Dapagliflozin Lowers Plasma Glucose Concentration and Improves Beta Cell Function

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Background: Beta cell dysfunction is a core defect in T2DM and chronic, sustained hyperglycemia has been implicated in progressive beta cell failure, i.e. glucotoxicity. The aim of the present study was to examine the effect of lowering the plasma glucose concentration with dapagliflozin, a glucosuric agent, on beta cell function in T2DM individuals.

Research Design and Methods: 24 subjects with T2DM received dapagliflozin (n=16) or placebo (n=8) for 2 weeks, and a 75-gram OGTT and insulin clamp were performed before and after treatment. Plasma glucose, insulin, and C-peptide concentrations were measured during the OGTT.

Results: Dapagliflozin significantly lowered both the fasting and 2-hour plasma glucose concentrations and the incremental area under the plasma glucose concentration curve (ΔG_{0-120}) during OGTT by -33±5 mg/dl, -73±9mg/dl and -60±12 mg/dlmin, respectively, compared to -13±9, -33±13 and -18±9 reductions in placebo-treated subjects (both $p<0.01$). The incremental area under the plasma C-peptide concentration curve tended to increase in dapagliflozin-treated subjects, while it did not change in placebo-treated subjects. Thus, $\Delta C\text{-}Pep_{0-120}/\Delta G_{0-120}$ increased significantly in dapagliflozin-treated subjects, while it did not change in placebo-treated subjects (0.019±0.005 vs 0.002±0.006, $p<0.01$). Dapagliflozin significantly improved whole body insulin sensitivity (insulin clamp). Thus, beta cell function, measured as $\Delta C\text{-}Pep_{0-120}/\Delta G_{0-120} \div$ insulin resistance, increased by 2-fold ($p<0.01$) in dapagliflozin-treated versus placebo-treated subjects.

Conclusion: Lowering the plasma glucose concentration with dapagliflozin markedly improves beta cell function, providing strong support in man for the glucotoxic effect of hyperglycemia on beta cell function.

Beta cell failure is a core defect in type 2 diabetes mellitus (T2DM) and is the major factor responsible for the development and progression of hyperglycemia (1). Multiple factors including advancing age, genes, insulin resistance, beta cell incretin resistance, incretin deficiency, islet-associated amylin polypeptide, lipotoxicity and others (1) have been implicated in development of beta cell failure in T2DM. Chronic elevation of the plasma glucose concentration also impairs insulin secretion, ie, glucose toxicity (2), although proof of the glucotoxicity hypothesis in man has yet to conclusively be established (3). The toxic effect of chronic hyperglycemia on beta cell function was demonstrated in experimental animals more than 60 years ago (4). Chronic (>4 days) elevation of plasma glucose concentration to 29 mM in cats and dogs completely obliterated the beta cell response to a glucose stimulus (5–7). Moreover, the severity of the beta cell defect and the time required for recovery of beta cell function following correction of the hyperglycemia were directly related to the level of hyperglycemia produced (5–7). Using the hyperglycemic clamp technique, Rossetti and colleagues (2) demonstrated that even a small (16 mg/dl) persistent in-

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Abbreviations:

