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Dapagliflozin, a Novel SGLT2 Inhibitor, Induces Dose-Dependent Glucosuria in Healthy Subjects

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Dapagliflozin selectively inhibits renal glucose reabsorption by inhibiting sodium–glucose cotransporter-2 (SGLT2). It was developed as an insulin-independent treatment approach for type 2 diabetes mellitus (T2DM). The safety, tolerability, pharmacokinetics, and pharmacodynamics of the drug were evaluated in single-ascending-dose (SAD; 2.5–500 mg) and multiple-ascending-dose (MAD; 2.5–100 mg daily for 14 days) studies in healthy subjects. Dapagliflozin exhibited dose-proportional plasma concentrations with a half-life of ~17 h. The amount of glucosuria was also dose-dependent. Cumulative amounts of glucose excreted on day 1, relating to doses from 2.5–100 mg (MAD), ranged from 18 to 62 g; day 14 values were comparable to day 1 values, with no apparent changes in glycemic parameters. Doses of ~20–50 mg provided close-to-maximal SGLT2 inhibition for at least 24 h. Dapagliflozin demonstrates pharmacokinetic (PK) characteristics and dose-dependent glucosuria that are sustained over 24 h, which indicates that it is suitable for administration in once-daily doses and suggests that further investigation of its efficacy in T2DM patients is warranted.

Type 2 diabetes (T2DM) is a chronic disease that presents a growing worldwide problem.[1,2] Currently there are an estimated 246 million people with diabetes, and this number is expected to increase to 380 million by the year 2025.[3] T2DM is associated with serious complications and comorbidity and is quickly becoming one of the leading causes of death and disability in the world.[1,2,4] Complications of diabetes arise from chronic hyperglycemia, which can cause damage to large and small blood vessels and peripheral nerves, potentially leading to heart attack, stroke, blindness, the need for limb amputation, and kidney failure.[5,6] Current therapies act to improve metabolism by increasing insulin secretion, improving insulin sensitivity, or replacing insulin altogether.[7] Most of these agents lose their glycemic efficacy over time.[1,8] For example, in a prospective study of insulin, sulfonylurea, and metformin monotherapy, 50% of patients were unable to maintain glycemic goals after 3 years.[9] Moreover, after 9 years, only 25% of patients were able to maintain glycemic control.[9] Therefore, additional agents, especially those that work independently of insulin, are needed for the successful management of T2DM.

The kidneys contribute to maintaining normal blood glucose levels by reabsorbing ~180 g of glucose each day.[10] In the context of diabetes, blocking the reabsorption of glucose has become an intriguing therapeutic strategy, one based on the inhibition of sodium–glucose cotransporter-2 (SGLT2). SGLT2 is localized to the brush border in the S1 segments of the proximal tubule in the renal cortex and is purportedly the major transporter involved in glucose reabsorption, as shown in expression and loss-of-function studies (**Figure 1a**).[11] Glucose is transported against a concentration gradient in proximal tubules by a secondary active transport system involving the co-transport of glucose and sodium ions.[12] Increased renal glucose transporter expression and activity have been associated with T2DM in a human cellular model.[13] Mutations in the gene that codes for SGLT2 (*SLCA5*) cause renal glucosuria, a predominantly benign condition in which patients have normal kidney function, are not hypoglycemic, and generally have no significant clinical manifestations,[11] although a few subjects with mutational variations may experience renal sodium wasting and/or mild volume depletion.[14] The idea that pharmacologic inhibition of SGLT2 may provide a noninsulin-dependent option toward glycemic control for T2DM patients is promising.[15] Preclinical studies indicate that inhibitors of SGLT2 can induce renal glucose excretion and consequently lower plasma glucose levels (**Figure 1b**).[16–20]

Dapagliflozin is a potent and highly selective SGLT2 inhibitor with a distinct chemical structure containing a C-glucoside[21] (**Figure 2**), which preclinical studies predict will provide a longer half-life because of increased metabolic stability, thereby allowing once-daily dosing. Dapagliflozin is unlikely to significantly affect the pharmacokinetics of concurrently administered medications that are cytochrome P450 (CYP) or P-glycoprotein

