The Journal of Clinical Pharmacology
53(6) 601–610

DOI: 10.1002/jcph.88

Pharmacokinetics and Pharmacodynamics of Canagliflozin, a Sodium Glucose Co-Transporter 2 Inhibitor, in Subjects With Type 2 Diabetes Mellitus

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Abstract

This study characterized single- and multiple-dose pharmacokinetics of canagliflozin and its *O*-glucuronide metabolites (M5 and M7) and pharmacodynamics (renal threshold for glucose [RT_G], urinary glucose excretion [UGE_{0-24h}], and 24-hour mean plasma glucose [MPG_{0-24h}]) of canagliflozin in subjects with type 2 diabetes. Thirty-six randomized subjects received canagliflozin 50, 100, or 300 mg/day or placebo for 7 days. On Days 1 and 7, area under the plasma concentration-time curve and maximum observed plasma concentration (C_{max}) for canagliflozin and its metabolites increased dose-dependently. Half-life and time at which C_{max} was observed were dose-independent. Systemic molar M5 exposure was half that of canagliflozin; M7 exposure was similar to canagliflozin. Steady-state plasma canagliflozin concentrations were reached by Day 4 in all active treatment groups. Pharmacodynamic effects were dose- and exposure-dependent. All canagliflozin doses decreased RT_G, increased UGE_{0-24h}, and reduced MPG_{0-24h} versus placebo on Days 1 and 7. On Day 7, placebo-subtracted least-squares mean decreases in MPG_{0-24h} ranged from 42–57 mg/dL with canagliflozin treatment. Adverse events (AEs) were balanced between treatments; no treatment-related serious AEs, AE-related discontinuations, or clinically meaningful adverse changes in routine safety evaluations occurred. The observed pharmacokinetic/pharmacodynamic profile of canagliflozin in subjects with type 2 diabetes supports a once-daily dosing regimen.

Keywords

canagliflozin, sodium glucose co-transporter 2 inhibitor, type 2 diabetes mellitus, pharmacokinetics, pharmacodynamics

In humans, glucose is freely filtered through the renal glomerulus and then reabsorbed in the proximal tubules. The renal threshold for glucose (RT_G) is the plasma glucose (PG) concentration at which tubular reabsorption of glucose begins to saturate; glucose is excreted into the urine in direct proportion to the glucose concentration above this threshold. The sodium glucose co-transporter 2 (SGLT2) is responsible for the majority of filtered glucose reabsorption from the lumen.[1,2] Patients with diabetes have been shown to have elevated renal glucose reabsorption, which may contribute to persistent elevated PG concentrations.[3,4]

Canagliflozin, an orally active inhibitor of SGLT2, is currently in development for the treatment of patients with type 2 diabetes mellitus.[5,6] By inhibiting SGLT2, canagliflozin inhibits glucose reabsorption in renal proximal tubular cells, thereby reducing the RT_G.[7,8] In preclinical models of diabetes, canagliflozin reduces RT_G, increases urinary glucose excretion (UGE), decreases PG, reduces weight gain, and improves β-cell function.[9]

In a multiple-dose clinical study in healthy subjects, once-daily, orally administered canagliflozin decreased the 24-hour mean RT_G and increased UGE in a dose-dependent manner while 24-hour mean PG (MPG_{0-24h}) levels were not affected by canagliflozin treatment.[6] Maximal lowering of the 24-hour mean RT_G to approximately 60 mg/dL and increases in mean 24-hour UGE to up to approximately 70 g were seen with canagliflozin treatment.[6]

O-glucuronidation is the major metabolic elimination pathway for canagliflozin, and the two major metabolites are the inactive M5 and M7 *O*-glucuronide conjugates of unchanged drug (unpublished data). The purpose of the current double-blind, placebo-controlled, randomized, parallel-group, multicenter, Phase 1 study was to

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Submitted for publication 27 October 2012; accepted 18 March 2013.

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characterize the pharmacokinetics, pharmacodynamics, and safety of canagliflozin and its inactive *O*-glucuronide metabolites (M5 and M7) after multiple oral doses in subjects with type 2 diabetes mellitus (ClinicalTrials.gov: NCT01128985).

Methods

Study Population

This study was conducted from April 6, 2010, to July 12, 2010 at two sites in Fort Myers and Miramar, FL, USA. The study protocol and all amendments were reviewed and approved by IntegReview Ethical Review Board, Austin, TX, USA. This study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki and that are consistent with Good Clinical Practices and applicable regulatory requirements. All subjects provided written informed consent to participate in the study after having been informed about the nature and purpose of the study, participation/termination conditions, and the possible risks and benefits of treatment.

This study enrolled men and women who were between 25 and 65 years of age, had a body mass index between 18 and 39.9 kg/m^2, had a body weight of at least 50 kg, and had a history of type 2 diabetes mellitus for at least 1 year, but not more than 12 years, at the time of screening. Eligible subjects were medically stable based on physical examination, medical history, laboratory results, vital sign measurements, and 12-lead electrocardiogram (ECG) at screening. In addition, eligible subjects were on a stable regimen of antihyperglycemic therapy for at least 2 months prior to screening and had a fasting plasma glucose (FPG) concentration between 140 and 270 mg/dL at baseline. Stable antihyperglycemic regimens could include a single oral agent (eg, metformin, a sulfonylurea, a meglitinide, a dipeptidyl peptidase-4 inhibitor, or an α-glucosidase inhibitor) with glycated hemoglobin (HbA_{1c}) $\geq$6.5% and $\leq$9.5%, low-dose dual oral antihyperglycemic therapy (ie, $<$50% maximum labeled doses of both agents) with HbA_{1c} $\geq$6.5% and $\leq$9.5%, or no current antihyperglycemic therapy with HbA_{1c} $\geq$7.0% and $\leq$10.0%. Subjects with a history of having taken insulin, thiazolidinediones, exenatide, thiazide diuretics, or β-blockers within 3 months of the screening visit were not eligible for participation in the study. Subjects were excluded if they had a history of clinically significant diabetic complications, type 1 diabetes mellitus, or repeated severe hypoglycemic episodes. Subjects were also excluded if they had an estimated glomerular filtration rate (eGFR) $<$70 mL/min/1.73 m^2 based on the Modification of Diet in Renal Disease (MDRD) equation[10] or a history of, or currently active renal diseases, nephrolithiasis, or repeated upper or lower urinary tract infections including prostatitis.

