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Safety and Pharmacokinetics of High-Dose TAS-303 in Healthy Japanese Volunteers: A Single-Center, Single-Blind, Randomized, Placebo-Controlled, Parallel-Group, Multiple-Ascending-Dose Study

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Abstract

Preclinical data of TAS-303 (4-piperidinyl 2,2-diphenyl-2-[propoxy-1,1,2,2,3,3,3-d_7] acetate hydrochloride), a noradrenaline reuptake inhibitor, show that it increases urethral contraction in rats and may therefore benefit stress urinary incontinence patients. In this single-blind, randomized, placebo-controlled, parallel-group, multiple-ascending-dose phase 1 study, we evaluated the safety and tolerability of once-daily TAS-303 8, 10, 12, 15, or 18 mg administered for 16 days in healthy subjects. In addition, we investigated the pharmacokinetics and inhibitory effect of TAS-303 on hepatic cytochrome P450 (CYP) 3A activity. Rates of adverse events, adverse drug reactions, and pharmacokinetic parameters of TAS-303 were evaluated. Fifty subjects were randomized: 7 subjects each were assigned to receive TAS-303 8-18 mg, and 3 subjects each were assigned to receive placebo at each dose. The overall incidences of adverse events and adverse drug reactions in all subjects administered TAS-303 (n = 35) was 25.7% and 2.9%, respectively, and those for the placebo groups (n = 15) were 46.7% and 0%, respectively. No deaths or serious adverse events occurred. TAS-303 displayed a dose-proportional pharmacokinetic profile across doses of 8-18 mg over the 16-day multiple administration period, and TAS-303 might inhibit hepatic CYP3A activity within this dose range. TAS-303 at a dose of 8-18 mg was confirmed to be safe and tolerable.

Keywords

pharmacokinetics, phase 1 study, safety, TAS-303, multiple-ascending-dose study

Urinary incontinence has been reported in 13.1% of women, for whom stress urinary incontinence (SUI; 6.4%) is the most common type (48.9%).[1] SUI is the observation of involuntary leakage from the urethral orifice synchronous with effort or physical exertion or on sneezing or coughing.[2,3] This condition severely compromises the quality of life of women worldwide.[4] Therefore, treatment of SUI is expected to have a notable social impact on women. The development of effective and safe drugs for SUI is currently awaited.

TAS-303 (4-piperidinyl 2,2-diphenyl-2-[propoxy-1,1,2,2,3,3,3-d_7] acetate hydrochloride) is a noradrenaline reuptake inhibitor developed by Taiho Pharmaceutical Co., Ltd.; its structural formula is shown in Figure 1. In an ex vivo study,[5] TAS-303 increased urethral contraction concentration-dependently in rats. In an in vivo study, TAS-303 (0.3, 1, and 3 mg/kg) increased urethral pressure as well as leak point pressure in a dose-dependent manner in rats.[5] Based on these results, TAS-303 is expected to contribute to the remission of incontinence and

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[Correction added on 8 July 2020, after first online publication: a comma is added between "Parallel-Group" and "Multiple-Ascending" in the title.]

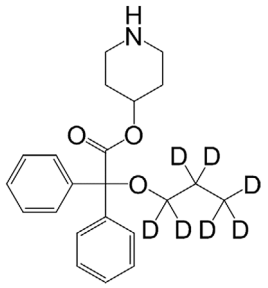

Figure 1. Structural formula of TAS-303 (4-piperidinyl 2,2-diphenyl-2-[propoxy-1,1,2,2,3,3,3-d_7] acetate hydrochloride). D, deuterium.

improve the quality of life in SUI patients by selectively inhibiting the reuptake of noradrenaline in the lower urinary system, thereby increasing urethral resistance.

A single-dose phase 1 study was conducted in healthy subjects, the results of which suggested that single oral administration of TAS-303 was safe at doses of 0.25, 0.75, 2, 6, 12, and 18 mg. A repeated-dose phase 1 study was conducted in healthy subjects, and it confirmed the safety and tolerability of TAS-303 at doses of 1, 3, and 6 mg when it was repeatedly administered once daily for 16 days. Based on these 2 studies, the pharmacokinetic (PK) profile of TAS-303 was confirmed as linear within a single-administration dose range of 0.25 to 18 mg and within the repeated-administration dose range of 1 to 6 mg. Furthermore, TAS-303 was confirmed to be safe within these tested dose ranges. However, the safety and PK of TAS-303 administered in repeated doses greater than 6 mg have not yet been investigated.

Based on the results of nonclinical studies investigating drug interactions using human liver microsomes, TAS-303 was found to inhibit midazolam 1′-hydroxylation mediated by cytochrome P450 (CYP) 3A in a time-dependent manner with a half-maximal inactivation of 1024 ng/mL and a maximal inactivation rate constant of 4.68 h^{-1} (unpublished data). A drug-drug interaction study was conducted to evaluate drug interactions between this compound and CYP3A substrate medicines.[6] In this study, interactions between TAS-303 3 mg and oral simvastatin 5 mg or intravenous midazolam 1 mg were investigated in healthy subjects. The results showed that TAS-303 had a weak inhibitory effect on CYP3A, and the inhibition was limited to small intestinal CYP3A; thus, it was concluded that TAS-303 3 mg had no effect on hepatic CYP3A activity. The effects of TAS-303 on hepatic CYP3A activity administered in multiple doses greater than 3 mg have not yet been investigated.

