Comparison of the effects of empagliflozin and glimepiride on endothelial function in patients with type 2 diabetes: A randomized controlled study

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Abstract

Background: Among patients with type 2 diabetes and established cardiovascular disease, those receiving empagliflozin have a lower rate of primary composite cardiovascular outcomes and death from any cause. Alternatively, treatment with sulfonylurea reduces microvascular complications in diabetes but appears to increase cardiovascular hospitalization or mortality in combination with metformin. Therefore, in the present study, we assessed the effects of empagliflozin and glimepiride, a sulfonylurea, on endothelial function using flow-mediated dilation (FMD) to estimate arteriosclerosis and cardiovascular events in patients with type 2 diabetes. **Methods:** In this prospective, open-label, randomized, parallel-group comparison, 58 patients with type 2 diabetes were administered metformin and glargine before bedtime for 12 weeks, followed by the random addition of 10 mg empagliflozin or 0.5 mg glimepiride for 12 weeks. The primary outcome was the change in the FMD measurement (DFMDs), which was measured prior to and following 12 weeks of additional treatment. Secondary outcomes comprised changes in metabolic markers and body composition. **Results:** Analysis of the empagliflozin group (n = 30) and glimepiride group (n = 28) showed no significant differences in DFMDs (empagliflozin, $-0.19 \pm 2.34\%$; glimepiride, $-0.37 \pm 2.77\%$; $P = 0.79$). Likewise, glycated hemoglobin (HbA1c) changes were similar between the two groups. Body weight changes significantly differed (empagliflozin, -0.59 ± 2.5 kg; glimepiride, 1.2 ± 3.0 kg; $P = 0.02$). However, analysis of the body composition revealed that body fluid volume significantly decreased only after empagliflozin treatment (baseline, 35.8 ± 6.8 L; after 12 weeks, -0.33 ± 0.72 L; $P = 0.03$). **Conclusions:** Empagliflozin did not improve endothelial function compared with glimepiride in patients with type 2 diabetes, but decreased their body fluid volume. This suggested that the coronary protective effect of empagliflozin is not derived by protecting endothelial function but rather from reducing the risk of heart failure.

Background

In the treatment of coronary artery disease, the assessment of coronary endothelial vasoreactivity has important diagnostic and prognostic implications. Specifically, an estimate of arteriosclerosis and cardiovascular events can be made by measuring endothelial dysfunction [1]; thus, this metric may be useful for assessing cardiovascular risk in diabetes as well. Flow-mediated dilation (FMD) constitutes a method that assesses endothelial function in a noninvasive manner, based on the intrinsic ability of blood vessels to respond to blood flow [2]. FMD involves high-frequency ultrasonographic imaging of the brachial artery and reflects nitric oxide production within. As FMD can predict cardiovascular events, it has been utilized in numerous investigations of arteriosclerosis [3-5]. However, variables such as age, systolic blood pressure, body mass index (BMI), sex, diabetes mellitus, lipid-lowering medication, smoking, and a decrease in visceral adipose tissue mass can impact FMD measurements [6-8].

Blood glucose levels in diabetes can be decreased by preventing proximal tubular glucose reabsorption and increasing urinary glucose excretion using an inhibitor of sodium glucose cotransporter 2 (SGLT2). Notably, such inhibitors can also reduce blood pressure [9] and body weight [9, 10]. The Empagliflozin Cardiovascular Outcome Event Trial in Type 2 Diabetes Mellitus Patients-Removing Excess Glucose

(EMPA-REG OUTCOME) trial revealed that treatment with empagliflozin, an inhibitor of SGLT2, reduced the risk of cardiovascular outcomes and death from all causes in patients with pre-existing atherosclerotic cardiovascular disease. However, the incidence of myocardial infarction or stroke did not significantly differ between empagliflozin and placebo[11].