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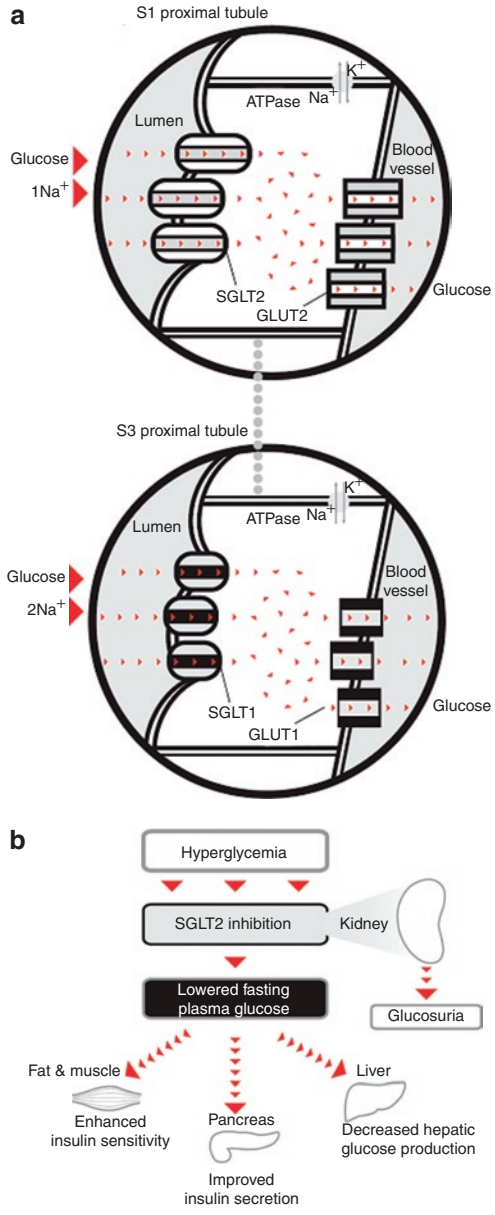

Figure 1 Sodium–glucose cotransporter-2 (SGLT2) reabsorbs glucose in the proximal tubule. (**a**) Renal glucose reabsorption by SGLT1 and SGLT2 occurs in the S3 and S1 segments of the proximal tubule, respectively. (**b**) The physiologic outcome of SGLT2 inhibition is increased renal glucose excretion.

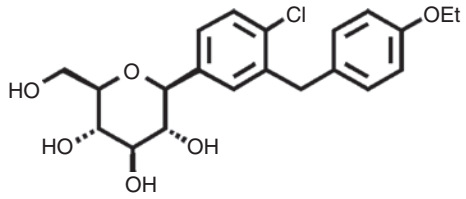

Figure 2 Chemical structure of dapagliflozin.

substrates. *In vitro* studies with recombinant CYP isoforms indicate that the metabolism of dapagliflozin may be catalyzed by multiple CYP enzymes, including CYP1A1, CYP1A2, CYP2A6, CYP2C9, CYP2D6, and CYP3A4, although turnover was low in these experiments. *In vitro* metabolic profiling experiments identified an o-de-ethylated metabolite, BMS-511926, as being an active metabolite with SGLT2 IC50 values similar to those of the parent dapagliflozin; this metabolite was quantitated in the single- and multiple-ascending-dose (SAD and MAD) studies reported here. Data from subsequent *in vitro* studies indicated that dapagliflozin is predominantly metabolized via UGT1A9, a member of the phase II enzyme UGT family, to another (inactive) metabolite, which will be measured in future studies.

The SAD and MAD studies with dapagliflozin were designed to confirm that it has a pharmacokinetic (PK) profile consistent with once-daily dosing and produces a dose-dependent increase in glucosuria in humans. We describe the first two clinical studies that have assessed the initial safety and the PK and pharmacodynamic (PD) parameters of both single and multiple doses of dapagliflozin in healthy subjects.

RESULTS

Subjects

A total of 64 subjects enrolled and completed the SAD study, and 40 subjects enrolled and completed the MAD study conducted at the Bristol-Myers Squibb Clinical Research Center, Hamilton, NJ. The subject demographics were comparable between groups. In both studies, the mean age of subjects ranged from 28 to 37 years, and the mean body weight ranged from 74 to 84 kg. The subjects were men of various races, including white (Hispanic and non-Hispanic/Latino), black/African American, Asian, and American Indian/Alaskan.

Safety outcomes

Single and multiple doses of dapagliflozin appeared to be well tolerated. Adverse events (AEs) were reported in 10 (21%) and 11 (37%) subjects who received single and multiple doses of dapagliflozin, respectively, and in 9 (35%) subjects who received placebo. Incidents of AEs did not appear to be dose-related. In both studies, treatment-emergent AEs included upper abdominal pain, contact dermatitis, dizziness, ecchymosis, erythema, fatigue, a feeling of abnormality, flank pain, headache, hyperhidrosis, hypotension, pallor, pruritic rash, other rash, stress symptoms, and swelling of the face. Two events of hypoglycemia that were mild and asymptomatic (one in placebo) were reported in the SAD study. There were no deaths or discontinuations because of AEs during these studies.

