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Pharmacokinetics and Pharmacodynamics of Tofogliflozin (a Selective SGLT2 Inhibitor) in Healthy Male Subjects

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ABSTRACT

Purpose Tofogliflozin is a selective oral inhibitor of sodium-glucose co-transporter 2 for treatment of type 2 diabetes mellitus. The pharmacokinetics, pharmacodynamics, and safety of tofogliflozin were investigated in healthy male subjects.

Methods Three studies were conducted: single-ascending dose study (10–640 mg) in 56 Japanese and 24 Caucasian subjects; multiple-ascending dose study (2.5–80 mg once daily for 7 days) in 24 Japanese subjects; and food-effect study (20–40 mg) in 30 Japanese subjects.

Results Tofogliflozin was absorbed rapidly and eliminated from the systemic circulation with a $t_{1/2}$ of 5–6 h. Exposure increased dose-proportionally up to 320 mg. Body weight-corrected exposure was similar between Japanese and Caucasian subjects. Urinary excretion of tofogliflozin ranged from 17.1 to 27.4 % of dose. Tofogliflozin did not accumulate with once daily administration. Food intake decreased C_{max} by approximately 30 % but did not change $AUC_{0\text{-}inf}$. Tofogliflozin caused dose-dependent daily urinary glucose excretion ($UGE_{0\text{-}24h}$), but food intake condition at administration did not affect it. The exposure-response relationship between plasma average concentration of tofogliflozin (C_{avg}) and $UGE_{0\text{-}24h}$ fitted E_{max} model well. There were no serious adverse events leading to discontinuation or episodes of hypoglycemia.

Conclusions Single and multiple administration of tofogliflozin were generally well tolerated. Exposure to tofogliflozin was dose-proportional up to 320 mg and did not accumulate with multiple once-a-day administration. The model suggests more than 100 ng/mL C_{avg} corresponding to the dose of between 20 and 40 mg leads to almost maximum effect of tofogliflozin.

Introduction

With the increase in obesity due to changes in eating habits and lack of exercise, in addition to genetic susceptibility, the number of patients with type 2 diabetes mellitus (T2DM) is increasing steadily worldwide. Sodium-glucose co-transporter 2 (SGLT2) inhibitors reduce blood glucose levels by inhibiting renal glucose reabsorption via SGLT2 and increasing urinary excretion of excess glucose [1]. In the world, six SGLT2 inhibitors marketed over the past few years including tofogliflozin [2] provide a new armamentarium for the treatment of T2DM patients due to the following characteristics: (1) their pharmacological action is insulin-independent, and they can therefore be administered either as monotherapy or in combination with any other anti-hyperglycemic medication; (2) the urinary glucose excretion (UGE) induced by SGLT2 inhibition causes a corresponding loss of calories, leading to a reduction in body weight and (3) the frequency of hypoglycemic events is suggested to be low since SGLT1, in intestine and renal tubule, still functions when renal glucose reabsorption is inhibited by selective SGLT2 inhibitors.

Tofogliflozin [CAS no: 903565-83-3; (1S,3′R,4′S,5′S,6′R)-6-[(4-ethylphenyl)methyl]-3′,4′,5′,6′-tetrahydro-6′-(hydroxymethyl)-spiro{isobenzofuran-1(3H),2′-[2H]pyran}-3′,4′,5′-triol], which was discovered and synthesized by Chugai Pharmaceutical Co., Ltd. (Tokyo, Japan), is one of the most selective SGLT2 inhibitors available [3]. Monotherapy of tofogliflozin 10, 20, or 40 mg/day for 12 weeks reduced HbA1c up to 0.990 % as placebo-adjusted mean change in Japanese T2DM patients [4]. Tofogliflozin was well tolerated and the severity of adverse events related to hypoglycemia was mild or moderate, and all events resolved within a day [4]. Moreover, tofogliflozin in combination with other anti-T2DM drugs except sulfonylurea did not cause an increase in the incidence of hypoglycemia compared to those in monotherapy [4–5], which is related to the third advantage shared with SGLT2 inhibitors.

Some of basic pharmacokinetic (PK) characteristics of tofogliflozin have been reported [6–8]. The in vitro study has suggested that tofogliflozin is metabolized by CYP2C18, 3A4/5, 4A11, and 4F3B, and tofogliflozin-derived substances are mainly eliminated by urinary excretion [6]. A human mass balance study combined with intravenous microdosing has demonstrated high oral bioavailability (BA) (97.5 %) of tofogliflozin [7], and single PK profile of tofogliflozin with/without representative anti-T2DM drugs was evaluated in drug-drug interaction study [8]. However, the clinical pharmacodynamic (PD) profile based on the PK of tofogliflozin as its background mechanism has not been clarified yet. To provide comprehensive information for the PK/PD of tofogliflozin, we now report its detailed PK profile (linearity of exposure, PK in multiple dosing, food effect, comparison of exposure between Japanese and Caucasian subjects, and exposure ratio of metabolites), PD profile (UGE rate, and $UGE_{0\text{-}24h}$), and their relationship in healthy male subjects.

