

## Inhibition of Theophylline Metabolism by Suplatast and Its Metabolites in Rats

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The inhibitory effect of suplatast (ST), an anti-allergic drug, on theophylline (TP) metabolism was investigated in rats *in vivo* and *in vitro*. Intravenous injection of aminophylline (AP) at 10 mg/kg of TP equivalent was performed with or without pretreatment by oral administration of 100 mg/kg of ST 2.5 h prior to AP. In the ST-pretreated group, plasma concentration (C<sub>p</sub>), the area under C<sub>p</sub>-time profile (AUC) and urinary excretion of TP increased significantly, and urinary excretion of TP metabolites, 1,3-dimethyluric acid (DMU) and 1-methyluric acid (1MU) decreased significantly. Metabolic clearance of DMU (CL<sub>DMU</sub>) and that of 1MU (CL<sub>1MU</sub>) were remarkably suppressed by ST pretreatment, however, renal clearance (CL<sub>r</sub>) of TP did not change. To compare the inhibitory effect of ST on TP metabolism with that of its main metabolite (M1) *in vivo*, a concomitant intravenous injection of AP (10 mg/kg of TP equivalent) with ST or M1 (40 mg/kg of ST equivalent) was carried out. In the M1 group, C<sub>p</sub> and AUC of TP increased significantly, and the total body clearance of TP decreased significantly. In contrast, ST did not induce these changes. Then, the inhibitory effect of ST and M1 on TP metabolism *in vitro* was evaluated using rat-liver microsomes. ST and M1 suppressed DMU formation in a competitively inhibitory manner, and their equilibrium dissociation constants (K<sub>i</sub>) were 822 and 731 μM, respectively. In conclusion, inhibition of TP metabolism by ST was demonstrated *in vivo* and *in vitro*, and the involvement of M1 and/or other metabolites in this drug interaction was suggested.

**Key words** theophylline; suplatast; drug interaction; metabolism; rat; microsome

In pharmacotherapy for bronchitis and asthma, anti-histamines and anti-allergic drugs can be used concomitantly with theophylline (TP) to avoid induction of bronchoconstriction and inflammation in the respiratory tract. Some anti-histamines such as azelastine, ketotifen, mequitazine, mizolastine, pemiropast potassium, picumast dihydrochloride, repirinast, temelastine and terfenadine have been reported not to interact adversely with TP.<sup>1)</sup> However, doxalast accelerated elimination of TP by increasing the free fraction in blood, and ketotifen also decreased the serum level of TP.<sup>2)</sup> TP has a narrow therapeutic range in serum level of 10–20 μg/ml and can interact with other drugs in its metabolic process.<sup>1,3)</sup> Since many kinds of anti-allergic drugs have been developed and are commercially available, pharmacokinetic evaluation of the possible interaction between anti-allergic drugs and TP is important.

Suplatast (ST), (±)-[2-[4-(3-ethoxy-2-hydroxypropoxy)-phenylcarbamoyl]ethyl]dimethylsulfonium *p*-toluenesulfonate, is a novel anti-allergic drug and can inhibit not only production of IgE antibody but also type I allergic reaction.<sup>4)</sup> That means ST can block both the first and second steps in an allergic reaction. In clinical practice, ST has been used for the treatment of asthma, atopic dermatitis and allergic rhinitis. Anti-allergic drugs can be administered in association with TP, however, no interaction between ST and TP has been reported. In this study, we investigated the effect of ST on pharmacokinetics of TP in rats. A pharmacokinetic study *in vivo* was performed, and TP metabolism was also examined in rat-liver microsomes *in vitro*. Finally, the inhibitory effect of ST and its main metabolite, M1, on TP metabolism was demonstrated.

### MATERIALS AND METHODS

**Animals** Male Wistar rats weighing 250–350 g (Sankyo

Labo Service Corporation, Inc., Tokyo, Japan) were housed in a cage maintained at 24±2 °C. Animals were fed a cube diet (MF, Oriental Yeast Co., Tokyo) and tap water *ad libitum*.

