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ORIGINAL ARTICLE

Arterial pressure lability is improved by sodium-glucose cotransporter 2 inhibitor in streptozotocin-induced diabetic rats

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To prevent cardiovascular events in patients with diabetes mellitus (DM), it is essential to reduce arterial pressure (AP). Sodium-glucose cotransporter 2 inhibitor (SGLT2i) prevents cardiovascular events via the depressor response in patients with DM. In the present study, we examined whether SGLT2i ameliorates AP lability in DM rats. Ten-week-old male Sprague–Dawley rats were administered a single intravenous injection of streptozotocin (50 mg kg^{-1}) and were divided into three groups treated with low-dose SGLT2i, vehicle (VEH) or subcutaneously implanted insulin pellets (SGLT2i, VEH and Insulin group, respectively) for 14 days. SGLT2i reduced blood glucose, but its effect was lower than that of insulin. The telemetered mean AP at the end of the experiment did not differ among the SGLT2i, Insulin and VEH groups (83 ± 7 *vs.* 98 ± 9 *vs.* 90 ± 8 mm Hg, respectively, $n = 5$ for each). The standard deviation of AP as the index of lability was significantly smaller during the active period in the SGLT2i group than in the VEH group (5.6 ± 0.5 *vs.* 7.0 ± 0.7 mm Hg, $n = 5$ for each, $P < 0.05$). Sympathetic nerve activity during the active period was significantly lower in the SGLT2i group than in the VEH group. Baroreflex sensitivity (BRS) was significantly higher in the SGLT2i group than in the VEH group. The standard deviation of AP and sympathoexcitation did not differ between the Insulin and VEH groups. In conclusion, SGLT2i at a non-depressor dose ameliorated the AP lability associated with sympathoinhibition during the active period and improved the BRS in streptozotocin-induced DM rats.
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Keywords: arterial pressure; diabetes mellitus; SGLT2 inhibitor; sympathetic nerve activity

INTRODUCTION

There is a strong relationship between diabetes mellitus (DM) and cardiovascular events.[1,2] A meta-analysis of more than 100 prospective studies has shown that the risk of developing cardiovascular diseases in patients with DM is twice as high as that in those without DM.[2] Although the benefits of optimal glycemic control with regard to microvascular complications have been established, the prevention of macrovascular complications by tight glycemic control has not been determined.[3] Cardiovascular events and all-cause mortality were increased by tight glycemic control in patients with type 2 DM.[4–6] Available and widely used anti-DM agents increased the risk of hospitalization for heart failure.[7,8] By contrast, lowering the arterial pressure (AP) has been shown to prevent cardiovascular events in patients with DM.[9,10] Moreover, these patients tend to have impaired AP variability in both the short and long term, even when they are normotensive.[11–15] Impaired AP variability has been shown to increase cardiovascular events.[16–20] These previous studies suggest that the improvement of impaired AP variability would be crucial to reducing cardiovascular events in patients with DM.

In the prevention of cardiovascular diseases, a new class of anti-DM agent, sodium-glucose cotransporter 2 inhibitor (SGLT2i), has recently become an investigational focus. SGLT2i significantly decreased cardiovascular death via a depressor response in patients with DM who were at high and well-treated cardiovascular risk.[21] Other anti-DM agents used before SGLT2i did not show such an obvious reduction in cardiovascular events. While the most important pharmacological characteristic of SGLT2i is its insulin-independent hypoglycemic effect,[22] its unique AP-lowering effect has been in the spotlight.[23–28] Interestingly, SGLT2i decreases AP and changes its circadian rhythm from a non-dipper to a dipper pattern in rats with metabolic syndrome.[25] To gain deeper insights into the cardiovascular regulatory system, in this decade, experiments have been performed to

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measure sympathetic nerve activity, baroreflex function and AP lability mediated by baroreflex in rats.[29,30]

To elucidate the mechanisms by which SGLT2i could prevent cardiovascular events, we hypothesized that SGLT2i would ameliorate AP lability independent of the depressor response in DM rats. To archive the aim of the present study, we recorded telemetered AP in the conscious state and assessed the AP lability using a histogram of AP in DM rats treated with SGLT2i at a non-depressor dose.