Materials and Methods

The following three phase 1 studies of tofogliflozin were conducted in Japan. (1) A single-ascending dose (SAD) study: a double-blind, randomized, placebo-controlled study in healthy male Japanese and Caucasian subjects. (2) A multiple-ascending dose (MAD) study: a double-blind, randomized, placebo-controlled study in healthy male Japanese subjects. (3) A food-effect study: an open-label, randomized, three-period, crossover study in healthy male Japanese subjects. All studies were conducted in accordance with the Declaration of Helsinki [9], the Good Clinical Practice, and the International Conference on Harmonization guidelines. The SAD and food-effect studies were approved by the Institutional Review Board of the CPC Clinical Trial Hospital, Medipolis Medical Research Institute (Kagoshima, Japan) and were conducted in September–December 2007 and May–June 2012, respectively. The MAD study was approved by the Institutional Review Board of the Kyushu Clinical Pharmacology Research Clinic (Fukuoka, Japan) and was conducted in April–June 2008. All subjects gave written informed consent prior to participation.

Subjects

Subjects were healthy men aged ≥ 20 and < 40 years (for the SAD and MAD studies) or ≤ 45 years (for the food-effect study) at consent, with a body mass index ≥ 18.5 and < 25.0 kg/m^2 for the Japanese subjects or ≥ 18.5 and < 30.0 kg/m^2 for the Caucasian subjects at screening and who were judged to be medically suitable for enrollment. Major exclusion criteria included history or presence of renal, hepatic, circulatory, and/or respiratory disorders that may interfere with the study.

Study design

Tofogliflozin was administered with 200 mL of water under the following food intake condition after a fasting period of ≥ 10 h.

SAD study

Forty-two healthy male Japanese subjects were orally administered a single dose of tofogliflozin (10, 20, 40, 80, 160, 320, or 640 mg) and 18 healthy male Caucasian subjects were orally administered a single dose of tofogliflozin (10, 20, or 80 mg). Blood samples were collected before administration and at 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 24, 36, and 48 h after administration. Urine samples for PK assessment were collected for 48 h after administration. Tofogliflozin was administered under a fasting condition. Each cohort of both ethnicities consisted of 6 subjects treated with tofogliflozin and 2 subjects treated with placebo.

MAD study

Eighteen healthy male Japanese subjects were administered a once-a-day dose of tofogliflozin (2.5, 20, or 80 mg) for 7 days. Blood samples were collected before administration and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16 (until this time on Day 1), 24, 36, 48, 60, 72, 84, and 96 h (until this time on Day 7) after administration and were also collected prior to dosing from Days 2 to 6 to assess the attainment of a steady state on Day 7. Urine samples for PK assessment were collected every dosing day and up to 96 h after the last administration. Tofogliflozin was administered 15 min before breakfast. Each cohort consisted of 6 subjects treated with tofogliflozin and 2 subjects treated with placebo.

Food-effect study

Thirty healthy male Japanese subjects were orally administered 20 or 40 mg tofogliflozin in each cohort consisting of 15 healthy male Japanese subjects. A three-way crossover study was designed to investigate the effect of food on the PK, PD, and tolerability of tofogliflozin. A single dose of tofogliflozin was administered in a pre-meal condition (15 min before breakfast), post-meal condition (30 min after breakfast), or fasting condition in each period. The breakfast in the pre- or post-meal condition was classed as high fat (approximately 50 % of total caloric content of the meal) and high calorie (approximately 800–1 000 calories) according to FDA guidance [10]. Blood samples were collected before administration and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, 24, 36, and 48 h after single administration of tofogliflozin. Urine samples for PK assessment were collected for 48 h after administration.

PK analysis

In vitro profiling of the metabolism of tofogliflozin using human hepatocytes demonstrated that carboxylated form was the main metabolite and the productions of other metabolites were very small [6]. However, because ketone form is one of main metabolites in rats and acyl-glucuronide is regarded as reactive species involved in toxicity [11], the concentrations of tofogliflozin and its 3 metabolites (carboxylated, ketone, and acyl-glucuronide forms)

in human plasma or urine were measured using liquid chromatography–tandem mass spectrometry that met the appropriate validation criteria [12].

In the SAD study, the plasma and urine concentrations of tofogliflozin were measured by Chugai Pharmaceutical Co., Ltd. (Tokyo, Japan). The plasma and urine quantification range (lower limit to upper limit of quantification) and the between-run variability for each assay were 0.200–200 ng/mL and ≤ 7.9 % and 10.0–1 800 ng/mL and ≤ 3.8 % for tofogliflozin, respectively. In the MAD study, the plasma and urine concentrations of tofogliflozin and its metabolites (i.e., the carboxylated and ketone forms) were measured by F. Hoffman-La Roche, Co., Ltd. (Basel, Switzerland). The plasma and urine quantification range and the between-run variability for each assay were 0.200–500 ng/mL and ≤ 6.6 % and 5.00–10 000 ng/mL and ≤ 9.4 % for tofogliflozin, 0.200–500 ng/mL and ≤ 11.0 % and 5.00–10 000 ng/mL and ≤ 9.4 % for the carboxylated form, and 0.500–500 ng/mL and ≤ 7.6 % and 10.0–10 000 ng/mL and ≤ 8.4 % for the ketone form, respectively. In the food-effect study, the plasma concentrations of tofogliflozin and its metabolites (i.e., the carboxylate and acyl-glucuronide forms) were measured by Chugai Pharmaceutical Co., Ltd., and the plasma quantification range and the between-run variability for each assay were 0.200–200 ng/mL and ≤ 8.2 % for tofogliflozin, 0.500–500 ng/mL and ≤ 8.5 % for the carboxylated form, and 1.00–500 ng/mL and ≤ 5.1 % for the acyl-glucuronide form, respectively.