Study Design

Following a 3-week screening phase and a 3-week washout of antihyperglycemic agents that included dietary counseling for all subjects, eligible subjects entered the clinical research center on Day −2 and underwent baseline safety and FPG assessments. On Day −1, all subjects received a single-blind dose of placebo and underwent baseline pharmacodynamic assessments. Subjects were then randomized in a 1:1:1:1 ratio to receive oral canagliflozin 50, 100, or 300 mg/day (supplied as over-encapsulated tablets) or matching placebo for 7 days. Subjects were stratified by FPG level measured on Day −2 ($\leq$200 mg/dL or $>$200 mg/dL) to ensure that within each FPG stratum a comparable number of subjects were assigned to each treatment group. Randomization was based on a computer-generated schedule using randomly permuted blocks.

On Days 1–7, a daily dose of canagliflozin or matching placebo was administered in a double-blind fashion. Each dose was given at approximately the same time each morning with 240 mL of water, approximately 10 minutes before a standardized breakfast. Standardized lunch and dinner were provided at 4.5 and 10.5 hours postdose, respectively. The standard meals contained 660–670 Calories (total), and each meal was approximately 55–59% carbohydrate, 15–16% protein, and 26–29% fat. On Days 8–12, no study medication was administered, and subjects remained at the study center for pharmacokinetic, pharmacodynamic, and safety evaluations prior to discharge on Day 12. A follow-up visit occurred 7–10 days after the last dose of study drug was administered.

Clinical Evaluations

Pharmacokinetics. Venous blood samples (3 mL, with dipotassium ethylenediaminetetraacetic acid [K_2EDTA] as an anticoagulant) were taken at predetermined time points up to 24 hours after the Day 1 dose or up to 120 hours after the Day 7 dose for determination of plasma concentrations of canagliflozin and its major metabolites, M5 and M7. Blood samples (3 mL) were also collected predose on Days 3–6 to determine trough plasma concentrations (C_{trough}) of canagliflozin (ie, the concentration measured at the end of a dosing interval). Urine samples were collected at the time intervals 0–4, 4–10, 10–13, and 13–24 hours on Days 1 and 7 and from 24–48 hours after the Day 7 dose for determination of urine canagliflozin and metabolite concentrations.

Pharmacodynamics. Blood samples (2 mL) were taken at predetermined time points on Days −1, 1, 2, 7, and 8 for the measurement of PG concentrations. Urine samples were collected at the time intervals 0–4, 4–10, 10–13, and 13–24 hours on Days −1, 1, 2, 7, 8, and 9 for the assessment of UGE.

Bioanalytical Analyses

K_2EDTA plasma canagliflozin, M5, and M7 concentrations were determined with $^{13}C_6$-canagliflozin as an internal standard for all compounds using validated liquid chromatography–tandem mass spectrometry (LC–MS/MS) methods. The high-performance liquid chromatography system consisted of a Shimadzu LC-20AD pump with Shimadzu SIL-HTC autosampler (Shimadzu Corporation, Kyoto, Japan). An API4000 mass spectrometer with a Turbo-Ionspray™ Interface (AB SCIEX, Framingham, MA, USA) in the positive ion mode was used for mass spectrometric determination. Multiple reaction monitoring transitions were m/z 462.1 → 267.0 for canagliflozin, 468.1 → 273.0 for the internal standard, and 638.2 → 427.0 for both M5 and M7. The method for canagliflozin plasma determination used a liquid–liquid extraction with *tert*-butylmethylether, followed by chromatography with 30% ammonium acetate (0.01 M) and 70% methanol as the mobile phase on a Waters XBridge™ C18 column (5 cm × 4.6 mm, 3.5 μm particle size). The validated quantification range was 5.0–10,000 ng/mL. The method for M5 and M7 determination used a protein precipitation with acetonitrile, followed by chromatography with a gradient of ammonium formate (0.01 M, pH 4.0 with formic acid) and methanol using the same column as above. The validated quantification range was 5.0–10,000 ng/mL. For both assays, the validation was performed according to US Food and Drug Administration (FDA) guidance for bioanalysis.[11–13] This included within- and between-run precision and accuracy, selectivity, matrix effect, recovery, incurred sample reproducibility, and stability (blood, plasma, processed sample). All validation results were within predefined acceptance criteria. The storage period between sample collection and analysis was covered by the available long-term stability data for the analytes (the validated storage period is 454 days for canagliflozin and 424 days for M5 and M7 at −20°C). Urine canagliflozin, M5, and M7 concentrations were determined using the same sample preparation and LC–MS/MS methods as validated for plasma determination; these methods were considered qualified methods without further validation for urine. Run acceptance criteria for the qualified urine assays and the validated plasma assays were in accordance with FDA guidance for bioanalysis.[11–13] These plasma and urine analyses were performed by Frontage Laboratories Co. Ltd. (Shanghai, China) under the supervision of the Sponsor's Bioanalytical Department at Janssen Research & Development, LLC.

Safety Evaluations

Clinical laboratory tests, 12-lead ECGs, vital sign measurements, and physical examinations were performed at predefined time points throughout the study. Adverse events (AEs) were monitored on a daily basis from the signing of informed consent until the last study procedure. Treatment-emergent adverse events (TEAEs) were defined as AEs that were new in onset or increased in severity or frequency following administration of study drug. All TEAEs were categorized by the investigator according to intensity and relationship to study drug.