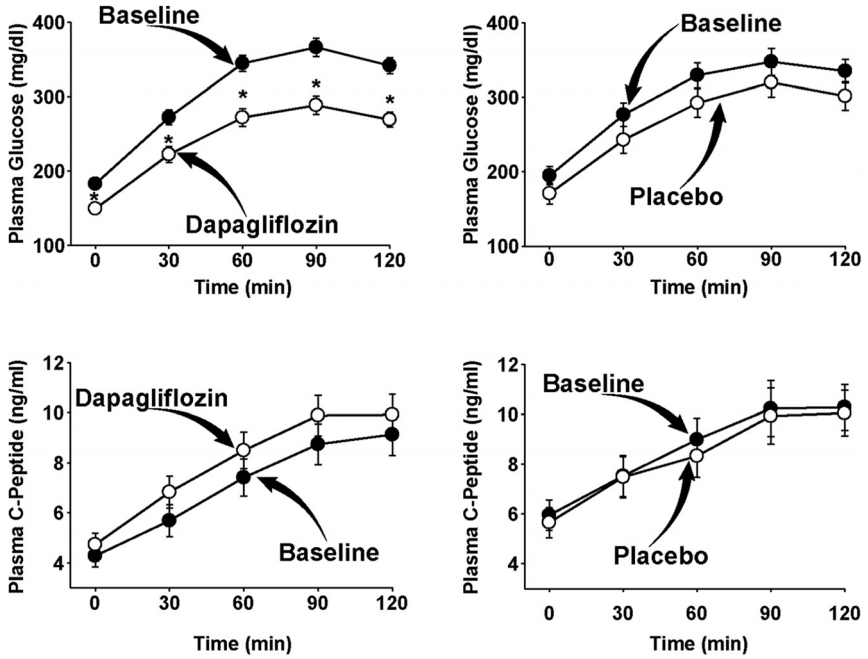

Figure 1. Plasma glucose and C-peptide concentrations during the OGTT in dapagliflozin-treated and placebo-treated T2DM patients at baseline and after2 weeks of treatment. *= $P <$ .05

crease in the plasma glucose concentration impairs both first and second phase insulin secretion in partially prancreatectomized diabetic rats. Further, correction of the hyperglycemia with phlorin restored glucose-stimulated insulin secretion to normal (8). Although the glucotoxic effect of hyperglycemia is well established in in vitro and in vivo studies in experimental animals, conclusive evidence for the detrimental effect of chronic hyperglycemia on beta cell function in T2DM has yet to be provided. In NGT individuals, a modest elevation in day-long plasma glucose concentration for 24 hours caused a 24% decrease in beta cell function (9). Conversely, lowering the plasma glucose concentration with insulin therapy in T2DM patients significantly improved insulin secretion (10, 11). Although insulin is very effective in lowering the plasma glucose concentration in T2DM, it has many other metabolic effects which also could lead to an improvement in beta cell function. For example, insulin is a powerful inhibitor of lipolysis and markedly lowers the plasma FFA concentration (12), which could lead to improved beta cell function (13).

To examine the glucotoxicity hypothesis in man, we used dapagliflozin, a potent and specific SGLT2 inhibitor (14), to lower the plasma glucose concentration and examined the effect of this intervention on beta cell function. Since the primary effect of dapagliflozin is on the kidney to inhibit renal glucose reabsorption and produce glucosuria, this provides a novel approach to examine the glucotoxicity hypothesis with regard to the development of beta cell failure in T2DM individuals.

Research Design and Methods

Subjects

24 T2DM males treated with metformin (n = 17) or metformin plus sulfonylurea (n = 7) participated in the study. The mean HbA1c was 8.5 ± 0.4 (range 7.0–11.0%). Other than diabetes, subjects were in general good health as determined by medical history, physical examination, screening lab tests, urinalysis and EKG. Table 1 summarizes the clinical characteristics of the study participants. Body weight was stable (±3 pounds) in all subjects for ≥ 3 months prior to study and no subject participated in any excessively heavy exercise program. No subjects were taking any medications (other than metformin or sulfonylurea) known to affect glucose metabolism. The study protocol was approved by the IRB of the UTHSCSA and all subjects gave their written voluntary consent prior to participation.

Research design

At baseline all subjects received a 75-g OGTT at 8 AM after a 10 hour overnight fast. Plasma glucose, insulin and C-peptide measured at –30, –15 and 0 minutes and every 30 minutes after glucose ingestion. On a separate day, subjects received a 4 hour euglycemic hyperinsulinemic clamp with 3-^{3}H-glucose infusion to quantitate whole body insulin-mediated glucose disposal and endogenous glucose production (EGP) (see following description). After completing the baseline studies, subjects received dapagliflozin, 10 mg, or placebo in randomized double-blind fashion (2:1 randomization) for 14 days. On days 13 and 14, the OGTT and euglycemic hyperinsulinemic clamp were repeated.