For all analytes, the PK parameters of C_{max} (maximum plasma drug concentration), T_{max} (time to reach C_{max}), $t_{1/2}$ (elimination half-life), $AUC_{0\text{-}inf}$ (area under the plasma concentration–time curve from time zero to infinity), $AUC_{0\text{-}24h}$ (area under the plasma concentration–time curve from time zero to 24 h after administration), and f_e (fraction of dose excreted in the urine) were determined using WinNonlin ver. 6.1 software (Pharsight Corporation, Mountain View, CA, USA).

PD analysis

The PD profile of tofogliflozin was evaluated by UGE rate in the SAD study, and $UGE_{0–24h}$, i.e., cumulative UGE for 24 h after administration, in all 3 studies. To investigate the relationship between the exposure of tofogliflozin and $UGE_{0–24h}$, we adopted the plasma average concentration of tofogliflozin (C_{avg}) which was calculated from $AUC_{0\text{-}24h}$ divided by dosing interval that is 24 h as an index of exposure. Since $UGE_{0–24h}$ increased relative to an increase in C_{avg} and it seemed to reach the plateau at higher exposure based on the physiological mechanisms, the following E_{max} model was used for this exposure-response (E-R) analysis.

$$UGE_{0–24h}\ (g) = \frac{E_{max} \times C_{avg}}{EC_{50} + C_{avg}}$$

C_{avg}: plasma average concentration of tofogliflozin (ng/mL)

E_{max}: maximum amount of $UGE_{0–24h}$ attributable to tofogliflozin (g)

EC_{50}: C_{avg} that produces half of E_{max} (ng/mL)

Each parameter in this model was fitted by SAS ver. 9.4 software (SAS Institute, Inc., Cary, NC, USA).

Safety assessments

Safety was evaluated via adverse events, clinical laboratory tests, vital signs, and standard 12-lead electrocardiogram (ECG) examinations. Adverse events that developed during the study period after tofogliflozin administration were recorded. In the SAD study, laboratory tests were performed before administration, at 24 and 48 h after administration, and at the last observation. Vital signs were measured at screening, before administration, at 1, 2, 3, 4, 5, 6, 12, 24, 36, and 48 h after administration, and at the last observation. Standard 12-lead ECG examinations were measured at screening, before administration, at 1, 2, 3, 4, 6, 8, 10, 24, 36, and 48 h after administration, and at the last observation. In the MAD study, laboratory tests were performed before the initial administration and at Day 2 (immediately before the 2nd administration), Day 4 (immediately before the 4th administration), Day 8 (24 h after the final administration), and the last observation. Vital signs and standard 12-lead ECG examinations were performed before the initial administration and at Days 2–7 (1 h before each administration), Days 8–11 (23, 48, 72, and 96 h after the final administration), and the last observation. Moreover, taking the results of the SAD study into consideration, urinary tract infection, serum ketone bodies, renin activity, aldosterone levels, and fluid balance were added as special safety assessments. The decision to escalate to the next higher dose was made following review of the safety information available from the preceding dose level in the SAD and MAD studies. In the food-effect study, laboratory tests, vital signs, and standard 12-lead ECG examinations were performed before the initial administration and at Day 2, Day 3, and the last observation.

Statistical methods

All subjects administered tofogliflozin or placebo were included in the safety analysis, those in whom the plasma concentration of tofogliflozin or its metabolites was measured were included in the PK analysis set, and those in whom PD endpoints were measured were included in the PD analysis set. Summary statistics on the subjects' baseline characteristics were calculated in each study. In addition, the characteristics of the subject populations in the Japanese and Caucasian groups were compared in the SAD study. Summary statistics of the PK/PD data were calculated and time course graphs were prepared for each dose group. The dose-proportionality of exposure (C_{max} and $AUC_{0\text{-}inf}$) after administration under the fasting condition in the SAD study was judged in a comprehensive manner based on assessments with two models: (1) the power model, where two-sided 95 % confidence intervals (CIs) of the slope that included 1 suggested dose-proportionality, was applied to log-transformed C_{max} and $AUC_{0\text{-}inf}$ versus log-transformed dose; and (2) the linear regression model, where two-sided 95 % CIs of the intercept that included 0 suggested dose-proportionality, was applied to C_{max} and $AUC_{0\text{-}inf}$ versus dose. To assess the effect of food intake on the PK of tofogliflozin, the ratios of the geometric means and their 90 % CIs for C_{max} and $AUC_{0\text{-}inf}$ of each analyte in the food intake conditions at administration (15 min before breakfast or 30 min after breakfast) relative to those in the fasting condition were calculated using a linear mixed-effects model with period, group, and food intake condition at administration as the fixed effects and subject as the random effect. These studies were exploratory in nature; therefore, multiple comparisons were not applied.

▶ **Table 1** Demographic and baseline characteristics of the subjects.

Study	Ethnicity	N	Age (years)	Body weight (kg)	Blood glucose (mg/dL)	eGFR (mL/min/1.73 m^2)
SAD	Japanese	56	24.8 ± 4.36	61.2 ± 5.91	92.4 ± 5.26	107 ± 12.8
	Caucasian	24	31.0 ± 5.27	73.2 ± 10.1	93.9 ± 4.98	121 ± 14.6
MAD	Japanese	24	27.4 ± 5.55	62.9 ± 5.46	70.2 ± 8.29	116 ± 16.2
Food effect	Japanese	30	27.0 ± 3.83	62.9 ± 8.28	90.0 ± 4.95	98.7 ± 9.61

Mean ± SD. eGFR: estimated glomerular filtration rate

SAS ver. 9.4 software (SAS Institute, Inc., Cary, NC, USA) was used for all analyses and calculations.