Forty-nine and 54 marked laboratory abnormalities were reported in the SAD and MAD studies, respectively. The most frequent abnormality in both studies was low absolute neutrophils + bands (five total incidents in the SAD study measured as lowest level $\times 10^3$ cells/μl: 1.43 (placebo), 1.49 (2.5 mg), 1.50 (10 mg), 1.46 (50 mg), and 1.43 (100 mg); and six in the MAD study: 1.04 (placebo), 1.32 (placebo), 1.41 (2.5 mg), 1.31 (10 mg), 1.38 (20 mg), and 1.08 (100 mg). None of these was considered clinically significant. In the two studies, a total of 17 serum chemistry abnormalities occurred. The highest reported values for individual subjects included total bilirubin: 1.4 mg/dl (10 mg), 2.7 mg/dl (20 mg), and 1.5 mg/dl (500 mg); direct bilirubin: 0.40 mg/dl (20 mg); aspartate aminotransferase (AST): 57 U/l (10 mg); alanine aminotransferase (ALT): 93 U/l

(placebo), 71 U/l (2.5 mg), and 65 U/l (50 mg); blood urea nitrogen (BUN): 24 mg/dl (10 mg), 26 mg/dl (10 mg), and 25 mg/dl (20 mg); potassium: 5.8 mEq/l (placebo), 5.8 mEq/l (2.5 mg), 5.8 mEq/l (10 mg), and 6.3 mEq/l (20 mg); and creatinine: 1.1 mg/dl (100 mg). After receiving a 20-mg dose of dapagliflozin in the SAD study, one subject had an elevated total bilirubin (2.7 mg/dl at study discharge on day 3 and 2.1 mg/dl after the 20-day follow-up) before returning to the normal range (0.1–1.2 mg/dl) at day 36. The investigator did not consider this abnormality to be clinically significant. There were no clinically relevant changes in vital signs, electrocardiograms, or physical examination findings in either study. Overall, there was no apparent relationship between the frequency of any laboratory abnormality and the dose of dapagliflozin in either study.

In the MAD study, 24-h urinary excretion of sodium was measured at baseline and on days 8 and 13. There were no notable increases in excreted sodium relative to baseline values on days 8 and 13 and no apparent differences between the placebo group and the dapagliflozin dose groups. The SAD study did not assess this parameter. Neither study evaluated plasma renin, serum aldosterone, or changes in body weight.

There were no treatment-related serious AEs in these studies. One unrelated serious AE occurred in a subject in the 20-mg dapagliflozin group who was hospitalized for severe stress symptoms. This subject later revealed a history of anxiety episodes.

Pharmacokinetics

SAD study. Dapagliflozin was rapidly absorbed after oral administration, and maximum plasma concentrations (C_{max}) were observed within 2 h of administration. Key PK parameters are reported in **Table 1**. Total exposure of dapagliflozin, as measured in terms of the area under the plasma concentration–time curve (AUC), increased dose-proportionally, up to the 100-mg dose. Between the 100- and 500-mg doses AUC increase slightly greater than dose-propotionally. C_{max} values increased slightly less than dose-propotionally. After a high-fat meal, the median T_{max} was delayed by 2.5 h (**Figure 3** and **Table 1**), C_{max} was reduced by 39%, and $AUC_{(INF)}$ was reduced by 7% as compared to values during fasting. The active metabolite of dapagliflozin was observed only with doses of >50 mg because of its substantially lower AUC values compared with those of the parent

Table 1 Summary of dapagliflozin PK parameters for a dose of 250 mg administered to fasted and fed subjects (PK population)

Pharmacokinetic parameter	n[a]	Fasted	Fed
Geometric mean C_{max}, ng/ml (CV%)	5	2,510 (31)	1,532 (24)
Geometric mean $AUC_{(INF)}$, ng·h/ml (CV%)	5	13,337 (28)	12,455 (31)
Median T_{max}, hours (min, max)	5	1.50 (1.00, 2.00)	4.00 (4.00, 4.00)
Mean $T_{1/2}$, hours (SD)	5	17.33 (19.75)	18.25 (15.94)
Mean urinary recovery, % (SD)	6	1.54 (0.40)	1.90 (0.60)
Mean CLR, ml/min (SD)	6	4.99 (0.91)	6.33 (1.19)

$AUC_{(INF)}$, area under concentration–time curve; CLR, renal clearance; C_{max}, maximum observed concentration; CV, coefficient of variation; PK, pharmacokinetic; $T_{1/2}$, half-life; T_{max}, time to C_{max}.