Euglycemic Insulin Clamp

Subjects remained fasting after 10 PM and at 6 AM on the following day a catheter was placed into an antecubital vein for the infusion of all test substances. A second catheter was inserted retrogradely into a vein on the dorsum of the hand, and the hand was placed into a thermoregulated box heated to 70°C. At 6 AM, a prime(25 μCi x FPG/100)-continuous (0.25 μCi/min) infusion of 3-^{3}H-glucose (DuPont NEN Life Science Products, Boston, MA) was started as described above and continued for 7 hours. After a 3-hour basal tracer equilibration period (9 AM), sub-

Table 1. Clinical and metabolic characteristics of the study participants at baseline and at 14 days

	Dapagliflozin		Placebo	
	Baseline	**Treatment**	**Baseline**	**Treatment**
Age (years)	51 ± 2	-	55 ± 2	-
Weight (kg)	99.5 ± 4.4	98.3 ± 4.4	96.5 ± 5.4	95.5±
BMI (kg/m^2)	32.3 ± 1.3	31.9 ± 1.3	33.1 ± 1.7	32.7 ± 1.7
SBP (mmHg)	133 ± 3	127 ± 2	135 ± 3	131 ± 4
HbA1c (%)	8.4 ± 0.3		8.6 ± 0.4	
Serum creatinine (mg/dl)	0.99 ± 0.05	1.03 ± 0.05	0.87 ± 0.04	0.87 ± 0.04
Fasting FFA (meq/liter)	0.49 ± 0.03	0.47 ± 0.03	0.44 ± 0.03	0.41 ± 0.03
FPG (mg/dl)	182 ± 8	149 ± 8*#	184 ± 19	171 ± 9
2-h PG (mg/dl)	342 ± 12	269 ± 14*#	334 ± 16	301 ± 16
ΔG_{0-120} (ng/ml.h)	257 ± 12	197 ± 16*#	220 ± 22	203 ± 18
Fasting C-Pep (ng/ml)	4.3 ± 0.4	4.7 ± 0.4	5.9 ± 0.8	5.7 ± 0.6
2-h C-Pep (ng/ml)	9.1 ± 1.1	9.9 ± 0.8	10.3 ± 1.3	10.0 ± 0.9
ΔC-Pep$_{0-120}$ (ng/ml.h)	5.72 ± 0.94	6.81 ± 0.8	5.53 ± 0.7	5.22 ± 0.9
$(\Delta C\text{-}Pep/\Delta G)_{0-120}$	0.022 ± 0.003	0.041 ± 0.006*#	0.026 ± 0.006	0.028 ± 0.006
TGD (mg/kg·min)	4.5 ± 0.5	5.2 ± 0.6*#	4.3 ± 0.8	4.4 ± 0.6
$(\Delta C\text{-}Pep_{0-120}/\Delta G)_{0-120} \div IR$	0.12 ± 0.04	0.26 ± 0.05$^{*\#}$	0.14 ± 0.04	0.17 ± 0.03

SBP = systolic blood pressure; FPG = fasting plasma glucose; 2 h PG = 2 h plasma glucose; C-pep = C-peptide; TGD = total glucose disposal. IR = insulin resistance. * $P < 0.05$ *vs.* baseline. # $P < 0.05$ *vs.* placebo

jects received a prime-continuous insulin infusion (80 mU/m^{2}min) for 240 minutes (15). During the insulin infusion, plasma glucose concentration was measured every 5 minutes. After the start of insulin no glucose was infused until the plasma glucose concentration declined to 100 mg/dl, at which level it was maintained with a coefficient of variation < 5% by the adjustment of a variable glucose infusion based on the negative feedback principle (15). Plasma samples were collected every 15–30 minutes from 0 to 180 minutes after the start of insulin and every 5–10min from 180 to 240 minutes for the determination of plasma glucose and insulin concentrations and tritiated glucose specific activity. Urine was collected from 0–240 minutes and urinary volume and glucose concentration were measured. Urinary glucose loss was subtracted from the total rate of glucose disposal to determine insulin-mediated tissue glucose uptake.

Analytical Techniques:

Plasma glucose was measured by the glucose oxidase reaction (Glucose Oxidase Analyzer, Analox, Fullerton, CA). Plasma insulin and glucagon concentrations were measured by radioimmunoassay (RIA) (Linco Research, St. Louis, MO). Plasma 3-^{3}H-glucose radioactivity was measured in Somogyi precipitates.

