Studies on Interactions between Traditional Herbal and Western Medicines. I. Effects of Sho-seiryu-to on the Pharmacokinetics of Carbamazepine in Rats

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The effects of oral co- and pre-administration of Sho-seiryu-to extract powder (TJ-19, 1 g/kg), a widely used Kampo (traditional Chinese herbal) medicine, on the pharmacokinetics of an anti-epileptic drug, carbamazepine (CBZ), and its active metabolite (carbamazepine-10,11-epoxide, CBZ-E) after oral administration of CBZ (50 mg/kg) were examined in male rats. The simultaneous administration of TJ-19 significantly lengthened the time to reach the peak plasma concentration ($T_{\max}$), but did not influence the peak plasma concentration, area under the plasma concentration–time curve or terminal elimination half-life ($t_{1/2}$). Each parameter for CBZ or CBZ-E with a single pretreatment with TJ-19 was not significantly different from that with the vehicle. $T_{\max}$ and the elimination rate constant for CBZ were significantly increased by 1-week repeated pretreatment with TJ-19, by 83% ($p < 0.01$) and 88% ($p < 0.001$), respectively. $t_{1/2}$ and the mean residence time from zero to infinity ($MRT_{0-\infty}$) in the TJ-19 pretreatment group were significantly shortened, by 52 and 34% ($p < 0.005$), respectively. No significant difference in the bound fraction of each drug at two concentrations (1 and 10 $\mu\text{g/ml}$) was observed between the control and TJ-19 pretreatment groups. These results indicate that simultaneous oral administration of TJ-19 delays the oral absorption of CBZ, while 1-week repeated pretreatment with TJ-19 accelerates the metabolism of CBZ in rats, without affecting the protein binding of CBZ.

Key words Sho-seiryu-to (TJ-19); carbamazepine; carbamazepine-10,11-epoxide; pharmacokinetic interaction; protein binding; rat

Kampo (traditional Chinese herbal) medicines have been frequently prescribed recently with various Western drugs for the treatment of many chronic diseases in Japan. However, there are far fewer reports about the pharmacokinetic interactions between Kampo and Western medicines in humans than those between pairs of Western drugs.^{1–6)} Therefore, in order to prevent an adverse reaction based on their possible interaction in advance, research on the interactions between them and the accumulation of information on them are urgently needed.

Sho-seiryu-to (Tin Chuan Tang) is one of the most widely used traditional Chinese herbal medicines for the treatment of cold syndrome, bronchitis, bronchial asthma, nasal allergy, etc.⁷⁾ In particular, the clinical efficacy and safety of Sho-seiryu-to extract granules for ethical use for perennial nasal allergy have been demonstrated in a joint double-blind trial in comparison with a placebo in Japan.⁸⁾

Carbamazepine (CBZ) is a broadly used anti-epileptic, and prescribed for many epileptic patients over a long period.⁹⁾ CBZ administered orally is known to be almost epoxidized to its efficacious but adverse reaction-inducing metabolite (carbamazepine-10,11-epoxide, CBZ-E), by cytochrome P-450 (CYP) 3A4, which is one of the most important isoforms responsible for drug metabolism in humans because it is the major or an abundant enzyme in critical tissues such as the gastrointestinal tract and liver, and is involved in the oxidative biotransformation of numerous useful therapeutic agents.^{9–12)} For this reason, the pharmacokinetic interactions of CBZ with many other Western drugs which either inhibit or induce CYP3A4 have been reported.¹³⁾ Recently, grapefruit juice has been reported to increase the bioavailability of CBZ by inhibiting CYP3A4 enzymes in the gut

wall and the liver in patients with epilepsy.¹⁴⁾

Sho-seiryu-to and CBZ may be administered concomitantly in many clinical situations, e.g., in patients with both perennial nasal allergy and epilepsy. Kimura *et al.*¹⁵⁾ reported that Sho-seiryu-to extract powder (TJ-19; Tsumura & Co.) and two of its herbal constituents, Ephedrae Herba and Cinnamomi Cortex, strongly inhibit the activity of erythromycin *N*-demethylating enzymes in hepatic microsomes obtained from male rats *in vitro*, suggesting that TJ-19 might inhibit the activity of CYP3A. However, the possibility of their pharmacokinetic interaction *in vivo* remained unclear.