Results

Demographics of the subjects

All subjects completed the study and their demographics and baseline characteristics are shown in ▶ **Table 1**. Body weight differed between the Japanese and Caucasian groups, but no imbalance was seen in the other demographics or baseline characteristics between the two groups.

PK profile

Tofogliflozin was absorbed rapidly into the blood and reached the peak at 1 h then eliminated after single administration (▶ **Table 2**). Thereafter, tofogliflozin plasma concentration declined in a biphasic manner indicative of a rapid distribution phase and a slower elimination phase with a $t_{1/2}$ of 5–6 h (▶ **Fig. 1**).

For Japanese subjects, systemic exposure of tofogliflozin increased in a dose-dependent manner over the dose range 10 to 640 mg with C_{max} values of 310 ± 63.7 (mean ± standard deviation [SD]) to 11 900 ± 1 130 ng/mL, and with AUC_{0-inf} values of 1 330 ± 444 to 99 100 ± 26 800 ng × h/mL (▶ **Table 2**). Assessment of dose-proportionality using the power model showed that both C_{max} and AUC_{0-inf} increased in a dose-proportional manner up to 320 mg. The corresponding 95 % CIs of the estimates of the slope were 0.894–1.01 and 0.965–1.11, respectively. The results of the assessment based on the linear regression model suggested that C_{max} increased in a dose-proportional manner up to 320 mg and AUC_{0-inf} did so up to 640 mg. The corresponding 95 % CIs of the estimates of the intercept were − 63.2 to 288 and − 5 150 to 646, respectively.

As for the comparison of the PK profiles between Japanese and Caucasian subjects, the plasma tofogliflozin concentration profile of the Caucasian subjects tended to be slightly lower than that of the Japanese subjects. Systemic exposure of tofogliflozin in the Caucasian subjects was also slightly lower than in the Japanese subjects (▶ **Table 2**). The geometric mean ratios and their 90 % CIs of Japanese to Caucasian subjects of C_{max} and AUC_{0-inf} standardized by dose-adjusted for body weight (C_{max}/D_w and AUC_{0-inf}/D_w) were 1.11 (1.00–1.23) and 1.01 (0.875–1.17), respectively.

When tofogliflozin was administered multiple times, the concentration profile was comparable to that for single administration. The accumulation ratio was approximately 1, indicating virtually no accumulation by multiple dosing. The trough plasma concentration of tofogliflozin was maintained and nearly constant during the treatment period (▶ **Fig. 2**). The cumulative percentage of the dose excreted into urine (f_e) was 17.1–18.4 % of the total administered dose.

C_{max} decreased by approximately 30 % and T_{max} tended to delay under post-meal condition compared to fasting condition; however, it had little effect on AUC_{0-inf}. There were no remarkable differences in PK parameters between under pre-meal and fasting condition. PK parameters and the ratios of the geometric means for C_{max} and AUC_{0-inf} and their 90 % CIs are shown in ▶ **Table 2**.

The PK profiles of the 3 metabolites of tofogliflozin (i.e., carboxylated, ketone, and acyl-glucuronide forms) were evaluated in the MAD and food-effect studies. The AUC_{0-24h} ratios at the steady state of the carboxylated and ketone forms to unchanged were 116–131 %, and 4.76–6.12 %, respectively. The AUC_{0-inf} ratio of the acyl-glucuronide form to unchanged was 4.64–5.50 % at a dose of 40 mg (Supplemental **Table S1** given in Online Resource 1). The f_e of the carboxylated metabolite was approximately 40 % of the total dose (Supplemental **Table S2** given in Online Resource 2).

PD profile

UGE and other glycemic parameters

Single administration of tofogliflozin to Japanese subjects caused UGE within 2 h of administration at all doses (▶ **Fig. 3**). The time course of UGE rate for up to 12 h after administration was similar among the doses tested, and thereafter the duration and degree of the effect was dose-dependent.

Mean UGE_{0-24h} increased in a dose-dependent manner, ranging from 22.1 to 78.8 g (▶ **Table 3**). UGE_{0-24h} at doses of 10–80 mg was comparable between Japanese and Caucasian healthy male subjects. In the MAD study, daily UGE_{0-24h} increased in a dose-dependent manner for doses of 2.5–80 mg and its degree was maintained during the administration period, and thus UGE_{0-24h} did not change on Day 1 and Day 7 in each dose group.

Multiple dosing of tofogliflozin did not cause any clinically relevant changes in glycemic parameters, such as daily glucose excursion, fasting plasma glucose, plasma glucose AUC_{0-24h}, or postprandial glucose AUC_{0-4h} in healthy male subjects.

