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Feasibility Study to Assess Canagliflozin Distribution and Sodium-Glucose Co-Transporter 2 Occupancy Using [^{18}F]Canagliflozin in Patients with Type 2 Diabetes

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Sodium-glucose co-transporter 2 (SGLT2) inhibitors, including canagliflozin, reduce the risk of cardiovascular and kidney outcomes in patients with and without type 2 diabetes, albeit with a large interindividual variation. The underlying mechanisms for this variation in response might be attributed to differences in SGLT2 occupancy, resulting from individual variation in plasma and tissue drug exposure and receptor availability. We performed a feasibility study for the use of [^{18}F]canagliflozin positron emission tomography (PET) imaging to determine the association between clinical canagliflozin doses and SGLT2 occupancy in patients with type 2 diabetes. We obtained two 90-minute dynamic PET scans with diagnostic intravenous [^{18}F]canagliflozin administration and a full kinetic analysis in 7 patients with type 2 diabetes. Patients received 50, 100, or 300 mg oral canagliflozin (n=2:4:1) 2.5 hours before the second scan. Canagliflozin pharmacokinetics and urinary glucose excretion were measured. The apparent SGLT2 occupancy was derived from the difference between the apparent volume of distribution of [^{18}F]canagliflozin in the baseline and post-drug PET scans. Individual canagliflozin area under the curve from oral dosing until 24-hours ($AUC_{P0\text{-}24h}$) varied largely (range 1,715–25,747 μg/L*hour, mean 10,580 μg/L*hour) and increased dose dependently with mean values of 4,543, 6,525, and 20,012 μg/L*hour for 50, 100, and 300 mg, respectively (P=0.046). SGLT2 occupancy ranged between 65% and 87%, but did not correlate with canagliflozin dose, plasma exposure, or urinary glucose excretion. We report the feasibility of [^{18}F]canagliflozin PET imaging to determine canagliflozin kidney disposition and SGLT2 occupancy. This suggests the potential of [^{18}F]canagliflozin as a tool to visualize and quantify clinically SGLT2 tissue binding.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THIS TOPIC?

☑ Sodium-glucose co-transporter 2 (SGLT2) inhibitors, including canagliflozin, improve cardiovascular and kidney outcomes in patients with and without type 2 diabetes, but a large and unexplained interindividual response variability exists.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ We assessed whether [^{18}F]canagliflozin positron emission tomography (PET) imaging can be used to determine canagliflozin tissue disposition and SGLT2 occupancy.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ [^{18}F]Canagliflozin PET imaging is feasible in patients with type 2 diabetes and able to detect changes in SGLT2 occupancy induced by canagliflozin.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ [^{18}F]Canagliflozin PET imaging has the potential to be used as a tool to visualize and quantify clinically SGLT2 tissue binding and pave the way to study SGLT2 density and response to treatment in health and disease.

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Sodium-glucose co-transporter 2 (SGLT2) inhibitors increase urinary glucose excretion and were initially developed as glucose lowering medication for patients with type 2 diabetes. Subsequent large cardiovascular outcome trials demonstrated that SGLT2 inhibitors reduce the risk of cardiovascular, heart failure, and kidney outcomes.[1–5] These clinical benefits appear to be present in patients with and without type 2 diabetes and are largely independent of their glucose lowering effects.[6] SGLT2 inhibitors also inhibit sodium re-absorption in the proximal tubule, which may contribute to natriuretic effects. This effect may explain, at least in part, the beneficial effects of SGLT2 inhibitors on heart failure and kidney failure.[7]

However, the precise underlying mechanisms for how SGLT2 inhibitors confer clinical benefits are incompletely understood, as is the response variability between and within patients. The response to SGLT2 inhibitors in markers of kidney function (i.e., albuminuria, estimated glomerular filtration rate (eGFR)) varies between individuals, such that ~20% of patients do not show a satisfactory improvement in albuminuria.[8] Understanding the underlying mechanisms for the individual variation in response could lead to more effective treatment strategies for these high-risk individuals. Imaging modalities might contribute to this understanding as they allow *in vivo* quantitative assessment of physiological, pathophysiological, and pharmacological processes at the (kidney) tissue level and are therefore increasingly used in nephrology. They provide unique insights into the nephroprotective effects of SGLT2 inhibitors and the variability in response.[9] To quantitatively and use minimal invasive methods to investigate the tissue distribution of SGLT2 inhibitors and SGLT2 density in patients, we previously developed a good manufacturing practice automated synthesis method for the ^{18}F-isotopologue of the extensively characterized, selective SGLT2 inhibitor canagliflozin, which thus shares its toxicological and pharmacological characteristics, enabling its immediate use in patients.[10]

We hypothesize that the underlying mechanisms of the varying response in multiple parameters within an individual can be attributed to variability in the causal path among drug administration, plasma exposure, drug tissue distribution, and tissue receptor interaction. Our aim in this clinical study was to assess the feasibility of positron emission tomography (PET) imaging using [^{18}F]canagliflozin and to determine the association between clinical canagliflozin doses and SGLT2 occupancy in patients with type 2 diabetes.