Therefore, in this study, the effects of the simultaneous administration of TJ-19, and single and repeated oral pretreatment with TJ-19 on the pharmacokinetics of CBZ and CBZ-E after oral administration of CBZ were examined in rats. We also examined the effect of TJ-19 on the animals' protein binding *in vitro*.

MATERIALS AND METHODS

Chemicals TJ-19 (Lot No. 260019030) was a kind gift from Tsumura & Co. (Tokyo, Japan). It comprises a mixed powder of 8 herbal drugs, i.e., 6 parts of Pinelliae Tuber, 3 parts of Glycyrrhizae Radix, 3 parts of Cinnamomi Cortex, 3 parts of Schisandrae Fructus, 3 parts of Asiasari Radix, 3 parts of Paeoniae Radix, 3 parts of Ephedrae Herba, and 3 parts of Zingiberis Siccata Rhizoma. The CBZ and CBZ-E powders used were purchased from Wako Pure Chemical Ind., Ltd. (Osaka, Japan) and Sigma Chemical Co. (St. Louis, MO, U.S.A.), respectively. Propentofylline (PRF) powder (an internal standard for HPLC analysis; Lot No. A118) was supplied by Hoechst Japan, Ltd. (Tokyo). All

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other chemicals were reagent- or HPLC-grade commercial products.

Animals 9-week-old male Wistar rats (Japan SLC, Inc., Hamamatsu, Japan), weighing 220–290 g, were used throughout this study, and were fasted but allowed free access to water for 18 h before the administration of CBZ. The left carotid artery was cannulated with polyethylene tubing (PE-50; Clay Adams, Dickinson & Co., Parsippany, NJ, U.S.A.) under pentobarbital anesthesia (50 mg/kg, intraperitoneally) the day before the pharmacokinetic experiment.

Drug Administration and Sampling The rats were divided at random into 2 groups in each pharmacokinetic study, each consisting of 4–6 rats. TJ-19 and CBZ (50 mg/5 ml/kg) were administered orally *via* gastric intubation to unanesthetized rats as a suspension and solution, respectively. In simultaneous administration studies, a 5% (w/v) arabic gum aqueous solution (vehicle, 10 ml/kg) or TJ-19 suspended in a vehicle (1 g/10 ml/kg) was administered, and then CBZ dissolved in water containing 20% (v/v) ethanol and 50% (v/v) propylene glycol was continually administered. In single pretreatment studies, the rats received a single dose of the vehicle or TJ-19 suspension (1 g/10 ml/kg), and then the same dose of CBZ as in the simultaneous administration studies 3 h after administration of the vehicle or TJ-19. In repeated pretreatment studies, the rats were treated with either the vehicle or TJ-19 suspension (1 or 2 g/kg) once a day for 1 or 2 weeks, and then were given a CBZ solution 3 h after the last administration of the vehicle or TJ-19.

Blood samples (0.25 ml) were collected through the cannula in heparinized plastic microcentrifuge tubes (1.5 ml) before, and 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 4.0, 6.0 and 8.0 h after drug administration. The samples were centrifuged at 13000 rpm for 3 min at room temperature in a Centrifuge 5415C (Eppendorf GmbH, Germany), and then the plasma fraction was frozen at -80°C until assayed. The assays were performed within 1 week of collection. This experimental protocol was approved by the Experimental Animal Research Committee at Kyoto Pharmaceutical University.