**Chemicals and Reagents** ST was generously supplied by Taiho Pharmaceutical Co., Ltd. (Tokyo). Aminophylline (AP) injections were purchased from Eisai Co., Ltd. (Tokyo). TP and theobromine were purchased from Wako Pure Chemicals Industries, Ltd. (Osaka, Japan). 1-Methyluric acid (1MU), 3-methylxanthine (3MX), 3-propylxanthine and mexiletine hydrochloride (MX) were purchased from Sigma-Aldrich Japan K.K. (Tokyo), and 1,3-dimethyluric acid (DMU) was purchased from Nacalai Tesque, Inc. (Kyoto, Japan). M1, (±)-4-(3-ethoxy-2-hydroxypropoxy) acrylamide, was synthesized in our laboratory as follows: 0.2 g of ST and 0.1 g of Na<sub>2</sub>CO<sub>3</sub> were dissolved in distilled water and heated at 50 °C for 45 min. After addition of 1 ml of 1 M HCl and 5 ml of extract solvent, dichloromethane:ethyl acetate (1:5), mixture was shaken vigorously and then centrifuged to obtain the organic phase. The aqueous phase was washed with 3 ml of the extract solvent twice. The organic phase was gathered into another tube and then evaporated to dryness under a dry nitrogen stream at 45 °C to obtain a residue of white powder. M1 was confirmed by M<sup>+</sup> peak at 265 by mass spectroscopy. Purity of M1 was >95% as measured by reverse phase HPLC with octadecylsilyl-column at 254 nm detection.

**Pharmacokinetics of TP *in Vivo*** First, the effect of pretreatment by the oral administration of ST on pharmacokinetics of TP was investigated. Under anesthesia with isoflurane inhalation, polyethylene tubing (PE-50) was cannulated into the carotid artery and jugular vein and led subcutaneously to the outside of the occiput. The animal was placed in a metabolic cage and fed a cube diet and tap water *ad libitum*. The following day, 100 mg/kg ST or distilled water (for control

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rats) was orally administered at the volume of 1 ml/kg. After 2.5 h, 12.5 mg/kg AP (10 mg/kg of TP equivalent) was injected intravenously. About 120  $\mu$ l of arterial blood was collected into heparinized tubes at 0.5, 3, 5, 7 and 9 h after administration of AP and centrifuged to obtain plasma specimens. Urine was collected during 0—2, 2—4, 4—6, 6—12 and 12—24 h periods after dosing. Plasma and urine samples were stored at 4 °C until the following day and used to measure TP and its metabolites concentration.

Second, the effect of concomitantly intravenous administration of ST or M1 on the plasma disposition of TP was examined. Under anesthesia with isoflurane inhalation, polyethylene tubing (PE-50) was cannulated into the femoral artery and vein. ST or M1 dissolved in 20% polyethylene glycol 300 was intravenously injected at the dose of 80 mmol/kg (40 mg/kg of ST equivalent) concomitantly with 12.5 mg/kg AP. Blood samples were collected at 0.5, 1.5, 3, 4.5 and 6 h and treated as above.

For the pharmacokinetic analysis, the area under plasma concentration ( $C_p$ )—time profile (AUC), the elimination half-life ( $T_{1/2}$ ), the total body clearance ( $CL_{tot}$ ) and the volume of distribution at the steady state ( $V_{dss}$ ) of TP were calculated with the moment analysis using the method of Sato *et al.* (1996).<sup>5</sup> In our experiments, most of the administered TP was recovered in urine as TP, 1MU and DMU by 24 h, and 3MX was not detected. Therefore, the renal clearance of TP ( $CL_r$ ), the metabolic clearance of 1MU ( $CL_{1MU}$ ) and the metabolic clearance of DMU ( $CL_{DMU}$ ) were estimated as follows:

$$CL_r = CL_{tot} \cdot X_{u_{TP}} \quad (1)$$

$$CL_{1MU} = CL_{tot} \cdot X_{u_{1MU}} \quad (2)$$

$$CL_{DMU} = CL_{tot} \cdot X_{u_{DMU}} \quad (3)$$

where  $X_{u_{TP}}$ ,  $X_{u_{1MU}}$  and  $X_{u_{DMU}}$  are the urinary recovery ratio of TP, 1MU and DMU, respectively.