Calculations and Statistical Analysis

Under steady-state postabsorptive conditions, the basal rate of endogenous glucose appearance (Ra) equals the 3-^{3}H-glucose infusion rate divided by steady state plasma tritiated glucose specific activity. During the insulin clamp, nonsteady conditions for 3-^{3}H-glucose specific activity prevail and the rate of glucose appearance (R_a) was calculated with Steele's equation (16). The rate of residual EGP during the insulin clamp was calculated by subtracting the exogenous glucose infusion rate from the tracer-derived R_a. The insulin-stimulated rate of total body glucose disposal (TGD) was calculated by adding the rate of residual EGP to the exogenous glucose infusion rate, and was used as the insulin sensitivity index.

The incremental area under the plasma glucose, insulin, and C-peptide concentration curves was calculated with the trapezoid rule. Insulin secretion was measured as the ratio between the incremental area under the plasma C-peptide concentration to the incremental area under the plasma glucose concentration during the OGTT ($\Delta C\text{-}pep_{0-120}/\Delta G_{0-120}$). Beta cell function was measured as the insulin secretion/insulin resistance or disposition index, where insulin resistance was the inverse of TGD measured with the euglycemic insulin clamp ($\Delta C\text{-}pep_{0-120}/\Delta G_{0-120} \div IR$). This index is widely used for the assessment of beta cell health (17, 19–22). Beta cell sensitivity to glucose was calculated using the Mari model as previously described (23, 24).

Values are expressed as mean ± SEM. The change from baseline in TGD, EGP, $\Delta C\text{-}pep_{0-120}/\Delta G_{0-120}$, $\Delta C\text{-}pep_{0-120}/\Delta G_{0-120} \div IR$ in dapagliflozin-treated and placebo-

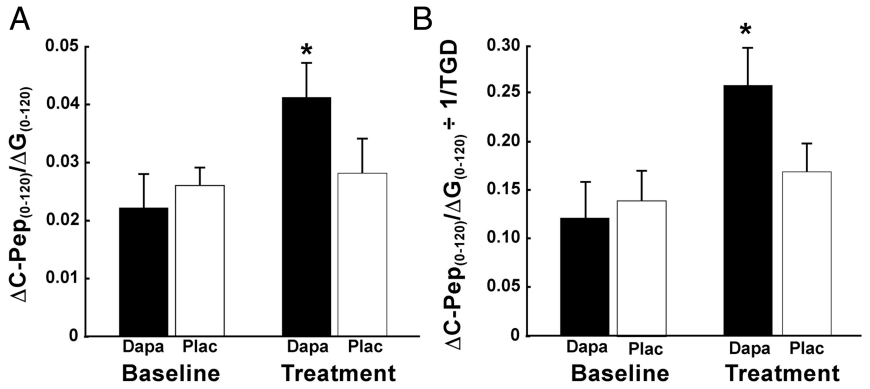

Figure 2. Beta cell function, measured as $\Delta C\text{-}Pep_{0-120}/\Delta G_{0-120} \div$ IR, in dapagliflozin-treated and placebo-treated T2DM patients at baseline and after2 weeks of treatment.* = $P < .05$ vs baseline and vs placebo.

treated groups was compared with ANOVA. Rates of TGD, EGP,ΔC-pep/ΔG, ΔC-pep/ΔG ÷ IR after dapagliflozin were compared to those before dapagliflozin with paired *t* test. Statistical significance was set at alpha < 0.05.

Results

Table 1 presents the clinical characteristics of the study participant before and after 2 weeks of treatment. Subjects were matched in age, sex and BMI. There was a small, statistically insignificant decrease in body weight at 2 weeks, which was similar in both groups. No significant difference was observed in lipid profile.

The plasma glucose concentration during the OGTT in dapagliflozin-treated and placebo-treated individuals is shown in figure 1A and 1B. Dapagliflozin caused a significant decrease in both the fasting (-33 ± 5 mg/dl) and 2-hour (-73 ± 9 mg/dl) plasma glucose concentrations (both $P < .001$ vs baseline) during the OGTT, while no significant change was observed in placebo-treated individuals (-13 ± 9[p=NS] and –33 ± 13 [p=NS], respectively). The decrease from baseline in both the FPG and 2-hour plasma glucose concentration was significantly greater ($P < .01$) in dapagliflozin-treated subjects compared to placebo-treated subjects. Similarly, The incremental area under the plasma glucose concentration curve (ΔG_{0-120}) decreased significantly in dapagliflozin-treated compared to placebo-treated subjects (-60 ± 12 vs –18 ± 9, $P < .01$).