Statistical Analyses

Sample Size Determination. Based on data from a previously completed study of canagliflozin pharmacokinetics,[14] the inter-subject coefficient of variation for the maximum observed plasma concentration during a dosing interval (C_{max}) and the area under the plasma concentration-time curve during a dosing interval (AUC_τ) of canagliflozin was estimated to be no more than 25%, both after a single dose and at steady state. Therefore, assuming a coefficient of variation of 25%, a sample size of nine subjects per treatment group was estimated to be sufficient for the point estimate of mean pharmacokinetic parameters to fall within the range of 82.5% and 121.2% of the true value with 95% confidence.

Based on data from a previous study of canagliflozin pharmacodynamics,[15] the inter-subject standard deviation (SD) for $MPG_{0–24h}$ was estimated to be approximately 20 mg/dL. Using this SD value, assuming equal SDs between canagliflozin doses and placebo, a sample size of nine subjects completing the study in each treatment group would be sufficient for the point estimate of the difference in the mean change from baseline in $MPG_{0–24h}$ between each canagliflozin dose and placebo to fall within ±16.5 mg/dL of the true value with 90% confidence.

Pharmacokinetics. Pharmacokinetic parameters were determined for each subject from plasma or urine data for canagliflozin and its inactive *O*-glucuronide metabolites (M5 and M7) using validated WinNonlin® (Pharsight Corporation) software, version 5.2.1. C_{max}, the time at which C_{max} was observed (t_{max}), and AUC_τ were calculated for each canagliflozin dose on Days 1 and 7. The terminal elimination half-life ($t_{1/2}$) and accumulation ratio (AUC_τ at steady state/AUC_τ following a single dose) were calculated on Day 7. C_{trough} was obtained from Days 3–7. The cumulative amount of canagliflozin, M5, and M7 excreted in urine (A_e) on Days 1 and 7 was determined from urine data. All estimated pharmacokinetic parameters for canagliflozin were summarized using descriptive statistics for each dose.

Pharmacodynamics. Pharmacodynamic parameters determined on Days −1, 1, and 7 included $MPG_{0–24h}$, $UGE_{0–24h}$, and 24-hour mean RT_G. RT_G was calculated over three separate time intervals (0–4, 4–10, and 10–24 h) using the measured PG profiles, UGE, and eGFR (calculated using the MDRD equation[10]), as described previously.[6,16] RT_G values obtained using this

methodology have recently been reported to agree well with values obtained using the multiple hyperglycemic clamp approach.[17] Twenty-four-hour mean RT_G was calculated as a weighted average of the values over these three time intervals. $MPG_{0–24h}$, $UGE_{0–24h}$, and 24-hour mean RT_G results were summarized with descriptive statistics; RT_G obtained at different intervals was used as a pharmacodynamic marker for determining the relationship between canagliflozin plasma concentration and the effects on renal glucose reabsorption. For each day of measurement (Days 1 and 7), analysis of covariance (ANCOVA) models with one of the pharmacodynamic parameters as the dependent variable, treatment as a fixed factor, and baseline value as a covariate were used to estimate the least-squares mean and inter-subject variance. Using the estimates from the model, point estimates and 90% confidence intervals (CIs) for the difference in the least-squares mean change from baseline between each canagliflozin dose and placebo were determined for $MPG_{0–24h}$, $UGE_{0–24h}$, and 24-hour mean RT_G on Days 1 and 7.

Exposure–response analysis was performed using RT_G as the pharmacodynamic variable. This was done because RT_G provides the most direct measurement of SGLT2 inhibition available, whereas the rate of UGE is dependent on both the filtered glucose load and the extent of SGLT2 inhibition. The exposure–response relationship between plasma drug concentration and RT_G was described using a maximum-exposure (E_{max}) model in which the pharmacodynamic effects of canagliflozin on RT_G depend on the current plasma drug concentration (often called a direct-response model). A direct-response model was chosen because there is no apparent time delay between increases in plasma drug concentrations and increases in UGE, and an E_{max} model (equation 1) was used based on several previous studies showing a plateau in the dose- and concentration-response relationships for both UGE and RT_G in healthy subjects and subjects with type 2 diabetes mellitus. This analysis was performed using the data obtained from both Days 1 and 7 together and separately using only the steady-state (Day 7) data.

$$\Delta RT_G(\%) = -\Delta RT_{G,max}(\%) \times \frac{[CANA]}{[CANA] + EC_{50}} \quad (1)$$

where ΔRT_G (the percent change from baseline in RT_G) and [CANA] (the mean plasma canagliflozin concentration in ng/mL during a given RT_G measurement interval) were measured. The parameters $\Delta RT_{G,max}$ (the maximal percentage reduction from baseline in RT_G) and EC_{50} (the plasma drug concentration giving half-maximal lowering of RT_G) were derived from fitting the model to the data. The best fit of equation 1 to the data was obtained using nonlinear regression in MATLAB® version 7.10 using the *nlinfit* command.

Results

Thirty-six subjects were randomly assigned to receive canagliflozin (50, 100, or 300 mg/day) or placebo for 7 days. All 36 subjects received at least one dose of study drug and completed the study. Subject demographic and baseline characteristics were comparable across treatment groups (Table 1). The majority of subjects were white (94% [34/36]) and Hispanic (97% [35/36]). Median subject age was 55.5 years (range, 33–64 years), and mean (SD) baseline body weight was 83.1 (16.7) kg. An equal number of men and women participated in the study. At baseline, mean (SD) FPG was 185(26) mg/dL and mean (SD) glycated hemoglobin was 8.1% (0.7%).