[a]In one subject, the 1-h dapagliflozin plasma concentration in the fasted state was greater than the upper limit of quantitation for the assay, with insufficient sample remaining to reanalyze. Because this data point was likely to contribute significantly to the systemic PK parameters, the values from this subject were excluded from the summary statistics for both the fed and fasted states.

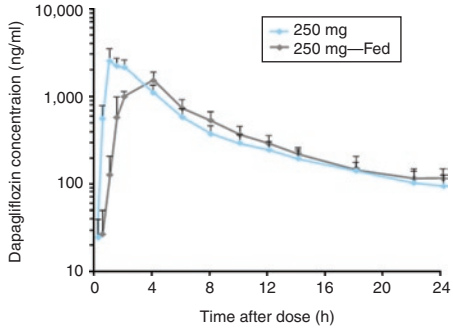

Figure 3 Single-ascending-dose study. Mean (SD) plasma concentration–time profiles for dapagliflozin on day 1 after a single 250-mg dose in fasted and fed subjects.

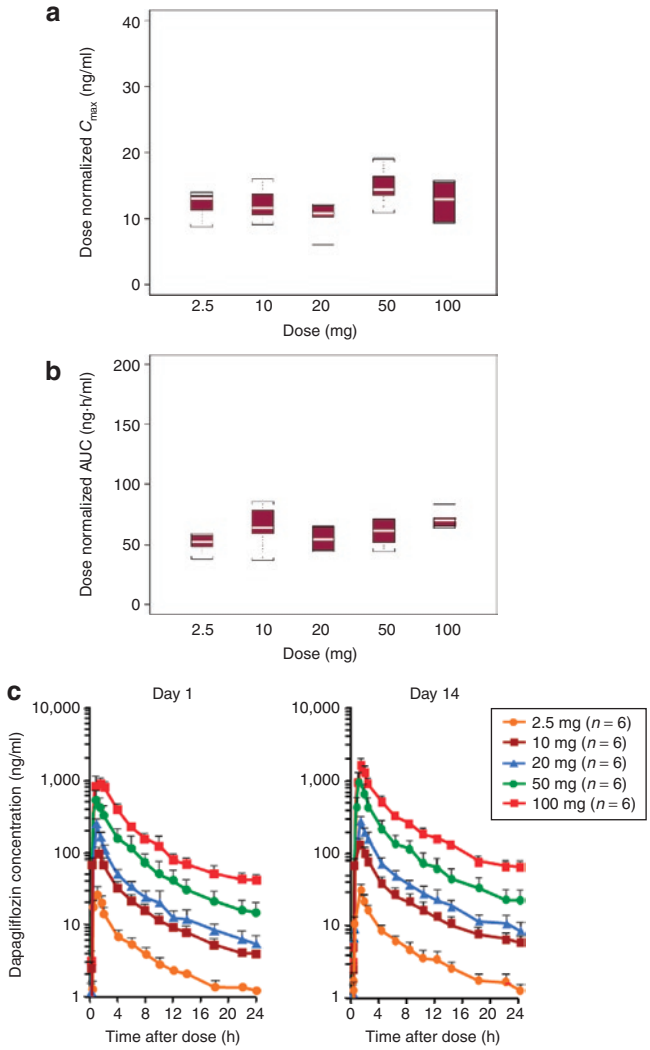

Figure 4 Multiple-ascending-dose study. Dose-normalized (**a**) C_{max} and (**b**) AUC for dapagliflozin at day 14; midlines of boxes are median values, boundaries are ~95% confidence limits for the median. (**c**) Mean (SD) plasma concentration–time profiles for dapagliflozin on days 1 and 14.

compound. Approximately 4% and 0.1% of the dose of dapagliflozin were excreted in the urine as parent compound and metabolite, respectively. The mean renal clearance ranged from 2 to 14 ml/min for the parent compound (all doses) and 30–52 ml/min for the metabolite (250- and 500-mg doses only).

