Studies on interactions between traditional herbal and Western medicines. III. Effects of Sho-seiryu-to (Tin Chuan Tang) on the pharmacokinetics of phenytoin in rats

Noriaki OHNISHI,[*a)] Sachiko UMEHARA,[a)] Yusuke NOGUCHI,[a)] Shinji NAKASAKO,[a)] Koji TAKARA,[a)] Kazuki NAGASAWA,[a,1)] Mutsunobu YOSHIOKA,[b)] Kazuo KURODA,[b)] Teruyoshi YOKOYAMA[a)]

[a)]*Department of Hospital Pharmacy, Faculty of Pharmaceutical Sciences, Kyoto Pharmaceutical University,* [b)]*Department of Pharmacy, Takarazuka City Hospital*

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Abstract

The possibility of pharmacokinetic interactions between Sho-seiryu-to (Tin Chuan Tang) extract powder (TJ-19), a widely used traditional Chinese herbal (Kampo) medicine, and phenytoin (PHT), an important anti-epileptic drug, was examined in rats. There were no significant differences in the serum protein binding of PHT at two concentrations (1 and 10 μg/ml) between two groups pretreated orally with the vehicle and TJ-19 suspension (1 g/kg/d) for 1 week. One-week repeated pretreatment with TJ-19 (1 g/kg/d) did not affect the liver weight or contents of cytochromes P450 and b_5 in hepatic microsomes, while that with phenobarbital (80 mg/kg/d, i.p.) induced significant increases in these parameters. Neither a single nor 1-week repeated oral pretreatment with TJ-19 (1 g/kg/d) altered the plasma concentration-time curve or any pharmacokinetic parameter of PHT after intraperitoneal administration at a dose of 20 mg/kg. These results demonstrated that co-administration of TJ-19 with PHT has no effect on the pharmacokinetics of PHT in rats.

Key words Sho-seiryu-to (Tin Chuan Tang; 小青竜湯), phenytoin, pharmacokinetic interaction, protein binding, cytochrome P450, metabolism, rat.

Abbreviations CBZ, carbamazepine; CYP, cytochrome P450; PB, phenobarbital; PHT, phenytoin; PRF, propentofylline; TJ-19, Sho-seiryu-to.

Introduction

Sho-seiryu-to (Tin Chuan Tang) is one of the most widely used traditional Chinese herbal (Kampo) medicines for the treatment of cold symptoms, bronchitis, bronchial asthma, nasal allergy, *etc.*[2] The clinical efficacy and safety of Sho-seiryu-to extract granules for treatment of perennial nasal allergy have been demonstrated in a joint double-blind trial in comparison with a placebo in Japan.[3]

We previously found that the pharmacokinetics of carbamazepine (CBZ), one of the most commonly prescribed drugs for epilepsy, after its oral administration were not affected by single oral pretreatment of Sho-seiryu-to. However, 1-week repeated oral pretreatment with Sho-seiryu-to accelerated the elimination of CBZ after oral administration to rats.[4]

Phenytoin (PHT) has also been prescribed for treatment of epileptic patients over long periods, and has frequently been shown to have clinically significant interactions with other modern drugs,[5,6] because

*〒607-8414 京都市山科区御陵中内町5
京都薬科大学病院薬学教室 大西 憲明
5 Nakauchi-cho, Misasagi, Yamashina-ku, Kyoto 607-8414, Japan

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of its narrow therapeutic index, slow absorption and saturable metabolism.[7] Kanamoto *et al.*[8] and Hosoya *et al.*[9] reported that the total clearance of PHT in rabbits was increased by repeated oral pretreatment with Kampo medicines, Saiko-keishi-to and Sho-saiko-to, respectively. Also, PHT may be concomitantly administered with Sho-seiryu-to in many clinical situations, *e.g.,* in patients with both epilepsy and perennial nasal allergy. However, it has not yet been clarified whether pharmacokinetic interactions occur between PHT and Sho-seiryu-to.

Therefore, in this study, the effects of oral pretreatment with Sho-seiryu-to on the serum protein binding of and the contents of hepatic cytochromes involved in metabolism of PHT were examined *in vitro* using rats. We also examined the effects of a single and 1-week repeated oral pretreatment with Sho-seiryu-to on the pharmacokinetics of PHT after its intraperitoneal administration in rats.