Determination of Serum Protein Binding of CBZ and CBZ-E *in Vitro* The protein binding of CBZ and CBZ-E was determined using serum obtained from rats pretreated with and without TJ-19 by means of ultrafiltration techniques. Briefly, two groups of 3 rats each received the vehicle and TJ-19 suspension (1 g/10 ml/kg) orally once a day for 1 week, respectively, and 3 h after the last administration, a blood sample (8–9 ml/rat) was rapidly withdrawn from the abdominal aorta under ether anesthesia. The sample was kept for 2 h at room temperature and then centrifuged at 3000 rpm for 10 min in a centrifuge (H-108NA; Kokusan Enshinki Co., Ltd., Tokyo). The theoretical concentrations of CBZ and CBZ-E in the serum obtained through the above procedures were 1 and 10 $\mu\text{g/ml}$, respectively. Each serum sample (1 ml) was vortexed for 5 s and then shaken in a water bath at 37°C for 1 h. Immediately after shaking, half of the serum (0.5 ml) was ultrafiltered with an MPS micro partition kit (Amicon, Inc., Beverly, MA, U.S.A.) at 1000 *g* for 10 min in a thermostatted centrifuge (RL-101; Tomy, Tokyo) held at the same temperature as the water bath, the remainder (0.5 ml) being retained for determination of the total concentrations. The CBZ and CBZ-E concentrations were measured in whole serum (total drug) and in the ultrafiltrate (unbound

drug). The bound fraction (%) of each drug in serum was calculated.

Assaying of Albumin Concentration in Serum The albumin concentration in serum was determined by the method reported by Doumas *et al.*¹⁶⁾ with an Albumin B-Test (Wako Pure Chemical Ind., Ltd., Osaka).

Assaying of CBZ and CBZ-E in Plasma The CBZ and CBZ-E concentrations in plasma were determined by the method of Shinoda *et al.*¹⁷⁾ using HPLC with a slight modification as follows: 0.1 ml of plasma was placed in a plastic centrifugation tube (1.5 ml), and then 0.5 ml of acetonitrile containing PRF (internal standard) (1 $\mu\text{g/ml}$) was added. After vortex mixing for 30 s, the mixture was centrifuged at 13000 rpm for 5 min. Then, 20 μl of the upper liquid phase was injected into an HPLC apparatus (LC-6A; Shimadzu, Kyoto, Japan) equipped with a column oven (CTO-6A; Shimadzu) and an ultraviolet detector (SPD-6A; Shimadzu). The conditions for analysis were as follows: column size, 250×4.0 mm i.d.; packing, STR ODS-II (Shinwa Chemical Industries, Ltd., Kyoto); mobile phase, acetonitrile–deionized double-distilled water (24:76); column temperature, 40°C ; flow rate, 1.0 ml/min; wavelength, 210 nm; and sensitivity, 0.00125 a.u.f.s. The retention times of CBZ-E, PRF and CBZ were about 10, 14 and 25 min, respectively. The coefficient of variation of assay was less than 3% and the recovery rate of CBZ or CBZ-E in plasma averaged over 90%. The calibration curves for CBZ and CBZ-E (0.5–20.0 $\mu\text{g/ml}$) showed good linearity ($r^2 > 0.999$). The limits of detection of CBZ and CBZ-E were approximately 0.5 and 0.2 $\mu\text{g/ml}$, respectively.