METHODS

This study was reviewed and approved by the Committee on the Ethics of Animal Experiments, Kyushu University Graduate School of Medical Sciences, and it was performed according to the Guidelines for Animal Experiments of Kyushu University.

Animals

We used Sprague–Dawley rats purchased from Japan SLC (Shizuoka, Japan) that were kept in individual cages in temperature-controlled cabinets (23 ± 2 °C) on a 12- h light–dark cycle, fed standard chow and provided with water *ad libitum*.

Drugs

Ipragliflozin, obtained from MedChemExpress (Monmouth Junction, NJ, USA), was suspended in 0.5% methyl cellulose (Sigma-Aldrich, St Louis, MO, USA) solution. We used the 'Linplant' insulin pellet (Linshin Canada, Toronto, ON, Canada), which sustainably releases insulin at ~2 U per 24 h for each implant for more than 40 days. Streptozotocin (STZ; Sigma-Aldrich) was used to induce DM.

Experimental protocols

We implanted the telemetry system in 9-week-old male Sprague–Dawley rats. After a 1-week recovery period when the rats were 10 weeks old, we established STZ-induced DM rats (STZ rats) by administering a single intravenous injection of STZ (50 mg kg^{-1}) dissolved in a 0.05 M citrate buffer (pH 4.5) via the tail vein of overnight-fasted rats. To minimize the influence of diet on the blood glucose level, we instituted a 6-h fast during the rest period. Next, 4 h after the fast ended, we checked the casual blood glucose level using Accu-Check Nano (Roche Diagnostics Australia, North Ryde, NSW, Australia) and determined the establishment of DM when the blood glucose was ⩾250 mg dl^{-1}.

We divided the STZ rats into three groups ($n=5$, each) that were treated with low-dose SGLT2i (ipragliflozin 1 mg kg^{-1} per day) (SGLT2i group), vehicle (VEH group) or insulin pellets subcutaneously inserted beneath the upper abdominal skin (Insulin group) for 14 days. The SGLT2i and vehicle were administered by oral gavage once daily. The non-depressor dose of the ipragliflozin was determined by a previous study,[31] which assessed the effects of the ipragliflozin at various doses (0.1–10 mg kg^{-1}) for 14 days (same as the present experiment) on DM profiles and AP in STZ (50 mg kg^{-1}, same as the present experiment)-induced DM rats.

Measurement of hemodynamic parameters

We used a radiotelemetry system (Data Science International, Saint Paul, MN, USA) to evaluate the AP and heart rate (HR). Telemetry catheters were inserted into the abdominal aorta of 9-week-old Sprague–Dawley rats, and 24-h monitoring of the AP and HR was initiated in the conscious state. Throughout the experimental period, the AP of the light (rest) and dark (active) periods was separately recorded at a 500-Hz sampling frequency, and the data were averaged data using 1-s bins (86 400 data points per day). We created daily AP histograms during the active and rest periods, and calculated the mean ± s.d. of the AP as the index of the AP lability, as described in our previous study.[30] In addition, spectral analysis was performed using an adaptive autoregressive model to obtain the power spectra for the systolic AP (SAP) and HR.[29] The beat-to-beat pulse frequencies and SAP were converted into data points at 20 Hz using a spline interpolation. We segmented them into 15 sets of half-overlapping bins of 2048 data points. For each data set, after the linear trend was removed and a 4-term Blackman–Harris window was applied, we performed a fast Fourier transform analysis to obtain the power spectral density.

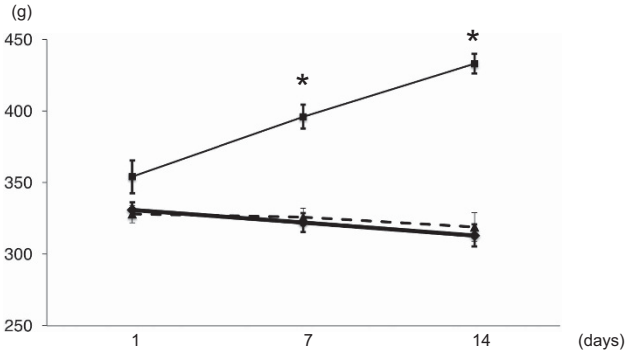

Figure 1 Serial changes in the body weights in each group ($n=5$ for each). Bold solid line, diabetic rats administered sodium-glucose cotransporter 2 inhibitor; solid line, diabetic rats treated with insulin; dotted line, diabetic rats administered vehicle. *$P<0.05$ *vs.* vehicle.