Materials and Methods

Chemicals : Sho-seiryu-to extract powder (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 Siccatum Rhizoma. Phenytoin sodium salt (PHT-Na) and phenobarbital (PB) powders used were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Propentofylline (PRF) powder (an internal standard for HPLC analysis; Lot No. A118) was supplied by Hoechst Japan, Ltd. (Tokyo). NADH was purchased from Oriental Yeast Co., Ltd. (Tokyo). All other chemicals were reagent- or HPLC-grade commercial products.

Animals : Nine-week-old male Wistar rats (Japan SLC, Inc., Hamamatsu, Japan), weighing 220-290 g, were used throughout this study.

Determination of serum protein binding of PHT in vitro : The protein binding of PHT was determined using serum obtained from rats pretreated with or without TJ-19 by ultrafiltration. Briefly, two groups of 3 rats each received a 2% (w/v) arabic gum aqueous solution (vehicle, 10 ml/kg) and TJ-19 suspended in vehicle (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 (H-108NA ; Kokusan Enshinki Co., Ltd., Tokyo). The theoretical concentrations of PHT in the serum obtained through the above procedures were 1 and 10 μg/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 ultrafiltrated with an MPS micro partition kit (Amicon, Inc., Beverly, MA, U.S.A.) at 1000 g for 10 min in a thermostatically regulated centrifuge (RL-101 ; Tomy, Tokyo) held at the same temperature as the water bath, the remainder (0.5 ml) being retained for determination of total concentrations. The PHT concentrations were measured in whole serum (total drug) and in the ultrafiltrate (unbound drug). The bound fraction (%) of each drug in serum was calculated. The albumin concentration in serum was determined by the method reported by Doumas *et al.*[10] with an Albumin B-Test (Wako Pure Chemical Ind., Ltd., Osaka, Japan).

Preparation of liver microsomes : The preparation of hepatic microsomes was carried out using rats pretreated with the vehicle, TJ-19 suspension or PB-saline solution. Briefly, the vehicle (p.o.), TJ-19 (1 g/10 ml/kg, p.o.) and PB (positive control, 80 mg/2 ml/kg, i.p.) were administered once a day for 1 week, and the liver was excised 3 h after the last drug pretreatment. Microsomes were prepared according to a slight modification of the conventional methodology of Omura and Sato[11] by differential centrifugation. Protein concentrations in microsomes were determined by the method of Lowry *et al.* with bovine serum albumin as a standard.[12]

Hepatic enzyme assays : The contents of cytochrome P450 (CYP) and cytochrome b_5 in hepatic microsomes of vehicle-, TJ-19- and PB-pretreated rats described above were estimated by the method of Omura and Sato.[11]

Pharmacokinetic experiments : The rats were

divided at random into 2 groups in each pharmacokinetic study, each consisting of 4 or 5 rats. 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. They were fasted but allowed free access to water for 18 h before the administration of drugs. In single pretreatment studies, the vehicle or TJ-19 suspension (1 g/10 ml/kg) was administered to unanesthetized rats, followed 1 h later by intraperitoneal administration of PHT-Na dissolved in water containing 20 % (v/v) ethanol and 50 % (v/v) propylene glycol (20 mg/2 ml/kg, PHT equivalent). In repeated pretreatment studies, rats were treated with either the vehicle or TJ-19 (1 g/kg) once a day for 1 week, and then given PHT solution 1 h after the last administration. The vehicle and TJ-19 were orally administered by gastric intubation. Blood samples (0.25 ml) were collected through the cannula in heparinized plastic microcentrifuge tubes (1.5 ml) before, and 0.25, 0.5, 1, 2, 3, 4, 6 and 8 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 assay. The assays were performed within 1 week of collection. Referring to clinically effective PHT concentrations in the plasma at steady state (10-20 μg/ml), we established a bolus dose of 20 mg/kg in this study. Also, the dose of TJ-19 (1 g/kg) was determined with reference to the ratio of PHT dose in rats to that in humans. All experimental protocols using animals were approved by the Experimental Animal Research Committee of Kyoto Pharmaceutical University.