Furthermore, a meta-analysis of SGLT2 inhibitors showed that they improved the composite outcome of myocardial infarction, stroke, or cardiovascular death[12]. However, the effects varied as per the presence of atherosclerotic cardiovascular disease. These inhibitors had the effect of reducing the composite of major adverse cardiovascular events, albeit only in patients with existing atherosclerotic cardiovascular disease. In addition, hospitalization consequent to a heart failure was reduced with SGLT2 inhibitor treatment regardless of the presence of atherosclerotic cardiovascular disease or heart failure at baseline. Thus, the composite of cardiovascular death and hospitalization for heart failure reduced with SGLT2 inhibitor treatment regardless of existing atherosclerotic cardiovascular disease. However, the anti-atherosclerotic mechanism of SGLT2 inhibitors remains unclear [12].

In the UK Prospective Diabetes Study (UKPDS) 33 Group, microvascular complications but not macrovascular disease, were reduced with sulfonylurea therapy [13]. However, the observation that a combination therapy of sulfonylureas and metformin increased cardiovascular hospitalization or mortality was concerning [14]. The lack of effect on macrovascular disease could be a result of non-action on endothelial function. The effect of reduced microvascular complication was derived from strict control of blood glucose and decreased glycated hemoglobin (HbA1c). We thus hypothesized that sulfonylureas may not improve endothelial function. Consequently, in this study we used FMD to compare endothelial function in patients with type 2 diabetes treated with either an SGLT2 inhibitor (empagliflozin) or a sulfonylurea (glimepiride).

Methods

Study design

This study was registered with the University Hospital Medical Information Network Clinical Trial Registry (UMIN000024001) as a prospective, open-label, randomized, parallel-group comparison study. Approval for this study was obtained from the ethics committee of Chigasaki Municipal Hospital, approval No. 2016-04. The study protocol conforms to the provisions of the Declaration of Helsinki as revised in 2013.

Written, informed consent was obtained from all patients that were hospitalized for 1-2 weeks.

Inclusion and exclusion criteria

This study enrolled patients with type 2 diabetes, between 20–80 years of age, who were hospitalized at Chigasaki Municipal Hospital for education and blood glucose control. Such patients underwent metformin and basal insulin therapy prior to discharge and had a BMI $\leq$ 45 kg/m².

Patients were excluded if they showed severe renal (estimated glomerular filtration rate [eGFR] < 45 ml min⁻¹ 1.73 m⁻²) or liver dysfunction; were on steroid therapy; experienced cardiovascular disease and a cerebral infarction within 24 weeks of the study; had cancer, a severe infection, or were traumatized; were or could become pregnant; or were allergic to empagliflozin, glargine, glimepiride, or metformin; or if the supervising doctor decided that the patient did not qualify for this study.

Treatment and interventions

Patients treated with metformin and glargine for 12 weeks from discharge were randomized to receive 10 mg empagliflozin or 0.5 mg glimepiride daily. Randomization was stratified by age, glycated hemoglobin (HbA1c), and FMD. The blood sample was obtained in a fasting state. Postprandial plasma glucose (PPG) was measured by self-monitoring blood glucose system (ONE TOUCH Verio IQ; Johnson and Johnson Co., New Brunswick, NJ, USA). First time measurements of FMD were made prior to additional treatment and randomization. Second time measurements were made following additional treatment for 12 weeks. Treatment was not changed in general after randomization, although glargine was decreased by one unit, once weekly, if the fasting plasma glucose was maintained under 90 mg/dL. Hypoglycemia (i.e., blood glucose < 70 mg/dL) was ascertained using the values recorded by the patients.

Endpoints and assessments

The primary outcome was a change in FMD and was measured prior to and following 12 weeks of additional treatment (Fig. 1). Secondary outcomes included changes in the levels of metabolic markers in plasma during fasting state, and were measured prior to and following 12 weeks about metabolism of treatment: Body composition components, such as skeletal muscle and total fat mass, and body fluid volume were assessed using a Multifrequency Bioelectrical Impedance Analyzer (BIA) (InBody720; InBody Co., Ltd., Seoul, South Korea).

Sample size and statistical analysis

The effect of SGLT2 inhibitors on endothelial function as measured using FMD was unknown at the time this protocol was developed. We estimated that the Δ FMD with empagliflozin treatment for 12 weeks would be $1.5 \pm 2.0\%$ after referring to a previous study about another drug[15]. Based on a two-sided *P*-value of 5% and a power of 80%, we needed 58 patients to detect a significant difference in comparisons of the two groups.