Figure 1C and 1D depict the plasma C-peptide concentration during the OGTT. The incremental area under plasma C-peptide concentration curve in subjects treated with placebo decreased slightly but not significantly (-0.31 ± 0.6, P=NS), while the incremental area under plasma C-peptide concentration curve in dapagliflozin-treated subjects increased slightly but not significantly (+1.1 ± 0.8, P=NS) at 2 weeks. The ratio between the incremental area under the plasma C-peptide to the plasma glucose concentration curves ($\Delta C\text{-}Pep_{0-120}$ AUC/ΔG_{0-120} AUC) increased significantly in dapagliflozin-treated (0.022 ± 0.003 to 0.041 ± 0.006, $P < .05$ vs baseline and placebo) compared to placebo-treated (0.026 ± 0.006 to 0.028 ± 0.006, P=NS) subjects (Table 1).

Insulin-stimulated total body glucose disposal (TGD) measured with the euglycemic-hyperinsulinemic clamp was comparable at baseline in both groups (4.5 ± 0.5 and 4.3 ± 0.8, p=NS). TGD increased significantly in dapagliflozin-treated (5.2 ± 0.6, $P < .05$) subjects, while no significant change was observed in placebo-treated subjects (4.4 ± 0.6, $P < .05$ vs dapagliflozin). Thus, the insulin secretion/insulin resistance index ($\Delta C\text{-}Pep_{0-120}/\Delta G_{0-120} \div 1/TGD$), which represents the gold standard measure of beta cell function, increased more than two-fold in dapagliflozin-treated subjects (0.12 ± 0.04 to 0.26 ± 0.05, $P < .01$), while no significant change was observed in placebo-treated subjects (0.14 ± 0.04 to 0.17 ± 0.03, $P < .01$ vs dapagliflozin); the increase in $\Delta C\text{-}Pep_{0-120}/\Delta G_{0-120} \div$ IR at 2 weeks relative to baseline in dapagliflozin-treated subjects (0.15 ± 0.03) was significantly greater ($P < .05$) than that in placebo-treated subjects (0.03 ± 0.03). Lastly, we measured beta cell glucose sensitivity before and after dapagliflozin treatment. Dapagliflozin caused a significant increase in beta cell glucose sensitivity from 23 ± 5 to 35 ± 5 pmol/min.mM ($P < .01$) (Figure 3).

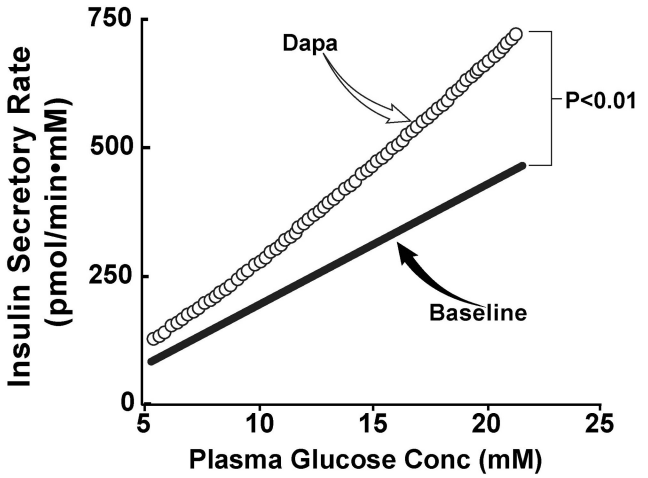

Figure 3. Beta cell glucose sensitivity measured with the Mari Model before and after treatment with dapagliflozin.

Discussion

The major finding of the present study is that reduction of the plasma glucose concentration by inducing glucosuria in T2DM individuals significantly improved beta cell function (Figure 1). Because the primary action of dapagliflozin is on the kidney and the drug has no known direct action on the beta cell, these findings demonstrate that dapagliflozin treatment improves beta cell function in T2DM by correcting hyperglycemia, ie, reversal of glucotoxicity. Thus, the present study provides the first conclusive evidence in man that chronic hyperglycemia exerts a deleterious effect on beta cell function in T2DM. Consistent with previous results, improvement in hyperglycemia with dapagliflozin for only 2 weeks also improved insulin sensitivity (25). Thus, improvement in the plasma glucose profile corrected two of the core defects present in T2DM individuals.