We conducted the present study, which was aimed primarily at evaluating the safety and tolerability of TAS-303 given as multiple oral doses of 8 to 18 mg once daily for 16 days, in healthy subjects. The secondary objectives were: (1) to evaluate the PK of TAS-303 following multiple oral doses of 8 mg or more and (2) to investigate the effects of TAS-303 on hepatic CYP3A activity within the dose range of 8 to 18 mg by reference to a study in which 6β-hydroxylation metabolic clearance of intrinsic cortisol was used.[7]

Subjects and Methods

Study Design

This was a single-blind, randomized, placebo-controlled, parallel-group, multiple-ascending-dose phase 1 study conducted in SOUSEIKAI Sumida Hospital in Tokyo, Japan, from March 14, 2017, to December 23, 2017. Initially, this study was planned to be conducted in 3 steps (with TAS-303 administered at doses of 8, 12, and 18 mg). However, on further consideration by the study investigators, it was determined that the dose increments for subsequent steps should be reduced for safety reasons because the mean accumulation factor (R) of the area under the curve from time zero to 24 hours ($AUC_{0\text{-}24\,h}$) on day 16 versus day 1 of multiple doses of 8 mg in step 1 exceeded 5.5. The common ratio for the incremented dose of TAS-303 for the subsequent step was reduced from 1.5 to 1.25. As a result, the study was conducted in 5 steps. TAS-303 or placebo was repeatedly administered once daily at doses of 8, 10, 12, 15, or 18 mg under fed conditions (after breakfast) in a single-blind manner in steps 1, 2, 3, 4, and 5, respectively. Subjects fasted for 4 hours after dosing and were not allowed to drink water during the same period except at the time of dosing. In addition, they were not allowed to lie down for 4 hours after dosing unless needed for examinations. The study design and procedures in each step are shown in Supplementary Figure 1. In each step, subjects who had provided written informed consent and had been deemed eligible after screening (days −28 to −3) were hospitalized at the study site on day −2 (2 days before administration) and received the study drug once daily 30 minutes after breakfast for 16 days (days 1 to 16). After 72 hours of observation following the last dose on day 16, subjects were discharged from the study site on day 19. Subjects returned to the study site for follow-up twice in total, once between 7 and 11 days after the last dose (days 23 to 27) and once between 14 and 21 days after the last dose (days 30 to 37). If an adverse event (AE) occurred before completion of the follow-up period, a follow-up examination was performed until the symptom (including laboratory abnormalities) was confirmed to have recovered to the preonset status or to be resolving.

The dose increments were determined by comparing the predicted maximum concentration in plasma

(C_{max}) and AUC in the no-observed-adverse-effect level (NOAEL) and lowest-observed-adverse-effect level (LOAEL) in a 13-week multiple-dose toxicity study in rats and dogs (unpublished data).

This study was approved by the Hakata Clinic Institutional Review Board. The study protocol was created in accordance with the ethical principles that have their origin in the Declaration of Helsinki, the Pharmaceutical and Medical Device Act, and the Ministerial Ordinance on Good Clinical Practice for Drugs. All subjects provided written informed consent prior to initiation of the study.

Study Subjects

Subjects who met all the following inclusion criteria were included in the study: male subjects aged 20 to <40 years at the time of informed consent with a body weight of ≥50 kg and body mass index between 18.5 and <25.0 kg/m^2 at screening, and deemed healthy by the investigators based on physical findings (subjective symptoms and objective findings), blood pressure, pulse rate, body temperature, 12-lead electrocardiogram, and laboratory test results at screening and baseline.

Subjects who met any of the following criteria were excluded from the study: previous medical history, such as severe hepatic, renal/urological, cardiovascular, respiratory, gastrointestinal, neuropsychiatric, immunological, or metabolic/endocrine disease, blood dyscrasia, or malignancy; current or previous drug abuse or alcoholism; previous history of angle-closure glaucoma, ileus or abdominal operation or dysuria; current or previous hypersensitivity or allergy to drugs; risk factors for torsade de pointes; previous treatment with TAS-303 hydrochloride; treatment with another study drug within 180 days before study drug administration; tested positive for hepatitis B surface antigen, hepatitis C virus antibody, HIV antigen/antibody, syphilis, or urine drug test during screening; or deemed ineligible for the study by the investigator for any other reason.

Interventions

Subjects were randomly assigned to TAS-303 and placebo at a ratio of 7:3 in each step and received multiple doses of the study drug in a single-blind manner. TAS-303 8, 10, 12, 15, or 18 mg (steps 1, 2, 3, 4, and 5, respectively) or placebo was repeatedly administered once daily under fed conditions for 16 days to subjects. On the basis of the results of safety and pharmacokinetics in each step, the sponsor discussed the dose increment for the subsequent steps with the investigator and decided to increase the dose of TAS-303 using a common ratio of 1.25. The study drug was taken with 150–-250 mL of water 30 minutes after breakfast. Concomitant use of the following medications and therapies was prohibited, except in case of emergency or if considered necessary by investigators: any medications, study drugs, supplements other than the study drug, or foods/beverages containing grapefruit or St. John's Wort from 7 days before the initial dose to the second follow-up visit; alcoholic and caffeine-containing beverages from 3 days before the initial dose to the second follow-up visit; and vaccinations from screening to the second follow-up visit.

Randomization and Blinding

The study drug allocation manager generated random numbers corresponding to subject ID codes in each step using the data analysis and random number generation functions of Microsoft Office Excel. As this study was conducted in a single-blind manner, during the study, subjects did not know the type of study drug they received. The study drug allocation manager confirmed that all study drugs were identical in appearance (shape and color) before and after allocation.

End Points

The primary end point was the occurrence of AEs and adverse drug reactions (ADRs). The secondary end points were PK parameters of plasma TAS-303 concentration, including the time course of mean plasma concentration of TAS-303 and R for C_{max} and $AUC_{0\text{-}24\ h}$ (day 16/day 1).

As the formation of hydroxycortisol (6β-OHF) from cortisol is catalyzed by hepatic CYP3A, the 6β-OHF/cortisol ratio and the formation clearance (CL_f) of 6β-OHF have been utilized as endogenous hepatic CYP3A activity markers. Changes in the urinary 6β-OHF/cortisol ratio and CL_f of 6β-OHF from baseline, which can reflect the impact of the addition of TAS-303 (inhibitory effect) on hepatic CYP3A, were evaluated to investigate the inhibitory effect of TAS-303 on hepatic CYP3A.[7]

For measuring plasma TAS-303 concentrations, on day 1, blood was collected immediately before dosing and 1, 2, 4, 6, 8, 12, and 24 (day 2) hours after dosing. On days 9 to 15, blood was only collected immediately before dosing. On day 16, blood was collected immediately before dosing and 1, 2, 4, 6, 8, 12, 24 (day 17), 48 (day 18), and 72 (day 19) hours after dosing. In addition, blood was collected at the first follow-up visit (between days 23 and 27). At each blood sampling time, 3 mL of whole blood was collected per sample. In total, 78 mL of blood was collected per subject in this study for measuring TAS-303 concentrations.