We used Students' t-test to compare the averages of Δ FMD between the two groups, before and after additional treatment (Table 2).

Results

Patients

Between June 2016 and 2018, 69 patients with type 2 diabetes agreed to participate in this study. A total of 63 patients were randomized to an empagliflozin or glimepiride group. Ultimately, 30 patients in the empagliflozin group and 28 patients in the glimepiride group were analyzed (Fig. 2). Baseline clinical and biochemical characteristics were all statistically similar (Table 1).

Table 1 Baseline characteristics of patients

	Empagliflozin group (±SD)	Glimepiride group (±SD)	<i>P</i> value
Age [years]	58.6±8.5	54.3±12.2	0.13
Gender (male/female)	18 / 12	18 / 10	0.74
Estimated duration of diabetes [years]	6.1±7.2	4.9±4.9	0.44
Past smoker [%]	60.0	60.7	0.96
Current smoker [%]	20.0	39.3	0.11
FMD [%]	5.49±2.05	5.46±2.2	0.96
HbA1c [%]	6.9±1.1	6.6±0.7	1.0
FPG [mg/dL]	136.9±65.2	127.1±52.6	0.37
GA [%]	17.0±3.7	16.3±3.4	0.48
Body weight [kg]	70.0±11.3	69.6±17.1	0.92
BMI [kg/m ²]	26.1±3.7	25.9±5.4	0.9
Body fluid volume [L]	35.8±6.8	36.6±8.6	0.69
Skeletal muscle mass [kg]	26.7±5.7	27.1±7.1	0.81
Total fat mass [kg]	21.1±6.8	20.5±11.0	0.8
Waist circumference [cm]	91.6±9.2	91.2±15.7	0.27
Cr [mg/dL]	0.76±0.16	0.73±0.16	0.54
eGFR [mL min ⁻¹ 1.73 m ⁻²]	74.9±12.9	81.9±19.7	0.11
CysC [mg/L]	0.8±0.12	0.79±0.16	0.78
eGFRcys [mL min ⁻¹ 1.73 m ⁻²]	96.4±20.1	99.3±21.6	0.59
F-CPR [ng/mL]	3.1±1.7	2.5±1.9	0.23
Log U-Alb	2.9±1.7	2.5±1.6	0.34
Log L-FABP	0.96±0.85	0.6±0.85	0.11
UA [mg/dL]	5.5±1.2	5.4±1.5	0.96
LDL-C [mg/dL]	94.0±26.7	89.2±28.1	0.51
HDL-C [mg/dL]	54.7±16.0	57.4±16.4	0.52
TG [mg/dL]	197.7±101.1	176.0±131.5	0.2
CRP [mg/dL]	0.17±0.11	0.23±0.39	0.54
sBP [mmHg]	129.6±14.9	130.1±20.5	0.9

dBP [mmHg]	80.8±10.0	78.0±9.3	0.28
HR [bpm]	79.4±14.8	77.5±14.3	0.64
HOMA2%B	217.8±92.3	205.4±105.1	0.63
HOMA2%S	21.5±28.5	25.5±16.2	0.07
HOMA2IR	8.8±8.9	6.5±6.1	0.07
Metformin [mg]	883.3±375.6	991.1±473.8	0.34
Glargine [U]	9.4±4.6	11.7±9.0	0.6
ARB/ACE-I [%]	23.3	32.1	0.46
Statin [%]	40.0	53.6	0.31

Values are shown as the means ± standard deviation (SD). Paired Student's *t*-tests were used to compare values between different groups.

FMD, flow-mediated dilation; HbA1c, glycated hemoglobin; FPG, fasting plasma glucose; GA, glycated albumin; BMI, body mass index; Cr, serum creatinine; eGFR, estimated glomerular filtration rate; CysC, cystatin C; eGFRcys, estimated glomerular filtration rate by cystatin C; F-CPR, fasting C-peptide immunoreactivity; U-Alb, urine albumin; L-FABP, liver-type fatty acid binding protein; UA, uric acid; LDL, low-density lipoprotein; HDL, high-density lipoprotein; TG, triglycerides; CRP, C-reactive protein; sBP, systolic blood pressure; dBP, diastolic blood pressure; HR, heart rate; HOMA2%B, Homeostasis Model Assessment 2 steady-state beta cell (%B) function; HOMA2%S, Homeostasis Model Assessment 2 insulin sensitivity (%S); HOMA2IR, Homeostasis Model Assessment 2 insulin resistance (IR); ARB/ACE-I, angiotensin type II receptor blocker/angiotensin converting enzyme-I