METHODS

The Canagliflozin REnal Distribution Intervention Trial was performed in accordance with the Declaration of Helsinki and Good Clinical Practice and approved by the local medical ethics committee. All participants signed written informed consent before any study-specific procedure commenced. The study was registered in the Netherlands Trial Register (accessible via the International Clinical Trial Registry Platform; NL7707) and the EU Clinical Trials Register (EUCTR2019-001835-29-NL).

Participants

Patients with type 2 diabetes and aged between 40 and 75 years were recruited via general practitioner practices and via the outpatient clinic of the department of internal medicine of the University Medical Center Groningen (UMCG). Most important exclusion criteria were an eGFR < 30 mL/minute/1.73 m^2, a cardiovascular event within 3 months prior to inclusion, established peripheral arterial disease, an active malignancy, significant prior radiation exposure, or a pregnancy or child-bearing potential without using reliable contraception. Use of a stable dosage of diuretics for at least 4 weeks prior to screening was allowed. Patients already using an SGLT2 inhibitor were allowed to participate, but SGLT2 inhibitor use should be interrupted 1 week prior to both PET visits because of interference with binding of [^{18}F]canagliflozin and canagliflozin used in the study.

Study design

The study consisted of one screening- and two study visits. At all visits, participants entered the clinic fasted and blood was collected for clinical chemistry assessment (central laboratory of the UMCG) after which a standardized breakfast low in sugar and fat content was offered. At screening, a physical examination was performed, and demographics were collected, including self-reported sex by the participant. On the first study visit, after intravenous 200 MBq [^{18}F]canagliflozin administration, a baseline 90-minute dynamic PET scan (Biograph Vision, Siemens Healthcare GmbH, Erlangen, Germany) was taken to measure selective uptake and accumulation of [^{18}F]canagliflozin, with both kidneys, abdominal aorta, and part of the liver in the field of view. On the second study visit, scheduled 1 week after the first visit, and after oral administration of 50, 100, or 300 mg canagliflozin, a second intravenous 200 MBq [^{18}F]canagliflozin dose was administered at the time of the reported maximal canagliflozin plasma concentration (T_{max}: 2.5 h)[11] followed by a second 90-minute dynamic PET scan. In this post-drug scan, receptor binding sites were (partly) occupied by canagliflozin, hence the reduction of [^{18}F]canagliflozin uptake compared with the baseline scan can be used to determine the apparent receptor occupancy (RO) based on the volume of distribution of the tracer obtained from both scans.[12] In all participants, arterial plasma samples were taken after radiotracer administration to derive the metabolite-corrected arterial input function. In addition, on the second study visit, plasma- and 24-hour urine samples were taken to quantify unlabeled concentrations of canagliflozin and its O-glucuronide metabolites (M5 and M7)[11] for the purpose of determining the pharmacokinetic parameters for each subject.

Study medication

Canagliflozin was commercially bought and packaged by the hospital pharmacy of the UMCG and given as one tablet of 100 or 300 mg. The 50 mg dose is not commercially available and therefore a tablet of 100 mg was split and the actual weight of the administered dose was reported. The synthesis and labeling of [^{18}F]canagliflozin was performed as described previously.[10] In short, [^{18}F]canagliflozin was obtained via a Cu-mediated [^{18}F]-fluorination of its boronic ester precursor. For this study, the mean ± SD radiochemical purity of [^{18}F]canagliflozin was 99.4% ± 0.45% (range 98.8–100%, n = 19). The mean ± SD administered dose for the baseline PET scan was 161 ± 28.3 MBq (range 86.3–181 MBq), with a molar activity of 305,176 ± 231,082 GBq/mmol, and for the post-drug PET scan 165 ± 32.4 MBq (range 79.5–190 MBq), with a molar activity of 322,146 ± 226,738 GBq/mmol.

Arterial sampling

Blood was sampled continuously through an arterial canula placed in the radial artery during the first 30 minutes of both PET scans using an online blood sampler and 8 manual whole blood and plasma samples were collected at t = 5, 10, 20, 30, 45, 60, 75, and 90 minutes after tracer administration. Activity concentration, metabolites, and parent fractions were measured to obtain the [^{18}F]canagliflozin metabolite corrected arterial input function.