For measuring the urinary excretion of cortisol and 6β-OHF, time-matched urine pooling was performed on the day before study drug administration (day −1) and 16 days after the start of study drug administration (day 16).

Assay Method

Concentrations of TAS-303 in plasma were determined by validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) assays. Plasma samples were spiked with stable isotope-labeled internal standard and were subjected to liquid-liquid extraction using dichloromethane. After the organic layer was evaporated under a stream of nitrogen gas, the residue was dissolved in 10 mmol/L ammonium acetate (pH 4.2)/methanol (70:30, v/v). The processed sample was injected into an LC-MS/MS system, equipped with Capcell Pak C18 UG120 (2.0 mm i.d. × 150 mm, particle size 5 μm; Shiseido Co., Ltd., Tokyo, Japan) as the analytical column, using methanol and 10 mmol/L ammonium acetate solution (pH 4.2) as the mobile phase. Electrospray ionization was performed in positive ion detection mode with a multiple reaction monitoring (MRM) mode. MRM transitions were m/z 361 to 294 for TAS-303 and m/z 364 to 304 for the internal standard. The lower limit of quantification for TAS-303 in plasma was 0.2 ng/mL. Within- and between-day variability (% coefficient of variation) was ≤2.2% and ≤4.1%, respectively.

Concentrations of cortisol in plasma and urinary concentrations of cortisol and 6β-OHF were determined by validated LC-MS/MS assays. Plasma and urine samples were spiked with cortisol-d4 and 6β-OHF-d4 as internal standards and were subjected to solid-phase extraction. After the eluate was evaporated, the reconstituted sample was injected into an LC-MS/MS system, equipped with XBridge C18 (2.1 mm i.d. × 50 mm, particle size 2.5 μm; Waters Corp., Milford, Massachusetts) as the analytical column, using acetonitrile/formic acid (1000:1, v/v) and 10 mmol/L aqueous ammonium formate/formic acid (1000:1, v/v) as the mobile phase. Electrospray ionization was performed in negative ion detection mode with an MRM mode. MRM transitions were m/z 407 to 331 for cortisol, m/z 411 to 335 for cortisol-d4, m/z 423 to 347 for 6β-OHF, and m/z 427 to 351 for 6β-OHF-d4. The lower limits of quantification were 2 ng/mL for cortisol and 4 ng/mL for 6β-OHF. Within-day variability was ≤2.6% for cortisol and ≤1.6% for 6β-OHF. Between-day variability (% coefficient of variation) was ≤4.7% for cortisol and ≤3.9% for 6β-OHF.

Statistical Analysis

The target sample size of a maximum of 60 subjects was not statistically determined. The safety analysis set was defined as all randomized subjects who received at least 1 dose of the study drug. The PK analysis set was defined as all subjects in the safety analysis set who were eligible for the study, did not use prohibited concomitant medications and therapies, and were evaluated for PK. Descriptive statistics were used for baseline demographic and clinical characteristics, with n (%) for categorical variables and mean ± standard deviation (SD) for continuous variables. The incidence of individual AEs, ADRs, and the incidence by severity were calculated in the safety analysis set. PK parameters were calculated and summarized using the measured concentrations of TAS-303, plasma cortisol, urinary cortisol, and urinary 6-OHF, and urine volume, as appropriate, in the PK analysis set. In the tabulation of AEs, the diagnoses described on the eCRFs were coded by System Organ Class and Preferred Term using the Medical Dictionary for Regulatory Activities version 20.1.

The statistical software used for the statistical analysis was SAS version 9.4 (SAS Institute Inc., Cary, North Carolina). SAS version 9.4 (SAS Institute Inc., Cary, North Carolina) was also used for the safety analysis. For PK analyses, Phoenix WinNonlin version 7.0 (Certara LP, Princeton, New Jersey) was used for calculations of PK parameter, and SAS version 9.2 (SAS Institute Inc., Cary, North Carolina) for dose-dependent analyses. JMP 13.2.0 (SAS Institute Inc., Cary, North Carolina) was used to perform the analysis of covariance.

Results

Subjects

The disposition of subjects is shown in Figure 2. A total of 129 subjects provided consent and underwent screening, and 79 subjects dropped out at screening. Fifty subjects were randomized. Of these, 7 subjects each (a total of 35) were assigned to receive 8, 10, 12, 15, or 18 mg (steps 1, 2, 3, 4, or 5, respectively) of TAS-303, and 3 subjects each (a total of 15) were assigned to receive placebo in each step. All subjects took the study drug as planned, and no subjects discontinued the study.

Because all randomized subjects received at least 1 dose of the study drug, 50 subjects were included in the safety analysis set. Of the 50 subjects in the safety analysis set, 1 subject who vomited after administration of TAS-303 10 mg was excluded from the PK analysis set as it affected the evaluation, and 49 subjects were included in the PK analysis set (TAS-303 group: 34 subjects [7 subjects each receiving 8, 12, 15, and 18 mg and 6 subjects receiving 10 mg]; placebo group: 15 subjects [3 subjects each receiving each dose]).

Demographic and clinical characteristics were well balanced between the 2 treatment groups (Table 1). The mean age of subjects was 27.5 and 28.5 years in the TAS-303 and placebo groups, respectively. Most patients in both groups had no medical history and received no concomitant medication (94.3% and 93.3%, respectively).