Pharmacokinetic Analysis The peak plasma concentration (C_{max}) and the time to reach C_{max} (T_{max}) of CBZ and CBZ-E were obtained from the actual observed data after oral administration. The elimination rate constant (λ) was calculated by fitting individual data for several terminal points of the plasma CBZ concentration profile with a log-linear regression equation using the least-squares method. The corresponding elimination half-life ($t_{1/2}$) was calculated by dividing $\ln 2$ by λ . The λ value for CBZ-E could not be estimated because of the lack of the terminal time points needed for fitting. The areas under the plasma concentration–time curves from zero to 8 h ($AUC_{0-8\text{h}}$) for CBZ and CBZ-E, and from zero to infinity ($AUC_{0-\infty}$) for CBZ were calculated by means of the trapezoidal rule without and with extrapolation to infinity with λ , respectively. The mean residence time from zero to infinity ($MRT_{0-\infty}$) for CBZ was estimated by moment analysis.¹⁸⁾

Statistical Analysis Data are expressed as means $\pm$ S.E. Comparisons between two groups and among more than three groups were made using Student's unpaired *t*-test and one-way analysis of variance (ANOVA) followed by Scheffé's test, respectively, with StatView J4.02 for Macintosh (Abacus Concepts Inc., Berkeley, CA, U.S.A.), and differences were considered to be statistically significant when $p < 0.05$.

RESULTS

Effect of Simultaneous Administration of TJ-19 Figure 1 shows the mean plasma CBZ and CBZ-E concentration–time curves after simultaneous administration of CBZ

Fig. 1. Effects of Simultaneous Oral Administration of TJ-19 on the Plasma CBZ and CBZ-E Concentrations after Oral Administration of CBZ to Rats

Each point represents the mean $\pm$ S.E. of 4 or 5 rats. *a*) $p < 0.05$ and $p < 0.01$ vs. each control value at the corresponding time point, respectively (Student's *t*-test). The vehicle or TJ-19 suspension (1 g/10 ml/kg) was administered orally to unanesthetized rats, and then the CBZ solution (50 mg/5 ml/kg, *p.o.*) was continually administered orally. $\circ$, control (CBZ); $\bullet$, TJ-19 (CBZ); $\triangle$, control (CBZ-E); $\blacktriangle$, TJ-19 (CBZ-E).

Table 1. Effects of Simultaneous Oral Administration of TJ-19 on the Pharmacokinetic Parameters for CBZ and CBZ-E after Oral Administration of CBZ to Rats

Parameter	CBZ		CBZ-E	
	Control	TJ-19	Control	TJ-19
C_{max} ($\mu\text{g/ml}$)	16.14 $\pm$ 2.74	14.06 $\pm$ 0.81	12.48 $\pm$ 0.99	12.57 $\pm$ 0.36
T_{max} (h)	0.5 $\pm$ 0.2	1.8 $\pm$ 0.1 ^{a)}	4	4
λ (h^{-1})	0.404 $\pm$ 0.060	0.452 $\pm$ 0.042	—	—
$t_{1/2}$ (h)	1.9 $\pm$ 0.3	1.6 $\pm$ 0.2	—	—
$AUC_{0-8\text{h}}$ ($\mu\text{g} \cdot \text{h/ml}$)	51.7 $\pm$ 7.1	57.7 $\pm$ 3.0	76.1 $\pm$ 6.0	73.5 $\pm$ 3.4
$AUC_{0-\infty}$ ($\mu\text{g} \cdot \text{h/ml}$)	54.6 $\pm$ 6.9	60.7 $\pm$ 3.2	—	—
$MRT_{0-\infty}$ (h)	3.0 $\pm$ 0.3	3.3 $\pm$ 0.1	—	—

Each value represents the mean $\pm$ S.E. of 4 or 5 rats. *a*) $p < 0.001$ vs. each control value.

with the vehicle (control) or TJ-19 suspension. The CBZ concentrations in the TJ-19 administration group were lower in the absorption phase (0.25–1 h) than those in the control group. The concentration at 0.25 h in the former was about one half that in the latter ($p < 0.05$). In the elimination phase (4 and 6 h), inversely, the CBZ levels in the TJ-19 administration group were significantly higher ($p < 0.05$ and 0.01, respectively). In the case of CBZ-E, the concentrations at 0.5 and 0.75 h in the TJ-19 administration group were lower than those in the control group ($p < 0.05$).