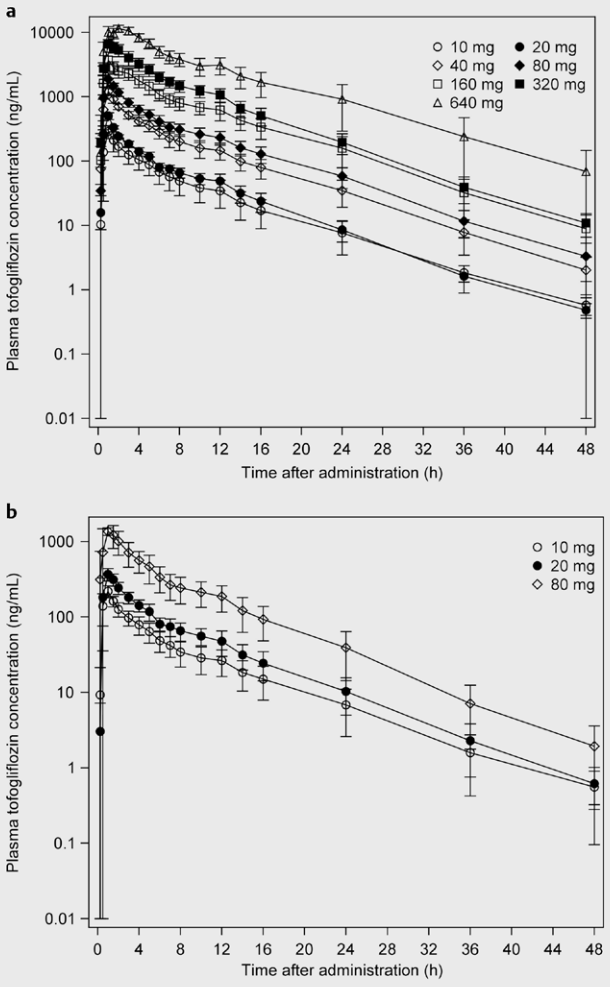
In the food-effect study, UGE_{0-24h} after administration of tofogliflozin 20–40 mg under the fasting, pre-meal, and post-meal conditions were 42.4–50.7 g, 47.0–54.5 g, and 47.5–53.5 g, respectively; therefore, UGE_{0-24h} was not dependent on the food intake condition at administration.

The relationship between C_{avg} of tofogliflozin and UGE_{0-24h} with a fitting curve from the E_{max} model is shown in ▶ **Fig. 4**. There was no obvious difference in the relationship between Japanese (shown as open circles) and Caucasian (shown as solid circles) subjects. E_{max} and EC_{50} were estimated by 67.8 g, and 29.2 ng/mL, respectively.

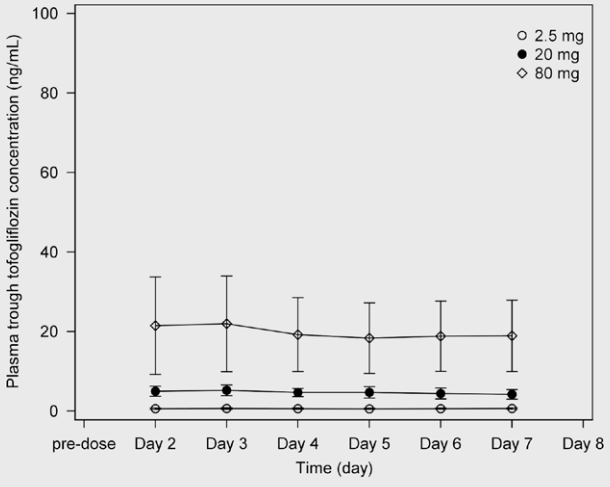
▶ **Table 2** PK parameters of tofogliflozin.

SAD study								
Ethnicity	**Dose (mg)**	**N**	**C_{max} (ng/mL)**	**AUC_{0-inf} (h × ng/mL)**	**T_{max}[a] (h)**	**$t_{1/2}$ (h)**	**f_e[b] (%)**	
Japanese	10	6	310 ± 63.7	1 330 ± 444	1.00 (0.50–1.50)	5.71 ± 0.682	24.5 ± 6.13	
Caucasian		6	220 ± 39.6	1 040 ± 329	1.00 (1.00–1.00)	6.09 ± 0.729	19.1 ± 3.83	
Japanese	20	6	506 ± 61.4	1 900 ± 264	1.00 (1.00–1.00)	5.29 ± 0.508	18.2 ± 2.56	
Caucasian		6	394 ± 52.4	1 820 ± 394	1.00 (0.50–1.50)	5.70 ± 0.325	19.4 ± 4.98	
Japanese	40	6	1 210 ± 133	5 640 ± 1 170	1.00 (1.00–1.00)	5.77 ± 0.600	25.5 ± 5.81	
Japanese	80	6	1 930 ± 420	8 830 ± 1 670	1.00 (0.50–1.50)	5.73 ± 0.701	23.2 ± 4.72	
Caucasian		6	1 570 ± 310	7 090 ± 2 260	1.00 (0.50–1.50)	5.36 ± 0.577	17.1 ± 1.72	
Japanese	160	6	3 710 ± 1 240	21 800 ± 5 580	1.00 (1.00–1.00)	5.63 ± 0.522	26.6 ± 4.46	
Japanese	320	6	6 740 ± 598	38 100 ± 7 680	1.00 (1.00–2.00)	5.53 ± 0.357	24.7 ± 3.29	
Japanese	640	6	11 900 ± 1 130	99 100 ± 26 800	2.00 (1.00–3.00)	6.06 ± 0.666	27.4 ± 3.77	
MAD study								
Ethnicity	**Dose (mg)**	**Day**	**N**	**C_{max} (ng/mL)**	**AUC_{0-24h} (h × ng/mL)**	**T_{max}[a] (h)**	**$t_{1/2}$[c] (h)**	**f_e[b] (%)**
Japanese	2.5	1	6	69.3 ± 21.2	204 ± 34.8	0.500 (0.500–1.00)	4.37 ± 0.324	–
	20		6	484 ± 186	1 680 ± 211	0.500 (0.500–3.00)	4.14 ± 0.342	–
	80		6	1 810 ± 504	7 240 ± 1 640	1.00 (0.500–2.00)	4.07 ± 0.383	–
	2.5	7	6	59.9 ± 20.0	192 ± 41.7	0.500 (0.50–1.00)	4.35 ± 0.290	18.4 ± 2.90
	20		6	391 ± 164	1 550 ± 244	0.750 (0.50–3.00)	3.81 ± 0.206	18.1 ± 3.77
	80		6	1 660 ± 641	6 740 ± 1 680	0.750 (0.50–4.00)	3.98 ± 0.520	17.1 ± 2.29
Food effect study								
Ethnicity	**Dose (mg)**	**Food**	**N**	**C_{max} (ng/mL)**	**AUC_{0-inf} (ng × h/mL)**	**T_{max}[a] (h)**	**$t_{1/2}$ (h)**	
Japanese	20	Fasting	15	509 ± 118	2 140 ± 656	1.00 (0.50–2.00)	5.40 ± 0.622	
		Pre-meal	15	444 ± 106, 0.879 [0.763–1.01][d]	1 890 ± 543, 0.886 [0.846–0.927][d]	1.00 (0.50–1.50)	5.65 ± 0.855	
		Post-meal	15	344 ± 108, 0.672 [0.566–0.797][d]	1 990 ± 650, 0.926 [0.886–0.969][d]	2.00 (1.00–4.00)	5.82 ± 0.744	
	40	Fasting	15	1 020 ± 336	4 190 ± 1 000	1.50 (0.50–2.00)	5.39 ± 0.376	
		Pre-meal	15	1 050 ± 289, 1.07 [0.963–1.18][d]	3 730 ± 603, 0.923 [0.882–0.966][d]	1.00 (0.50–1.00)	5.65 ± 0.590	
		Post-meal	15	742 ± 211, 0.748 [0.664–0.843][d]	3 680 ± 613, 0.908 [0.856–0.962][d]	1.50 (0.50–4.00)	5.48 ± 0.506	