MAD study. **Figure 4a,b** shows analyses of C_{max} and AUC dose-proportionality after normalization for differences in dose. The flatness of the slopes demonstrates that increases in C_{max} and AUC were proportional to increments in dosage. The mean $T_{1/2}$ after the last dose ranged from 11.2 to 16.6 h and the data were similar for the SAD study high dose (**Figure 4c**). Consistent with these half-life values, the mean day 14:day 1 AUC accumulation index for dapagliflozin in each dose panel ranged from 1.20 to 1.30; these values appeared to be independent of dose. As in the SAD study, the active metabolite had substantially lower AUC values than the parent compound. For example, after 14 days of daily dosing with 100 mg dapagliflozin, the $AUC_{(TAU)}$ (AUC over a dose interval of 24 h) of the parent compound was 5,599 ng·h/ml, whereas the $AUC_{(TAU)}$ of the metabolite was 46 ng·h/ml (0.008% of that of the parent compound). Neither the parent compound nor the metabolite was extensively excreted in the urine (<3.0 and 0.2% of the dapagliflozin dose were excreted in the urine as parent compound and metabolite, respectively). The mean renal clearances of dapagliflozin and its metabolite were comparable with the clearance observed with single doses of dapagliflozin in the SAD study.

Pharmacodynamics

SAD study. The amount of glucosuria was dose-dependent. Doses on the order of 20–50 mg maintained a close-to-maximal rate of glucose excretion of ~3 g/h for at least 24 h (**Figure 5**). The mean serum glucose AUC 0–4 h after lunch ranged from 413 to 446 mg·h/dl in subjects in the placebo group and from 393 to 405 mg·h/dl in subjects in the dapagliflozin group. Single oral doses of dapagliflozin did not alter urinary calcium excretion. Other than the expected elevations in urinary glucose observed in the dapagliflozin group, there were no apparent changes in clinical laboratory parameters that were attributable to dapagliflozin.

MAD study. The cumulative amount of glucose excreted per day was dose-dependent on days 1 and 14 (**Figure 6a,b**, respectively). Close-to-maximum glucose excretion per day was achieved with doses of 20 mg and higher. Cumulative amounts of glucose excreted over 24 h on day 1 with the 2.5-, 10-, 20-, 50-, and 100-mg doses of dapagliflozin were 17.7, 40.0, 58.0, 62.0, and 58.3 g, respectively, and were dose-dependent. This translates to ~20–30% inhibition of renal glucose reabsorption. By day 14, the 2.5-, 10-, 20-, 50-, and 100-mg doses of dapagliflozin produced 20.4, 33.6, 49.2, 53.3, and 55.4 g, respectively, of glucose excreted over 24 h. Inhibition of renal glucose reabsorption at day 14 was ~16–50%. No clear change in daily glucose excretion was observed at day 14 as compared with day 1, which suggests there were no meaningful changes in filtered glucose load in this study of healthy adult men. Consistent with this finding, serum glucose, serum insulin, and serum C-peptide concentrations were unchanged. In addition, dapagliflozin had no apparent effect on urinary calcium, magnesium, sodium, potassium, phosphate, chloride, uric acid, oxalate, citrate, total protein, albumin, osmolality, or renal tubular markers such as N-acetyl-b-D-glucosaminidase and β2-microglobulin. Furthermore, there were no observed effects on serum osteocalcin, parathyroid hormone, 25-hydroxy vitamin D, 1,25dihydroxy-vitamin D, deoxypyridinoline crosslinks, and C-telopeptide.

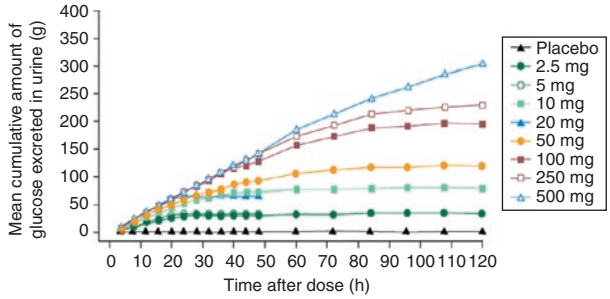

Figure 5 Single-ascending-dose study. Total mean cumulative amount of urinary glucose after a single dose of dapagliflozin. The mean cumulative amount of glucose excreted in the urine was dose-dependent. Data are shown for up to 120 h after a single dose.