The pharmacokinetic parameters are listed in Table 1. The control parameters for CBZ were similar to those reported by Levy *et al.*⁹⁾ and Turner and Renton.¹⁹⁾ C_{max} , λ , $t_{1/2}$, $AUC_{0-8\text{h}}$, $AUC_{0-\infty}$ and $MRT_{0-\infty}$ for CBZ, and C_{max} , T_{max} and $AUC_{0-8\text{h}}$ for CBZ-E in the TJ-19 administration group were comparable with those in the control group ($p > 0.05$). In the TJ-19 administration group, T_{max} for CBZ was significantly increased by 260% ($p < 0.001$).

Effect of Single Pretreatment with TJ-19 The plasma CBZ and CBZ-E concentrations after the oral administration of CBZ with or without single oral pretreatment with TJ-19 are shown in Fig. 2. Table 2 summarizes the pharmacokinetic parameters. No significant differences in the concentrations of CBZ and CBZ-E at any time point or in the parameters for each drug were observed between the control and TJ-19 pre-

Fig. 2. Effects of Single Oral Pretreatment with TJ-19 on the Plasma CBZ and CBZ-E Concentrations after Oral Administration of CBZ to Rats

Each point represents the mean $\pm$ S.E. of 5 or 6 rats. The vehicle or TJ-19 suspension (1 g/10 ml/kg) was administered orally to unanesthetized rats, and then the CBZ solution (50 mg/5 ml/kg, *p.o.*) was administered orally 3 h after a single pretreatment with TJ-19. $\circ$, control (CBZ); $\bullet$, TJ-19 (CBZ); $\triangle$, control (CBZ-E); $\blacktriangle$, TJ-19 (CBZ-E).

Table 2. Effects of Single Oral Pretreatment with TJ-19 on the Pharmacokinetic Parameters for CBZ and CBZ-E after Oral Administration of CBZ to Rats

Parameter	CBZ		CBZ-E	
	Control	TJ-19	Control	TJ-19
C_{max} ($\mu\text{g/ml}$)	9.98 $\pm$ 1.80	13.71 $\pm$ 2.27	11.16 $\pm$ 1.09	10.51 $\pm$ 0.81
T_{max} (h)	0.9 $\pm$ 0.2	1.3 $\pm$ 0.1	4.4 $\pm$ 0.4	4
λ (h^{-1})	0.283 $\pm$ 0.045	0.339 $\pm$ 0.034	—	—
$t_{1/2}$ (h)	2.7 $\pm$ 0.4	2.2 $\pm$ 0.2	—	—
$AUC_{0-8\text{h}}$ ($\mu\text{g} \cdot \text{h/ml}$)	43.3 $\pm$ 6.0	52.3 $\pm$ 6.6	68.1 $\pm$ 6.4	64.3 $\pm$ 5.0
$AUC_{0-\infty}$ ($\mu\text{g} \cdot \text{h/ml}$)	50.0 $\pm$ 5.1	56.5 $\pm$ 6.3	—	—
$MRT_{0-\infty}$ (h)	4.3 $\pm$ 0.5	3.4 $\pm$ 0.3	—	—

Each value represents the mean $\pm$ S.E. of 5 or 6 rats.

Fig. 3. Effects of 1-Week Repeated Oral Pretreatment with TJ-19 on the Plasma CBZ and CBZ-E Concentrations after Oral Administration of CBZ to Rats

Each point represents the mean $\pm$ S.E. of 5 or 6 rats. *a*) $p < 0.01$ vs. control value at the corresponding time point (Student's *t*-test). The vehicle or TJ-19 suspension (1 g/10 ml/kg) was administered orally once a day for 1 week to unanesthetized rats, and then the CBZ solution (50 mg/5 ml/kg, *p.o.*) was administered orally 3 h after the last pretreatment with TJ-19. $\circ$, control (CBZ); $\bullet$, TJ-19 (CBZ); $\triangle$, control (CBZ-E); $\blacktriangle$, TJ-19 (CBZ-E).

treatment groups.

