Acute and Direct Effects of Sodium–Glucose Cotransporter 2 Inhibition on Glomerular Filtration Rate in Spontaneously Diabetic Torii Fatty Rats

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Recent clinical studies indicate that sodium–glucose cotransporter 2 (SGLT2) inhibitors exhibit a renoprotective effect. While studies at the single nephron level suggest that direct effects of SGLT2 inhibitors on renal hemodynamics may be a possible mechanism underlying their renoprotective effect, few studies have focused on such direct effects at the whole-kidney level. In the present study, we investigated the acute and direct effect of SGLT2 inhibition on creatinine clearance, an index of whole-kidney glomerular filtration rate (GFR), in a rat model of type 2 diabetes. Twelve to fifteen-week-old male Spontaneously Diabetic Torii (SDT) fatty rats and Sprague-Dawley rats were used as diabetic animals and non-diabetic controls, respectively. Under general anesthesia, baseline urine samples were collected from the left and right ureters for 1 h. The selective SGLT2 inhibitor ipragliflozin or vehicle was subsequently administered intravenously and post-drug urine was collected for 1 h. Baseline and post-drug blood samples were collected immediately before baseline urine collection and immediately after post-drug urine collection, respectively. Plasma glucose, urine volume, urinary glucose and albumin excretion were measured, and creatinine clearance was calculated. Blood pressure and heart rate were monitored continuously throughout the experiment. A single intravenous injection of ipragliflozin increased both urine output and glucose excretion, but reduced creatinine clearance without affecting systemic blood pressure. These results suggest that SGLT2 inhibition directly reduced whole-kidney GFR, most likely due to a reduction in intraglomerular pressure, by altering local renal hemodynamics, which may contribute to the renoprotective effects demonstrated in clinical studies.

Key words type 2 diabetes mellitus; diabetic nephropathy; glomerular filtration rate; tubuloglomerular feedback; sodium–glucose cotransporter 2; ipragliflozin l-proline

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is strongly associated with both macrovascular and microvascular complications.1,2 Patients with microvascular complications show greater diabetes-related impairment of QOL than those without such complications.3 Diabetic nephropathy (DN) is a serious and progressive microvascular complication that can cause renal failure.4 Given that hyperglycemia and hypertension are the most prominent risk factors for DN,5 current standard renal protective therapies for T2DM have focused on controlling glycemia and blood pressure.4,5

Sodium–glucose cotransporter 2 (SGLT2) inhibitors were recently developed to manage glycemic control in T2DM patients.6 SGLT2 is a low-affinity and high-capacity SGLT that is primarily expressed in the early proximal tubules, where it acts to reabsorb approximately 90% of glucose in glomerular filtrates.7,8 Therefore, SGLT2 inhibition prevents this absorption and leads to substantial glucosuria, thereby reducing plasma glucose levels.9,10 Several recent non-clinical and clinical studies have reported the benefits of SGLT2 inhibitors for DN. Repeated administration of SGLT2 inhibitors decreased glomerular hyperfiltration11,12 and delayed progression of DN in various animal models of diabetes.13–19 Large clinical studies have demonstrated that the SGLT2 inhibitor empagliflozin reduces albuminuria, a marker of glomerular damage, in patients with T2DM and chronic kidney disease,20 and that adding empagliflozin to standard care slows the progression of kidney disease in patients with T2DM and mild-to-moderate renal insufficiency.21 Although the improvement in glycemic control by SGLT2 inhibitors is essential for preventing the progression of DN, the blood pressure-lowering effects of these compounds may also contribute to slowing the disease progression.22 A recent study reported renal hemodynamic effects as an additional mechanism of action of SGLT2 inhibition in patients with T1DM.23 In an experimental study, Thomson et al. demonstrated that the SGLT2 inhibitor dapagliflozin acutely reduced proximal reabsorption of glucose and sodium. This led to a significant increase in early distal chloride levels, a saturated tubuloglomerular feedback (TGF) response, and a marked reduction in the single nephron glomerular filtration rate (SNGFR) in streptozotocin-induced diabetic rats, an animal model of type 1 diabetes (T1DM), using a micropuncture technique.24 Recently, Kidokoro et al. demonstrated that acute inhibition of SGLT2 led to constriction of afferent glomerular arterioles in a mouse model of T1DM using an in vivo multiphoton microscope imaging technique,11 suggesting that the acute inhibition of SGLT2 reduces SNGFR via a TGF response. While a reduction in SNGFR should reduce whole-kidney GFR, to our knowledge, few studies have examined such direct effects at the whole-kidney level.