Assay of PHT: The PHT concentrations were determined by HPLC according to a slight modification of the method used for CBZ assay, which we reported previously:[13] Briefly, 0.1 ml of plasma or serum sample was placed in a plastic centrifugation tube (1.5 ml), and then 0.5 ml of acetonitrile containing PRF (internal standard; 0.5 μg) was added. After vortex mixing for 30 s, the mixture was centrifuged at 13000 rpm for 5 min. Then, the upper liquid phase (0.5 ml) was transferred to another tube and evaporated to dryness at 40°C under a stream of nitrogen. The residue was completely reconstituted with 0.1 ml of acetonitrile-water solution (7 : 3) and 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 PRF and PHT were about 14 and 25 min, respectively. The coefficient of variation of the assay was less than 5 % and the recovery rate of PHT in plasma averaged over 90 %. The calibration curves for PHT (0.1-20.0 μg/ml) showed good linearity ($r^2>0.999$). The limits of detection of PHT were approximately 0.05 μg/ml.

Pharmacokinetic Analysis: The peak plasma concentration (C_{max}) and the time to reach C_{max} (T_{max}) of PHT were obtained from the actual data observed after oral administration. The terminal elimination rate constant (λ) was calculated by fitting individual data for three terminal points of the plasma PHT concentration profile with a log-linear regression equation using the least-squares method. The corresponding elimination half-life ($t_{1/2(\lambda)}$) was calculated by dividing ln 2 by λ. The areas under the plasma concentration-time curves from zero to infinity ($AUC_{0-\infty}$) were calculated by the trapezoidal rule with extrapolation to infinity with λ. The mean residence time from zero to infinity (MRT) was estimated by moment analysis.[14]

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

Results

Effects of pretreatment with TJ-19 on serum protein binding of PHT in vitro

We examined the protein binding of PHT at two concentrations (1 and 10 μg/ml) *in vitro* using serum obtained from rats pretreated with the vehicle or TJ-19 for 1 week. The serum-bound fractions at 1 μg/ml in the control and TJ-19 group were 88.5±1.3 and 89.7±0.9 %, respectively. At a dose of 10 μg/ml, these values were 90.0±0.4 and 89.3±0.5 %, respectively. No significant differences were observed in their values between the two groups. The binding rates of PHT were similar to those reported previously by Colburn and Gibaldi.[15] The serum albumin concentrations in the control and TJ-19 groups were 3.4±0.04 and 3.4±0.1 g/dl, respectively, and thus were not significantly different. These levels were consistent with those reported by Yoshinaga *et al.*[16]

Effects of pretreatment with TJ-19 on contents of microsomal cytochromes in the liver

Next, we examined the effects of 1-week repeated pretreatment with vehicle, TJ-19 or PB on the liver weight and the contents of CYP and cytochrome b_5 in rat liver microsomes (Table I). There were no significant differences in any of these parameters between the control and TJ-19 groups. The con-

Table I Effects of 1-week repeated pretreatment with TJ-19 or PB on liver weight and contents of CYP and cytochrome b_5 in hepatic microsomes in rats.

	Control	TJ-19	PB
Wet liver/body weight ratio×100 (g/g %)	3.87±0.05	3.98±0.25	5.55±0.05[a)]
CYP content (nmol/mg protein)	0.81±0.05	0.57±0.06	2.61±0.46[a)]
Cytochrome b_5 content (nmol/mg protein)	0.41±0.01	0.38±0.02	0.53±0.02[a)]

Each value represents the mean±S.E. of 3 rats. a) $p<0.01$ *vs.* control value (ANOVA; Scheffe's F-test). Rats were pretreated once a day with the vehicle (control, p.o.), TJ-19 suspension (1 g/10 ml/kg, p.o.) or PB solution (80 mg/2 ml/kg, i.p.) for 1 week, and then hepatic microsomes were prepared from each rat 3 h after the last drug pretreatment.