Effect of Repeated Pretreatment with TJ-19 Figure 3 depicts the CBZ and CBZ-E concentrations in plasma after oral administration of CBZ with 1-week repeated oral pretreatment with the vehicle or TJ-19 (1 g/kg/d). The plasma CBZ levels at 4–8 h in the TJ-19 pretreatment group were lower than those in the control group. There was a significant difference ($p < 0.01$) in the concentration of CBZ at the last

Table 3. Effects of 1-Week Repeated Oral Pretreatment with TJ-19 on the Pharmacokinetic Parameters for CBZ and CBZ-E after Oral Administration of CBZ to Rats

Parameter	CBZ		CBZ-E	
	Control	TJ-19	Control	TJ-19
$C_{\max}$ ($\mu\text{g/ml}$)	16.79 $\pm$ 1.67	17.44 $\pm$ 1.78	10.87 $\pm$ 0.75	12.43 $\pm$ 1.02
$T_{\max}$ (h)	0.6 $\pm$ 0.1	1.1 $\pm$ 0.1 ^{a)}	4.3 $\pm$ 0.3	4
λ (h^{-1})	0.276 $\pm$ 0.037	0.520 $\pm$ 0.023 ^{c)}	—	—
$t_{1/2}$ (h)	2.7 $\pm$ 0.3	1.3 $\pm$ 0.1 ^{b)}	—	—
$AUC_{0-8\text{h}}$ ($\mu\text{g} \cdot \text{h/ml}$)	59.2 $\pm$ 6.2	57.7 $\pm$ 4.8	68.5 $\pm$ 5.4	76.0 $\pm$ 6.1
$AUC_{0-\infty}$ ($\mu\text{g} \cdot \text{h/ml}$)	68.0 $\pm$ 7.9	59.0 $\pm$ 4.9	—	—
$MRT_{0-\infty}$ (h)	3.8 $\pm$ 0.3	2.5 $\pm$ 0.1 ^{b)}	—	—

Each value represents the mean $\pm$ S.E. of 5 or 6 rats. a), b) and c) $p < 0.01$, 0.005 and 0.001 vs. each control value, respectively.

Table 4. *In Vitro* Protein Binding of CBZ and CBZ-E Determined Using Serum Obtained from Rats Pretreated with and without TJ-19 for a Week

Drug concentration tested ($\mu\text{g/ml}$)	Bound fraction (%)			
	CBZ		CBZ-E	
	Control	TJ-19	Control	TJ-19
1.0	71.1 $\pm$ 0.6	76.5 $\pm$ 2.6	42.6 $\pm$ 5.0	42.9 $\pm$ 2.5
10.0	73.0 $\pm$ 1.4	75.2 $\pm$ 0.8	38.5 $\pm$ 2.7	38.8 $\pm$ 2.1

Each value represents the mean $\pm$ S.E. of the results obtained for 3 rats.

time point, 8 h, between two groups. The CBZ-E concentration-time curves for the two groups were apparently comparable.

The pharmacokinetic parameters are summarized in Table 3. $C_{\max}$, $AUC_{0-8\text{h}}$ and $AUC_{0-\infty}$ for CBZ in the TJ-19 pretreatment group were not significantly different from those in the control group, respectively. $T_{\max}$ and λ for CBZ were significantly increased by 1-week pretreatment, by 83% ($p < 0.01$) and 88% ($p < 0.001$), respectively. $t_{1/2}$ and $MRT_{0-\infty}$ in the TJ-19 pretreatment group were significantly decreased, by 52 and 34% ($p < 0.005$), respectively. The value of each parameter for CBZ-E in the TJ-19 pretreatment group was not significantly different from that in the control group.