Endothelial function

Average baseline FMD values were $5.49 \pm 2.05\%$ and $5.46 \pm 2.2\%$ for empagliflozin and glimepiride groups, respectively (Tables 1 & 2). FMD values following treatment with empagliflozin and glimepiride were $5.3 \pm 2.28\%$ and $5.09 \pm 1.86\%$, respectively (Table 2). Changes in FMD were $-0.19 \pm 2.34\%$ and $-0.37 \pm 2.77\%$ for empagliflozin and glimepiride groups, respectively, without a significant difference ($P = 0.79$).

Table 2 FMD (%±SD) with treatment

	Empagliflozin	Glimepiride	
FMD (0)	5.49±2.05	5.46±2.2	<i>P</i> = 0.96
FMD (12)	5.3±2.28	5.09±1.86	<i>P</i> = 0.71
	<i>P</i> = 0.66	<i>P</i> = 0.49	
ΔFMD (12) – (0)	-0.19±2.34	-0.37±2.77	<i>P</i> = 0.79

Values are shown as the means ± standard deviation (SD).

FMD, flow-mediated dilation

FMD (0) means the baseline FMD value at 0 week. FMD (12) means the FMD value at 12 weeks after additional treatment.

Δ indicates the change in the FMD value between 0 and 12 week.

P values in each row show the comparison of FMD values at the baseline and at week 12. *P* value in each line shows the comparison of changes in FMD values for both the groups.

Metabolic markers

Fasting plasma glucose remained unchanged, with no significant difference evident between the two groups following treatment (*P* = 0.69) (Table 3). However, HbA1c and glycated albumin (GA) levels significantly decreased per group following treatment (*P* ≤ 0.05), with the ΔHbA1c (*P* = 0.75) and ΔGA (*P* = 0.42) showing no significant difference between the two groups. Uric acid (UA) significantly decreased in the empagliflozin group (*P* < 0.001), but significantly increased in the glimepiride group (*P* = 0.01). A significant difference between the two groups was also noted in the ΔUA (*P* < 0.001). Systolic (*P* = 0.59) and diastolic (*P* = 0.74) blood pressures along with the heart rate (*P* = 0.81) did not change significantly between the two groups. Empagliflozin treatment significantly increased low-density lipoprotein- C (LDL-C) (*P* < 0.001), whereas triglycerides (TG) (*P* = 0.31) and high-density lipoprotein- C (HDL-C) (*P* = 0.37) did not change significantly between the two groups. Glimepiride treatment led to a significant increase of body weight (*P* < 0.05) after treatment, with a significant difference observed in the Δbody weight between the two groups (*P* = 0.02). However, for the subgroup showing decreased body weight, ΔFMD was not significantly different between the two groups (*P* = 0.62) (Table 4). Glimepiride also led to a significant increase of waist circumference after treatment (*P* = 0.004). A significant difference was also observed in the Δwaist circumference between the two groups (*P* = 0.008). With regard to body

composition, glimepiride led to a significant increase of total fat mass ($P = 0.02$). In comparison, empagliflozin significant decreased body fluid volume ($P = 0.03$).