Pharmacokinetics of Canagliflozin and Its Inactive *O*-Glucuronide Metabolites (M5 and M7)

Single- and multiple-dose pharmacokinetic results for canagliflozin and its inactive *O*-glucuronide metabolites (M5 and M7) are summarized in Table 2. The mean plasma concentration-time profiles for the 50-, 100-, and 300-mg canagliflozin dose groups on Days 1 and 7 are shown in Figure 1. Mean plasma canagliflozin, M5, and M7 concentrations increased in a dose-dependent manner. Median t_{max} values were 1.5–2.0 hours for canagliflozin, 1.75–4.5 hours for M5, and 2.0–3.0 hours for M7 for all doses on Days 1 and 7. The terminal elimination half-life of canagliflozin was independent of dose and ranged from about 14–16 hours on Day 7; the terminal elimination half-lives of M5 and M7 ranged from about 14–15 and 14–17 hours, respectively. In all active treatment groups, steady-state plasma concentrations of canagliflozin, M5, and M7 were reached by Day 4. At steady state, mean canagliflozin accumulation ratios ranged from 1.29–1.36 for all three doses, mean M5 accumulation ratios ranged from 1.22–1.43, and mean M7 accumulation ratios ranged from 1.23–1.28. For all doses on Days 1 and 7, the ratio of metabolite to parent AUC ratio (corrected for the differences in molecular weights) ranged from 0.52–0.67 for M5 and from 0.70–1.04 for M7. Less than 1% of the administered canagliflozin dose was excreted unchanged in urine; approximately 7–10% was excreted in urine as M5 and approximately 21–32% was excreted as M7 (Table 2).

Canagliflozin Pharmacodynamics

Pharmacodynamic assessments were performed for all 36 subjects who completed the study. Changes in 24-hour mean RT_G, $UGE_{0–24h}$, and $MPG_{0–24h}$ are summarized in Table 3. Mean RT_G decreased to 119 mg/dL in the canagliflozin 50-mg group (~51% reduction from baseline value of 244 mg/dL), to 76.8 mg/dL in the canagliflozin 100-mg group (~64% reduction from baseline value of 212 mg/dL), and to 85.1 mg/dL in the canagliflozin 300-mg group (~64% reduction from baseline value of 237 mg/dL).

Table 1. Subject Demographic and Baseline Disease Characteristics

	Placebo (n = 9)	Canagliflozin 50 mg (n = 9)	Canagliflozin 100 mg (n = 8)	Canagliflozin 300 mg (n = 10)
Race, n (%)				
Black or African American	0	1 (11)	0	1 (10)
White	9 (100)	8 (89)	8 (100)	9 (90)
Ethnicity, n (%)				
Hispanic or Latino	9 (100)	9 (100)	8 (100)	9 (90)
Not Hispanic or Latino	0	0	0	1 (10)
Gender, n (%)[a]				
Female	5 (56)	4 (44)	3 (38)	6 (60)
Male	4 (44)	5 (56)	5 (63)	4 (40)
Age, years				
Mean (SD)	50.3 (9.8)	55.0 (9.3)	51.5 (4.7)	52.7 (7.5)
Median	53.0	58.0	50.5	55.0
Range	33–63	37–64	46–58	40–61
Weight, kg				
Mean (SD)	84.2 (15.1)	82.9 (19.9)	82.2 (11.5)	83.2 (20.5)
BMI, kg/m^2				
Mean (SD)	31.3 (4.8)	31.6 (5.2)	29.2 (3.0)	31.4 (5.4)
FPG level, n (%)				
≤200 mg/dL	7 (78)	7 (78)	6 (75)	7 (70)
>200 mg/dL	2 (22)	2 (22)	2 (25)	3 (30)
FPG, mg/dL[b]				
Mean (SD)	182 (24)	189 (28)	182 (35)	185 (20)
HbA_{1c}, %[b]				
Mean (SD)	7.8 (0.8)	8.2 (0.8)	8.2 (0.6)	8.0 (0.7)
$UGE_{0–24h}$, g[b]				
Mean (SD)	9.8 (6.6)	14.5 (19.1)	15.6 (12.4)	10.5 (9.2)
eGFR, mL/min/1.73 m^{2b}				
Mean (SD)	117.9 (25.2)	110.1 (9.4)	106.3 (25.5)	121.8 (15.1)
AHAs prior to washout, n (%)[a]				
0	0	2 (22)	3 (38)	4 (40)
1	9 (100)	7 (78)	5 (63)	6 (60)
2	0	0	0	0

Abbreviations: SD, standard deviation; BMI, body mass index; FPG, fasting plasma glucose; HbA_{1c}, glycated hemoglobin; UGE, urinary glucose excretion; eGFR, estimated glomerular filtration rate; AHA, antihyperglycemic agent.

[a]Percentages may not total 100% due to rounding.

[b]FPG refers to Day −2 measurement; HbA_{1c} refers to screening measurement; $UGE_{0–24h}$ refers to Day −1 measurement; eGFR refers to Day 1 predose measurement.

Increases in $UGE_{0–24h}$ were observed with all canagliflozin doses on both Days 1 and 7, with similar increases in $UGE_{0–24h}$ of approximately 100 g in the canagliflozin 100- and 300-mg groups, and with modestly smaller increases in the canagliflozin 50-mg group (Table 3).

PG concentration-time profiles on Days −1 (baseline), 1, and 7 are shown in Figure 2. Near maximal reductions in $MPG_{0–24h}$ were seen on Day 1 for all canagliflozin doses, with only small additional decreases seen after 7 days of treatment. Dose-dependent reductions in $MPG_{0–24h}$ were observed, and all canagliflozin doses provided statistically significant reductions in PG relative to placebo on both Days 1 and 7 (Table 3).

The relationship between canagliflozin plasma concentration and RT_G was described using an E_{max} model. Figure 3 shows this relationship and the parameter values identified by fitting data using results from both Days 1 and 7 and from analyses using only Day 1 or Day 7. The effects of canagliflozin on RT_G were generally similar on both Days 1 and 7, with a slightly greater maximal reduction in RT_G estimated on Day 7 compared with Day 1. In the combined analysis using data from both Days 1 and 7, the estimated maximal reduction in RT_G was 64% (95% CI = 61–67%) and the estimated EC_{50} value was 32 ng/mL (95% CI = 19–45 ng/mL).