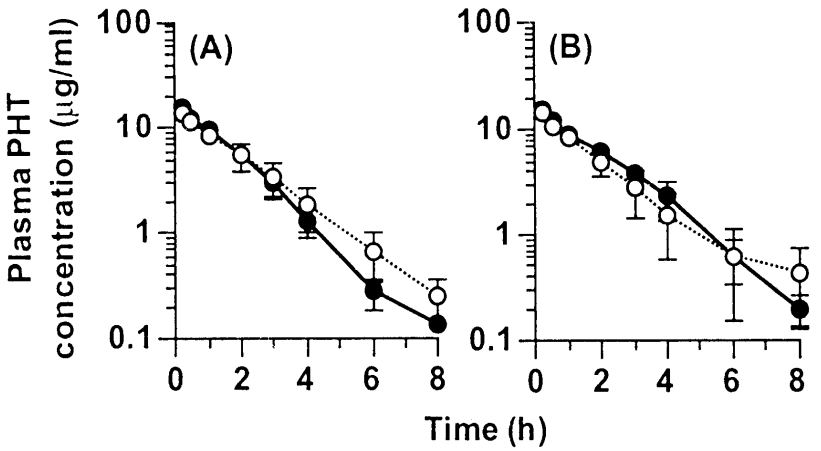

Fig. 1 Effects of single (A) and 1-week repeated (B) oral pretreatment with TJ-19 on the plasma PHT concentrations after intraperitoneal administration to rats. Each point represents the mean±S.E. of 4 or 5 rats. The vehicle (control) or TJ-19 suspension (1 g/10 ml/kg) was orally administered only once or once a day for 1 week to unanesthetized rats, and PHT solution (20 mg/2 ml/kg) was administered intraperitoneally 1 h after the last pretreatment. ○, control; ●, TJ-19.

Table II Effects of single and 1-week repeated oral pretreatment with TJ-19 on the pharmacokinetic parameters of PHT after intraperitoneal administration to rats.

Parameter	Single pretreatment		Repeated pretreatment	
	Control	TJ-19	Control	TJ-19
C_{max} (μg/mL)	14.0±1.0	15.5±0.6	14.9±1.2	16.0±0.7
T_{max} (h)	0.25	0.25	0.25	0.25
λ (h^{-1})	0.518±0.108	0.530±0.064	0.635±0.189	0.593±0.022
$t_{1/2(\lambda)}$ (h)	1.5±0.4	1.4±0.2	1.4±0.3	1.2±0.04
$AUC_{0-\infty}$ (μg · h/mL)	27.8±7.0	27.1±3.0	26.9±8.0	30.6±4.5
MRT (h)	1.8±0.3	1.6±0.1	1.8±0.2	1.8±0.2

Each point represents the mean±S.E. of 4 or 5 rats. The vehicle (control), TJ-19 suspension (1 g/10 ml/kg) was orally administered only once or once a day for 1 week to unanesthetized rat, and PHT solution (20 mg/2 ml/kg) was administered intraperitoneally 1 h after the last pretreatment.

tent of CYP in the control group was almost the same as those previously reported by Lin *et al.*[17] and Kang *et al.*[18] In contrast, pretreatment with PB (a representative enzyme inducer) significantly increased the liver weight, CYP and cytochrome b_5 by 43, 222 and 29 % ($p<0.05$), respectively, compared with controls.

Effects of Pretreatment with TJ-19 on pharmacokinetics of PHT

The plasma PHT concentration-time curves after intraperitoneal administration to rats pretreated orally once with vehicle and TJ-19 are shown in Fig. 1 (A). No significant differences were observed in the concentrations of PHT at any time point between the two groups. Table II lists the pharmacokinetic parameters. C_{max}, T_{max}, λ, $t_{1/2(\lambda)}$, $AUC_{0-\infty}$ and MRT for PHT in the TJ-19 administration group were comparable with those in the control group ($p>0.05$). The values of $t_{1/2(\lambda)}$ were similar to those reported by Kimura *et al.*[19]

Figure 1 (B) depicts the plasma concentration profiles of PHT after its intraperitoneal administration following 1-week repeated oral pretreatment with the vehicle or TJ-19. The PHT concentration profiles in the two groups were comparable. The pharmacokinetic parameters are summarized in Table II; no significant differences were observed in any of the parameters examined between the control and TJ-19 groups. Also, no significant differences were observed in any parameter between single and repeated treatment with the vehicle.

Discussion

PHT is highly bound to plasma proteins, essentially eliminated by hepatic metabolism, and is typically capacity-limited.[7] Accordingly, the total clearance (CL_{tot}) of PHT can be expressed approximately by the following equation:[20]

$$CL_{tot} = CL_H = f_p \cdot CL_{int,H}$$

where CL_H is the hepatic clearance, f_p is the plasma-unbound fraction and $CL_{int,H}$ is the intrinsic hepatic clearance. Hence, a change in CL_{tot} should be accounted for by an increase or decrease in f_p and/or $CL_{int,H}$.

Elevation in f_p will lead to the displacement of plasma protein binding. The bound fraction of PHT in serum was high (about 90 %) as described in the Result section. Valproate, in fact, has been reported to displace PHT from plasma protein binding sites, resulting in enhancement of the CL_{tot} of PHT *via* a significant increase of f_p.[21] However, in this study parent compounds and their various metabolites present in the serum obtained from rats pretreated with TJ-19 for 1 week were unable to displace PHT from serum protein binding sites, suggesting that there may be no interaction due to the change in protein binding.

