central compartment; C_5 , EPO in peripheral compartment. Amount of drug A_1 , CBZ in GI tract; A_3 , CBZ in peripheral compartment; A_5 , EPO in peripheral compartment. V_2 and V_4 are distribution volumes of CBZ and EPO, respectively, in the central compartment.

and EPO in humans based on these findings is given in Fig. 5 and five simultaneous differential equations based on this model were set up. The time courses of CBZ and EPO in serum after oral administration of the three tablet preparations and the solution were simultaneously fitted to this model by solving the differential equations by the Runge-Kutta-Gill method. The initial values for K_m and $V_{\max}$ values were obtained from the Lineweaver-Burk plot, those for disposition parameters were taken from Table II and those for absorption rate constants were taken from our previous report.¹⁵⁾ Table III summarizes the pharmacokinetic parameters obtained from the curve fitting. Figure 2 shows the calculated and observed serum concentration curves for CBZ and EPO after oral administration of the solution and the three tablet preparations (A, B, C). The good agreement between calculated and observed values suggests that the present model is appropriate to describe the formation of EPO from CBZ and its disposition. In addition, bioavailability of three tablet preparations used was set to 1.0 because relative bioavailability to a solution for these preparations was near unity.¹⁵⁾

TABLE III. Kinetic Parameters of CBZ and EPO Obtained by Simultaneous Curve-Fitting with the Pharmacokinetic Model in Fig. 5

$k_{a(S)} = 5.203$	$k_{23} = 0.183$	$V_{\max}^{a)} = 0.176$	$V_2 = 0.713$
$k_{a(A)} = 1.142$	$k_{32} = 0.862$	$K_m^{b)} = 15.691$	$V_4 = 0.580$
$k_{a(B)} = 0.584$	$k_{45} = 0.726$	$k_{e2} = 0.0078$	
$k_{a(C)} = 0.457$	$k_{54} = 1.690$	$k_{e4} = 0.225$	

k , rate constant (h^{-1}); k_a (S), k_a (A), k_a (B), and k_a (C) are the absorption rate constant for a solution, tablet A, tablet B, and tablet C, respectively. V_2 , and V_4 are the distribution volumes (l/kg) of the central compartment for CBZ and EPO, respectively. a) The maximum velocity of epoxidation (mg/h/kg). b) Michaelis constant of epoxidation ($\mu g/ml$).

DISCUSSION

Although most single dose studies of CBZ have been performed on volunteers using commercially available tablets, only a few trials have been done with solutions and suspensions.^{22,23)} Under these conditions the serum concentration-time curves showed monoexponential decay and the disposition of CBZ could be described by a one-compartment model. Further, it was suggested that there was no dose-independent kinetics within the dose range from 50 to 600 mg.²⁴⁾ In the present study, blood samples were frequently drawn during the initial stages after oral administration of the solution of CBZ or EPO to six subjects and the serum concentration time curves of CBZ and EPO each showed the characteristic "nose". This may be interpreted as a suggestion that the disposition of the drugs follows the two-compartment model.¹⁹⁾

Because of the lack of a preparation for intravenous injection of CBZ, the absolute bioavailability of CBZ in humans has not been determined.²³⁾ We assumed the absolute bioavailability of CBZ solution to be 1.0, and the pharmacokinetic parameters of CBZ were estimated from the serum concentration-time courses after oral administration of a solution. The values of apparent volume of distribution based on a one-compartment model and the total body clearance obtained after single oral doses of commercially available tablets of CBZ to human subjects ranged from 0.88 to 1.86 (1.46 ± 0.10 ,²⁵⁾ 0.99 ± 0.16 ,²⁶⁾ 1.20 ± 0.45 ,²⁷⁾ 0.91 ± 0.17 ,²⁸⁾ mean $\pm$ S.D.) l/kg and from 13.3 to 69.8 (29.7 ± 8.0 ,²⁵⁾ 18.5 ± 1.5 ,²⁶⁾ 30.4 ± 25.6 ,²⁷⁾ 22.2 ± 6.3 ²⁸⁾) ml/h/kg, respectively. As shown in Table II, the total body clearances (CL) after oral administration of CBZ solution ranged