The low- and high-frequency power of the SAP and R–R interval were computed by integrating the spectra in the range of 0.04–0.60 and 1.0–5.0 Hz, respectively. The sympathetic and parasympathetic nerve activities were presented as the normalized unit of the low-frequency component of the systolic AP variability (LFnuSAP), and the normalized unit of the high-frequency component of the R–R interval variability. The baroreflex sensitivity (BRS) was measured using the spontaneous sequence method as a parameter of autonomic control. Sequence analysis was performed to detect the sequences of three or more beats, which showed either an increase or a decrease in the SAP and pulse interval (up or down sequence, respectively). The BRS was estimated as the mean slope of the up and down sequences.

Metabolic data and sample collection

Body weight was measured once a week. Urine samples were collected before the STZ injections and 2 weeks after each treatment. We also monitored the urine volume and 24-h water intake when collecting the urine samples, which were all measured by SRL Japan. At the end of the experiment, we killed the rats and collected blood samples that were subsequently measured by Nagahama LSL (Shiga, Japan).

Statistical analysis

Normally distributed variables are expressed as means ± s.e.m. Data were analyzed using the statistical software (Ekuseru-Toukei 2013; Social Survey Research Information, Tokyo, Japan). Unpaired *t*-test and Mann–Whitney *U*-test were used to compare the differences between normally distributed and non-normally distributed variables, respectively. Data were also analyzed by a two-factor repeated-measures analysis of variance. Differences were considered to be statistically significant at $P<0.05$.

RESULTS

Diabetic profiles

The serial changes in the body weights were similar between the SGLT2i and VEH groups, and the body weights at the end of the experiment did not differ between the SGLT2i and VEH groups (313 ± 8 *vs.* 319 ± 10 g, respectively, $n=5$ for each; Figure 1). However, the serial changes in the body weights were significantly higher in the Insulin group low-frequency component of the systolic AP the VEH group, and the body weights at the end of the experiments were significantly higher in the Insulin group than in the VEH group (433 ± 7 *vs.* 319 ± 10 g, $n=5$ for each, $P<0.05$; Figure 1).

The blood glucose level at the end of the experiment was significantly lower in the SGLT2i group than in the VEH group

Table 1 Diabetic profiles of animals

	SGLT2i	*Insulin*	*VEH*
Body weight (g)	313±8	433±7*	319±10
Blood glucose (mg dl^{-1})	435±6*	373±34*	551±15
Glycoalbumin (%)	7.6±0.2	4.5±0.5*	8.4±0.5
Urine glucose (mg per day)	13 646±822	7220±1546*	13 562±1389
Urine volume (ml per day)	188±10	113±24	179±20
Water intake (ml per day)	220±9	154±30	208±27
Urinary sodium excretion (mEq per day)	5.22±0.18	4.53±0.29	5.11±0.16

Abbreviations: SGLT2i, sodium-glucose cotransporter 2 inhibitor; VEH, vehicle.
$N=5$ for each group, *$P<0.05$ *vs.* VEH.

(435±6 *vs.* 551±15 mg dl^{-1}, $n=5$ for each, $P<0.05$; Table 1). In addition, the blood glucose level was also significantly lower in the Insulin group than in the VEH group (373±34 *vs.* 551±15 mg dl^{-1}, $n=5$ for each, $P<0.05$; Table 1) and, to a greater extent, the SGLT2i group (Table 1). However, the glycoalbumin level did not differ between the SGLT2i and VEH groups (7.6±0.2% *vs.* 8.4±0.5%, $n=5$ for each) and was significantly lower in the Insulin group than in the VEH groups (4.5±0.5% *vs.* 8.4±0.5%, $n=5$ for each, $P<0.05$) (Table 1).

Water intake, urine volume, glucose excretion and sodium excretion

The urine volume and water intake at the end of the experiment were similar among the SGLT2i, Insulin and VEH groups (Table 1). Although urine glucose excretion at the end of the experiment did not differ between the SGLT2i and VEH groups, it was significantly lower in the Insulin group than in the VEH group (Table 1). Urine sodium excretion at the end of the experiment was similar among the SGLT2i, Insulin and VEH groups (Table 1).