Here, we investigated the acute and direct effects of the SGLT2 inhibitor ipragliflozin25–27 on creatinine clearance, an index of whole-kidney GFR, in Spontaneously Diabetic Torii (SDT) fatty rats, an animal model of T2DM, which is characterized by hyperglycemia, hypertension, and early develop-
ment of diabetes-associated complications such as nephropathy.28-31 Ipragliflozin has been shown to slow the development of DN in SDT fatty rats,19 and to delay the progression of overt DN in other T2DM animal models.17,18

MATERIALS AND METHODS

Compounds Ipragliflozin L-proline [(1S)-1, 5-anhydro-1-C-[3-[(1-benzothiophen-2-yl)methyl]-4-fluorophenyl]-α-glucitol compound with L-proline (1:1)] (PubChem CID: 57339444) was synthesized at Astellas Pharma Inc. (Tsukuba, Japan). The compound was dissolved in 5% hydroxypropyl-β-cyclodextrin (HP-β-CD) in saline to prepare a 0.6 mg/mL ipragliflozin solution before use. The ipragliflozin dose is expressed as a proline-free substance.

Animals and Experimental Protocol All animal experimental procedures were approved by the Institutional Animal Care and Use Committee of the Tsukuba Research Center of Astellas Pharma Inc., which is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International. All animal experiments were conducted in the Hashima Laboratories of Nihon Bioresearch Inc., which is accredited by the Center for Accreditation of Laboratory Animal Care and Use, Japan Health Sciences Foundation. Ten- to twelve-week-old male Jcl: SD (Sprague-Dawley (SD) rats) and SDT.Cg-Leprfa/JttJcl rats (SDT fatty rats) were purchased from CLEA Japan (Tokyo, Japan). Rats were housed in plastic cages with floors comprising autoclaved wood chips in a temperature- and humidity-controlled room with a 12-hour light and dark cycle (lights on 6:00–18:00). Water and standard laboratory chow were available ad libitum. The animals were acclimatized to the laboratory conditions for more than 12d. SD rats were allocated to the non-diabetic control group. SDT fatty rats were allocated to either the diabetic control group or ipragliflozin-treated group according to body weight and non-fasting plasma glucose levels measured the morning of the experiment.

One animal at 12 to 15 weeks old was used per day for the study. SD and SDT fatty rats were anesthetized with intraperitoneal injections of thiobutabarbital sodium at 100 and 110 mg/mL/kg, respectively. Under complete anesthesia, a polyethylene cannula was inserted into the trachea. Polyethylene catheters (Intramedic PE-50, Becton & Dickinson Japan, Tokyo, Japan) filled with physiological saline containing 20 units/mL of sodium heparin were inserted into the femoral artery of the right hind limb and the femoral veins of the right and left hind limbs. A polyethylene catheter (Intramedic PE-10, Becton & Dickinson Japan) was inserted into the left and right ureters of each animal. The polyethylene catheter inserted into the femoral artery was connected to a pressure transducer (TP-300T, Nihon Kohden Corporation, Tokyo, Japan), and the blood pressure waveform was amplified using a strain pressure amplifier (AP-601G, Nihon Kohden Corporation) and recorded using a recording/analysis system (PowerLab, ADInstruments, Nagoya, Japan). The body temperature of the animals was maintained at 36.5°C throughout the experiment using a thermal insulation pad (Animal Blanket Controller ATB-1100, Nihon Kohden Corporation).