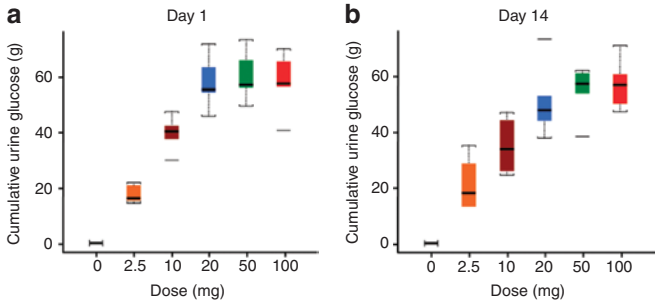

Figure 6 Multiple-ascending-dose study. The cumulative amount of glucose (g/day) in the urine 0–20 h after multiple doses of dapagliflozin at (**a**) day 1 and (**b**) day 14. The mean amount of glucose excreted in the urine at dapagliflozin doses 20–100 mg was comparable after days 1 and 14. The cumulative 24-h glucose excretion after dapagliflozin doses of 2.5 and 10 mg was ~40 and ~70%, respectively, of the glucose excreted after dapagliflozin doses of 20–100 mg.

DISCUSSION

The results of these first-in-human studies in healthy subjects support the evidence from preclinical models that SGLT2 inhibition produces dose-dependent, sustained glucosuria.[20,21] In these normoglycemic subjects, dapagliflozin doses of 20 mg and higher inhibited up to 50% of filtered glucose from being reabsorbed by the kidney, which resulted in glucose excretion of ~60 g/day (**Figure 5**) and up to 3 g/h. The dose–response curve for glucosuria may be shifted to higher doses in hyperglycemic patients. Therefore doses up to 100 mg will be tested in T2DM patients to ensure that the entire dose–response curve for glucosuria is characterized.

In humans, mutations in the SGLT2 gene, *SLC5A2*, result in familial renal glucosuria and produce varying degrees of

glucosuria depending on the type of mutation and its zygosity.[22,23] In the MAD study, doses between 20 and 100 mg produced urine glucose levels similar to those seen in some individuals with moderate renal glucosuria (between 50 and 60 g glucose/day, i.e., inhibition of only 25–50%). These glucosuria levels are approximately half of those observed in individuals with severe renal glucosuria, in whom glucose excretion can exceed 125 g glucose/day.[23,24] Generally, even severe forms of renal glucosuria do not produce other clinically relevant effects and are considered to be benign conditions. In these studies, healthy subjects who exhibited moderate glucosuria (i.e., ~60 g glucose/day), likewise demonstrated no other clinically remarkable findings.

Study volunteers did not experience reduction in serum glucose after receiving dapagliflozin. This is an expected finding, given that healthy individuals typically maintain glucose homeostasis through elaborate endocrine feedback loops and counter-regulatory pathways.[25] In contrast, T2DM patients have an excess glucose load because glycemic regulation is impaired by defects in homeostatic pathways, and preclinical studies support the hypothesis that inducing glucosuria by inhibition of SGLT2 reduces serum glucose and improves glycemic parameters in diabetic animals. An early study of the SGLT inhibitor phlorizin demonstrated that phlorizin treatment normalized insulin sensitivity in diabetic rats but had no effect on insulin action in nondiabetic controls.[26] A later study found that T-1095, another SGLT2 inhibitor, effectively suppressed postprandial hyperglycemia in diabetic rats, leading investigators to conclude that saturation of the SGLT reabsorptive mechanism increases excretion of urinary glucose more effectively under hyperglycemic than normoglycemic conditions.[27] Consistent with these reports, a significant increase in urine glucose excretion was seen within 6 h after administration of dapagliflozin in diabetic rats, whereas in normal rats on the same dose no significant increase was observed over 24 h.[20]

Over the full range of doses studied, dapagliflozin C_{max} and $AUC_{(TAU)}$ increased in proportion to the increment in dose. The PK parameters of dapagliflozin, such as its half-life of ~17 h, and its PD characteristic of maintaining maximal glucosuria over 24 h support the viability of a once-daily dosing regimen. This is in line with preclinical data attributing the enhanced glucosuric potency of dapagliflozin to the C-glucoside linkage that confers metabolic stability.[21] Renal excretion of both analytes was minimal because they are highly bound to protein in the plasma (to an extent of ~97%). Food had only a modest effect on the PK and PD parameters of a single 250-mg dose of dapagliflozin and slightly delayed and reduced C_{max}; however, the overall daily exposure was almost unchanged. Furthermore, subjects who received this dose after a high-fat meal did not have a substantially different maximal rate of urinary glucose excretion, duration of maximal rate of urinary glucose excretion, or cumulative amount of glucose excreted in the urine.