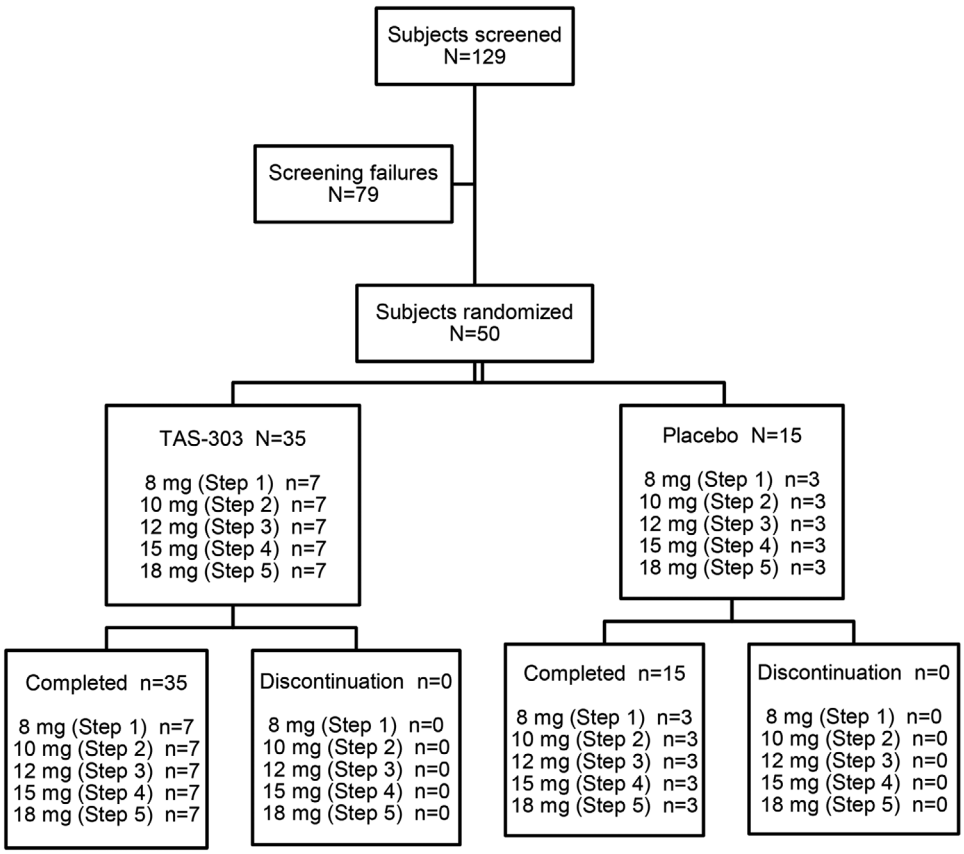

Figure 2. Flow diagram of subject disposition.

Table 1. Baseline Demographic and Clinical Characteristics

	TAS-303 n = 35	Placebo n = 15
Sex, male	35 (100.0)	15 (100.0)
Age, years	27.5 ± 4.4	28.5 ± 6.3
	27.0 (20-38)	28.0 (20-39)
Height, cm	172.28 ± 5.56	172.50 ± 5.24
	171.5 (159.2-181.3)	171.2 (165.6-183.3)
Weight, kg	62.99 ± 7.19	64.20 ± 7.31
	61.2 (51.9-77.7)	63.8 (52.5-76.9)
Body mass index, kg/m^2	21.19 ± 1.84	21.53 ± 1.84
	20.6 (19.0-24.9)	21.6 (18.5-24.6)
No medical history	33 (94.3)	14 (93.3)
No concomitant medication	33 (94.3)	14 (93.3)

Data are presented as n (%), mean ± standard deviation, or median (range).
Safety analysis set.

Safety

The results of the primary end point are shown in Table 2. The incidence of AEs was comparable in the TAS-303 (8, 10, 12, 15, and 18 mg) and placebo groups, and no increase in the incidence of AEs was observed with increasing dose.

In the TAS-303 groups (n = 35), the overall incidence of AEs was 25.7%, and in the placebo group (n = 15), the incidence of AEs was 46.7%. The most common AE (frequency ≥ 10%) was diarrhea, with an incidence of 14.3% in the TAS-303 groups and 26.7% in the placebo group. There was only 1 case of a treatment-related AE (diarrhea), in a patient administered TAS-303 18 mg (2.9%). No treatment-related AEs occurred in the placebo group. This event of diarrhea was mild, resolved in 241 hours and 30 minutes without treatment and did not lead to treatment discontinuation. No death or serious AEs were reported, and no safety concern was raised in the study.

Pharmacokinetics

The mean plasma concentration of TAS-303 increased with dose and reached a steady state between day 11 and day 13 (Figure 3). The plasma TAS-303 concentration accumulated by repeated administration of once-daily doses of 8, 10, 12, 15, and 18 mg, and the Rs for C_{max} and $AUC_{0\text{-}24\ h}$ were both consistent, regardless of the dose (R [C_{max}], 5.01 to 6.00; R [$AUC_{0\text{-}24\ h}$], 5.64 to 6.77; Table 3). The dose-proportionality of PK