from 12.23 to 19.83 (mean 15.43) ml/h/kg. This range of values of CL is slightly lower than those reported previously, as described above. This difference of CL between the present and previously reported values may be explained by the fact that in the present study, blood samples were frequently drawn during the initial stages. Further, it is expected to be completely bioavailable since a solution was given in the present study. Therefore the present value of CL may be considered to be close to a real value. The distribution volume ($V_{d(\lambda_2)}$) extrapolated from the λ_2 phase of CBZ (dose/ C_2 /body weight, 0.878 ± 0.108 l/kg) is also slightly lower. These present values of $V_{d(\lambda_2)}$ and CL are reasonably similar to the values after oral administration of solution, as reported by Cotter *et al.* (0.83 ± 0.10 l/kg for V_d , 16.3 ± 6.1 ml/h/kg for CL).²⁸⁾ The half-life ($t_{1/2(\lambda_2)}$) of the terminal log-linear phase for CBZ obtained in the present study ranged from 33.97 to 45.00 (mean 39.98) h and this range of values are in good agreement with those reported by others.²⁵⁻²⁸⁾ Therefore, the present values estimated from serum levels after oral administration of solution are considered to be appropriate as disposition parameters of CBZ.

EPO has been found to decompose in the gastric juice *in vitro*. However, there was no indication of decomposition of EPO in the juice of the small intestine or water.¹³⁾ Administration of 10 ml of the antacid Maalox raised the intragastric pH to above 5 for at least 15 min.²⁹⁾ By giving this dose three times, the intragastric pH was expected to be kept sufficiently high during the passage of the EPO solution through the stomach. Thus, when EPO solution and antacid are given simultaneously, it is considered that EPO is absorbed almost completely. This complete absorption was confirmed by the experiments of

Tomson *et al.*,¹³⁾ who observed that $90 \pm 11\%$ (mean $\pm$ S.D.) of the end metabolite (*trans*-10, 11-dihydroxy-10, 11-dihydro-CBZ) was recovered in urine after administration of EPO with antacid. Therefore, the present value of 0.81 for absolute bioavailability is considered to be reasonable for oral administration of the solution alone. An antacid delays gastric emptying, which alters the rate of drug absorption.³⁰⁾ Therefore, the pharmacokinetic parameters of EPO were determined from the serum concentration curves after oral administration of the solution alone, since coadministration of EPO solution with antacid was expected to cause difficulty in the accurate determination of the distribution phase of EPO. The half-life of the terminal phase $t_{1/2(\lambda_2)}$ (6.44 ± 0.67 h, mean $\pm$ S.D.) of EPO based on the two-compartment model agreed with the half-life (6.10 ± 0.88 h) based on the one-compartment model reported by Tomson *et al.*¹³⁾ who gave EPO with the antacid. However, the present value of total body clearance CL (112.56 ± 36.59 ml/h/kg) is larger than the total body clearance (86.4 ± 24.9 ml/h/kg) reported previously.¹³⁾ This difference in CL between the present and previously reported values might be due to the difference in blood sampling schedules and numbers as discussed in CBZ or to the assumed value of absolute bioavailability. However, as mentioned above, the present value of absolute bioavailability appears to be reasonable. Therefore, this may be a small factor to be considered in calculating total body clearance.

The contribution of the distribution volume in the peripheral compartment to the steady-state volume of distribution (V_{ss}) is 14% for CBZ, since the difference in distribution volume between V_1 and V_{ss} is minimal, as shown in Table II. On the other hand, the contribution of the distribution volume in the peripheral compartment to the V_{ss} is about 31% for EPO. This difference between CBZ and EPO may be partly explained by the fact that CBZ is 73 to 78% bound to plasma protein in humans^{25,31,32)} and that the free fraction of EPO in human plasma is twice that of CBZ.^{7,33)} Based on these facts, there may be a slight problem if one applies the one-compartment model for convenience to predict the serum levels of CBZ. However, it would not be reasonable to apply the one-compartment model for EPO.

Recently, we calculated the rates of sulfation and glucuronidation of acetaminophen by using a deconvolution method and proposed a pharmacokinetic model for the fate of acetaminophen and its conjugates in rats.¹⁸⁾ This deconvolution method was also applied in the present study to obtain the formation rate of EPO in humans. The formation kinetics of EPO obeyed the Michaelis-Menten equation. Chang and Levy³⁴⁾ also showed that, for the isolated perfused rat liver, the formation of EPO followed Michaelis-Menten kinetics. The K_m value obtained by deconvolution for epoxidation in human is similar to that obtained in the *in situ* rat liver perfusion experiments ($11.25 \mu\text{g/ml}$) but the V_{max} for epoxidation in humans was very much smaller than that for the perfused rat liver (20.47 mg/h/kg , calculated by assuming the rat body weight to be 250 g). Therefore, it may be considered that the affinity of CBZ for the mono-oxygenase system in the endoplasmic reticulum of hepatocytes is similar in humans and rats but the apparent activity or amount of enzyme responsible for the epoxidation of CBZ in humans may be much smaller than that in the rat.