**Metabolism of TP *in Vitro*** Metabolism of TP was evaluated by DMU formation in rat-liver microsomes. Microsomal fractions were obtained from rat livers by a differential centrifugation technique according to Mizuki *et al.*<sup>6</sup> and stored at -20 °C. Protein concentration in microsomal fractions was determined by the Lowry method<sup>7</sup> using bovine serum albumin as standard. Incubation conditions of TP in liver microsomes were according to the reports of Zhang and Kaminsky<sup>8</sup> and Ha *et al.*<sup>9</sup> with minor modification. A reaction mixture (500  $\mu$ l) containing 0.1—1 mM TP, 0—0.4 mM ST or M1, 1.2 mM NADPH and 0.05 M Tris-HCl (pH 7.4) was prepared at 4 °C. After 3 min pre-incubation at 37 °C, reactions were initiated by adding microsomes (1 mg/tube) and after 5—60 min terminated by adding 0.5 ml of 2% ZnSO<sub>4</sub> at 4 °C. Samples were used immediately to measure DMU concentrations. MX was used as a positive control for the inhibition of TP metabolism to compare with ST and M1.

Based on the concentration of TP, DMU and inhibitors (ST, M1 or MX) in the reaction mixture, Lineweaver-Burk plot was carried out, and the maximum velocity ( $V_{max}$ ) and Michaelis constant ( $K_m$ ) of TP were evaluated.  $K_i$  values of inhibitors were calculated by the following equation:

$$K_i = K_m \cdot [I] / (K'_m - K_m) \quad (4)$$

where [I] is concentration of inhibitors, and  $K_m$  and  $K'_m$  is

Michaelis constant of TP in the absence and presence of inhibitors, respectively.

**Plasma Free Fraction of TP** Effect of ST on plasma free fraction (fp) of TP was determined *in vivo* and *in vitro*. To investigate fp *in vivo*, plasma samples at 5 h after intravenous administration of AP in rats were used, and ST was orally administered 2.5 h prior to AP injection. For fp *in vitro*, AP (10  $\mu$ g/ml) and ST (0—2500 ng/ml) were added to blank plasma. Free fractions in plasma were obtained by ultrafiltration method (Amicon Centrifree; Millipore Corporation, MA, U.S.A.). Samples were used immediately to measure TP concentration.

**Quantitative Determination of TP and Its Metabolites** TP concentration in plasma was measured by fluorescence polarization immunoassay (Abbott Laboratories, IL, U.S.A.). TP, DMU, 1MU and 1MX in urine and in microsomal fractions were analyzed by HPLC with UV detection according to Robson *et al.*<sup>10</sup>

**Statistical Analysis** Student's *t*-test was used for the statistical evaluation between control and ST groups after oral pretreatment with ST in pharmacokinetic study. Bonferroni's test in multiple comparison was used for the concomitantly intravenous administration *in vivo* and the metabolism study *in vitro*. Statistically significant differences were considered based on *p* values of <0.05.

## RESULTS

Time profiles of  $C_p$  of TP and urinary excretion of TP and its metabolites after intravenous administration of AP to rats with or without ST pretreatment are illustrated in Fig. 1 and Fig. 2, respectively. In Fig. 1,  $C_p$  of TP increased significantly 3, 5 and 7 h after AP administration. In control rats (Fig. 2A), the main compound excreted in urine was DMU, and 1MU and TP were also excreted but not 3MX. In ST-pretreated rats, urinary excretion of TP increased and that of DMU and 1MU decreased compared with control rats. Pharmacokinetic parameters were calculated based on these data (Table 1). AUC and  $T_{1/2}$  of TP increased significantly in ST-pretreated rats. Concerning clearance parameters,  $CL_{tot}$ ,  $CL_{1MU}$  and  $CL_{DMU}$  decreased to 52%, 25% and 40%, respectively, with ST pretreatment.  $CL_r$  did not change in ST-pretreated rats.

$C_p$ —time profiles and pharmacokinetic parameters of TP after concomitant intravenous administration with ST or M1 are shown in Fig. 3 and Table 2, respectively. In Fig. 3,  $C_p$  of TP in the ST group was slightly but not significantly higher than that in the control at all sampling points. In the M1 group,  $C_p$  of TP increased significantly from 1.5 h after concomitant administration compared with that in control and ST groups. In Table 2,  $CL_{tot}$  of TP in the ST group decreased to 88% of the control, however, this change was not a significant difference.  $CL_{tot}$  in the M1 group decreased significantly to 42% that of the control and to 48% that of the ST group. AUC and  $T_{1/2}$  in the M1 group increased significantly from that in ST and control groups. In contrast, there was no significant difference between ST and the control groups.  $V_{dss}$  of TP was almost equal among control, ST and M1 groups.

In metabolic experiments with liver microsomes *in vitro*, the duration of incubation was investigated preliminarily.