Furthermore, we examined the effects of an increased daily dose of TJ-19 or duration of pretreatment with TJ-19. One-week pretreatment with TJ-19 (2 g/kg/d) and 2-week pretreatment with TJ-19 (1 g/kg/d) also significantly ($p < 0.005$ and 0.05, respectively) shortened $t_{1/2}$ for CBZ (2 g/kg/d treatment, 1.4 $\pm$ 0.2 h; and 2-week treatment, 1.6 $\pm$ 0.1 h; these values not being significantly different from the value in the 1-week pretreatment with 1 g/kg/d of TJ-19 (1.3 $\pm$ 0.1 h): data not shown in Tables), compared with the control (2.7 $\pm$ 0.3 h, Table 3).

Effect of TJ-19 on Protein Binding *in Vitro* The protein binding of CBZ and CBZ-E *in vitro* determined using serum obtained from rats pretreated with and without TJ-19 for 1 week is shown in Table 4. No significant difference in the bound fraction of each drug at two concentrations (1 and 10 $\mu\text{g/ml}$) was observed between the control and TJ-19 pretreatment groups. The rates of CBZ protein binding are similar to those reported by Kimura *et al.*²⁰⁾ The serum albumin concentrations in the control and TJ-19 groups were 3.4 $\pm$ 0.04 and 3.4 $\pm$ 0.1 g/dl, respectively, and thus were not significantly different. These levels are consistent with those

reported by Yoshinaga *et al.*²¹⁾

DISCUSSION

TJ-19 should generally be administered before meals or between meals, although CBZ is commonly administered after meals in the case of the oral dosage form. Like CBZ, however, TJ-19 must sometimes be taken after meals for certain clinical reasons, *e.g.*, greater compliance. Therefore, we examined the effects of simultaneous administration of TJ-19 on the plasma CBZ and CBZ-E concentrations after oral administration of CBZ. TJ-19 co-administration significantly increased $T_{\max}$ without affecting the elimination rate or the extent of bioavailability of CBZ (Table 1), apparently indicating slowed absorption of CBZ when co-administered with TJ-19. TJ-19 is a mixture of 8 crude herbal drugs. Schisandrae Fructus, Paeoniae Radix and Zingiberis Siccata Rhizoma in TJ-19 are known to have inhibitory effects on the gastric emptying rate (GER).²²⁾ Although the mechanism is unclear at present, TJ-19 is likely to decrease GER, in contrast with the case of Sho-saiko-to-tolbutamide interaction in rats.²³⁾ With CBZ-E, the concentrations at 0.5 and 0.75 h in the TJ-19 administration group were significantly lower than those in the control group. This may be due to the lateness of the start of metabolism of CBZ to CBZ-E, resulting from the delay of CBZ absorption.

Next, to confirm more clearly whether or not TJ-19 affects the elimination of CBZ and CBZ-E, CBZ was orally administered 3 h after a single oral administration of TJ-19. The pretreatment with TJ-19 had no effect on λ or $t_{1/2}$ for CBZ (Table 2), found in simultaneous administration studies. Unlike in the co-administration studies, there were no significant differences in $T_{\max}$ for CBZ between the two groups, showing that the duration of action for the reduction of the CBZ absorption rate by TJ-19 appears to be relatively transient. CBZ undergoes almost complete hepatic and intestinal biotransformations, less than 2% of the dose excreted being unchanged, but the absolute bioavailability after its oral administration is more than 70% in rats, as in humans^{9,17)}; CBZ is believed to be one of the capacity-limited and flow-independent drugs. Its several metabolites are formed through parallel or sequential reactions, the main pathway being conversion to CBZ-E, which is extensively hydrolyzed to a *trans*-dihydrodiol prior to excretion in the urine.⁹⁾ In recent years there has been an increase in the knowledge of the different CYP isoenzymes involved in drug metabolism. The hepatic isoenzymes responsible for CBZ-E formation in humans have been identified as CYP3A4 and CYP2C8,¹⁰⁾ the former playing the most important role and being inhibited or induced by numerous drugs.⁹⁾ In rats, as well as in humans, CYP3A is known to metabolize CBZ.²⁴⁾ From our results and these facts, it is suggested that TJ-19 administered orally might not inhibit the CYP-mediated monooxygenase system, particularly the activity of CYP3A.