When the pharmacological effects of the parent drug are to be discussed, it is essential to consider the kinetics of EPO in the serum since a relatively high EPO to CBZ concentration ratio in cerebrospinal (CSF) has been reported because of low binding of EPO to human plasma. The present study was undertaken to develop a pharmacokinetic model to predict the behavior of EPO in the serum after oral administration of CBZ preparations. For pharmacokinetic analysis of the fate of CBZ and EPO, it is assumed *a priori* that the epoxidation of CBZ and hydration of EPO occur in the central compartment, which includes blood, since the liver appears to be a major organ responsible for the metabolism of CBZ^{12,35)} and EPO,¹²⁾ and is a well-perfused organ which is usually classified as an organ of the central compartment.³⁶⁾ This is a reasonable assumption. The other necessary assumptions are that the elimination of CBZ by routes other than biotransformation to EPO is a first-order rate process, and furthermore, that enterohepatic circulation of CBZ is negligible, since only about 1% of the administered dose is excreted into the bile.³⁷⁾ Based on these assumptions, a very good coincidence between simulated and observed

serum levels of CBZ and EPO was obtained (Fig. 2). Although the epoxidation of CBZ is represented by saturation enzyme kinetics, as shown in Fig. 5, no indication of nonlinear kinetics of CBZ has been reported²⁴⁾ and the elimination rate of CBZ did not change appreciably. This is because the elimination rate of CBZ equals the sum of the epoxidation rate and elimination rate by routes other than epoxidation and the K_m value of epoxidation is approximately twice the therapeutic concentration range of CBZ (about 5 to 10 $\mu\text{g/ml}$).^{22,23)} Furthermore, a simulation based on Fig. 5 was done by using mean disposition values of CBZ or EPO taken from Table II and values of absorption rate constant taken from a previous study¹⁵⁾ and by assuming the epoxidation of CBZ to be linear, i.e., $V_{\max}/K_m/V_1$. As can be seen in Fig. 2 the differences in calculated serum levels is minimal between solid lines (the epoxidation of CBZ obeyed by Michaelis-Menten kinetics) and dotted lines (the epoxidation of CBZ assumed to be linear). This further confirmed the above discussion. It is therefore reasonable to assume that the apparent elimination rate of CBZ is first-order. Therefore, the disposition parameter of CBZ estimated from serum levels after oral administration would be appropriate. In addition, the sum of $V_{\max}/K_m/V_1$ plus k_{e2} for Fig. 5 is 0.0228 h^{-1} and this value is comparable to the value of k_{10} of CBZ (Table II) estimated from serum levels after oral administration.

The serum concentration-time course of EPO is governed by both the formation rate and the elimination rate and is rate-limiting by the smaller of these two rate constants.³⁸⁾ When the epoxidation rate constant of CBZ is approximated by the value of $V_{\max}/K_m/V_1$, the value of $V_{\max}/K_m/V_1$ (0.015 h^{-1}) is approximately one-fifteenth of that of the elimination rate constant of EPO (k_{e4}). This indicates that the disposition of EPO after CBZ administration follows a flip-flop model in which the formation rate of EPO is rate-limiting. Further, CL and k_{10} of CBZ were approximately 8-fold smaller than those of EPO (Table II). Therefore, the EPO concentration in serum after administration of CBZ should be very low compared to that of the parent drug. In fact, EPO levels in the serum after administration of CBZ were one-tenth to one-twentieth of those of CBZ, as can be seen in Fig. 2. This difference in serum level between

CBZ and EPO is well explained by flip-flop kinetics of EPO, as well as the large difference in total body clearance (CL) or elimination rate constant (k_{10}) between CBZ and EPO.

In the present study, although we applied the Michaelis-Menten kinetics for the epoxidation of CBZ, the difference in predicted serum levels was minimal between Michaelis-Menten and linear kinetics. However at doses of CBZ above 3.56 mg/kg, it may be better to use Michaelis-Menten kinetics for the epoxidation of CBZ.

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