Table 3 Changes in metabolic markers

	Empagliflozin	Glimepiride	
Fasting plasma glucose (mg/dL) [mean ± SD]			
Week 12	124.5±52.1	119.9±49.1	
	<i>P</i> = 0.11	<i>P</i> = 0.47	
ΔFPG	-12.4±44.4	-7.3±52.0	<i>P</i> = 0.69
Postprandial plasma glucose (mg/dL) [mean ± SD]			
Week 12	169.6±87.0	165.1±61.1	
	<i>P</i> = 0.07	<i>P</i> = 0.26	
ΔPPG	-16.5±43.9	-9.5±27.6	<i>P</i> = 0.63
HbA1c (%) [mean ± SD]			
Week 12	6.7±1.1	6.4±0.78	
	<i>P</i> = 0.001	<i>P</i> = 0.01	
ΔHbA1c	-0.22±0.36	-0.26±0.5	<i>P</i> = 0.75
GA (%) [mean ± SD]			
Week 12	16.0±3.2	15.7±3.1	
	<i>P</i> < 0.001	<i>P</i> = 0.05	
ΔGA	-0.97±1.3	-0.65±1.6	<i>P</i> = 0.42
Renal function			
Cr (mg/dL) [mean ± SD]			
Week 12	0.79±0.15	0.74±0.16	
	<i>P</i> = 0.03	<i>P</i> = 0.8	
eGFR (mL min ⁻¹ 1.73 m ⁻²) [mean ± SD]			
Week 12	72.1±11.7	81.5±20.2	

	<i>P</i> = 0.02	<i>P</i> = 0.8	
CysC (mg/dL) [mean ± SD]			
Week 12	0.84±0.13	0.82±0.17	
	<i>P</i> < 0.001	<i>P</i> = 0.03	
eGFRcys (mL min ⁻¹ 1.73 m ⁻²) [mean ± SD]			
Week 12	89.4±17.3	95.9±20.4	
	<i>P</i> = 0.01	<i>P</i> = 0.04	
UA (mg/dL) [mean ± SD]			
	-	-	-
Week 12	4.8±1.2	5.7±1.4	
	<i>P</i> < 0.001	<i>P</i> = 0.01	
ΔUA	-0.64±0.9	0.26±0.5	<i>P</i> < 0.001
Body weight (kg) [mean ± SD]			
Week 12	69.4±12.0	70.8±18.2	
	<i>P</i> = 0.22	<i>P</i> < 0.05	
ΔBody weight	-0.59±2.5	1.2±3.0	<i>P</i> = 0.02
Waist circumference (cm) [mean ± SD]			
Week 12	90.9±8.9	92.3±15.3	
	<i>P</i> = 0.07	<i>P</i> = 0.004	
ΔWaist circumference	-0.64±1.8	1.1±2.8	<i>P</i> = 0.008
Blood pressure (mmHg) [mean ± SD]			
sBP			
Week 12	130.2±14.2	128.9±19.3	
	<i>P</i> = 0.79	<i>P</i> = 0.62	

Δ sBP	0.69±13.7	-1.3±13.5	<i>P</i> = 0.59
dBP			
Week 12	80.6±7.6	78.6±12.0	
	<i>P</i> = 0.91	<i>P</i> = 0.72	
Δ dBP	-0.24±10.8	0.64±9.3	<i>P</i> = 0.74
Heart rate			
Week 12	80.1±15.4	79.0±12.8	
	<i>P</i> = 0.76	<i>P</i> = 0.55	
Δ Heart rate	0.68±11.2	1.5±11.6	<i>P</i> = 0.81
Lipids (TG, HDL-C, LDL-C) (mg/dL) [mean ± SD]			
LDL-C			
Week 12	107.5±33.0	93.9±29.6	
	<i>P</i> < 0.001	<i>P</i> = 0.33	
Δ LDL-C	13.5±19.3	4.7±25.2	<i>P</i> = 0.14
HDL-C			
Week 12	56.0±12.5	56.4±13.1	
	<i>P</i> = 0.43	<i>P</i> = 0.61	
Δ HDL-C	1.4±9.4	-1.1±10.9	<i>P</i> = 0.37
TG			
Week 12	177.1±86.9	185.7±123.7	
	<i>P</i> = 0.29	<i>P</i> = 0.8	
Δ TG	-20.6±103.8	9.6±121.5	<i>P</i> = 0.31
Body fluid volume (L) [mean ± SD]			