Safety

There were no treatment-related serious AEs and no subjects discontinued from the study. TEAEs were reported by 22% (2/9) of subjects in the placebo group and by 30% (8/27) of subjects treated with canagliflozin (all doses), with no apparent dose relationship. Constipation (11% [3/27]) and headache (7% [2/27]) were the most common TEAEs in the canagliflozin groups. There were

Table 2. Mean (SD) Pharmacokinetic Parameters Following Single and Multiple Doses of Canagliflozin

	C_{max} (ng/mL)	C_{max} (Metabolite/ Parent)[a]	t_{max} (h)[b]	AUC_τ (ng · h/mL)	AUC_τ (Metabolite/ Parent)[a]	$t_{1/2}$ (h)	Accumulation Ratio[c]	Ae_{24} (% of Dose)[d]
Canagliflozin 50 mg (n = 9)								
Day 1	426 (106)	—	2.0 (1.0–4.0)	3,139 (935)	—	—	—	0.46 (0.14)
Day 7	536 (174)	—	2.0 (1.0–5.0)	4,059 (1,105)	—	16.3 (4.8)	1.30 (0.11)	0.83 (0.29)
Canagliflozin 100 mg (n = 8)								
Day 1	1,096 (444)	—	1.5 (1.0–5.0)	6,357 (1,431)	—	—	—	0.55 (0.10)
Day 7	1,227 (481)	—	1.5 (1.0–5.0)	8,225 (1,947)	—	13.7 (2.1)	1.29 (0.11)	0.75 (0.23)
Canagliflozin 300 mg (n = 10)								
Day 1	3,480 (844)	—	1.5 (1.0–6.0)	22,583 (7,343)	—	—	—	0.40 (0.13)
Day 7	4,678 (1,685)	—	1.5 (1.0–2.0)	30,995 (11,146)	—	14.9 (4.8)	1.36 (0.12)	0.75 (0.32)
M5 metabolite (50-mg group; n = 9)								
Day 1	290 (144)	0.50 (0.17)	4.0 (2.0–5.0)	2,933 (1,796)	0.67 (0.23)	—	—	6.99 (2.20)
Day 7	324 (132)	0.47 (0.19)	4.0 (1.5–6.0)	3,607 (2,109)	0.64 (0.25)	14.8 (3.9)	1.25 (0.28)	10.10 (2.62)
M5 metabolite (100-mg group; n = 8)								
Day 1	503 (177)	0.38 (0.20)	4.5 (2.0–5.0)	4,871 (1,303)	0.57 (0.15)	—	—	8.11 (1.61)
Day 7	559 (191)	0.37 (0.20)	3.0 (1.5–6.0)	6,003 (1,943)	0.54 (0.15)	14.2 (2.6)	1.22 (0.24)	9.57 (2.38)
M5 metabolite (300-mg group; n = 10)								
Day 1	1,472 (474)	0.33 (0.11)	4.5 (1.5–8.0)	15,307 (4,296)	0.52 (0.11)	—	—	7.67 (1.56)
Day 7	1,900 (534)	0.31 (0.08)	2.0 (1.0–4.0)	21,911 (7,865)	0.54 (0.15)	13.8 (4.6)	1.43 (0.34)	10.49 (2.03)
M7 metabolite (50-mg group; n = 9)								
Day 1	547 (255)	0.95 (0.29)	3.0 (3.0–4.0)	4,637 (3,048)	1.04 (0.36)	—	—	21.24 (4.39)
Day 7	608 (305)	0.82 (0.18)	3.0 (2.0–5.0)	5,765 (3,989)	1.00 (0.44)	17.2 (5.0)	1.23 (0.15)	30.68 (6.97)
M7 metabolite (100-mg group; n = 8)								
Day 1	1,126 (547)	0.80 (0.41)	3.0 (2.0–6.0)	8,721 (4,445)	1.02 (0.50)	—	—	25.06 (6.40)
Day 7	1,276 (588)	0.80 (0.33)	2.5 (2.0–5.0)	10,819 (5,216)	0.98 (0.47)	13.9 (2.4)	1.25 (0.12)	31.88 (10.98)
M7 metabolite (300-mg group; n = 10)								
Day 1	2,591 (631)	0.57 (0.17)	3.0 (1.5–6.0)	22,036 (5,941)	0.75 (0.22)	—	—	20.85 (2.87)
Day 7	3,122 (542)	0.53 (0.15)	2.0 (1.5–3.0)	28,110 (7,655)	0.70 (0.18)	15.0 (4.7)	1.28 (0.19)	27.04 (4.09)

Abbreviations: SD, standard deviation; C_{max}, maximum plasma concentration; t_{max}, time to maximum plasma concentration; AUC_τ, area under the plasma concentration-time curve during the dosing interval; $t_{1/2}$, elimination half-life; Ae_{24}, amount of unchanged drug excreted in urine from time 0 to 24 hours; MW, molecular weight.

[a]Calculated as (parameter [metabolite]/MW [metabolite])/(parameter [parent]/MW [parent]); MW: canagliflozin, 454 g/mol; M5 and M7, 620.6 g/mol.

[b]Median (range).

[c]Calculated as AUC_τ (Day 7)/AUC_τ (Day 1).

[d]Calculated as 100 · (Ae · [MW canagliflozin/MW metabolite]/dose).

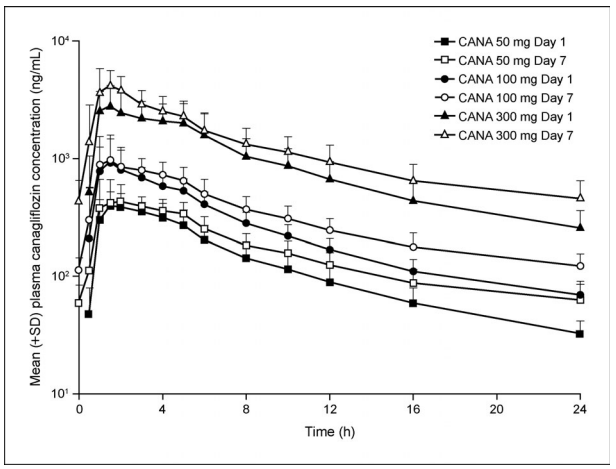


Figure 1. Mean (+SD) plasma concentration-time profiles on Day 1 (single dose) and Day 7 (multiple doses) for canagliflozin. SD, standard deviation; CANA, canagliflozin. n = 9 for canagliflozin 50 mg, n = 8 for canagliflozin 100 mg, and n = 10 for canagliflozin 300 mg.

no infection-related TEAEs. No clinically notable imbalances were seen among treatment groups in reports of specific TEAEs. All TEAEs were considered mild in intensity. No episodes of hypoglycemia were reported. None of the TEAEs reported during the study were considered by the investigator to be very likely or probably related to the study drug. One report of pollakiuria in the canagliflozin 50-mg group and one report of headache in the canagliflozin 100-mg group were considered possibly related to the study drug.