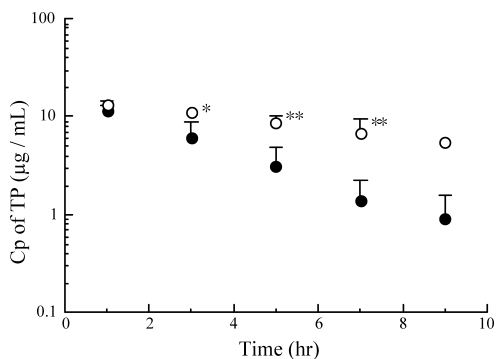


Fig. 1. Cp–Time Profile of TP after Intravenous Administration of AP (10 mg/kg of TP equivalent) in Rats with or without 2.5 h Pretreatment with Oral ST (100 mg/kg)

Each value presents the mean±S.D. of 3–4 experiments in control (open circles) and ST-treated (closed circles) rats. Statistical significance when compared with the control: \*  $p < 0.05$  and \*\*  $p < 0.01$ .

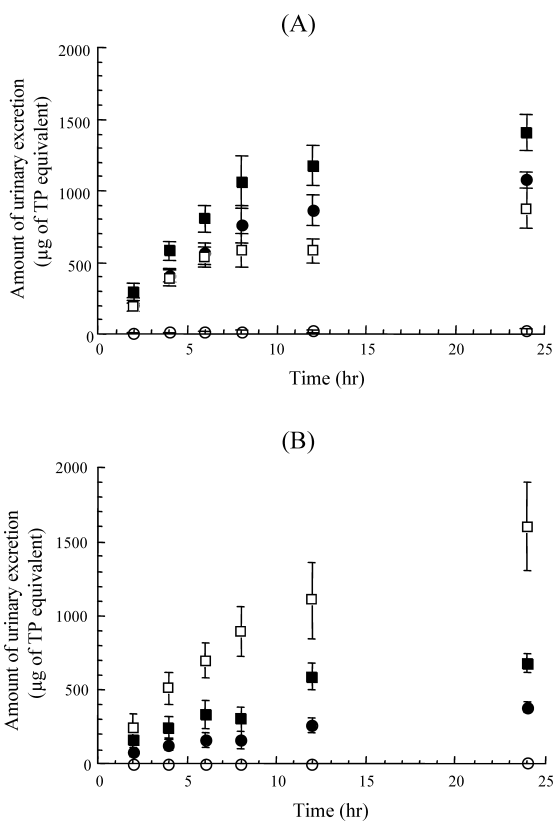


Fig. 2. Cumulative Amount of Excretion of TP (open squares), DMU (closed squares), 1MU (closed circles) and 3MX (open circles) in Urine after Intravenous Administration of AP (10 mg/kg of TP equivalent) in Rats with (B) or without (A) 2.5 h Pretreatment with Oral ST (100 mg/kg)

Each value presents the mean±S.E.M. of 3–5 experiments.

Linear formation of DMU in the presence or absence of ST or M1 was observed for 45 min, and the inhibitory ratio of ST and M1 did not depend on the incubation time (data not shown). Thus the incubation time was fixed as 30 min thereafter. Figures 4A and B show the Lineweaver–Burk plot for DMU formation in the presence or absence of ST and M1, respectively. A linear relationship between the inverse of substrate concentration and the inverse of the velocity of DMU formation was observed.  $V_{max}$  and  $K_m$  of TP were  $222 \pm 38$  pmol/min/mg protein and  $1.40 \pm 0.30$  mM, respectively

Table 1. Pharmacokinetic Parameters of TP after Intravenous Administration of AP (10 mg/kg of TP equivalent) in Rats with or without 2.5 h Pretreatment with Oral ST (100 mg/kg)

Parameters		Control	ST
AUC	( $\mu\text{g} \cdot \text{h/ml}$ )	$63.8 \pm 3.9$	$127.3 \pm 35.7^*$
$T_{1/2}$	(h)	$2.48 \pm 0.29$	$5.88 \pm 1.52^*$
CL <sub>tot</sub>	(ml/h/kg)	$162.2 \pm 15.6$	$84.1 \pm 27.2^*$
CL <sub>r</sub>	(ml/h/kg)	$41.8 \pm 11.3$	$51.7 \pm 16.1$
CL <sub>1MU</sub>	(ml/h/kg)	$53.6 \pm 8.6$	$13.3 \pm 9.2^*$
CL <sub>DMU</sub>	(ml/h/kg)	$70.3 \pm 7.6$	$28.1 \pm 14.8^*$

Each value presents the mean±S.D. of 3–4 experiments. Statistical significance when compared with the control: \*  $p < 0.05$ .