A schematic representation of the experimental protocol is shown in Fig. 1. With the aim of maintaining urine output, physiological saline was continuously infused at 100 µL/kg/min (6 mL/kg/hr) using a syringe pump into the left femoral vein throughout the experiment. After confirming that the mean blood pressure and urine output had stabilized, 0.3 mL of blood (baseline) was collected from the right femoral vein, and a urine sample was collected from the left and right ureters for 1 h (baseline). Subsequently, 5% HP-β-CD in saline or ipragliflozin solution (0.3 mg/kg) was intravenously administered at 0.5 mL/kg. Plasma concentrations of ipragliflozin measured 15, 30 and 60 min after intravenous administration at 0.3 mg/kg were mostly comparable to concentrations obtained after oral administration at 1 mg/kg in SD rats (data not shown), a dose that showed anti-hyperglycemic effects in various diabetic animal models.19,26,27 Urine sample collection was resumed immediately after 5% HP-β-CD or ipragliflozin administration. One hour after administration, 1-h urine samples and 0.3 mL of blood were collected (post-drug). Blood samples were collected into a lithium heparin-treated blood collection tube. Plasma was separated from blood samples for the measurement of glucose, creatinine, and electrolyte levels. Mean, systolic and diastolic blood pressure (MBP, SBP and DBP, respectively), and heart rate were measured for 6 min at two time points, immediately before drug administration (baseline) and after the post-drug urine collection, and averaged.

Blood Chemical Analysis and Urinalysis Plasma levels of glucose, creatinine, and electrolytes were measured using an automatic biochemical analyzer (AU 480, Beckman Coulter Inc., Tokyo, Japan). Urine volume was measured using a microsyringe. Urinary glucose and creatinine levels, and electrolyte levels were measured using an automatic biochemical analyzer (AU 480, Beckman Coulter Inc.) and an automatic electrolyte analyzer (EA07, A&T Corporation, Yokohama, Japan), respectively. Urinary albumin levels were determined.
using a rat albumin enzyme-linked immunosorbent assay (ELISA) kit (AKRAL-120, Shibayagi Co., Ltd., Shibukawa, Gunma, Japan) and the albumin to creatinine ratio (ACR) was calculated. Creatinine clearance (Ccr) was calculated from plasma creatinine, urinary creatinine excretion, and body weight using the following formula: 

\[
Ccr = \frac{(\text{Creatinine concentration in 1 h urine (mg/dL)} \times 1 \text{ h total urine volume (mL/h)})}{(\text{Plasma creatinine at the respective blood sampling point (mg/dL)} \times \text{body weight (kg)})}
\]

**Statistical Analysis**

The results are expressed as

### Table 1. Blood Pressure Levels and Heart Rate

<table>
<thead>
<tr>
<th>Group</th>
<th>MBP (mm Hg)</th>
<th>SBP (mm Hg)</th>
<th>DBP (mm Hg)</th>
<th>HR (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-diabetic control</td>
<td>102 ± 4</td>
<td>131 ± 4</td>
<td>88 ± 4</td>
<td>355 ± 12</td>
</tr>
<tr>
<td>Post-drug</td>
<td>95 ± 4</td>
<td>124 ± 4</td>
<td>81 ± 4</td>
<td>352 ± 9</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>126 ± 7</td>
<td>161 ± 7</td>
<td>108 ± 7</td>
<td>357 ± 9</td>
</tr>
<tr>
<td>Post-drug</td>
<td>114 ± 9</td>
<td>149 ± 10</td>
<td>97 ± 9</td>
<td>363 ± 5</td>
</tr>
<tr>
<td>Ipragliflozin</td>
<td>134 ± 4</td>
<td>168 ± 4</td>
<td>117 ± 3</td>
<td>341 ± 9</td>
</tr>
<tr>
<td>Post-drug</td>
<td>128 ± 4</td>
<td>160 ± 5</td>
<td>112 ± 4</td>
<td>347 ± 11</td>
</tr>
</tbody>
</table>

Non-diabetic control, non-diabetic control group; Diabetic control, diabetic control group; Ipragliflozin, ipragliflozin-treated diabetic group. MBP, mean blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate. Baseline, before vehicle or ipragliflozin administration; Post-drug, immediately after the post-drug urine collection. Vehicle or ipragliflozin (0.3 mg/kg) was administered intravenously. MBP, SBP, DBP, and HR were measured for 6 min and averaged. Mean ± S.E.M., n = 6. 

\( ^p < 0.05 \) and \( ^{##}p < 0.01 \), compared to the non-diabetic control group at the respective time point.