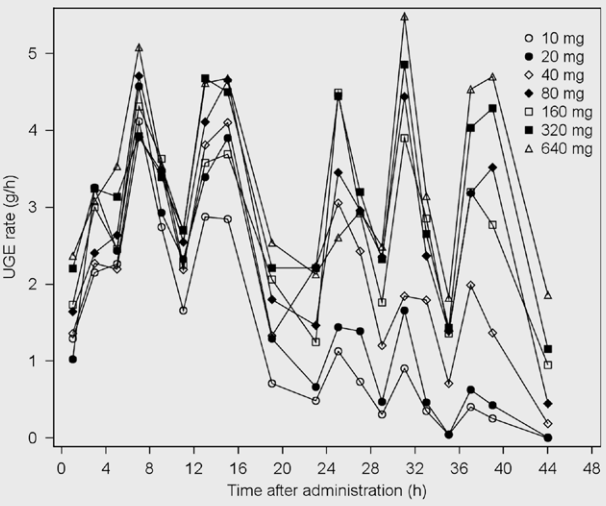
Mean ± SD; [a] T_{max} are shown as median (minimum–maximum); [b] Cumulative urinary excretion ratio until final observation; [c] $t_{1/2}$ are calculated using data collected 0–24 h post-administration; [d] Geometric mean ratio [90 % CI] against fasting condition



▶ **Fig. 1** Plasma concentration profile of tofogliflozin (mean ± SD) after single administration to healthy male Japanese **a** and Caucasian **b** subjects.



▶ **Fig. 2** Trough plasma concentration profile of tofogliflozin (mean ± SD) after multiple once-a-day administration for 7 days.

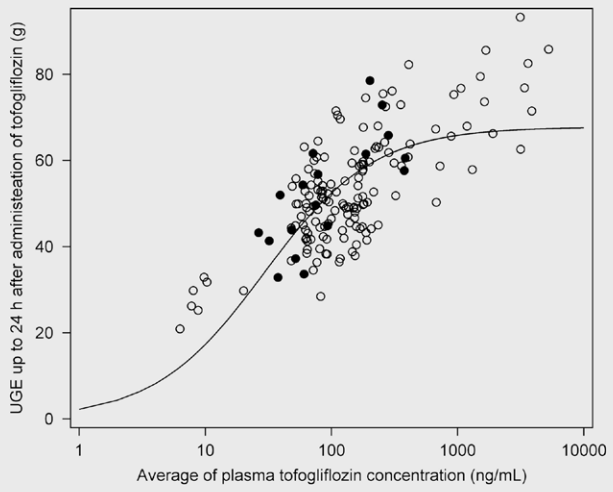


▶ **Fig. 3** UGE rate following single administration of tofogliflozin.

▶ **Table 3** Cumulative UGE up to 24 h after tofogliflozin administration.