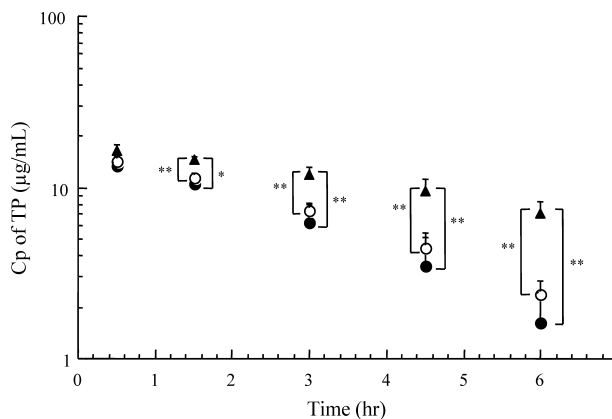


Fig. 3. Cp–Time Profile of TP after Intravenous Administration of AP (10 mg/kg of TP equivalent) in Rats with or without Concomitantly Intravenous Administration of ST or M1 (40 mg/kg of ST equivalent)

Each value presents the mean±S.D. of 3 experiments in control (closed circles), ST (open circles) and M1 (closed triangles) rats. Statistical significance when compared with the control: \*  $p < 0.05$  and \*\*  $p < 0.01$ .

Table 2. Pharmacokinetic Parameters of TP after Intravenous Administration of AP (10 mg/kg of TP equivalent) in Rats with or without Concomitant Intravenous Administration of ST or M1 (40 mg/kg of ST equivalent)

Parameters	Control	ST	M1	
AUC	( $\mu\text{g} \cdot \text{h/ml}$ )	$46.4 \pm 11.3$	$54.6 \pm 6.9$	$116 \pm 15^*$
$T_{1/2}$	(h)	$1.49 \pm 0.24$	$1.84 \pm 0.18$	$3.98 \pm 0.78^*$
CL <sub>tot</sub>	(ml/h/kg)	$223 \pm 148$	$185 \pm 22$	$87.3 \pm 12.4^*$
V <sub>dss</sub>	(ml/kg)	$535 \pm 45$	$529 \pm 30$	$509 \pm 35$

Each value presents the mean±S.D. of 3 experiments. Statistical significance when compared with control and ST groups: \*  $p < 0.05$ .

( $n=3$ , mean±S.D.). In both panel A and B of Fig. 4, ST and M1 inhibited DMU formation in a competitive manner.  $K_i$  values of ST and M1 were  $822 \pm 162 \mu\text{M}$  and  $731 \pm 182 \mu\text{M}$ , respectively ( $n=3$ , mean±S.D.). MX was also tested in this study as a positive control. Competitive inhibition for DMU formation by MX was observed, and its  $K_i$  value was about  $494 \mu\text{M}$ .

Effect of ST on fp of TP was investigated both *in vivo* and *in vitro*. Values of fp in control and ST-pretreated rats *in vivo* were  $37.4 \pm 4.2\%$  and  $35.8 \pm 6.6\%$ , respectively ( $n=3$ , mean±S.D.), and there was no significant difference between these two groups. Values of fp in the presence of 0, 100, 500 and 2500 ng/ml of ST *in vitro* were  $26.8 \pm 2.6\%$ ,  $26.1 \pm 2.4\%$ ,  $26.3 \pm 5.0\%$  and  $26.3 \pm 6.8\%$ , respectively ( $n=3$ , mean±S.D.), and there was no significant difference among these