Treatment with canagliflozin had no clinically meaningful effects on laboratory test results, vital signs, ECG parameters, or physical examination measurements. On Days 7 and 9, the mean plasma creatinine concentration in the placebo group was unchanged compared with baseline, while in the canagliflozin 50-, 100-, and 300-mg groups, mean serum creatinine was slightly elevated (by about 0.05–0.07 mg/dL or 10%) in a non-dose-dependent fashion compared with baseline values. In all three canagliflozin groups, mean plasma creatinine values

Table 3. Mean (SD) Pharmacodynamic Parameters Following Single and Multiple Doses of Canagliflozin Compared With Placebo

	24-h Mean RT_G (mg/dL)	UGE_{0-24h} (g)	MPG_{0-24h} (mg/dL)
Placebo (n = 9)			
Baseline	235 (27)	9.8 (6.6)	207 (35)
Day 1	244 (26)	12.4 (8.6)	214 (41)
Day 7	235 (32)	16.2 (14.0)	215 (46)
Canagliflozin 50 mg (n = 9)			
Baseline	244 (35)	14.5 (19.1)	217 (40)
Day 1	143 (30)	80.8 (26.8)	190 (33)
Day 7	119 (33)	99.3 (17.5)	185 (38)
Day 1 difference of LS means (90% CI)[a]	−106.0 (−120.7; −91.3)[b]	63.0 (49.3; 76.6)[c]	−35.9 (−51.2; −20.6)[d]
Day 7 difference of LS means (90% CI)[a]	−119.7 (−137.1; −102.4)[b]	79.6 (62.9; 96.3)[c]	−42.2 (−64.0; −20.4)[d]
Canagliflozin 100 mg (n = 8)			
Baseline	212 (22)	15.6 (12.4)	195 (23)
Day 1	97.5 (21)	117.3 (18.8)	169 (19)
Day 7	76.8 (16)	119.1 (30.7)	158 (18)
Day 1 difference of LS means (90% CI)[a]	−131.3 (−147.0; −115.6)[b]	98.2 (84.2; 112.3)[c]	−36.1 (−52.0; −20.2)[d]
Day 7 difference of LS means (90% CI)[a]	−145.6 (−164.1; −127.1)[b]	98.6 (81.4; 115.9)[c]	−48.7 (−71.3; −26.1)[d]
Canagliflozin 300 mg (n = 10)			
Baseline	237 (33)	10.5 (9.2)	197 (35)
Day 1	104 (27)	113.1 (27.2)	164 (21)
Day 7	85 (20)	111.5 (24.3)	151 (23)
Day 1 difference of LS means (90% CI)[a]	−140.4 (−154.7; −126.2)[b]	99.8 (86.7; 113.0)[c]	−42.8 (−57.9; −27.6)[d]
Day 7 difference of LS means (90% CI)[a]	−150.3 (−167.1; −133.5)[b]	94.8 (78.7; 110.8)[c]	−57.3 (−78.9; −35.6)[d]

Abbreviations: SD, standard deviation; RT_G, renal threshold for glucose; UGE, urinary glucose excretion; MPG, mean plasma glucose; LS, least-squares; CI, confidence interval; ANCOVA, analysis of covariance; FPG, fasting plasma glucose.
No adjustments were made for multiplicity.
[a]Pairwise comparisons of LS mean changes from baseline between each canagliflozin dose and placebo.
[b]CIs are based on the pairwise comparison of LS means from an ANCOVA model including treatment as a factor and baseline RT_G as a covariate.
[c]CIs are based on the pairwise comparison of LS means from an ANCOVA model including treatment as a factor and baseline (Day −1) UGE as a covariate.
[d]CIs are based on the pairwise comparison of LS means from an ANCOVA model including treatment as a factor and FPG at baseline as a covariate.

returned toward baseline values at the follow-up visit. Relative to placebo, dose-dependent decreases in serum urate (5–16%) were observed in the canagliflozin treatment groups. Across all treatment groups, no changes in body weight were observed. Mean sitting systolic blood pressure (BP) remained relatively stable in the placebo group at approximately 121 mmHg from baseline to Day 9. In the canagliflozin treatment groups on Day 1, mean sitting systolic BP ranged from 121–127 mmHg at baseline (predose) and decreased by about 4 mmHg following treatment; that reduction persisted until Day 7 and returned to baseline at the follow-up visit after discontinuation of canagliflozin. The small decrease in systolic BP was not dose-dependent. There were no apparent differences among treatment groups in mean diastolic BP or pulse rate changes from baseline. Relative to Day –1, median 24-hour urine volumes on Day 7 were decreased in all groups, with no notable differences between the canagliflozin and placebo groups.

Discussion

In this study of subjects with type 2 diabetes mellitus, canagliflozin lowered RT_G, increased UGE, and lowered 24-hour mean PG levels, with reductions in both FPG and post-meal glucose values. A comparison of the canagliflozin 50-, 100-, and 300-mg doses showed that 24-hour mean RT_G changes from baseline decreased in a dose-dependent manner from 50 to 100 mg and appeared to plateau from 100 to 300 mg. Clinically meaningful, dose-dependent reductions from baseline in MPG_{0-24h} were observed with all three canagliflozin doses compared with placebo. A decrease in MPG_{0-24h} of nearly 60 mg/dL was observed with the 300-mg dose. Although similar reductions in 24-hour mean RT_G were observed with the 100- and 300-mg doses in this study, in other clinical studies, canagliflozin doses higher than 100 mg provided greater RT_G lowering than the 100-mg dose.[6,18] The relatively small sample size in the current study and the between-group differences in baseline RT_G values may have limited the ability to detect differences in RT_G lowering between these doses. Nearly maximal changes in RT_G, UGE, and PG were seen after the first dose of canagliflozin.