Table 2. Incidence of Adverse Events and Adverse Drug Reactions

		Adverse Events				Adverse Drug Reactions			
Dose (Step)	Preferred Term, MedDRA (version 20.1)	TAS-303 n (%)	95%CI	Placebo n (%)	95%CI	TAS-303 n (%)	95%CI	Placebo n (%)	95%CI
8 mg (step 1)		n = 7		n = 3		n = 7		n = 3	
	Any adverse event	0 (0.0)	0.0-41.0	0 (0.0)	0.0-70.8	0 (0.0)	0.0-41.0	0 (0.0)	0.0-70.8
10 mg (step 2)		n = 7		n = 3		n = 7		n = 3	
	Any adverse event	3 (42.9)	9.9-81.6	2 (66.7)	9.4-99.2	0 (0.0)	0.0-41.0	0 (0.0)	0.0-70.8
	Diarrhea	1 (14.3)	0.4-57.9	2 (66.7)	9.4-99.2	0 (0.0)	0.0-41.0	0 (0.0)	0.0-70.8
	Vomiting	1 (14.3)	0.4-57.9	0 (0.0)	0.0-70.8	0 (0.0)	0.0-41.0	0 (0.0)	0.0-70.8
	Wound	0 (0.0)	0.0-41.0	1 (33.3)	0.8-90.6	0 (0.0)	0.0-41.0	0 (0.0)	0.0-70.8
	Headache	1 (14.3)	0.4-57.9	0 (0.0)	0.0-70.8	0 (0.0)	0.0-41.0	0 (0.0)	0.0-70.8
	Boredom	1 (14.3)	0.4-57.9	0 (0.0)	0.0-70.8	0 (0.0)	0.0-41.0	0 (0.0)	0.0-70.8
12 mg (step 3)		n = 7		n = 3		n = 7		n = 3	
	Any adverse event	1 (14.3)	0.4-57.9	1 (33.3)	0.8-90.6	0 (0.0)	0.0-41.0	0 (0.0)	0.0-70.8
	Nasopharyngitis	0 (0.0)	0.0-41.0	1 (33.3)	0.8-90.6	0 (0.0)	0.0-41.0	0 (0.0)	0.0-70.8
	Otitis media	1 (14.3)	0.4-57.9	0 (0.0)	0.0-70.8	0 (0.0)	0.0-41.0	0 (0.0)	0.0-70.8
	Upper respiratory tract infection	1 (14.3)	0.4-57.9	0 (0.0)	0.0-70.8	0 (0.0)	0.0-41.0	0 (0.0)	0.0-70.8
	Aspartate aminotransferase increased	1 (14.3)	0.4-57.9	0 (0.0)	0.0-70.8	0 (0.0)	0.0-41.0	0 (0.0)	0.0-70.8
	Blood creatine phosphokinase increased	1 (14.3)	0.4-57.9	0 (0.0)	0.0-70.8	0 (0.0)	0.0-41.0	0 (0.0)	0.0-70.8
	C-reactive protein increased	1 (14.3)	0.4-57.9	0 (0.0)	0.0-70.8	0 (0.0)	0.0-41.0	0 (0.0)	0.0-70.8
	Acne	1 (14.3)	0.4-57.9	0 (0.0)	0.0-70.8	0 (0.0)	0.0-41.0	0 (0.0)	0.0-70.8
15 mg (step 4)		n = 7		n = 3		n = 7		n = 3	
	Any adverse event	1 (14.3)	0.4-57.9	2 (66.7)	9.4-99.2	0 (0.0)	0.0-41.0	0 (0.0)	0.0-70.8
	Oropharyngeal pain	1 (14.3)	0.4-57.9	1 (33.3)	0.8-90.6	0 (0.0)	0.0-41.0	0 (0.0)	0.0-70.8
	Rhinorrhea	0 (0.0)	0.0-41.0	1 (33.3)	0.8-90.6	0 (0.0)	0.0-41.0	0 (0.0)	0.0-70.8
18 mg (step 5)		n = 7		n = 3		n = 7		n = 3	
	Any adverse event	4 (57.1)	18.4-90.1	2 (66.7)	9.4-99.2	1 (14.3)	0.4-57.9	0 (0.0)	0.0-70.8
	Diarrhea	4 (57.1)	18.4-90.1	2 (66.7)	9.4-99.2	1 (14.3)	0.4-57.9	0 (0.0)	0.0-70.8
	Tonsillitis	1 (14.3)	0.4-57.9	0 (0.0)	0.0-70.8	0 (0.0)	0.0-41.0	0 (0.0)	0.0-70.8

CI, confidence interval.
Safety analysis set.

parameters (C_{max} and $AUC_{0\text{-}24\,h}$) following multiple-administration (day 16) was evaluated using linear regression with the following power model (log [PK parameter] = a + b × log [dose], where a is the intercept and b is the slope). Regression curves obtained by power model analysis and 95% confidence intervals are shown in Figure 4. The C_{max} and $AUC_{0\text{-}24\,h}$ of TAS-303 after multiple doses met the acceptance criteria (95% confidence interval for the estimate of b contained 1: 0.6579-1.1491 for C_{max} and 0.6725-1.1745 for $AUC_{0\text{-}24\,h}$) for dose proportionality, and the plasma exposure of TAS-303 was demonstrated to increase dose-proportionally in the range of 8 to 18 mg.

Effect on Hepatic CYP3A Activity

Analysis of covariance was performed to statistically evaluate the effects of TAS-303 given as multiple doses on the urinary 6β-OHF/cortisol ratio and CL_f, with the baseline value as a covariate. The arithmetic mean (on day -1 and day 16) and geometric least-squares mean of the urinary 6β-OHF/cortisol ratio (treatment/placebo) on day 16 and that of CL_f of 6β-OHF are shown in Tables 4 and 5, respectively. Significant decreases were observed in the urinary 6β-OHF/cortisol ratio at TAS-303 doses ranging from 10 to 18 mg and in CL_f at TAS-303 doses ranging from 8 to 18 mg.