Week 12	35.4±6.9	36.3±8.7	
	<i>P</i> = 0.03	<i>P</i> = 0.32	
ΔBody fluid volume	-0.33±0.72	-0.35±1.8	<i>P</i> = 0.94
Skeletal muscle mass (kg) [mean ± SD]			
Week 12	26.4±5.7	27.1±7.2	
	<i>P</i> = 0.08	<i>P</i> = 0.72	
ΔSkeletal muscle mass	-0.21±0.6	0.05±0.7	<i>P</i> = 0.15
Total fat mass (kg) [mean ± SD]			
Week 12	20.6±6.8	21.7±11.6	
	<i>P</i> = 0.16	<i>P</i> = 0.02	
ΔTotal fat mass	-0.58±2.1	1.2±2.3	<i>P</i> = 0.006
Adverse events (%)			
	rash on both arms (3.3%)	hypoglycemia (3.6%)	
Glargine (U) [mean ± SD]			
Week 12	8.4±5.1	9.6±8.9	
	<i>P</i> = 0.02	<i>P</i> < 0.001	
ΔGlargine	-1.0±2.4	-2.1±3.5	<i>P</i> = 0.16

Values are shown as the means ± standard deviation (SD).

Δ indicates the changes in the FMD values between 0 and 12 week.

P values in each row show the comparison of FMD values at the baseline and at week 12. P value in each line shows the comparison of changes in FMD values for both the groups.

FMD, flow-mediated dilation; HbA1c, glycated hemoglobin; GA, glycated albumin; Cr, serum creatinine; eGRF, estimated glomerular filtration rate; CysC, cystatin C; eGFRcys,

estimated glomerular filtration rate by cystatin C; UA, uric acid; sBP, systolic blood pressure; dBP, diastolic blood pressure; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides

Table 4 Change of FMD (%±SD) with treatment with subgroup of decreasing body weight for observation period

	Empagliflozin N=17	Glimepiride N=8	
ΔFMD (12) – (0)	0.41±2.55	0.94±2.16	<i>P</i> = 0.62

FMD, flow-mediated dilation

Δ indicates the change in FMD value between 0 and 12 week.

P value in each line shows the comparison of changes in FMD values of both the groups.

Fasting C-peptide immunoreactivity (CPR) (*P* = 0.55) Homeostasis Model Assessment 2 steady state beta cell (%B) function (*P* = 0.2), Homeostasis Model Assessment 2 insulin sensitivity (%S) (*P* = 0.09), and Homeostasis Model Assessment 2 insulin resistance (*P* = 0.22) were not significantly changed between the two groups (Table S1). In this study, empagliflozin and glimepiride did not affect insulin secretion, insulin sensitivity, pancreatic β-cell function, or insulin resistance. Table 5 showed associations between arterial sclerosis markers and ΔFMD. ΔHbA1c, Δbody weight, ΔHDL-C, and ΔTG were related to the ΔFMD. Additionally, the ΔFMD may have been greater if the baseline FMD was lower.

Table 5 Associations between arterial sclerosis markers and ΔFMD in all patients (n = 58)

	Raw β	Std β	<i>P</i> value
Age	0.01	0.05	0.7
Gender	-0.02	-0.004	0.98
Duration	0.02	0.06	0.67
FMD (0)	-0.74	-0.61	0.0001***
HbA1c (0)	-0.47	-0.18	0.18
FPG (0)	-0.005	-0.11	0.4
GA (0)	-0.17	-0.24	0.07
BW (0)	0.03	0.18	0.18
BMI (0)	0.12	0.2	0.15
UA (0)	0.31	0.16	0.22
LDL (0)	-0.02	-0.23	0.09
HDL (0)	-0.04	-0.26	0.05
TG (0)	0.004	0.2	0.11
Past smoker	-0.79	-0.15	0.25
sBP (0)	-0.03	-0.18	0.17
dBP (0)	-0.06	-0.21	0.11
Δ HbA1c	-1.85	-0.31	0.02*
Δ GA	-0.16	-0.1	0.48
Δ FPG	-0.005	-0.09	0.49
Δ BW	-0.33	-0.38	0.003**
Δ LDL-C	0.01	0.11	0.43
Δ HDL-C	0.08	0.31	0.02*
Δ TG	-0.009	-0.38	0.003**
Δ sBP	0.01	0.06	0.63
Δ dBP	0.05	0.18	0.18
ARB/ACE-I	0.41	0.07	0.59
Statin	0.34	0.07	0.62

Values are shown as the means. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ indicate a significant association between arterial sclerosis markers and Δ FMD.