Effects of SGLT2i or insulin on AP and HR

Serial changes in the mean AP during the active and rest periods were similar among the SGLT2i, Insulin and VEH groups (Figures 2a and b). At the end of the experiment, the mean AP did not differ among the SGLT2i, Insulin and VEH groups during the active period (86±6 *vs.* 101±8 *vs.* 93±7 mm Hg, respectively, $n=5$ for each; Table 2) and rest period (80±6 *vs.* 95±8 *vs.* 88±7 mm Hg, respectively, $n=5$ for each; Table 2). The serial changes in the HR were similar between the SGLT2i and VEH groups (Figures 2c and d), and the HR at the end of the experiment was similar between the SGLT2i and VEH groups during the active and rest periods (Table 2). However, the serial changes in the HR during the active period were significantly higher in the Insulin group than in the VEH group (Figure 2c), and the HR at the end of the experiment was significantly higher in the Insulin group than in the VEH group (349±29 *vs.* 300±32b.p.m., $n=5$ for each, $P<0.05$; Table 2). During the rest period, the serial changes of the HR were similar between the Insulin and VEH groups (Figure 2d).

Effects of SGLT2i and insulin on AP lability and autonomic function

The s.d. of the AP was significantly smaller in the SGLT2i group than that in the VEH group during the active period (5.6±0.5 *vs.* 7.0±0.7 mm Hg, $n=5$ for each, $P<0.05$; Figure 3) but not during the rest period (Figure 3). However, the s.d. of the AP did not differ between the Insulin and VEH groups during the active and rest periods (Figure 3).

The LFnuSAP was significantly smaller in the SGLT2i group than in the VEH group during the active period, but not during the rest period (Table 3). However, the LFnuSAP was similar between the Insulin and VEH groups during the active and rest periods (Table 3). The high-frequency component of the R–R interval variability did not differ among the SGLT2i, Insulin and VEH groups during the active and rest periods (Table 3). The BRS was significantly higher in the SGLT2i group than in the VEH group during the active and rest periods (Table 3). However, the BRS did not differ between the Insulin and VEH groups during the active and rest periods (Table 3).

DISCUSSION

In the present study, we assessed the effects of a non-depressor dose of the SGLT2i on the AP lability in DM rats and observed the following major findings: (1) the s.d. of the AP was significantly smaller and the low-frequency component of the SAP variability significantly lower during the active period, but not during the rest period, in the SGLT2i group than in the VEH group; (2) the BRS was significantly higher in the SGLT2i group than in the VEH group; and (3) the s.d. of the AP, low-frequency component of the SAP variability and BRS did not differ between the Insulin and VEH groups. Considering these results, we concluded that SGLT2i at the non-depressor dose ameliorated the AP lability associated with sympathoinhibition during the active period and improved the baroreflex in the DM rats.

The most significant finding was that the SGLT2i improved the AP lability without inducing a depressor effect in the DM rats. The impaired AP lability indicates the disruption of circulatory homeostasis and causes cardiovascular events.[18,29,30] It has been previously determined that SGLT2i agents improve the circadian rhythm of the AP by changing it from a non-dipper to a dipper pattern.[25] We previously examined the AP lability associated with baroreflex control,[30] and to the best of our knowledge, this is the first study to demonstrate the beneficial effect of SGLT2i on the AP lability. Interestingly, the present benefit occurred without a depressor response. Because the s.d. of the AP is associated with the mean AP,[32] we could exclude the possibility that the improved AP lability would be accompanied by a depressor response.