Discussion

In this study, the safety and tolerability of TAS-303 administered in repeated doses up to 18 mg once daily were confirmed. In addition, no subjects discontinued the study due to an AE. We did not observe an increase in the incidence of AEs with increasing TAS-303 dose. The most common AE reported in this study in all subjects treated with TAS-303 or placebo was diarrhea, and the only reported treatment-related AE was diarrhea

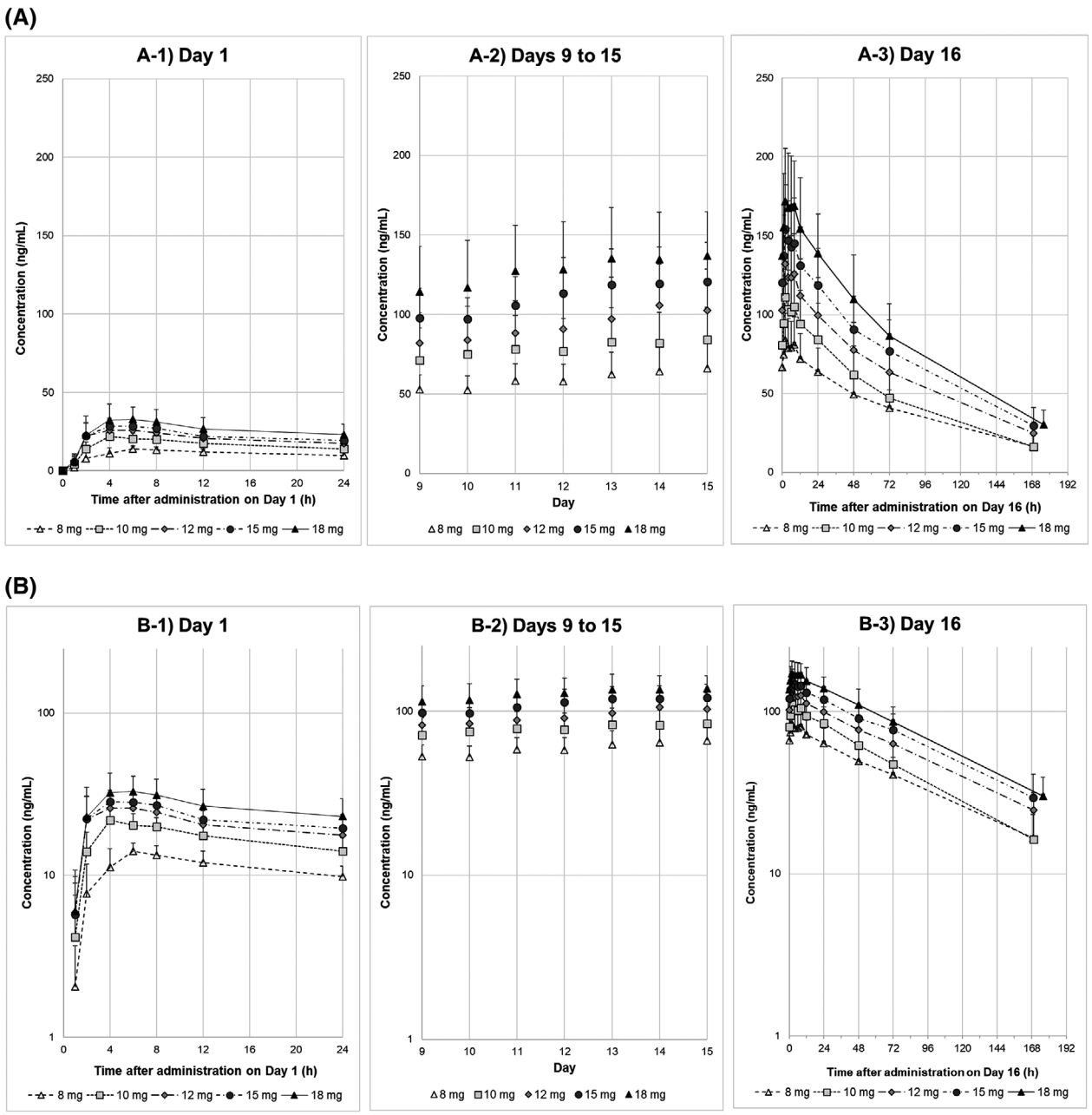

Figure 3. Time course of mean plasma concentration of TAS-303 at each dose. (A) Linear plots for (1) day 1, (2) days 9 to 15, and (3) day 16. (B) Semilog plots for (1) day 1, (2) days 9 to 15, and (3) day 16. Data are presented as arithmetic mean ± standard deviation (n = 7 for 8, 12, 15, and 18 mg). Mean actual sampling times were used for plotting.

in 1 subject in the TAS-303 18 mg group, which was mild and resolved without treatment. These results were generally consistent with previous single- and repeated-dose studies of TAS-303. ADRs affecting the circulatory system that could potentially occur with TAS-303 administration, based on its mechanism of action, were not observed with repeated administration of doses up to 18 mg. Based on the above results, repeated doses of TAS-303 once daily within the dose range of 8 to 18 mg for 16 days were considered safe and well tolerated.

TAS-303 is a deuterated compound. One of the objectives of deuteration is to prolong half-life through to the stabilization of metabolic conversion. Generally, deuteration is expected to achieve greater efficacy and/or reduce ADRs by prolonging the plasma retention time of the active pharmaceutical ingredient.[8] This effect can be attributed to the greater atomic mass of deuterium, as cleavage of the carbon-deuterium covalent bond requires greater energy than that of the carbon-hydrogen bond.[9] During once-daily repeated

Table 3. Summary Statistics of PK Parameters of TAS-303

Dose (mg)	Summary Statistics	C_{max} (ng/mL) Day 1	C_{max} (ng/mL) Day 16	R (C_{max})	$AUC_{0\text{-}24\ h}$ (ng·h/mL) Day 1	$AUC_{0\text{-}24\ h}$ (ng·h/mL) Day 16	R ($AUC_{0\text{-}24\ h}$)	t_{max} (h) Day 1	t_{max} (h) Day 16	$t_{1/2}$ (h) Day 16
8	Arithmetic									
	mean	14.29	86.36	6.00	258	1753	6.77	6.0	3.1	74.2
	SD	1.73	19.47	0.79	43	395	0.68	1.2	2.3	10.1
	n	7	7	7	7	7	7	7	7	7
10	Arithmetic									
	mean	22.01	112.32	5.10	395	2284	5.76	4.3	4.0	60.8
	SD	3.59	22.37	0.51	66	529	0.62	0.8	2.5	9.7
	n	6	6	6	6	6	6	6	6	6
12	Arithmetic									
	mean	27.20	136.23	5.01	486	2733	5.64	4.0	4.0	75.7
	SD	5.56	32.19	0.72	112	613	0.32	1.6	2.8	19.7
	n	7	7	7	7	7	7	7	7	7
15	Arithmetic									
	mean	29.69	157.03	5.31	527	3205	6.10	4.9	3.4	75.1
	SD	4.77	25.47	0.49	92	591	0.68	2.0	2.2	20.1
	n	7	7	7	7	7	7	7	7	7
18	Arithmetic									
	mean	34.29	180.50	5.39	616	3730	6.17	4.9	4.9	70.0
	SD	9.42	33.82	0.86	163	706	0.83	1.1	2.5	17.2
	n	7	7	7	7	7	7	7	7	7