Kimura *et al.*¹⁵⁾ reported that TJ-19 strongly inhibits the activity of CYP3A in hepatic microsomes obtained from male rats *in vitro*. In this study, however, the rate of elimination of CBZ was unchanged by simultaneous administration of or single pretreatment with TJ-19 as described above. The reason the results are different between *in vivo* and *in vitro* might be that the final concentration of possible unknown in-

hibitory substance(s) at the site of action *in vivo* is much lower than that under experimental conditions *in vitro*, and that the inhibitor(s), which TJ-19 itself may originally include, is extensively decreased through presystemic first-pass metabolism when administered orally. Further investigation should be undertaken to examine this contradiction.

On the other hand, $t_{1/2}$ and $MRT_{0-\infty}$ for CBZ were significantly decreased by 1-week pretreatment with TJ-19, respectively, and $AUC_{0-\infty}$ tended to be decreased (Table 3), indicating that the metabolism of CBZ is accelerated by TJ-19. Kanamoto *et al.*²⁵⁾ reported that 10-day pretreatment of Saiko-keishi-to to rabbits, which is a most useful Kampo medicine like TJ-19, resulted in increases in the total plasma clearance of phenytoin (PHT) and antipyrine due to improvement of the liver function; 1-week pre-administration of Sho-saiko-to to rabbits has been speculated to increase PHT clearance by enhancing the stimulation of hepatic PHT-oxidizing metabolic enzyme activity.²⁶⁾ Sho-saiko-to has recently been reported to increase hepatic Cyp2d-9 mRNA level in six-week-old female mice by 3 months of pretreatment.²⁷⁾ Ohnishi *et al.* reported that Sho-saiko-to induces a 25% increase in the total content of CYP and enhances the metabolic rates for substrates of hepatic CYP2E1 in females with 2 weeks of pretreatment.²⁸⁾ These findings are similar to our results in the stimulation effect of drug-metabolizing enzyme by Kampo medicines. It has been found, however, that 1-week pretreatment with Sho-seiryu-to to male rats does not affect the total content of hepatic microsomal CYP,²⁹⁾ suggesting little possibility of CYP enzyme induction by pretreatment with TJ-19.

Valproic acid has been reported to displace CBZ from plasma protein binding sites,³⁰⁾ resulting in significant enhancement of the systemic clearance of the total drug. But it was confirmed in this study that parent compounds and their various metabolites surely existing in the serum obtained from rats pretreated with TJ-19 for a week are unable to displace CBZ or CBZ-E from serum protein binding sites (Table 4), indicating that the protein binding displacement is unlikely to be responsible for increased metabolism of CBZ. Further detailed studies are in progress to clarify the mechanism of action underlying the accelerated metabolism of CBZ by TJ-19 observed in this study; the effects of 1-week pretreatment with TJ-19 on the content and activity of CYPs and CYP-mediated monooxygenases (cytochrome b₅, *etc.*) in liver microsomes are being examined in rats.

In these 1-week pretreatment studies, as well as in the simultaneous administration studies, T_{max} for CBZ was significantly increased by the administration of TJ-19. This may be due to the pretreatment for 1 week with a much larger amount of TJ-19 than its clinical dose. In this study, we could not determine the effect of TJ-19 on the metabolism of CBZ-E as the blood sampling time was too short.

In conclusion, simultaneous oral administration of TJ-19 delays the oral absorption of CBZ, while 1-week repeated pretreatment with TJ-19 accelerates the metabolism of CBZ in rats, without affecting the protein binding of CBZ.

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