Plasma and urine measurements

From the start of oral canagliflozin administration, 11 venous blood samples were taken at t = predose, 15, 25, 40, 60, 100, 150, 180, 210, and 270 minutes, and 24 h and stored at −80°C until analysis. At both study visits, urine was collected at four intervals (t = 0–2, 2–5, 5–11.5, and 11.5–24 hours relative to oral canagliflozin administration). Urinary excretion of glucose, creatinine, albumin, protein, sodium, and potassium was immediately measured (central laboratory of the UMCG), and samples were stored at −80°C until further analysis. Plasma and urine concentrations of canagliflozin and metabolites M5 and M7 were measured in all available samples using a validated liquid chromatography with tandem mass spectroscopy method (canagliflozin: calibration range 10–5,000 µg/L, lower limit of quantification (LLOQ) 10 µg/L, interassay precision (coefficient of variation) 1.1–3.8% and accuracy 92.9–101.9%; M5: calibration range 10–5,000 µg/L, LLOQ 10 µg/L, interassay precision 0.0–7.6%, accuracy 91.7–101.0%; M7: calibration range 10–5,000 µg/L, LLOQ 10 µg/L, interassay precision 0.0–5.5%, accuracy 95.5–102.5%)[13] at the department of clinical pharmacy and pharmacology of the UMCG at the end of the trial. Serum creatinine and cystatin C were measured at the end of the trial in the plasma samples t = 24 h.

Kinetic analysis on PET data and metabolite corrected arterial input function

Analysis of the PET-images and kinetic modeling was performed using PMOD (v4.105, PMOD Technologies LLC, Zürich, Switzerland). PET images were reconstructed into a series of 26 frames (7 × 10, 2 × 30, 3 × 60, 2 × 120, 2 × 180, 5 × 300, and 5 × 600 seconds) with corrections for detector normalization, deadtime, isotope decay, photon attenuation, random, and scattered coincidences. For each baseline PET scan, a volume of interest (VOI) of the left and right kidneys individually was created using the summed PET-data acquired from 50 to 90 minutes. First, a three-dimensional ellipsoid was manually drawn covering the whole kidney. Any parts of the liver, bile ducts, renal pelvis, ureter, or colon with high activity compared with the cortex overlapping this ellipsoid were manually removed and the final VOI was defined as the isodensity contour within the ellipsoid at 50% of the range (i.e., 0.5·(max−min)). After matching the baseline and post-drug PET scan, the baseline VOI was also used for the post-drug scan. Applying the VOI to the dynamic scans resulted in time-activity curves for both baseline- and post-drug PET scans. Next, also for each scan, the arterial input function was calibrated by fitting the radioactivity data of the online blood sampler to the manual plasma- and whole blood samples and then multiplied by the parent tracer fraction at each sampling timepoint to generate the individual metabolite corrected input function. Finally, the kinetic analysis was performed on the time-activity curves of the individual kidneys and their arterial input function.

The apparent volume of distribution (V_T; i.e., the ratio of [^{18}F]canagliflozin concentration in the kidneys to that in plasma in mL/cm^3), was the primary parameter of interest. [^{18}F]Canagliflozin in the kidneys can be specifically bound to the SGLT2, nonspecifically bound, or free in tissue. Under the assumption that the administration of oral canagliflozin only affects the concentration specifically bound [^{18}F]canagliflozin to the SGLT2 and the non-displaceable (i.e., the nonspecifically bound and free) concentration is the same within one individual, the difference in V_T of the post-drug scan compared with the V_T of the baseline scan can be used as measure of changes in RO of canagliflozin.[12]

The apparent receptor occupancy was calculated as[14]:

$$\mathrm{RO}\,(\%) = \frac{V_{T,\mathrm{baseline}} - V_{T,\mathrm{post-drug}}}{V_{T,\mathrm{baseline}}} \cdot 100$$

One and two tissue compartment models and a graphical analysis using the Logan plot were explored to describe the data and to obtain V_T values. The left and right kidneys were analyzed separately and per individual, an RO for both kidneys was obtained from which the average was used in the exposure response analyses. In the compartment models, the apparent blood volume fraction in the kidneys (vB) and the delay in time between arrival of [^{18}F]canagliflozin in the radial artery and the kidneys were fitted individually per kidney and per scan. For the Logan plots, the time when the system reaches equilibrium between the tissue and plasma compartments, was fixed at 10 minutes, and data until 60 minutes were used. Weighting was applied to the residuals based on frame duration and decay. Kinetic model evaluation and selecting a single method to use in all participants, was based on visual inspection to appropriately describe all participants data, Akaike Information Criterion, and standard errors of the parameters.