$AUC_{0\text{-}24\ h}$, area under the curve from time zero to 24 hours; C_{max}, maximum concentration in plasma; PK, pharmacokinetic; R, accumulation factor; SD, standard deviation; $t_{1/2}$, elimination half-life; t_{max}, time to reach the maximum concentration.

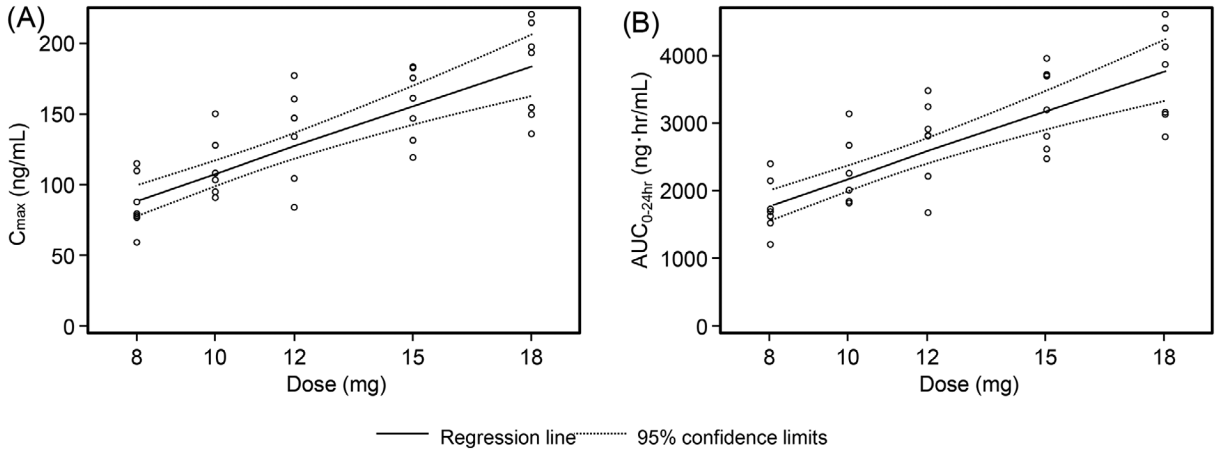

Figure 4. Regression curves for (A) C_{max} and (B) $AUC_{0\text{-}24\ h}$ obtained by power model analysis and 95% confidence intervals. C_{max}, maximum concentration in plasma; $AUC_{0\text{-}24\ h}$, area under the curve from time zero to 24 hours.

administration of TAS-303, drug accumulation was observed, probably because of prolongation of the duration of the plasma retention by deuteration. Because of this accumulation, plasma concentration after once-daily repeated administration was maintained at 5 times the concentration reported after single-dose administration at the same doses.

The steady-state plasma level with repeated administration of TAS-303 8 mg exceeded the C_{max} resulting from single dosing with 18 mg (unpublished data). The $AUC_{0\text{-}24\ h}$ after reaching the steady-state at multiple doses of 10 mg exceeded the NOAEL reported in a 13-week multiple-dose toxicity study in rats and dogs and reached the LOAEL for rats at 13 weeks (unpublished data). However, because the $AUC_{0\text{-}24\ h}$ did not reach the LOAEL in dogs at 13 weeks, we carefully monitored safety data and PK parameters in all subjects while gradually increasing the dose. Therefore, we

Table 4. Arithmetic Mean and Geometric Least-Squares Mean of Urinary 6β-Hydroxycortisol/Cortisol Ratio and Ratio of Geometric Least-Squares Means Between Each TAS-303 Group and the Placebo Group

	Arithmetic Mean of Urinary 6β-Hydroxycortisol/ Cortisol Ratio (SD)		Urinary 6β-Hydroxycortisol/ Cortisol Ratio (95%CI)[a]	Geometric LS Mean Ratios (95%CI) Treatment/ Placebo[b]	
Dose	Day −1	Day 16	Day 16	Day 16	*P*[c]
Placebo	7.11 (1.94)	6.90 (1.40)	6.61 (6.01-7.27)	Not applicable	Not applicable
8 mg	5.70 (1.98)	5.16 (0.54)	5.48 (4.76-6.32)	0.83 (0.66-1.04)	.1458
10 mg	5.99 (1.97)	4.53 (0.74)	4.69 (4.03-5.46)	0.71 (0.56-0.90)	.0019
12 mg	7.85 (2.38)	5.47 (1.24)	5.08 (4.36-5.91)	0.77 (0.61-0.97)	.0218
15 mg	6.32 (2.64)	4.37 (1.13)	4.39 (3.82-5.05)	0.66 (0.53-0.83)	< .0001
18 mg	8.09 (3.74)	5.61 (1.65)	5.17 (4.49-5.95)	0.78 (0.63-0.98)	.0247

CI, confidence interval; LS, least squares; SD, standard deviation.
Pharmacokinetic analysis set.
[a]Geometric LS mean and corresponding 95%CI constructed by analysis of covariance of common log-transformed urinary 6β-hydroxycortisol/cortisol ratio with treatment group as a fixed effect and the baseline value as a covariate.
[b]Geometric LS mean ratio and corresponding 95%CI constructed by Dunnett's test for the LS mean of common log-transformed urinary 6β-hydroxycortisol/cortisol ratio (versus placebo).
[c]Dunnett's test for the LS mean of common log-transformed urinary 6β-hydroxycortisol/cortisol ratio (versus placebo).