The incidence of AEs after single and multiple dapagliflozin doses did not appear to be dose-related in either study. Over the course of 2 weeks, only two mild episodes of hypoglycemia were reported, one in the placebo group and the other in the SAD 20-mg group, whereas none was reported in the MAD study. In addition, there was no increase in the occurrence of genitourinary infections in the dapagliflozin groups as compared with the placebo groups in either study; however, longer studies will be required to evaluate the effect of dapagliflozin in this context. Multiple oral doses of dapagliflozin over 2 weeks had no apparent effect on the safety and laboratory parameters measured, including renal tubular and bone turnover markers.

In summary, these first-in-human studies indicate that the SGLT2 inhibitor dapagliflozin induces sustained dose-dependent glucosuria without reducing serum glucose in healthy subjects. Further investigation in T2DM patients is needed to characterize the dose–response curve for glucosuria in hyperglycemic subjects and to demonstrate whether dose-dependent glucosuria with dapagliflozin will lead to clinically meaningful changes in glycemic parameters. A phase IIa study is also reported in this issue.[28]

METHODS

Subjects. In general, inclusion and exclusion criteria were similar for the two trials. Adult subjects (aged 18–45 years) with a body mass index in the range of 18–30 kg/m^2 were eligible for inclusion if they were deemed healthy in terms of medical history, physical examination findings, 12-lead electrocardiogram findings, and clinical laboratory evaluations. Women who were nursing, pregnant, or of childbearing age were excluded from the study. For the MAD study, subjects with urinary calcium >140 mg/g or creatinine or fasting serum glucose >110 mg/dl at screening were also excluded. Evidence of organ dysfunction or any clinically significant deviation from normal in physical examination, vital signs, electrocardiogram, or clinical laboratory determinations were criteria for exclusion. Subjects who had received calcium or vitamin D supplements within 2 weeks prior to enrollment, had undergone major surgery within 4 weeks prior to enrollment, had acute or chronic medical illness, or had a history of drug allergy or exposure to the study drug were also excluded from the trial. Studies were conducted in accordance with good clinical practice guidelines and were approved by an institutional review board. All subjects provided informed consent.

Design of the SAD study. This was a double-blind, randomized, placebo-controlled, two-period, sequential, ascending single-dose study (MB102001). Healthy subjects were randomly assigned in a 3:1 ratio to receive either a single dose of 2.5, 5, 10, 20, 50, 100, 250, or 500 mg dapagliflozin or placebo. Dapagliflozin or matching placebo was administered as an oral solution (2.5–5 mg vs. placebo) or capsule formulation (20–500 mg vs. placebo). If a dose regimen (beginning with the lowest dose) was found to be safe and well tolerated, then the succeeding panel of eight subjects received the next higher dose of dapagliflozin ($n = 6$) or placebo ($n = 2$). In period 1, subjects were given a single oral dose of dapagliflozin or placebo after a 10-h fast. Urine samples were collected for a minimum of 120 h (48 h for the 5- and 20-mg dose groups) after the dose. All subjects, excluding those in the 250-mg dapagliflozin group, were discharged from the study on day 6 (day 3 for 5- and 20-mg dose groups) if the morning urine voided was negative for glucose. If the morning urine was positive, 12-h urine samples continued to be tested for glucose and calcium excretion. In period 2, subjects who received 250 mg dapagliflozin entered a 7-day washout phase. After the washout interval, the subjects were given a high-fat breakfast and a second oral dose of 250 mg dapagliflozin or matched placebo. During the study, all subjects received diets containing fixed amounts of calcium and sodium chloride. The subjects were discharged on day 6 of period 2 if their urine was negative for glucose on the morning of that day. If the morning urine was positive, 12-h urine samples continued to be tested for glucose and

calcium excretion. The subjects were not discharged until the morning urine was negative for glucose.

Design of the MAD study. This was a double-blind, randomized, placebo-controlled, sequential, ascending multiple-dose study (MB102002). Healthy subjects were randomly assigned to one of the drug treatment groups (five sequential doses of 2.5, 10, 20, 50, or 100 mg of dapagliflozin) or to the placebo group in a ratio of 3:1. Dapagliflozin or matching placebo was administered as a capsule formulation. As in the SAD study, subjects were not enrolled at the next dose level until safety data from at least six subjects within the group had been reviewed by the sponsor in conjunction with the investigator. Beginning on day 1, subjects received a daily oral dose of dapagliflozin or matched placebo for 14 days. All subjects received diets containing fixed amounts of calcium and sodium chloride. The subjects were released from the clinical facility on day 20, and they returned on day 27 for discharge procedures.