Statistics

Data preparation, statistical analysis, and graphical presentation were performed in R V.3.6.3.[15] Data are presented as median and 25th–75th interquartile range, unless stated otherwise. As measure for total plasma exposure, the area under the concentration time curve (AUC) from the time of oral administration to the last data point at 24 hours ($AUC_{P0\text{-}24h}$) was calculated for canagliflozin, M5, and M7 per patient by noncompartmental analysis (trapezoidal rule). The metabolite to parent molar ratios, corrected for differences in molecular weight (molecular weight canagliflozin 454 g/mol, M5 and M7 620.6 g/mol) were calculated for $AUC_{P0\text{-}24h}$. As measures for plasma exposure during the course of the PET scan, the individual AUC from the time of oral administration to the time of the end of PET scan ($AUC_{P0\text{-}PET}$) and the mean plasma concentration during the course of the PET scan ($C_{mean,PET}$) were used. Last data point included for $AUC_{P0\text{-}PET}$ and $C_{mean,PET}$ calculations was the sample closest to the end of the PET data acquisition used for the kinetic analysis. In case there was not a sample available close to the end of the PET scan, the average of the 2 samples closest to 60 minutes was taken. To obtain $C_{mean,PET}$, the AUC from the sample closest to tracer administration until the 60-minute sample was divided by the time interval between these 2 samples. The amount of compound excreted in urine from the time of oral administration until 24 hours was calculated as the percentage of administered dose and corrected for molecular weight: 100*((absolute amount excreted*(molecular weight canagliflozin/molecular weight metabolite))/dose).

Correlations between plasma exposure and RO were assessed using Pearson correlation analysis. The relative change in serum creatinine was calculated from 24 hours after oral administration to the baseline value of the post-drug PET visit. The relative change in serum cystatin C was obtained from the 24-hour sample relative to the t = 0 sample for oral canagliflozin. The change in the 24-hour urinary glucose excretion was calculated as the absolute difference between the cumulative glucose excretion of the 4 collection intervals from the post-drug PET visit and the baseline PET visit. Associations between covariates and plasma and kidney canagliflozin exposure, and between covariates and response were tested using linear regression analysis. Differences among the three treatment groups in exposure and response parameters were assessed with one-way ANOVA and a *post hoc* Bonferroni if applicable. Two-sided P values < 0.05 were considered statistically significant.

RESULTS

Ten participants were included in the study and their demographics are listed in **Table 1**. Plasma and urine samples were collected in 9 participants (67% men) who received canagliflozin 50, 100, or 300 mg (n = 2:4:3), resulting in 89 plasma and 34 urine samples for canagliflozin concentration-time profiles (**Figure 1**; **Figure S1**). In eight participants, both baseline and post-drug PET scans were obtained (**Figure 1**).

Table 1 Clinical data and demographics of the participants

Dose, mg	None	50		100				300					
Participant	**10**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**Median**	**25% IQR**	**75% IQR**
Age, year	59	72	73	67	70	68	58	69	69	61	69	63	70
Sex, M or F	M	M	M	M	F	M	F	F	M	M			
Weight, kg	121.2	81.5	75.5	145.2	88.8	84.1	97.4	73.7	94.8	92.7	90.8	82.2	96.8
Body mass index, kg/m^2	35.2	26.9	26.3	45.1	38.2	26.8	36.0	25.7	29.9	30.4	30.2	26.9	35.8
Systolic blood pressure, mmHg	128.3	128.0	129.3	144.0	122.0	122.7	135.7	136.0	132.0	116.0	128.8	124.0	134.8
Diastolic blood pressure, mmHg	73.7	78.0	70.3	74.3	60.3	68.0	83.0	86.3	77.0	59.3	74.0	68.6	77.8
Fasting plasma glucose, mmol/L	11.2	8.6	5.1	9.6	9.7	7.9	6.2	6.9	10.7	8.4	8.5	7.2	9.7
HbA1c, %, mmol/mol	6.8 (51)	7.0 (53)	7.7 (61)	7.6 (60)	7.5 (58)	6.8 (51)	6.8 (51)	6.2 (44)	8.0 (64)	7.9 (63)	7.3 (56)	6.8 (51)	7.7 (61)
Creatinine, µmol/L	64	95	66	103	62	65	56	60	146	120	66	63	101
eGFR, mL/ minute*1.73m^2	101	68	90	64	87	95	99	89	41	56	88	65	94
NT-proBNP, ng/L	27	24	44	186	86	16	16	90	94	362	65	25	93
Urinary albumin excretion, mg/24 hours	55.5	12.7	24.1	3,386.3	0.0	0.0	4.7	10.1	248.2	2,422.7	18.4	6.1	200.0
Type 2 diabetes duration, years	17	7	13	18	7	15	4	4	25	11	12	7	17
ACEi/ARB use	Y	N	Y	Y	Y	Y	N	N	Y	Y			
Diuretic use	Y	N	N	Y	Y	N	N	N	Y	N			

ACEi, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; eGFR, estimated glomerular filtration rate; IQR, interquartile range; NT-proBNP, N-terminal pro-brain natriuretic peptide.