### Table 2. Plasma Glucose, Sodium, Chloride and Creatinine Levels

<table>
<thead>
<tr>
<th>Group</th>
<th>Glucose (mg/dL)</th>
<th>Sodium (mEq/L)</th>
<th>Chloride (mEq/L)</th>
<th>Creatinine (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-diabetic control</td>
<td>109.8 ± 5.9</td>
<td>148.9 ± 0.8</td>
<td>123.3 ± 1.5</td>
<td>0.33 ± 0.03</td>
</tr>
<tr>
<td>Post-drug</td>
<td>97.1 ± 5.5</td>
<td>149.6 ± 1.3</td>
<td>124.8 ± 2.4</td>
<td>0.31 ± 0.03</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>572.5 ± 21.8</td>
<td>147.1 ± 1.5</td>
<td>121.5 ± 1.6</td>
<td>0.27 ± 0.01</td>
</tr>
<tr>
<td>Post-drug</td>
<td>572.2 ± 28.2</td>
<td>147.0 ± 2.2</td>
<td>120.4 ± 2.9</td>
<td>0.27 ± 0.03</td>
</tr>
<tr>
<td>Ipragliflozin</td>
<td>542.7 ± 19.3</td>
<td>146.0 ± 1.4</td>
<td>120.0 ± 2.5</td>
<td>0.23 ± 0.02</td>
</tr>
<tr>
<td>Post-drug</td>
<td>450.4 ± 15.1</td>
<td>150.3 ± 1.1</td>
<td>121.8 ± 1.6</td>
<td>0.29 ± 0.01**</td>
</tr>
</tbody>
</table>

Non-diabetic control, non-diabetic control group; Diabetic control, diabetic control group; Ipragliflozin, ipragliflozin-treated diabetic group. Vehicle or ipragliflozin (0.3 mg/kg) was administered intravenously. Blood samples were collected 1 h prior to (baseline) and 1 h after vehicle or ipragliflozin administration (post-drug). Mean ± S.E.M., n = 6. 

\( ^p < 0.05 \) and \( ^{##}p < 0.01 \), compared to the non-diabetic control group; \( ^{##}p < 0.01 \), compared to the diabetic control group at the respective time point. **\( p < 0.01 \), compared to baseline.

Fig. 2. Urine Volume (A), Urinary Glucose Excretion (B), Albumin to Creatinine Ratio (ACR) (C), and Fractional Excretion of Sodium (FE Na) (D)

Non-diabetic control, non-diabetic control group; Diabetic control, diabetic control group; Ipragliflozin, ipragliflozin-treated diabetic group. Vehicle or ipragliflozin (0.3 mg/kg) was administered intravenously. Urine samples were collected for 1 h prior to (baseline) and for 1 h after vehicle or ipragliflozin administration (post-drug). A single intravenous administration of ipragliflozin increased both urine volume and urinary glucose excretion by 2-fold. The post-drug ACR decreased by 5% and 17% in the diabetic control group and ipragliflozin group, respectively, compared to baseline. Mean ± S.E.M., n = 6. 

\(^p = 0.061\), compared to the diabetic control group; \(^{##}p < 0.01\), compared to the non-diabetic control group; \(^{##}p < 0.01\), compared to the diabetic control group at the respective time point.
mean ± standard error of the mean (S.E.M.). A Student’s t-test was used to analyze differences at each of the two time points (baseline and post-drug). A paired t-test was used to compare plasma creatinine and Ccr values between baseline and post-drug time points as a secondary analysis. p < 0.05 was considered significant. Statistical analyses were conducted using SAS system (SAS Institute Japan, Tokyo, Japan).

RESULTS

Blood Pressure and Heart Rate Table 1 shows the blood pressure and heart rate measured before drug administration (baseline) and immediately after completion of post-drug urine collection (post-drug). At baseline, mean values of MBP, SBP and DBP were significantly higher in the diabetic control group than in the non-diabetic control group. Intravenous administration of ipragliflozin did not affect these blood pressure parameters. Heart rate at both time points was comparable among the three groups. The blood pressure and heart rate of all rats were approximately stable throughout the experiment.