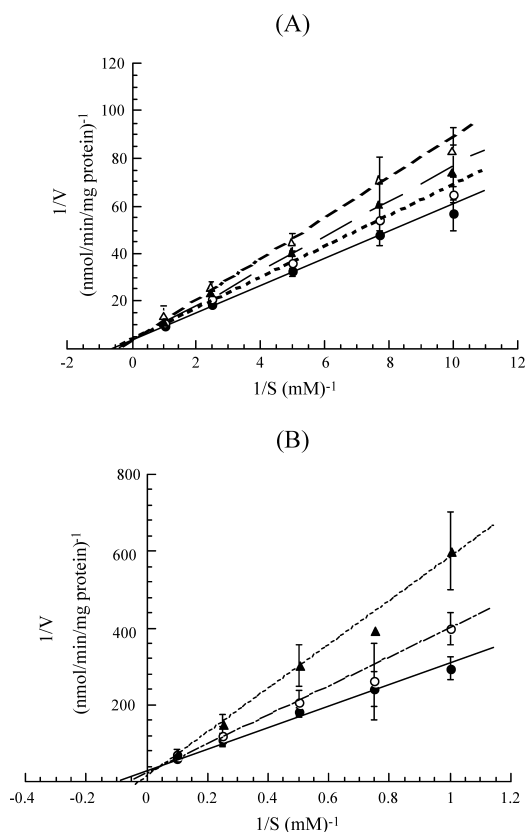


Fig. 4. Lineweaver-Burk Plot Showing Inhibition of Microsomal DMU Formation from TP by ST (A) and M1 (B)

S: TP concentration, V: the velocity of DMU formation. In panel A, control (closed circles), 0.1 mM ST (open circles), 0.2 mM ST (closed triangles), and 0.4 mM ST (open triangles). In panel B: control (closed circles), 0.2 mM M1 (open circles), and 0.4 mM M1 (closed triangles). Each value presents the mean  $\pm$  S.D. of 3 experiments.

groups.

## DISCUSSION

In the present study, the effect of ST on plasma disposition and metabolism of TP in rats was investigated *in vivo* and *in vitro*. In the pharmacokinetic study *in vivo*, AUC of TP increased 2-fold, and its CL<sub>tot</sub> decreased 48% in ST-pretreated rats (Table 1). To elucidate this ST-induced delay of TP elimination from plasma, CL<sub>r</sub> and metabolic clearance of TP were estimated separately based on urinary excretion. CL<sub>1MU</sub> and CL<sub>DMU</sub> decreased to 25% and 40%, however, CL<sub>r</sub> was not changed significantly by ST pretreatment. In the metabolic pathway of TP in rats, it was reported that 30–40% and 15–32% of administered TP was metabolized to DMU and 1MX, respectively, and 3MX was barely detectable.<sup>11,12</sup> Therefore, metabolic pathways from TP to DMU and 1MU can be interfered by ST and/or its metabolites. Details in pharmacokinetic profiles of ST and its metabolites in rats were reported as follows.<sup>13,14</sup> After oral administration, 47–70% of administered ST can be detected in systemic blood as unchanged ST or metabolites, however, the bioavailability of ST itself was only 5%, suggesting the formation of M1 and other metabolites in the first-pass process. ST appeared in plasma immediately after oral administration and was eliminated with 2.8 h T<sub>1/2</sub>. M1 and other metabolites, ( $\pm$ )-4-(3-ethoxy-2-hydroxypropoxy)acetanilide, cysteine conju-

gate of M1, ( $\pm$ )-4-(3-ethoxy-2-hydroxypropoxy)-[3-(methylsulfinyl)propion]anilide, glutathione conjugate of M1, and mercapturic acid conjugate of M1, were detected from 2 h after administration and remained in plasma due to enterohepatic recirculation. Our findings and these reports suggested the involvement of M1 and/or other metabolites in inhibition of TP metabolism.

To compare ST and M1 *in vivo*, concomitant intravenous administration of AP in rats was performed. In the ST group, Cp and pharmacokinetic parameters of TP did not change significantly (Fig. 3, Table 2). In contrast, M1 induced a 150% increase in AUC and a 60% decrease in CL<sub>tot</sub> (Table 2). Shindo *et al.*<sup>14</sup> reported plasma disposition of ST and M1 after intravenous administration to rats. In the case of ST, T<sub>1/2</sub> in the elimination phase was 3.7 h, and only a low level of M1 was detected among metabolites. After injection of M1, Cp of M1 decreased faster than ST (T<sub>1/2</sub>: 0.24 h), and other metabolites appeared in the plasma. Therefore, M1 and/or the other metabolites may have a stronger effect than ST to inhibit TP metabolism in an *in vivo* situation. This hypothesis based on concomitant intravenous administration can explain our findings in the previous experiment of pretreatment with oral ST.