Ethnicity	Study		Dose	N	$UGE_{0\text{-}24h}$ (g)
Japanese	SAD		Placebo	14	0.0644 ± 0.0124
			10 mg	6	45.2 ± 9.13
			20 mg	6	56.8 ± 5.43
			40 mg	6	59.1 ± 10.9
			80 mg	6	66.2 ± 11.2
			160 mg	6	64.2 ± 8.64
			320 mg	6	73.3 ± 9.88
			640 mg	6	78.8 ± 10.9
Caucasian	SAD		Placebo	6	0.0772 ± 0.0315
			10 mg	6	44.6 ± 7.74
			20 mg	6	47.3 ± 10.9
			80 mg	6	66.2 ± 8.05
Japanese	MAD	Day 1	Placebo	6	0.0582 ± 0.0905
			2.5 mg	6	27.8 ± 4.53
			20 mg	6	53.8 ± 9.37
			80 mg	6	66.1 ± 5.88
		Day 7	Placebo	6	0.0357 ± 0.0554
			2.5 mg	6	22.1 ± 4.82
			20 mg	6	46.0 ± 7.53
			80 mg	6	59.6 ± 11.0
Japanese	Food effect	Fasting	20 mg	15	42.4 ± 6.65
		Pre-meal		15	47.0 ± 5.13
		Post-meal		15	47.5 ± 6.71
		Fasting	40 mg	15	50.7 ± 8.38
		Pre-meal		15	54.5 ± 8.70
		Post-meal		15	53.5 ± 10.4

Mean ± SD



▶ **Fig. 4** E-R evaluation for $UGE_{0\text{-}24h}$ by tofogliflozin. Observations of Japanese subjects are marked as open circles, those of Caucasian subjects are marked as solid circles, and the E_{max} model (E_{max} = 67.8 g, EC_{50} = 29.2 ng/mL) is drawn to the best approximate relationship between the two variable.

Safety and tolerability

There were no serious adverse events, adverse events leading to discontinuation, or episodes of hypoglycemia in any of the 3 studies. No abnormalities in vital signs or standard 12-lead ECG examinations were seen during any of the studies. Most of the adverse events were increases of blood ketone bodies, which were reported due to beyond clinical site reference value (120 µmol/L). In the SAD study conducted under the fasting condition, the incidence of blood ketone bodies was 42.9–100 % (min–max) in the Japanese and Caucasian subjects, including the placebo groups. Although mean blood ketone bodies showed a dose-responsive increase: 181 ± 60.0, 296 ± 64.9, 379 ± 259, 362 ± 139, 325 ± 96.3, 473 ± 195, and 531 ± 138 µmol/L in the 10, 20, 40, 80, 160, 320, and 640 mg groups, respectively, no symptoms suggestive of ketoacidosis were reported. In the MAD study, conducted under the food intake condition, the frequency and the degree of the increase were much less than those in the SAD study, and only one case was observed in the 80 mg group (on Day 2, 269 µmol/L).

Discussion

We characterized the PK and PD profiles of tofogliflozin in detail through 3 clinical studies with healthy male subjects. For absorption, T_{max} of tofogliflozin was approximately 1 h for doses up to 320 mg. Evaluation of dose-proportionality using power and linear regression models showed that both C_{max} and $AUC_{0\text{-}inf}$ increased in a dose-proportional manner up to a dose of 320 mg. A human mass balance study combined with intravenous microdosing showed that the absolute BA of tofogliflozin was about 97.5 % [7]. All the above, tofogliflozin would be absorbed rapidly and close to 100 % of tofogliflozin would be absorbed into the systemic circulation. Multiple dosing of tofogliflozin did not cause accumulation, and the exposure of tofogliflozin at the final administration was almost the same as at the initial administration. The PK profile of tofogliflozin was affected by food intake, but a change in exposure was observed only for C_{max}; $AUC_{0\text{-}inf}$ was not changed, which implies that food intake delays the absorption of tofogliflozin but does not affect the extent of tofogliflozin absorbed. Therefore, from the perspective of PK, it is considered that tofogliflozin can be given independently of the timing of food intake.

Human mass balance study demonstrated the presence of several metabolites, and the AUC ratios of the metabolites (carboxylated, ketone, and acyl-glucuronide forms) to the unchanged form were 122, 7.50, and 5.62 %, respectively [13]. The results of the present study also confirmed similar AUC ratios of the metabolites, and the carboxylated form, an inactive metabolite of tofogliflozin [13], was the main circulating entities with comparable exposure to tofogliflozin. Tofogliflozin is thought to be metabolized to its carboxylated form by CYP2C18, 4A11, and 4F3B [6]. Due to little involvement of these enzymes in the metabolism of marketed medicines, the potential for drug-drug interactions is expected to be very low. From the results of the human mass balance study [13] and the present study, urinary excretion of unchanged drug and the carboxylated form amounted to about 16 % and 38 %, respectively, of the oral dose. Fifteen percent of the dose was recovered as the carboxylated form in feces [13]. These findings suggest that tofogliflozin would have multiple elimination pathways and sole renal or hepatic impairment would not cause a drastic change in PK profiles of tofogliflozin.

We found that exposure in the Japanese subjects was slightly higher than that in the Caucasian subjects. After body weight standardization, the ratio of the exposures was approximately 1. With consideration of the metabolic profile of tofogliflozin, this finding implies that the ethnic exposure difference was mainly attributable to body size rather than metabolic variability.

As the pharmacological mechanism of action of tofogliflozin is the inhibition of urinary glucose reabsorption, we evaluated $UGE_{0\text{-}24h}$. The relationship between tofogliflozin concentration and $UGE_{0\text{-}24h}$ was evaluated by E_{max} model. The model indicates that $UGE_{0\text{-}24h}$ reaches the maximum level when C_{avg} of tofogliflozin is approximately more than 100 ng/mL, corresponding to the dose of between 20 and 40 mg. As to ethnic difference, in a dose of 20 mg, there was a tendency to be smaller $UGE_{0\text{-}24h}$ in Caucasian subjects than that in Japanese subjects. It may be due to smaller exposure of tofogliflozin in Caucasian subjects. Supporting this explanation, the E-R relationship showed no distinct difference between both ethnicities. Therefore, in healthy subjects, there is no ethnic difference in PD response of tofogliflozin under the same systemic exposure condition.