The mechanisms by which SGLT2i improved the AP lability without a depressor effect should be assessed. Previous reports have suggested that SGLT2i reduces arterial stiffness, vascular resistance and vascular distensibility in patients with hypertensive type 2 DM and normotensive type 1 DM.[33,34] These changes were explained by the improvement in glycemic control and insulin sensitivity, weight loss, decreased dose of insulin, reduction of oxidative stress, direct effects on vascular smooth muscle and anti-inflammatory effects associated with SGLT2i.[33] It has been established that the AP lability is ameliorated by the improvement in arterial stiffness, vascular resistance and vascular distensibility.[35,36] Although we did not assess the arterial stiffness and distensibility, we consider that the present results regarding the AP lability are mainly explained by the improvement in arterial distensibility following SGLT2i administration. Furthermore, in the present study, the sympathetic nerve activity was decreased during the active period in the SGLT2i group. Previous studies have indicated that SGLT2i agents do not activate sympathetic nerve activity despite inducing a depressor response and diuresis.[28] In patients with type 1 DM, SGLT2i agents did not alter the plasma noradrenalin and adrenalin concentrations.[27] However, those previous studies assessed sympathetic nerve activity for 24 h. We showed, in the present study, that the LFnuSAP was decreased only during the active period, not during the rest period, and that insulin did not decrease the LFnuSAP as expected. These effects would contribute