Safety measurements. Safety assessments were similar in the two studies and were based on medical review of AE reports. This included vital signs, electrocardiograms, physical examinations, and clinical laboratory tests. AEs were recorded throughout the study period and defined as any new medical occurrence or worsening of a preexisting condition after administration of the study drug or placebo. Serious AEs were recorded for 30 days after the last dose of study medication; a serious AE was defined as an AE that resulted in death, hospitalization, or persistent or significant disability, or was life-threatening.

PK and PD assessments. Single-dose PK parameters (C_{max}, T_{max}, $AUC_{(INF)}$, $T_{1/2}$, % urinary recovery, and renal clearance) were derived from plasma concentration-vs.-time and urinary excretion data. The effect of food on dapagliflozin PK parameters was evaluated in the group receiving the 250-mg dose. PD measurements included serum glucose concentration and the amounts of glucose and calcium excreted in the urine. Samples were collected before and after lunch (within a 4-h window) on day 1 of period 1 and period 2. Urine samples were collected every 12 h for a minimum of 48 h (in the 5- and 20-mg dose groups) or 120 h (in all other groups) after the dose in period 1 and period 2 (for the 250-mg dose group). Multiple-dose PK parameters (C_{max}, T_{max}, $AUC_{(TAU)}$, accumulation index, $T_{1/2}$, % urinary recovery, and renal clearance) of dapagliflozin and its pharmacologically active metabolite were derived from plasma concentration-vs.-time data. PD parameters included urinary glucose, urinary calcium, serum glucose, serum insulin, serum C-peptide, and inhibition of renal glucose resorption. Samples of blood (0.25, 0.5, 1, 1.5, and 2 h after the dose and then every 2 h for a total of 24 h or hourly for 12 h) and urine (every 4 h for 24 h) were collected on days 1, 7, 8, 13, and 14, with the subjects in fasting condition for 8 h before collection. Urine samples were collected to calculate the excretion rate for glucose (ER). The filtered load (FL) is the product of estimated GFR and serum glucose concentrations, and the renal (tubular) glucose reabsorption rate (T_G) can be calculated as $T_G = FL - ER$.

Bioanalytical methods. Assays for plasma and urine concentrations of dapagliflozin and its metabolite, BMS-511926, were performed by Bristol-Myers Squibb using liquid chromatography atmospheric pressure ionization with tandem mass spectrometry detection in multiple-reaction monitoring mode within the period of known analyte stability. The between-run variability and within-run variability for the analytical quality controls of dapagliflozin were <7.5 and <10.1%, respectively, of the coefficient of variation, with deviations from the nominal concentrations of no more than ±3.5%. For dapagliflozin and BMS-511926, the assay range representing the lower and upper limits of quantitation in plasma and urine were 1–1,000 and 10–2,000 ng/ml, respectively.

Statistical methods. All subjects who received dapagliflozin or placebo were included in the safety and PD populations. Subjects who received dapagliflozin were included in the PK population; only subjects with data from both study periods were included in the tabulation of summary statistics for the food effect assessment. All statistical analyses were performed using SAS/STAT version 8.2 (SAS Institute, Cary, NC). Summary statistics were calculated for PK parameters by dose and period (fed, fasted), or study day, for each analyte. Scatter plots of C_{max} and $AUC_{(INF)}$ or $AUC_{(TAU)}$ relative to dose were analyzed by study day to assess the dependency on dose. Point estimates and 90% confidence intervals were calculated for the ratios of population geometric means (fed/fasted) for C_{max} and $AUC_{(INF)}$ to assess the effect of food on the PK of dapagliflozin. Geometric means and coefficients of variation were calculated for C_{max}, $AUC_{(TAU)}$, and accumulation index. Scatter plots of C_{max} and $AUC_{(INF)}$ were also provided, to further assess the effect of food on the PK of dapagliflozin. PK parameters were analyzed using noncompartmental methods. Summary statistics for PD measurements were tabulated for the total amount of glucose and calcium excreted in urine >120 h after dosing, by treatment group and period. Serum glucose concentrations were summarized by treatment group and by time elapsed since lunch for each period. Although the number of subjects included in the studies was not based on statistical power considerations, six subjects in each panel would have provided an 80% probability of observing at least one occurrence of any AE that occurred with an incidence of 24%.

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CONFLICT OF INTEREST

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