Dapagliflozin significantly lowered the fasting plasma glucose (by 33 mg/dl), the 2-hour plasma glucose (by 73 mg/dl), and the mean plasma glucose (by 60 mg/dl) during the OGTT. This reduction in plasma glucose levels resulted in a greater than 2-fold increase in beta cell function as measured with $\Delta C\text{-}pep_{0-120}/\Delta G_{0-120} \div IR$ ($P < .001$). No change in beta cell function was observed in placebo-treated subjects. These findings are consistent with previous publications from our group in rodents (2) and extend our prior observations by demonstrating that a reduction in plasma glucose concentration, by inhibiting renal sodium glucose reabsorption with a SGLT2 inhibitor, in T2DM patients improves two of the core defects (beta cell dysfunction and insulin resistance). Since SGLT2 is not expressed in the beta cell and since the plasma FFA concentration did not change in dapagliflozin-treated subjects, the improvement in beta cell function following dapagliflozin treatment is the direct result of amelioration of glucotoxicity (3). Importantly, these results demonstrate that the glucotoxic effect of chronic hyperglycemia on beta cell function in T2DM is reversible. Chronically elevated plasma glucose levels are the major risk factor for diabetic microvascular complications. The results of the present study demonstrate that hyperglycemia also worsens beta cell function, the major factor responsible for the progressive rise in plasma glucose levels in T2DM patients (1, 26). Thus, chronic hyperglycemia is a self-perpetuating cause of the progressive beta cell failure. These results underscore the importance of lowering and maintaining the plasma glucose concentration at or below the treatment goal (ie, HbA1c $<$ 6.5–7.0%) in T2DM individuals.

The glucotoxic effect of chronic hyperglycemia on insulin secretion is well established in experimental systems. In vitro studies using cell culture systems have shown that a small persistent increase in plasma glucose concentration has a deleterious effect on beta cell function (27). In partially pancreatectomized "normal glucose tolerant" rats, a 16 mg/dl increment in the mean day-long plasma glucose concentration has been shown to markedly impair first phase insulin secretion (8). Studies which have assessed the effect of elevated plasma glucose levels, created with glucose infusion, on beta cell function have yield conflicting results. An increase in plasma glucose concentration (+50 mg/dl) for 30 minutes in healthy, normal glucose tolerant subjects was shown to inhibit first phase insulin secretion, measured with the hyperglycemic clamp (28). More prolonged elevation of the fasting plasma glucose concentration for 62 hours decreased beta cell function by 36% in nondiabetic subjects (29). Other studies (30) have reported increased beta cell function after 42 hours of glucose infusion in healthy subjects. Methodological differences in the various protocols and differences in the study populations may, in part, explain these conflicting results. For example, in the study by Boden et al study (29) the plasma glucose concentration was allowed to return back to the preinfusion level for ~2 hours prior to the repeat hyperglycemic challenge. Thus, the glucotoxic effects of hyperglycemia may have been washed out, leaving the beta cell in a hypersensitive state when presented with a repeat glucose stimulus. It also is possible that the glucotoxic effect of chronic hyperglycemia only will manifest itself in genetically predisposed individuals, as we have shown for chronically elevated plasma FFA levels (31). Other evidence in support of a glucotoxic effect of chronic hyperglycemia on beta cell function includes the improvement in insulin secretion in type 2 diabetic subjects following correction of hyperglycaemia with insulin (32), and loss of the normal oscillatory beta cell response to glucose in NGT subjects following short term exposure to hyperglycemia (33).

In conclusion, our results demonstrate that, for the first time in man in vivo, glucotoxicity plays an important role in the beta cell dysfunction in T2DM and that reduction in plasma glucose levels with an intervention, glucosuria, that does not affect other metabolic parameters results in improved beta cell function.

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AM, CS, JX, GD, AC, DT, AC, SU generated the data, MAG analyzed the data and wrote the manuscript, RAD reviewed and revised the manuscript

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