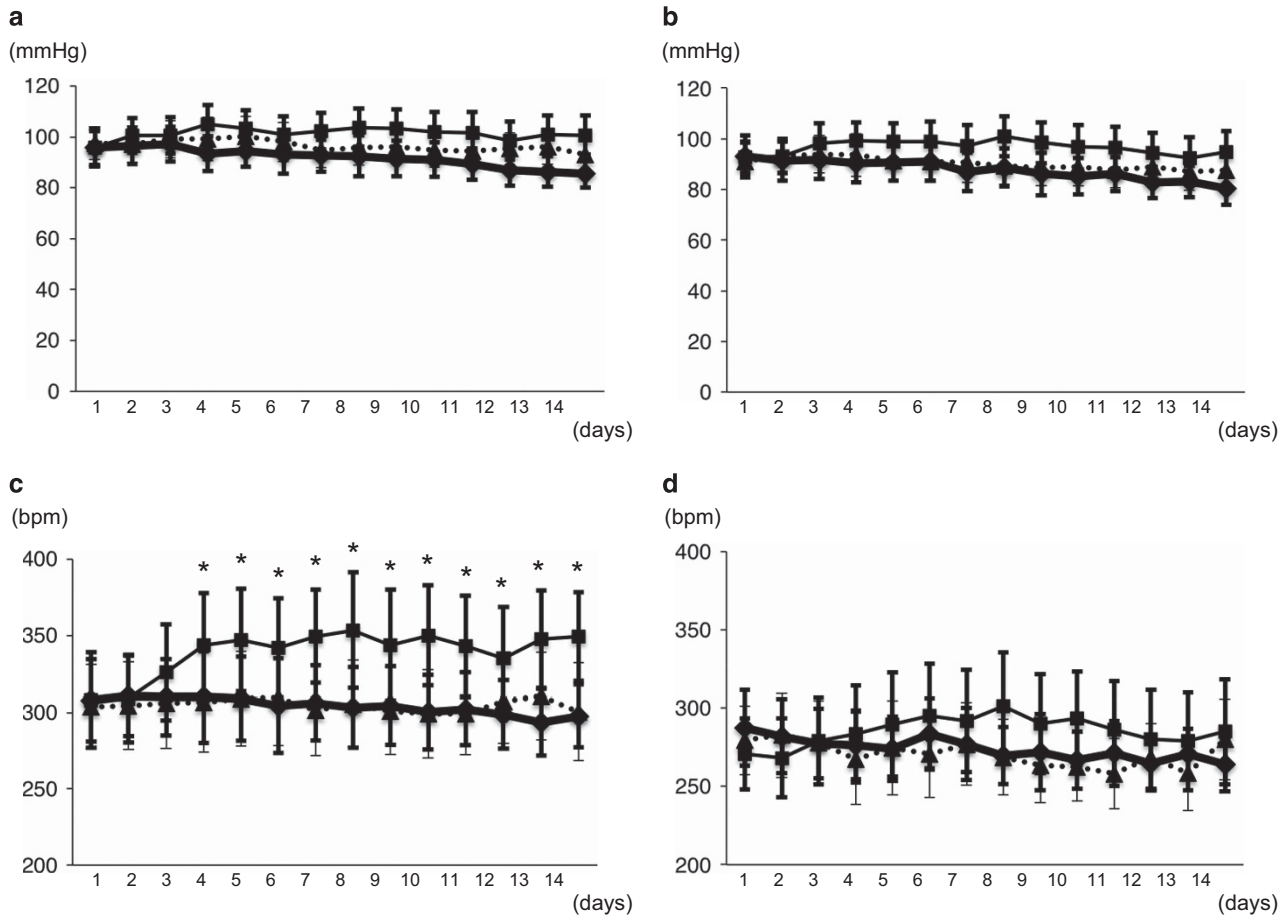

Figure 2 Serial changes in the mean arterial pressure (mAP) during the active period (**a**), mAP during the rest period (**b**), heart rate (HR) during the active period (**c**) and HR during the rest period (**d**) in each group ($n=5$ for each). Bold solid line, diabetic rats administered sodium-glucose cotransporter 2 inhibitor; solid line, diabetic rats treated with insulin; dotted line, diabetic rats administered vehicle. $^*P<0.05$ *vs.* vehicle.

Table 2 Arterial pressure and heart rate

	SGLT2i	*Insulin*	*VEH*
24-h mAP (mm Hg)	83±7	98±9	90±8
Rest (mm Hg)	80±6	95±8	88±7
Active (mm Hg)	86±6	101±8	93±7
24-h HR (b.p.m.)	281±25	317±46*	290±31
Rest (b.p.m.)	264±17	285±34	280±26
Active (b.p.m.)	297±20	349±29*	300±32

Abbreviations: HR, heart rate; mAP, mean arterial pressure; SGLT2i, sodium-glucose cotransporter 2 inhibitor; VEH, vehicle.
$N=5$ for each group, $^*P<0.05$ *vs.* VEH.

to the beneficial action of the SGLT2i in preventing cardiovascular events. Although the mechanisms by which SGLT2i decreases sympathetic nerve activity were not fully assessed in the present study, we successfully demonstrated that the sympathoinhibition was not due to the decrease in plasma glucose levels. We speculated that SGLT2i decreased sympathetic nerve activity by improving the renal–brain axis through the renal afferent nerve.[37,38] However, further examinations are clearly needed to confirm this speculation.

In addition, the BRS was also improved in the SGLT2i group. Because no study has examined the effect of SGLT2i on the baroreflex, our present results are of clinical importance. When the baroreflex function is impaired,[39] the BRS could predict cardiovascular events in patients with DM.[40] Previously, we have demonstrated that baroreflex failure strongly worsened the AP lability by inducing volume intolerance in normotensive rats.[30,41] The present results showed that SGLT2i improved the baroreflex, findings that are in agreement with the results of previous studies showing that SGLT2i agents successfully improved the AP circadian rhythm in DM rats.[25,42,43] Similar to the sympathoinhibition by SGLT2i, we could not show the mechanisms by which SGLT2i improved the baroreflex. However, we consider that baroreflex was mainly ameliorated by the SGLT2i-induced improvement in arterial stiffness and distensibility.[35,36] Moreover, in the central arc of the baroreflex from the AP to sympathetic nerve activity, we determined that oxidative stress, the inflammatory pathway or both associated with the renin–angiotensin system mainly damaged the central arc of the baroreflex and caused sympathoexcitation.[29,44] Considering these results, we speculated that SGLT2i indirectly ameliorated the activated central renin–angiotensin system and inflammation.

By contrast, the AP lability was not improved in the Insulin group compared with that in the SGLT2i group, although the Insulin group showed a greater improvement in the DM profile than in the SGLT2i group. It has been established that insulin causes sympathoexcitation and activation of the renin–angiotensin system by renal or central mechanisms or both.[29,45,46] Moreover, because insulin also activates sodium retention,[46] the AP lability could be worsened by insulin.

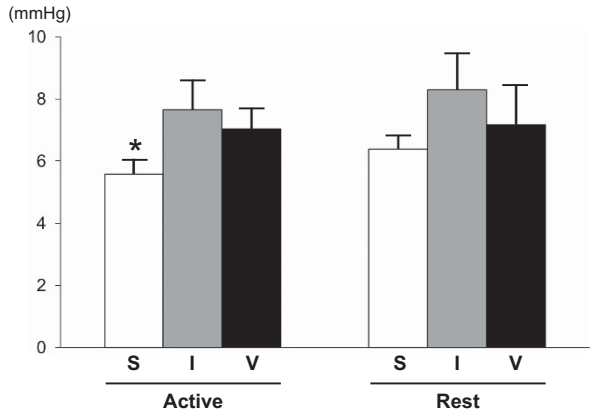

Figure 3 Arterial pressure lability in each group during the active or rest period ($n=5$ for each). S, sodium-glucose cotransporter 2 inhibitor; I, insulin; V, vehicle. $^*P<0.05$ *vs.* vehicle in each active or rest period.