In the subjects with type 2 diabetes mellitus evaluated in this study, baseline 24-hour mean RT_G values ranged from approximately 212–244 mg/dL, which is notably higher than the average RT_G value that has been reported

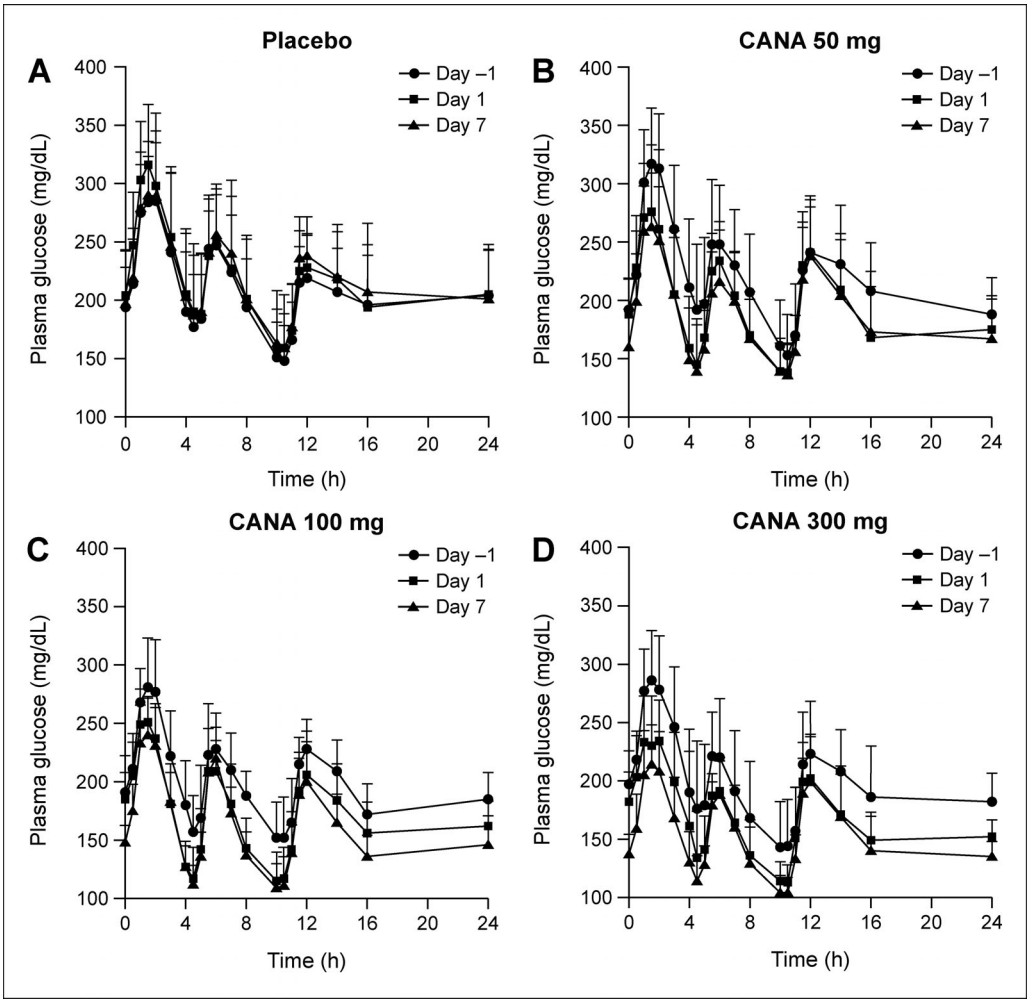
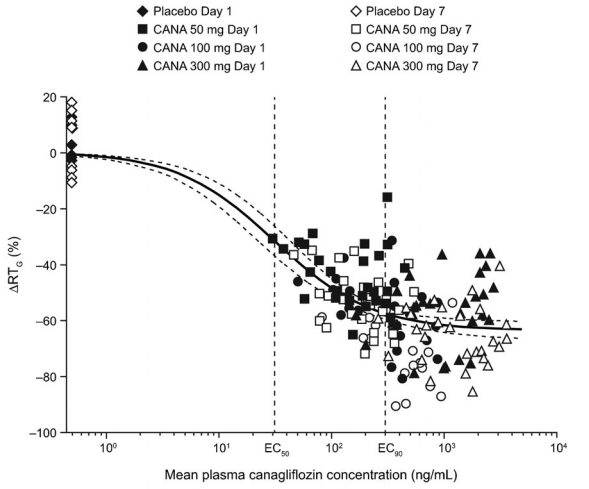


Figure 2. Mean (+SD) plasma glucose concentration-time profiles following single and multiple oral doses of placebo (A), canagliflozin 50 mg (B), canagliflozin 100 mg (C), or canagliflozin 300 mg (D). CANA, canagliflozin; SD, standard deviation. n = 9 for placebo, n = 9 for canagliflozin 50 mg, n = 8 for canagliflozin 100 mg, and n = 10 for canagliflozin 300 mg.

in nondiabetic individuals (~180 mg/dL).[19,20] The elevated baseline values of RT_G in subjects with type 2 diabetes are similar to values reported in an earlier canagliflozin study[16] and consistent with previous reports of increased renal glucose reabsorption in patients with diabetes relative to nondiabetic individuals.[3,4] In human studies and animal models of diabetes, increased proximal tubule expression of glucose transporters has been reported,[21–23] which could explain the increase in RT_G seen in individuals with diabetes. An increase in the renal glucose resorptive capacity in patients with diabetes may contribute to sustaining hyperglycemia in these patients.[24]

Maximum mean reductions in 24-hour RT_G to 77 mg/dL were seen with canagliflozin treatment. This value is above the PG level at which symptoms of hypoglycemia typically occur and thus, treatment with canagliflozin is not expected to be associated with an increased risk for hypoglycemia. Consistent with this observation, no AEs of hypoglycemia were reported in subjects receiving canagliflozin.