A large proportion of PHT administered to rats is exclusively eliminated by hepatic biotransformation from PHT to 5-(4-hydroxyphenyl)-5-phenylhydantoin (HPPH).[22] Thus, $CL_{int,H}$ is considered to be determined mainly by this enzymatic reaction in the liver. As shown in Table I, 1-week repeated pretreatment with TJ-19 did not influence the liver

weight or the contents of cytochromes in hepatic microsomes, whereas that with PB markedly increased these parameters. Based on these observations and results obtained *in vitro,* we speculated that orally administered TJ-19, unlike PB, would neither down-regurate nor induce the CYP-mediated monooxygenases, and that no interaction might occur in the process of hepatic metabolism.

Furthermore, we performed *in vivo* experiments to confirm whether TJ-19 affects the disposition and/or metabolism of PHT. Neither a single nor 1-week repeated oral pretreatment with TJ-19 altered the plasma concentration-time profile (Fig. 1) or any pharmacokinetic parameter of PHT examined after its intraperitoneal administration (Table II), demonstrating that there were no pharmacokinetic interactions *in vivo*. These *in vivo* results were completely consistent with those obtained *in vitro*. The findings of multiple TJ-19-treatment experiments in this study using PHT were similar to those obtained using theophylline (THP)[17] but not those of our previous study using CBZ;[4] 1-week repeated oral pretreatment with TJ-19 accelerated the elimination of CBZ after oral administration. In rats, PHT and THP are known to be biotransformed chiefly by CYP2C/2B[23] and CYP1A,[24] respectively, while CBZ is metabolized by CYP3A,[25] although all three drugs are the same in that they are mostly eliminated by metabolism in the livers and are capacity-limited. So, TJ-19 was speculated to have selective ability to significantly increase activities of limited CYP isoform(s) such as CYP3A or other enzyme(s) related to CYP3A. Further detailed investigations are currently in progress to clarify this mechanism of action. Furthermore, the results of this study were not consistent with those reported by Kanamoto *et al.*[8] or Hosoya *et al.*,[9] who showed that the total clearance of PHT in rabbits was increased by a repeated oral pretreatment with Saiko-keishi-to and Sho-saiko-to, respectively. This may have been due to the differences in constituents of each Kampo formulation or in the species studied. In addition, Bupleuri Radix, Scutellariae Radix, Zizyphi Fructus and/or Ginseng Radix present not in Sho-seiryu-to but in both Saiko-keishi-to and Sho-saiko-to may have ability to accelerate the metabolism of PHT.

The metabolic clearance of PHT in rats is markedly faster than that in humans.[21,26] The metabolites formed in the former, however, are essentially the same as those in the latter, and the major pathway is qualitatively similar in both species.[21,27] The hepatic isoenzymes responsible for the hydroxylation of PHT in humans have been identified as CYP2C9 and CYP2C19.[28] Therefore, the results of the present study using rats are likely to be applicable to humans.

In conclusion, co-administration of TJ-19 had no effect on the pharmacokinetics of PHT in rats. These observations suggested that concomitant use of TJ-19 and PHT should be pharmacokinetically safe in humans.

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和文抄録

汎用されている漢方薬の小青竜湯エキス末(TJ-19)と重要な抗てんかん薬のフェニトイン(PHT)の薬物動態学的相互作用の可能性についてラットを用いて検討した。ヴィークル及びTJ-19懸濁液(1 g/kg/day)を1週間反復経口投与した後のラット血清を用いて，PHT(1及び10 μg/ml)のタンパク結合を比較したところ，両群間で有意差は認められなかった。TJ-19(1 g/kg/day)の1週間反復処置は肝重量並びに肝ミクロソーム中シトクロムP450及びb_5量に影響しなかった。一方，フェノバルビタール(80 mg/kg/day)の腹腔内投与によりそれらは有意に増加した。さらに，TJ-19(1 g/kg)の単回及び1週間反復投与によりPHT(20 mg/kg)の腹腔内投与後の血漿中濃度-時間曲線及び動態パラメーターは変化しなかった。これらの結果は，ラットにおいてTJ-19の併用はPHTの体内動態に影響を及ぼさないことを示している。

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