Interestingly, SGLT2i agents have been reported to improve hyperinsulinemia.[47] The beneficial reduction of insulin by SGLT2i might, in part, ameliorate the AP lability in patients with DM.

In the present study, we used the SGLT2i at a low non-depressor dose because the s.d. of the AP is closely associated with the mean AP.[32] SGLT2i at a depressor dose could be expected to exhibit the beneficial effects on the AP, as shown in the present study. Therefore, the clinical benefit of the SGLT2i shown in the EMPA-REG OUTCOME trial[21] might have been partly due to the improvement in sympathetic nerve activity, baroreflex and AP lability.

There are several potential limitations in the present study. First, we performed basic experiments in type 1 DM model rats, whereas SGLT2i agents were used in patients with type 2 DM. The aim of the present study was to assess the effect of SGLT2i in normotensive DM model rats, and an ideal normotensive type 2 DM animal model is currently unavailable. However, the present beneficial effect of the SGLT2i may not be directly applicable to clinical practice and had no significant impact on the assessment of the benefits of SGLT2i. Second, we examined the effects of SGLT2i using only ipragliflozin at one low dose in the present study. Although we successfully showed that the SGLT2i at a non-depressor dose improved the AP lability independent of a depressor response, the dose-related or class-related effects of SGLT2i could not be assessed. The previous study using ipragliflozin at various doses (0.1–10 mg kg^{-1} per day) for 14 days (same as the present study) in STZ (50 mg kg^{-1}, same as the present study)-induced DM rats showed that ipragliflozin had dose-related antidiabetic benefits but not a dose-related depressor response.[31] In the clinical trial, SGLT2i did not have dose-related depressor responses.[21] Although we speculated that the present results would not have dose-related responses, further examinations are needed to confirm this speculation. Third, we performed the experiments for a relatively short duration (2 weeks) because of the tolerance of the telemetry device, although the most important targeted benefit of SGLT2i agents should be long-term survival without cardiovascular events. Fourth, we did not examine the histological changes in the brain, kidney, pancreas, lipid and other organs associated with the pathophysiology of DM. The histological assessments were not included in the aim of the present study because the usage of SGLT2i (1 mg kg^{-1} per day ipragliflozin for 14 days in 50 mg kg^{-1} STZ-induced DM rats) was already performed in a previous study that did not assess the histological changes.[31] Further long-term animal experiments with histological assessments should be conducted.

Table 3 Autonomic function

	SGLT2i	*Insulin*	*VEH*
LFnuSAP (%)	59±2	64±3	62±3
Rest (%)	57±2	62±4	60±2
Active (%)	61±2*	66±3	65±3
HFnuRRI (%)	49±2	45±3	47±4
Rest (%)	47±3	44±4	46±2
Active (%)	50±5	45±2	47±5
BRS (ms mm Hg^{-1})	1.91±0.13*	1.37±0.08	1.46±0.19
Rest (ms mm Hg^{-1})	1.78±0.12*	1.30±0.11	1.29±0.06
Active (ms mm Hg^{-1})	2.09±0.13*	1.49±0.07	1.66±0.26

Abbreviations: BRS, baroreflex sensitivity; HFnuRRI, normalized unit of the high-frequency component of R–R interval; LFnuSAP, normalized unit of the low-frequency component of systolic arterial pressure; SGLT2i, sodium-glucose cotransporter 2 inhibitor; VEH, vehicle. $N=5$ for each group. $^*P<0.05$ *vs.* VEH.

In conclusion, SGLT2i at a non-depressor dose ameliorated the AP lability associated with sympathoinhibition during the active phase and improved the baroreflex in DM rats. These effects occurred without excess body weight loss and sympathoexcitation. Although the present results were only from basic animal research, SGLT2i possibly improves the AP regulation in DM rats.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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