It is suggested that the dose which achieves the maximal level of $UGE_{0\text{-}24h}$ is similar between healthy and T2DM subjects by several reports of dapagliflozin. Simple E_{max} model analysis was applied to $UGE_{0\text{-}24h}$ in T2DM patients exposed by dapagliflozin and almost maximum $UGE_{0\text{-}24h}$ was observed with 5 to 10 mg dose of dapagliflozin [14]. When 10 mg of dapagliflozin was administered to healthy or T2DM subjects, clinically relevant $UGE_{0\text{-}24h}$ was observed for both, while T2DM patients showed relatively higher maximal $UGE_{0\text{-}24h}$ [15]. It implies that the E-R relationship of SGLT2 inhibitors in healthy subjects would be adapted to T2DM patients in respect of selecting the effective doses. In Phase 2 and 3 studies of tofogliflozin with Japanese T2DM patients, the dose of 20 mg once daily as monotherapy significantly decreased HbA1c by 0.990 % as

placebo-adjusted mean change and the dose of 40 mg did not produce any further decrease [4]. It is consistent with the recommended dose from our E-R analysis, which was estimated between 20 and 40 mg.

Based on available published data, tofogliflozin has higher BA (97.5 % [7]), lower plasma protein binding (83 % [7]), more sensible excretion ratio (16 % [13]), and shorter $t_{1/2}$ (5–6 h) than most of other SGLT2 inhibitors marketed in the world [2, 16]. However, such PK differences do not seem to affect the amount of UGE noticeably in the clinically recommended dose. Average $UGE_{0\text{-}24h}$ in healthy subjects among clinically recommended doses of other SGLT2 inhibitors was between 48.6 to 62.0 g [17–20] and that of tofogliflozin was 47.3–59.1 g following the single doses of 20 and 40 mg in the SAD study. Furthermore, the inhibition ratio in healthy subjects was estimated using in vitro IC_{50} against hSGLT2 [3] and average free concentration which was calculated with protein binding ratio [2, 7, 16] and C_{avg} using $AUC_{0\text{-}inf}$ divided by dosing interval for each SGLT2 inhibitor [15, 18–20]. The inhibition ratios attain more than approximately 80 % from all of the SGLT2 inhibitors, which suggests tofogliflozin as well as other SGLT2 inhibitors reaches the maximum effect on UGE even though some of PK characteristics were different from other SGLT2 inhibitors.

There were no serious adverse events when tofogliflozin was administered to healthy male subjects. Particularly, no occurrence of hypoglycemia can be explained mainly by the fact that tofogliflozin has the high selectivity against SGLT2 and glucose to maintain normoglycemia is reabsorbed via SGLT1 under the condition of SGLT2 inhibition [3]. A difference in the frequency and degree of an increase in blood ketone bodies after administration was observed between the SAD and MAD studies. As blood ketone bodies are greatly affected by daily food intake, this difference would reflect the food intake condition at administration, that is, fasting condition versus food intake condition. It implies that T2DM patients should take a meal just before or after administration of tofogliflozin.

In the present 3 studies, a comprehensive analysis of PK, PD, and their relationship of tofogliflozin in healthy volunteers were performed. These analysis would contribute to predict or confirm the efficacy across the dose levels and provide optimal treatment for the majority of T2DM patients.

Conclusions

Tofogliflozin was generally well tolerated by healthy male subjects. The study results provide a detailed PK and PD profile of tofogliflozin in healthy male subjects: tofogliflozin is absorbed rapidly and has a dose-proportional PK with a mean $t_{1/2}$ of 5–6 h for doses up to 320 mg; multiple-doses of tofogliflozin administered once daily caused no accumulation of exposure; tofogliflozin increased UGE in a dose-dependent manner; and the observed PK/PD profile suggests that tofogliflozin inhibits renal tubular reabsorption of glucose at maximum when the tofogliflozin concentration reaches approximately 100 ng/mL which corresponds to the dose of between 20 and 40 mg.

Author Contributions

All authors meet the criteria for authorship as recommended by the International Committee of Medical Journal Editors (ICMJE). H.F. contributed data acquisition of the SAD and the food-effect study, critically revised the draft manuscript. Y.Ogama contributed data acquisition of the MAD study, critically revised the draft manuscript. Y.T., T.I., and K.T. contributed the design of the SAD study, the MAD study, and the food-effect study, respectively. N.N. contributed the design of the SAD study and the MAD study, interpretation of the data, critically revised the draft manuscript. Y.K. contributed the design of the food-effect study and interpretation of the data, critically revised the draft manuscript. S.I. supervised the preparation of the manuscript, critically revised the draft manuscript. N.K., T.S., Y.Ohba, and S.S. analyzed all the data in the manuscript and wrote the draft manuscript. All authors approved the final version to be published.

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Conflict of Interest

N.K., T.S., Y.Ohba, S.S., K.T., Y.T., and S.I. are employees of Chugai Pharmaceutical Co., Ltd. T.I. is on loan to our associate company, Chugai Clinical Research Center Co. H.F., Y.Ogama, N.N. and Y.K. declare that they have no conflict of interest.

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