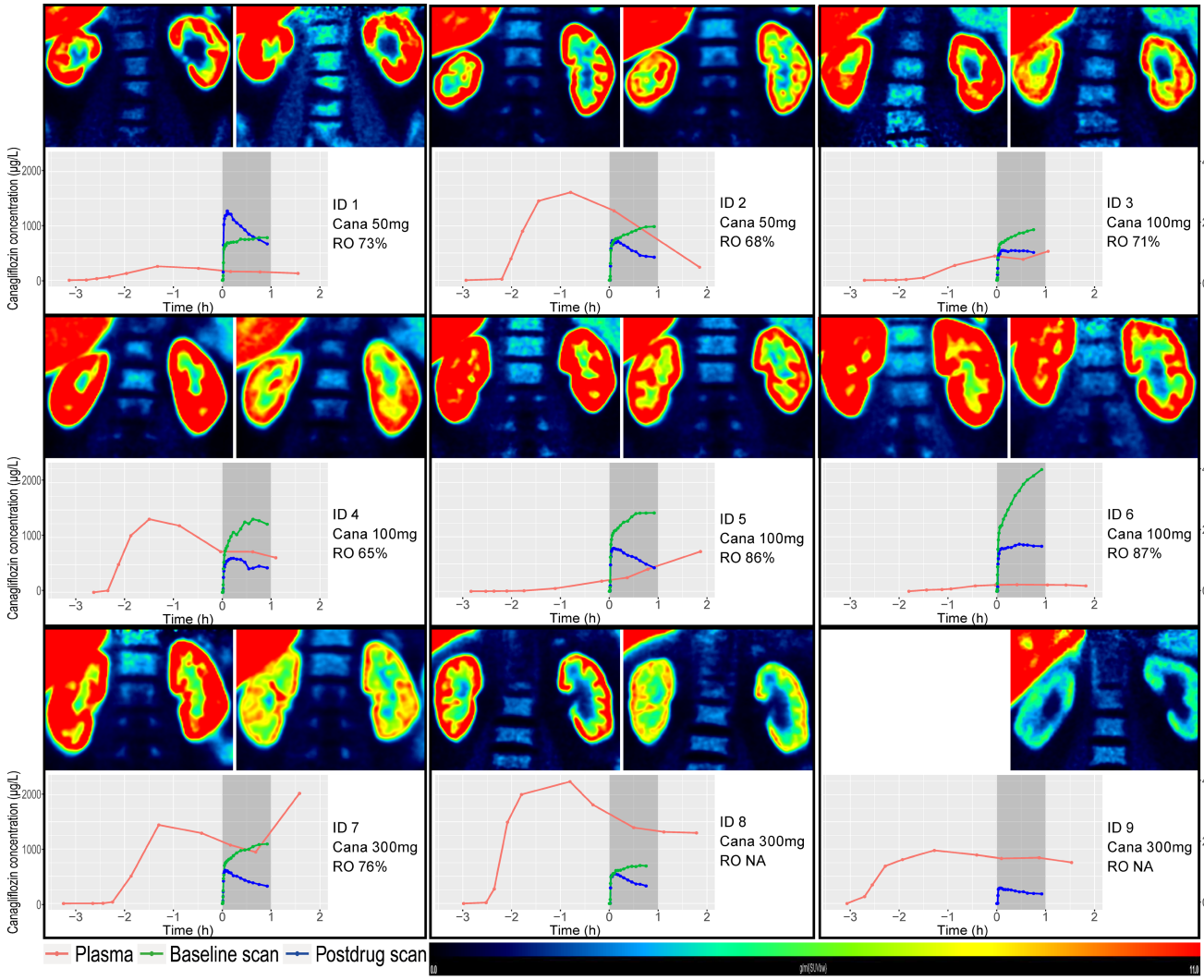

Figure 1 Combined PET and pharmacokinetic data per participant (ID). Top row: PET images of [^{18}F]canagliflozin uptake in part of the liver and both kidneys ~25 minutes after tracer administration (t = 0 hours) for the baseline (left) and post-drug scan (right). Bottom row: Plasma canagliflozin concentration (red, left y-axis) and individual time activity curves of [^{18}F]canagliflozin uptake (right y-axis) in both kidneys from the baseline (green) and post-drug (blue) PET scan. Gray bars: time frames of PET data acquisition used for kinetic analyses. PET images are scaled 0–11 SUVbw. NA, not applicable; PET, positron emission tomography; RO, apparent receptor occupancy; SUVbw, standardized uptake value, normalized to body weight, calculated as tissue radioactivity [KBq/g]/(injected dose [MBq]·body weight [kg]).