Table 5. Arithmetic Mean and Geometric Least-Squares Mean of Formation Clearance (CL_f) of 6β-Hydroxycortisol (6β-OHF) and Ratio of Geometric Least-Squares Means Between Each TAS-303 Group and the Placebo Group

	Arithmetic Mean of CL_f of 6β-OHF (SD)		Geometric LS Mean of CL_f of 6β-OHF (95%CI)[a]	Geometric LS Mean Ratios (95%CI) Treatment/Placebo[b]	
Dose	Day -1	Day 16	Day 16	Day 16	*P*[c]
Placebo	170 (35)	176 (34)	174 (161-189)	Not applicable	Not applicable
8 mg	182 (54)	122 (31)	116 (103-131)	0.67 (0.55-0.80)	< .0001
10 mg	164 (52)	118 (17)	122 (107-138)	0.70 (0.57-0.85)	< .0001
12 mg	162 (41)	115 (16)	112 (99-127)	0.64 (0.53-0.78)	< .0001
15 mg	186 (57)	116 (24)	111 (99-125)	0.64 (0.53-0.77)	< .0001
18 mg	166 (34)	127 (33)	126 (112-142)	0.72 (0.60-0.87)	.0002

CI, confidence interval; LS, least squares; SD, standard deviation.
Pharmacokinetic analysis set.
[a]Geometric LS mean and corresponding 95%CI constructed by analysis of covariance of common log-transformed CL_f of 6β-OHF with treatment group as a fixed effect and the baseline value as a covariate.
[b]Geometric LS mean ratio and corresponding 95%CI constructed by Dunnett's test for the LS mean of common log-transformed CL_f of 6β-OHF (versus placebo).
[c]Dunnett's test for the LS mean of common log-transformed CL_f of 6β-OHF (versus placebo).

were able to administer TAS-303 up to 18 mg and confirm its safety. In the case of gradually increasing the dose of reuptake inhibitors while carefully confirming safety data as well as the PK parameters, it would be possible to evaluate the safety and tolerability of high doses to provide a greater dose range for evaluating efficacy and safety in future clinical studies.

Measuring endogenous biomarkers of CYP3A-mediated drug-drug interactions in clinical studies in early-phase drug development could provide useful data to guide the optimal design of subsequent drug-drug interaction studies. Although the urinary 6β-OHF/cortisol ratio has been proposed as an endogenous biomarker for CYP3A4 activity, its sensitivity is controversial partly because this index is likely to be influenced by intra- and interindividual variations in the renal clearance of cortisol.[7] In addition, Furuta et al suggested the CL_f of 6β-OHF as a more accurate probe of CYP3A activity that is not affected by the variability of renal cortisol elimination.

To the best of our knowledge, several studies exhibited that strong CYP3A inhibitors such as clarithromycin, itraconazole, and ketoconazole reduced the CL_f value by approximately 60% to 80% and strong CYP3A inducer, rifampicin increased the CL_f by more than 3.8-fold,[10-12] suggesting the sensitivity of CL_f in identifying strong CYP3A inhibitors and inducers. As in the current study, these CYP3A indexes were evaluated in a repeated-dose phase 1 study in the TAS-303 dose range of 1 to 6 mg (unpublished data) and resulted in nonsignificant reductions of both the urinary 6β-OHF/cortisol ratio and CL_f, which is consistent with the lack of an inhibitory effect on intravenous midazolam PK in a clinical study with repeated TAS-303 doses of 3 mg.[6] In this study, however, statistically significant decreases were found in the urinary 6β-OHF/cortisol ratio at TAS-303 doses ranging from 10 to 18 mg and in CL_f at TAS-303 doses ranging from 8 to 18 mg.

Our findings suggest that inhibition of hepatic CYP3A activity may occur after repeated administration of TAS-303 8 to 18 mg. No dose-dependent decrease in the ratio of geometric least-squares means between each TAS-303 dose and placebo for both urinary 6β-OHF/cortisol ratio and CL_f was observed at doses from 8 to 18 mg in this study. This could be because of the narrow dose range tested and/or the limited dynamic range of the indexes. The latter possibility may be more pronounced in the case of interaction by a CYP3A inhibitor compared with a CYP3A inducer, most likely because of its nature of clearance as an index, as indicated in the above-mentioned clinical interaction studies with strong CYP3A inhibitors and inducers (ie, 60% to 80% reduction versus 3.8-fold increase[10-12]). In a nonclinical study, CYP3A at least partially contributed to the metabolism of TAS-303 (unpublished data). In this study, there were no effects on the metabolism of TAS-303 within the dose range of 8 to 18 mg because the elimination half-life was not prolonged with any of the doses administered. As the results suggest that TAS-303 8 to 18 mg had an effect on hepatic CYP3A activity, clinical drug-drug interaction studies of high doses of TAS-303 would provide detailed information of interactions between TAS-303 and CYP3A substrate.

The present study has some limitations. SUI is an important burden among women, yet the present study only included healthy male subjects. Furthermore, as the present study only included Japanese men, our results cannot be extrapolated to other ethnic populations. In addition, this was a single-blind study. Finally, as clinical drug-drug interaction studies of TAS-303 at doses ranging from 8 to 18 mg were not conducted, detailed information on TAS-303 interactions with the CYP3A substrate at doses ranging from 8 to 18 mg is not available.

In conclusion, as in the previous studies, TAS-303 at a dose of 8 to 18 mg was shown to be safe and well tolerated. TAS-303 exhibited a dose-proportional PK response after 16 days of multiple administration at doses ranging from 8 to 18 mg and reached a steady state between day 11 and day 13. Multiple doses of TAS-303 at doses ranging from 8 to 18 mg might inhibit hepatic CYP3A activity. Further confirmation of such interactions should be addressed in a drug-drug interaction study.

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Conflicts of Interest

R.H. received medical adviser fees from Taiho Pharmaceutical Co., Ltd.

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Supplemental Information

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