In this study, canagliflozin was generally well tolerated, with no clinically notable imbalances among treatments in the incidence or type of AEs reported or clinically relevant adverse changes in laboratory or ECG safety parameters. A small decrease in systolic BP was seen in the canagliflozin treatment groups relative to placebo without notable changes in diastolic BP or pulse. Although statistical comparisons were not performed, the reductions in systolic BP observed in this study are comparable in magnitude to those observed, and demonstrated to be statistically significant compared with placebo, in a Phase 3 study of canagliflozin in subjects with type 2 diabetes mellitus.[25] Similar decreases in systolic BP have been seen in previous studies with other SGLT2 inhibitors[26] and may be due, in part, to the osmotic diuresis that occurs



	Results using both Day 1 and Day 7 values	Results using only Day 7 (steady state) values	Results using only Day 1 values
$RT_{G,max}$ (%)	64.0 (61.0; 67.0)	68.9 (64.9; 72.9)	58.0 (53.8; 62.1)
EC_{50} (ng/mL)	32.1 (19.4; 44.8)	35.2 (17.3; 53.2)	24.9 (9.3; 40.4)
EC_{90} (ng/mL)	289.0 (174.7; 403.2)	317.1 (155.6; 478.5)	223.8 (83.7; 363.9)
r^2 for fit	0.71	0.76	0.70

Figure 3. Relationship between plasma canagliflozin concentrations and changes in RT_G. RT_G, renal threshold for glucose; CANA, canagliflozin; EC_{50}, half-maximal effective concentration; EC_{90}, 90% effective concentration; CI, confidence interval. The relationship between plasma drug concentrations was described using equation 1. Each dot represents an individual subject. The x-coordinate is the mean plasma concentration of canagliflozin over the time interval and the y-coordinate is the change in RT_G (relative to the Day −1 value). The lines show the best-fit relationship (solid), 95% CIs (dashed), and EC_{50} and EC_{90} values for the fit obtained using data from both Days 1 and 7. Results for subjects who received placebo are plotted with canagliflozin concentrations <1 ng/mL and appear on the left portion of the graph. The identified best-fit (95% CI) parameter values describing the relationship between mean plasma concentrations of canagliflozin and the effect of canagliflozin on RT_G are shown in the table below.

secondarily to increases in UGE. The findings from this study support further evaluation of the effects of canagliflozin with longer treatment durations, including assessment of other efficacy and safety parameters, such as body weight, BP, and risk of infection.

The pharmacokinetic results in this study in subjects with type 2 diabetes mellitus are similar to those in nondiabetic subjects[6] and support a once-daily dosing regimen. Canagliflozin, M5, and M7 concentrations rose in a dose-dependent fashion over the canagliflozin dose range examined and, consistent with the observed half-lives, steady-state levels were reached after 3 days of once-daily dosing. Minimal to modest accumulation of canagliflozin, M5, and M7 was observed at steady state for all doses. On a molar basis, systemic exposure to M5 was half that of canagliflozin and exposure to M7 was similar to canagliflozin over the dose range studied.

Only approximately 1% urinary excretion of canagliflozin was observed in this study, which is consistent with previous canagliflozin studies (unpublished data). Since SGLT2 is expressed on the luminal surface of proximal tubule cells and in vitro studies with other SGLT inhibitors suggest inhibition occurs from the luminal rather than the cytosolic side,[27] the relatively low renal excretion of canagliflozin raises the question as to the site of action of canagliflozin. The renal clearance of canagliflozin adjusted for protein binding is similar to eGFR, suggesting that unbound canagliflozin may be freely filtered and that canagliflozin concentrations in the lumen of the proximal tubule may be approximately equal to the unbound concentrations in the plasma. Adjusting the estimated in vivo EC_{50} values for protein binding (~99% [unpublished data]) gives estimated in vivo EC_{50} values based on unbound drug concentrations of 0.32–0.35 ng/mL (0.7–0.8 nM). These values are only modestly lower than the in vitro IC_{50} values for human SGLT2 determined under serum-free conditions reported for canagliflozin (2.4–4.4 nM).[5,9,28] Thus, the available data are generally compatible with the concept that unbound canagliflozin is freely filtered and acts on the luminal side of the proximal tubule to inhibit renal glucose reabsorption. This suggests that despite the relatively low observed renal excretion of canagliflozin (1%), sufficient free concentrations of canagliflozin may be present in the tubular lumen to provide effective inhibition of SGLT2-mediated glucose transport.

A limitation of this study is that the study population did not include racially or ethnically diverse subjects (almost all subjects were white and Hispanic). An additional limitation is that subjects with diminished renal function were not included in the study. As the efficacy of SGLT2 inhibitors is dependent on renal function, their efficacy will be diminished as renal function declines.

The pharmacokinetic and pharmacodynamic profiles of canagliflozin observed in this study of subjects with type 2 diabetes support a once-daily dosing regimen. If the results of this study showing that canagliflozin reduces RT_G, increases UGE, and reduces PG are confirmed in large-scale clinical trials, and if the long-term safety profile of canagliflozin is demonstrated to be acceptable, then canagliflozin may be a unique therapeutic option for the treatment of type 2 diabetes mellitus.

Declaration of Conflicting Interests

D. Devineni, C. R. Curtin, D. Polidori, J. Murphy, S. Rusch, and P. L. Rothenberg are employees of Janssen Research & Development. M. J. Gutierrez has no disclosures to report.

Funding

This study was supported by Janssen Research and Development, LLC. Editorial support for the writing of this manuscript was provided by Cherie Koch, PhD, of MedErgy, and was funded Janssen Global Services, LLC.

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