(0), baseline; FMD, flow-mediated dilation; HbA1c, glycated hemoglobin; FPG, fasting plasma glucose; GA, glycated albumin; BW, body weight; BMI, body mass index; UA, uric acid; LDL, low-density lipoprotein; HDL, high-density lipoprotein; TG, triglycerides; sBP, systolic blood pressure; dBP, diastolic blood pressure; LDL-C, LDL cholesterol; HDL-C, HDL cholesterol; ARB, angiotensin receptor blocker; ACE-I, angiotensin converting enzyme inhibitor

Discussion

We found that although fasting plasma glucose (FPG), HbA1c, and GA were equally improved in both empagliflozin and glimepiride treated patient groups, the Δ FMD did not show a significant difference. According to the previous study, glimepiride did not demonstrate an improvement in the endothelial function [15]. Empagliflozin had no effect toward improving the endothelial function irrespective of improvement in the glucose levels in this study. To observe the changes in endothelial function induced by drugs that lacked the ability to improve the glucose levels, this study was conducted with patients in a steady glucose-controlled state. Although the baseline HbA1c was targeted in this study, severe hypoglycemia did not occur after reducing glargine dose as the fasting plasma glucose was maintained under 90 mg/dL. Additionally, GA improved significantly in both groups. It is possible that any glucose variation was improved by the additional treatment. The insulin dose was also decreased significantly for both groups and this may have reduced any increase in body weight observed. The improvements of Δ HbA1c, body weight, HDL-C, and TG on the change of the FMD and the reduction of HbA1c in the empagliflozin group were not able to change FMD. These findings suggest that empagliflozin has no effect toward improving FMD although empagliflozin is effective in improving HbA1c.

Renal function was found to be worse in the empagliflozin group for a relatively short observation period, although empagliflozin has been previously shown to stably maintain the eGFR during long-term administration [16]. In a recent study, canagliflozin reduced the risk of kidney failure at a median follow up of 2.62 years [17], whereas empagliflozin decreased hyperfiltration by diuresis [18]. A previously reported study showed that a rapid initial decline in the renal function by losartan led to a slower decrease in long-term renal function [20]. However, it has been reported that an initial rapid decline of renal function leads to kidney failure [21]. Therefore, empagliflozin would be expected to prevent the progression of kidney disease over an extended period of time.

In Body720, an eight-polar BIA was shown to accurately estimate total and appendicular body composition independent of age and sex [19]. The body fluid volume was also found to have significantly decreased following 12 weeks of empagliflozin treatment. However, it is unclear whether the effect of empagliflozin on decreasing the body fluid volume is greater than that of glimepiride. Empagliflozin has

been shown to improve hospitalization rates after heart failure [20] and is thought to ameliorate this disease by decreasing the body fluid volume. In the observational period of our study, heart failure was not observed for patients in either treatment group. This suggests that empagliflozin may have had a coronary protective effect that is not derived from any impact on the endothelial function. The diuretic medication has an effect of decreasing congested heart failure, although its effect on reduces the cardiovascular event risk is unclear. It was reported that SGLT2 inhibitors reduced more interstitial fluid volume than blood volume compared with the loop diuretics[21]. It was hypothesized that improvement of systemic congestion and renal function which derived from decreasing hyperfiltration had effects of preventing cardiovascular event without reducing arterial filling and perfusion.

The FMD may be improved when the baseline FMD is low. However, in the subgroup showing a lower than median baseline FMD, the Δ FMD did not significantly differ between the two groups in both subgroup ($P = 0.83$) (Table 6). Notably, SGLT2 inhibitors have a secondary preventive role in adverse cardiovascular events but lack a primary preventive role [12]. However, DECLARE-TIMI 58 reported that dapagliflozin had both primary as well as a secondary preventive role in adverse cardiovascular events. Dapagliflozin decreased cardiovascular death and hospitalization for heart failure for patients without previous cardiovascular disease [22]. No patients in the present study had such a background; it would therefore be necessary to undertake a secondary intervention.