Plasma and urine exposure of canagliflozin and its inactive O-glucuronide metabolites

The individual concentration time profiles of canagliflozin and its O-glucuronide metabolites M5 and M7 in plasma and urine are presented in **Figure S1**. Individual plasma canagliflozin exposure in terms of AUC from the time of oral canagliflozin administration until the last measurement 24 hours later ($AUC_{PO\text{-}24h}$; **Table 2**) varied largely among participants, ranging from 1,715 to 25,747 µg/L*hour (mean 10,580 ± 7,909 µg/L*hour) and increased dose dependently with mean $AUC_{PO\text{-}24h}$ values of 4,543 (n = 2), 6,525 (n = 4), and 20,012 (n = 3) µg/L*hour, respectively, for the dose groups 50, 100, and 300 mg (P = 0.046, r^2 = 0.997). The $AUC_{PO\text{-}24h}$ of the 300 mg dose group was significantly higher compared with 50 mg (P = 0.049) and 100 mg (P = 0.041). There was no association among age, sex, weight, body mass index, eGFR, HbA1c, fasting plasma glucose, aspartate aminotransferase, alanine transaminase or total bilirubin, and $AUC_{PO\text{-}24h}$. Plasma canagliflozin exposure from time of oral canagliflozin administration until end of the PET scan ($AUC_{PO\text{-}PET}$) did not differ among groups (P = 0.11). The mean plasma canagliflozin concentration during time of oral canagliflozin administration until the end of the PET scan ($C_{mean,PET}$) was higher in the 300 mg (1,204.4 µg/L) compared with the 100 mg group (384.1 µg/L, P = 0.044), but not compared with the 50 mg group (457.4 µg/L, P = 0.13). Among participants with available plasma kidney exposure and RO data (n = 7), plasma canagliflozin exposure during the course of the PET scan (i.e., $AUC_{PO\text{-}PET}$ and $C_{mean,PET}$), did not differ among dose groups (P = 0.40 and P = 0.13, respectively).

Plasma exposure of M5 increased dose dependently with mean $AUC_{PO\text{-}24h}$ values of 7,989 (n = 2), 12,298 (n = 4), and 30,833 (n = 3) µg/L*hour, respectively, for the dose groups 50, 100, and 300 mg (r^2 = 1.000, P = 0.0074). Mean $AUC_{PO\text{-}24h}$ values of M7

Table 2 Pharmacokinetic and PET results

		Plasma exposure				Kidney exposure								
						L Kidney			R Kidney			Mean		
Dose (mg)	Participant	$AUC_{P0\text{-}24h}$ (μg/L*hour)	AUC_{P0_PET} (μg/L*hour)	$C_{mean,PET}$ (μg/L)	T_{max} (h)	$V_{T,baseline}$ [%SE] (mL/cm^3)	$V_{T,post\text{-}drug}$ [%SE] (mL/cm^3)	RO (%)	$V_{T,baseline}$ [%SE] (mL/cm^3)	$V_{T,post\text{-}drug}$ [%SE] (mL/cm^3)	RO (%)	$V_{T,baseline}$ (mL/cm^3)	$V_{T,post\text{-}drug}$ (mL/cm^3)	RO (%)
NA	10[a]	NA				23.7 [4.8]	NA	NA	28.9 [14.0]	NA	NA	26.3	NA	NA
50	1[b]	2,087	586	156	1.69	24.7 [11.5]	7.0 [1.8]	71.7	32.9 [10.6]	8.7 [1.5]	73.5	28.8	7.8	72.6
	2	7,000	3,686	759	1.68	20.1 [6.6]	6.5 [2.2]	67.4	20.9 [8.5]	6.7 [2.3]	68.1	20.5	6.6	67.7
Median		4,543	2,136	457	1.68			69.6			70.8			70.2
100	3[c]	6,139	883	435	3.60	25.4 [10.5]	7.2 [3.1]	71.5	23.8 [11.6]	7.2 [3.2]	69.8	24.6	7.2	70.6
	4	9,560	3,117	714	1.09	13.9 [13.0]	4.6 [4.9]	66.7	14.6 [14.7]	5.3 [3.7]	63.5	14.2	5.0	65.1
	5	8,685	388	267	4.66	54.3 [8.9]	7.3 [1.0]	86.5	51.4 [27.2]	7.2 [1.1]	86.0	52.8	7.3	86.2
	6	1,715	227	120	2.18	79.1 [15.3]	11.2 [8.8]	85.9	106.1 [12.9]	12.7 [2.4]	88.1	92.6	11.9	87.0
Median		7,412	636	351	2.89			78.7			77.9			78.4
300	7	25,747	3,790	1,279	4.46	17.3 [5.4]	4.0 [0.9]	76.9	16.1 [4.6]	4.1 [1.1]	74.7	16.7	4.0	75.8
	8[c,d,e]	22,569	6,002	1,498	2.00	NA	3.0 [2.0]	NA	NA	3.3 [2.0]	NA	NA	3.2	NA
	9[f]	11,720	2,926	836	1.71	NA	3.7 [1.7]	NA	NA	3.7 [1.1]		NA	3.7	NA
Median		22,569	3,790	1,279	2.00			76.9			74.7			75.8

AUC, area under the concentration time curve; $AUC_{P0\text{-}24h}$, AUC during 24 h after oral dosing; $AUC_{P0\text{-}PET}$, AUC from time of oral dosing until end of PET scan; $C_{mean,PET}$, mean concentration during first 60 minute of PET scan; NA, not applicable; PET, positron emission tomography; RO, apparent receptor occupancy; SE, standard error; T_{max}, time to maximum plasma concentration; $V_{T,baseline}$ and $V_{T,post\text{-}drug}$, volume of distribution of baseline or post-drug scan.