Increase in Cp of TP associated with ST was observed in *in vivo* studies, and inhibition of TP metabolism by ST and its metabolites was suggested. To clarify the mechanism of this drug interaction between ST and TP, the effect of ST and M1 on TP metabolism *in vitro* was evaluated using rat-liver microsomes. In preliminary study, the inhibitory ratio of ST and M1 for TP metabolism did not depend on the incubation time, suggesting that mechanism-based inhibition may not occur at least under our experimental conditions. Competitive inhibition by ST and M1 was observed (Fig. 4), and their K<sub>i</sub> values were 822  $\mu$ M and 731  $\mu$ M, respectively. In hepatic metabolism of TP, the involvement of cytochrome P-450 was reported: 80–90% of *N*-demethylation, the first step from TP to 1MU, and 50% of 8-hydroxylation, the step from TP to DMU, was catalyzed by CYP1A2, and the remainder of the 8-hydroxylation was by CYP2E1 and CYP3A.<sup>15,16</sup> DMU formation was measured in our microsomal study *in vitro*, and a decrease in CL<sub>1MU</sub> and CL<sub>DMU</sub> was observed in the pharmacokinetic studies *in vivo*. Therefore, ST and M1 may have an inhibitory effect on CYP1A2 and CYP2E1/CYP3A. In comparison with *in vivo* findings after concomitant intravenous injection, the inhibitory potency of ST and M1 in microsomal fractions was almost the same. However, ST did not suppress TP metabolism significantly after concomitant administration *in vivo*. The reason for this discrepancy could not be clarified in this study but may be due to the difference in distribution from blood to the liver between ST and M1.

The tissue concentration of inhibitors *in vivo* is also important in evaluating drug interactions associated with metabolic inhibition. Distribution data of radiolabeled ST was reported for the hepatic concentration of ST and its metabolites in rats.<sup>17</sup> In that report, <sup>14</sup>C-ST was administered orally at a dose of 100 mg/kg, and total concentration including metabolites in the liver during 2–8 h after dosing was about 20  $\mu$ g/ml. This value can be equivalent to 61–75  $\mu$ M based on the molecular weights of ST and M1 (328 and 265, respectively) and is less than the K<sub>i</sub> values of ST and M1 (822  $\mu$ M and 731  $\mu$ M, respectively) observed in our *in vitro*

experiments. This difference between the estimated level *in vivo* from the literature and our observed values *in vitro* could not be explained based on our findings. Possibilities of overestimation of  $K_i$  values under our experimental conditions and/or more potent inhibition by other metabolites should be considered.

In the interaction between doqualast, an anti-allergic drug, and TP, the change in plasma protein binding was a dominant mechanism.<sup>2)</sup> Therefore, the effect of ST on fp of TP was also examined in this study. As true in our results, fp of TP did not change *in vivo* or *in vitro*. The values of fp of ST and M1 are reported to be 0% and 66%, respectively.<sup>14)</sup> Possibility for the change in plasma protein binding can thus be excluded from mechanisms of this interaction between ST and TP.

Finally, clinical implications of this interaction between TP and ST should be considered. In humans, the therapeutic range of TP in the serum is 10–20  $\mu\text{g/ml}$ .<sup>3)</sup> In our study, TP was investigated at the same level as the clinical situations (Figs. 1 and 3). Concerning ST, the maximal plasma concentration ( $C_{\text{max}}$ ) of ST and M1 in rats after oral administration at the same 100 mg/kg was 2.33  $\mu\text{g/ml}$  (7.10  $\mu\text{M}$ ) and 122 ng/ml (460 nM), respectively.<sup>14)</sup> In contrast,  $C_{\text{max}}$  of ST and M1 in humans at the clinical dose was 43.2 ng/ml (131 nM) and 17.1 ng/ml (64.5 nM), respectively.<sup>18)</sup> ST and M1 in our experiments *in vivo* were thus examined at a higher concentration than their therapeutic level. At an M1 concentration 7-fold higher than that in humans, however, significant decrease of metabolic clearance of TP in ST-pretreated rats (25–40% of the control) was observed in this study (Table 1). Considering clinically practical situations for the possible occurrence of overdosing and/or slow metabolism of ST and M1, this interaction between TP and ST may not be clinically significant but should nonetheless be taken into account.

In conclusion, this is the first report on pharmacokinetic interaction between ST and TP *in vivo* and *in vitro*. Inhibition

of TP metabolism by M1 and/or other metabolites is suggested to be the dominant mechanism for the increase in Cp of TP associated with ST administration.

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