Table 6 Change of FMD (% $\pm$ SD) with treatment with subgroup of a lower than median baseline FMD

	Empagliflozin N=14	Glimepiride N=15	
Δ FMD(12)-(0)	0.66 $\pm$ 1.96	0.82 $\pm$ 1.92	$P = 0.83$

FMD, flow-mediated dilation

Δ indicates the change in the FMD value between 0 and 12 week.

P value in each line shows the comparison of changes in FMD values for both the groups.

In particular, the effects of other oral hypoglycemic agents like pioglitazone and glucagon-like peptide 1 (GLP–1) analogs on improving endothelial function have been previously reported [15, 23]. In comparison, a dipeptidyl peptidase 4 (DPP-4) inhibitor improved [24], had no effect [25], or worsened [26] endothelial function but did not affect cardiovascular events [27, 28]. Moreover, GLP–1 analog treatment itself enhanced [29, 30] or had no effect on [31] endothelial function. However, in patients with type 2 diabetes, liraglutide, a GLP–1 analog, was successful in preventing nonfatal myocardial infarction or

stroke along with death from cardiovascular causes [32]. Similar to these findings, significant heterogeneity existed between a meta-analysis study of a DPP-4 inhibitor and GLP-1 [33]. The size of the study, the duration of intervention, and the age or sex of participants did not affect the mean difference in FMD [33]. Rather, a change in FMD was found to be dependent on the baseline FMD. As the baseline FMD of our study was less than that in other reported studies [23, 24, 34], this may have affected our findings.

Several limitations were evident in this study. First, as the study participants were outpatients, it may not have been possible to completely exclude patients who smoked or had a meal before the FMD was examined. Some patients may also not have been very compliant with their diet, which may have affected the change in body weight observed. Second, the number of patients to detect a significant difference in comparisons of the two groups was adequate, but the study observation period was relatively short. A longer study period to monitor any adverse events is required in the future studies.

Conclusions

Overall, we found that empagliflozin, when compared to glimepiride, did not improve endothelial function after at least a 12-week treatment period, in patients with type 2 diabetes without previous cardiovascular disease. However, empagliflozin significantly reduced body fluid volume. Thus, the coronary protective effect of empagliflozin may be derived not from preventing endothelial dysfunction but rather from reducing heart failure.

Abbreviations

ARB/ACE-I, angiotensin type II receptor blocker/angiotensin converting enzyme I; BMI, body mass index; Cr, serum creatinine; CRP, C-reactive protein; CysC, cystatin C; dBp, diastolic blood pressure; DPP, dipeptidyl peptidase; eGFR, estimated glomerular filtration rate; eGFR_{cys}, estimated glomerular filtration rate by cystatin C; F-CPR, fasting C-peptide immunoreactivity; FMD, flow-mediated dilation; FPG, fasting plasma glucose; GA, glycated albumin; GLP, glucagon-like peptide; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; HOMA2%B, Homeostasis Model Assessment 2 steady state beta cell (%B) function; HOMA2%S, Homeostasis Model Assessment 2 insulin sensitivity (%S); HOMA2IR, Homeostasis Model Assessment 2 insulin resistance (IR); HR, heart rate; LDL, low-density lipoprotein; L-FABP, liver-type fatty acid binding protein; sBP, systolic blood pressure; TG, triglycerides; UA, uric acid; U-Alb, urine albumin

Declarations

Ethics approval and consent to participate

Approval for this study was obtained from the ethics committee of Chigasaki Municipal Hospital, approval No. 2016-04. Written, informed consent was obtained from all patients.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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We have not accepted any financial support for this study.

Authors' contributions

YK provided advice on study design; HT and YK designed the study. HT collected and analyzed the data. HT performed statistical analysis. HT, KI, and MH interpreted the data. HT wrote the manuscript. YK, SS, and YTDrafted the work or substantively revised it. All authors read and approved the final manuscript.

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Figures

Figure 1

Study flow chart. Patients who took metformin and glargine were randomized to empagliflozin or glimepiride groups. Flow-mediated dilation (FMD), blood examination, body weight, and blood pressure were checked at the study baseline and endpoints.

Figure 2

Consort 2010 flow diagram of patient selection. Ultimately, 30 patients in the empagliflozin group and 28 patients in the glimepiride group were analyzed

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