[a]No RO as only baseline PET scan was available for analysis. [b]Parent fraction of post-drug scan also used for correction arterial input function baseline scan, as metabolite data baseline scan were lacking. [c]Kinetic analysis performed on first 45 minutes due to obstruction of arterial canula thereafter. [d]Arterial canula placed in a. brachialis instead of a. radialis. [e]No $V_{T,baseline}$ as data blood monitor was not available. [f]No RO as only post-drug scan was available for analysis.

were 5,837 ($n=2$), 13,852 ($n=4$), and 28,055 ($n=3$) μg/L*hour for the dose groups 50, 100, and 300 mg. Less than 1% of the administered dose was excreted as parent canagliflozin over 24 hours (mean 0.58%, range (0.26–1.40)); 5.8% (3.3–9.9) as M5, and 14.0% (4.2–23.0) as M7.

Kidney canagliflozin exposure and receptor occupancy

Full kinetic analysis could be performed in seven participants (**Table 2**). From the individual PET images (**Figure 1**), there is a clear visual reduction in activity in the post-drug PET scan vs. the baseline PET scan in all participants. For the kinetic analysis, the Logan plot was most reliable to obtain a V_T for all participants and was therefore preferred. Within individuals, V_T values for the left and right kidneys were well comparable per scan and therefore the resulting RO values as well. Among individuals, V_T values varied largely (baseline range 14.2–92.6 mL/cm^3, median 25.4 mL/cm^3, $n=8$; post-drug range 3.2–11.9 mL/cm^3, median 6.6 mL/cm^3, $n=7$). In all participants, de V_T value of the post-drug scan was lower compared with the baseline scan, with an overall corresponding median RO value of 72.6% (69.2–81.0%, $n=7$). The RO did not differ significantly among the canagliflozin dose groups (70.2, 77.2 and 75.8 for the 50, 100 and 300 mg dose; $P=0.70$). There was no association between RO and canagliflozin exposure in terms of $AUC_{PO\text{-}PET}$ ($P=0.13$, $n=7$) and $C_{mean,PET}$ ($P=0.32$, $n=7$), urinary glucose excretion ($P=0.71$, $n=7$), or eGFR changes ($P=0.33$, $n=7$).

Effect of covariates on receptor occupancy

The mean baseline eGFR from both study visits did not correlate significantly with RO ($P=0.33$), nor did any of the other assessed clinical characteristics.

DISCUSSION

We performed a feasibility study for a novel [^{18}F]canagliflozin PET imaging method to assess the relation between clinical canagliflozin doses and SGLT2 occupancy in patients with type 2 diabetes. We showed reduced binding of [^{18}F]canagliflozin after oral canagliflozin administration supporting the feasibility of the [^{18}F]canagliflozin tracer to assess SGLT2 transporter occupancy with canagliflozin. This novel imaging approach has the potential to provide unique insights in the disposition of canagliflozin into the kidneys and may aid in understanding the observed interindividual response variability. Because the study was designed to assess feasibility aspects of the [^{18}F]canagliflozin tracer and the sample size was small, the results should be interpreted with caution and regarded as explorative.

Although the plasma canagliflozin exposure was in keeping with previous studies, we did not observe a clear association between canagliflozin dose and exposure.[16,17] Only the 300 mg dose group showed a significant increase in $AUC_{P0\text{-}24h}$ compared with the 50 and 100 mg groups. Moreover, no differences among dose groups were observed in $AUC_{P0\text{-}PET}$ and C_{mean}. Plasma exposure of the metabolites M5 and M7 was, however, higher compared with previous studies, and the contribution of parent canagliflozin to the total plasma parent and metabolites exposure was therefore substantial lower in our study; < 30% (~ 40% for M5 and ~ 35% for M7), in contrast to the previously reported 60%.[16] We do not have a clear explanation for this finding, but it seems that canagliflozin metabolism in this population is higher than expected. Urine excretion of canagliflozin was < 1%, consistent with literature.[16] Although the relatively small sample size may have hampered the ability to find dose-exposure associations, the pharmacokinetic data show that individual variability in canagliflozin plasma exposure results in overlapping exposure among the dose groups.

Despite the broad range of individual plasma canagliflozin exposures in our study, there was no relationship with the RO, nor with dose. Several aspects should be noted when interpreting this finding. First, we used the Logan plot to obtain the V_T values (from which the RO was calculated), and they therefore represent the total volume of distribution of [^{18}F]canagliflozin and no distinction can be made among specifically bound tracer to the SGLT2, non-specially bound, and free tracer in the tissue. However, the only difference between the baseline and post-drug scan was the administration of oral canagliflozin. We therefore believe it is reasonable to assume that this only affects the specifically bound concentration of the tracer to the SGLT2 and that the non-specifically bound and free concentrations are the same between the two PET scans. Accordingly, it is unlikely that the V_T values of the baseline and post-drug scans are differently affected, and thus can be used as a proxy for RO. Second, the RO values were relatively high and within a narrow range. Assuming free filtration of the unbound fraction of canagliflozin and a high protein binding,[18] the maximum, unbound canagliflozin concentration in the proximal tubules ranged between 2.8 and 33.4 nM. Five of the 7 participants would have expected to have full inhibition of the SGLT2 based on these concentrations and the reported Ki value of 4 nM for the SGLT2.[19] In these individuals, it is likely to observe high RO values in the same order of magnitude. However, in the two individuals with concentrations below the Ki value, no difference in RO was observed. Whether this lack of difference is due to the small sample size or reflects that other, unknown, factors determine the local concentration and its relation with RO, should be further assessed. Finally, the suggestion that for the entire canagliflozin exposure range in our study the maximal extent of SGLT2 saturation has been reached, would support findings from dose–response studies. These studies in patients with type 2 diabetes have been performed with daily dosages ranging from 25 up to 600 mg and all show a plateau in pharmacodynamic effect with increasing dosages. The effect on urinary glucose excretion increases clearly up to a dose of 100 mg, with little or no additional effect with higher doses.[20–22] Antihyperglycemic effects improve at least up to 100 mg dose-dependently, with some extra effect up to 300 mg and little thereafter.[21–23] Similar to our data, the maximal pharmacodynamic effects of canagliflozin seem to be exerted at a dose between 100 mg and 300 mg. This is the first study that provides exploratory evidence that a maximal RO might be reached in the 100–300 mg dose range.

Our findings support the further development and use of the novel [^{18}F]canagliflozin PET tracer to visualize and quantify clinically SGLT2 disposition. Future studies should more robustly assess the relationship among canagliflozin dose, exposure, and SGLT2

RO. Given the high RO values in our study and based on the expected canagliflozin concentrations in the proximal tubules within the used dose range of 50 mg to 300 mg, a lower oral canagliflozin dose, such as 25 mg, is recommended for future studies. For the timing of oral canagliflozin administration, it is important to consider the expected maximal plasma concentration as we assume that maximum SGLT2 blockage follows maximum plasma canagliflozin concentrations. In our study, the median T_{max} (2.0 h) was within the expected range and almost all participants had reached their maximum plasma canagliflozin concentration during the course of the PET scan (**Figure 1**). In the 2 participants with a longer T_{max} of ~4.5 hours, we cannot exclude the possibility that the RO may have been underestimated. Furthermore, PET models assume a steady-state condition and it is therefore important that the plasma canagliflozin concentration and RO are stable during the PET scan. Our results show that this condition was met in most participants. The large variability of plasma canagliflozin exposure and high metabolism in our study shows the need of obtaining PK samples and metabolite corrected arterial input function in future studies.

This study has limitations. The aim was to include three participants for each of the three oral doses. Unfortunately, RO could only be obtained in 7 participants, of which one received 300 mg and therefore our findings need to be taken with caution. Larger studies are required to confirm our findings. Another limitation is that we did not quantify unbound canagliflozin plasma concentration and assumed no variation in protein binding among participants. Although we may assume that unbound canagliflozin is freely filtered, and therefore the concentration at the transporter level may correspond with the plasma concentration, it is unknown if the local concentration is impacted by GFR, active secretion, or re-absorption of canagliflozin. Finally, canagliflozin is a selective SGLT2 inhibitor with minimal effect on the SGLT1, a high affinity but low-capacity glucose transporter. Therefore, enhanced glucose re-absorption mediated through SGLT1 in the setting of SGLT2 inhibition may explain the lack of a correlation among RO, canagliflozin dose, and urinary glucose response.

To conclude, we report on a novel approach of quantifying canagliflozin tissue distribution and SGLT2 occupancy and showed the feasibility of [^{18}F]canagliflozin PET imaging to clinically assess receptor occupancy of oral canagliflozin. In this small study, we did not find a relation between clinically used canagliflozin doses and RO. These data support the potential of [^{18}F]canagliflozin as a tool to visualize and quantify clinically SGLT2 disposition into the kidneys and tissue binding, and support larger, more robust, future studies.

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

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CONFLICT OF INTEREST

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AUTHOR CONTRIBUTION

S.vdH., A.T.M.W., T.V., A.H., D.J.M., R.P.H.B., R.H.J.A.S., P.H.E., H.J.L.H., and J.S. wrote the manuscript. S.vdH., R.H.J.A.S., P.H.E., H.J.L.H., and J.S. designed the research. S.vdH. performed the research. S.vdH., A.T.M.W., H.J.L.H., and J.S. analyzed the data.

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