Plasma Glucose, Sodium, Chloride and Creatinine Levels Table 2 shows the measured plasma glucose, sodium, chloride and creatinine levels 1h prior to drug administration (baseline) and 1h after drug administration (post-drug). The mean plasma glucose concentration at baseline was significantly higher in the diabetic control group than in the non-diabetic control group, and was maintained at approximately the same level throughout the study. The mean plasma glucose level was significantly reduced 1h after ipragliflozin administration (post-drug) compared to the diabetic control group. In the ipragliflozin-treated group, the mean post-drug plasma creatinine level was higher than that at baseline. Both plasma sodium and chloride levels were almost identical among the groups at both time points.

Urinalysis Figure 2 shows the urine volume, excreted urinary glucose, and albumin to creatinine ratio (ACR). At baseline, the urine volume (Fig. 2A), excreted glucose (Fig. 2B) and ACR (Fig. 2C) were significantly higher in the diabetic control group than in the non-diabetic control group (Fig. 2D). In contrast, the ACR during the post-drug period was lower in the ipragliflozin group than in the diabetic control group, but these differences were not statistically significant (Fig. 2C). Fractional excretion of sodium (FENa) values in all groups were comparable at both time points (Fig. 2D).

Figures 3A, B, and C show the individual Ccr values at baseline and post-drug time points in the non-diabetic control, diabetic control and ipragliflozin groups, respectively. Mean baseline Ccr was lower in the diabetic control than in the non-diabetic control group. Treatment with ipragliflozin reduced the Ccr in all individual rats. Figure 3D shows the differences in Ccr between baseline and post-drug time points. There was a significant reduction in Ccr in the ipragliflozin-treated group compared to the diabetic control group.
DISCUSSION

We investigated changes in Ccr in response to acute intravenous administration of the selective SGLT2 inhibitor ipragliflozin in SDT fatty rats, a model of T2DM that develops DN. A single intravenous injection of ipragliflozin increased both urine output and glucose excretion as expected, but reduced Ccr without affecting systemic blood pressure.

Given that acute inhibition of SGLT2 likely reduces SNGFR via constriction of afferent glomerular arterioles in rodent models of T1DM, we hypothesized that single administration of an SGLT2 inhibitor may also reduce whole-kidney GFR in an animal model of T2DM. We used ipragliflozin as a selective SGLT2 inhibitor because it prevents the progression of DN in various animal models of T2DM including SDT fatty rats following multiple oral dosing. In the present study, the acute and direct effects of SGLT2 inhibition on whole-kidney GFR were examined following intravenous administration of ipragliflozin. The dose of ipragliflozin used in the present study was based on plasma concentrations measured after an oral dose in SD rats that showed anti-hyperglycemic effects in various diabetes animal models. Further, the blood pressure and heart rate of each rat were continuously monitored throughout the experiment.

We demonstrated that ipragliflozin substantially inhibited SGLT2, indicated by a 2-fold increase in urinary glucose excretion, but reduced Ccr, an indicator of whole-kidney GFR, while the blood pressure and heart rate of each rat remained mostly stable. These findings suggest that SGLT2 inhibition directly altered local renal hemodynamics and reduced intraglomerular pressure, likely by affecting the TGF response. That is, inhibition of SGLT2 blocked glucose and sodium reabsorption at the proximal tubule, which resulted in increased distal sodium delivery to the macula densa. This consequently affected arterial tone (e.g. afferent arteriole constriction), which in turn reduced glomerular blood flow and subsequently reduced intraglomerular pressure. We measured FE Na as well as FE Cl (data not shown) but found no significant differences in either parameter among the groups. Urine sodium and chloride levels may not reflect changes in electrolyte levels at the proximal tubules, possibly due to compensatory reabsorption mechanisms in the loop of Henle, distal tubules and collecting duct.

Clinical studies have shown that estimated glomerular filtration rate (eGFR) is significantly reduced soon after initiation of treatment with SGLT2 inhibitors in patients with T2DM with moderate renal impairment. Importantly, this reduced eGFR is reversed within 3 weeks after discontinuation of treatment with an SGLT2 inhibitor, suggesting that the reduction in eGFR is a consequence of SGLT2 inhibition. The reduction in whole-kidney GFR following a single intravenous administration of ipragliflozin in the present study might explain, at least in part, the early reduction in eGFR observed in clinical studies. In addition, ipragliflozin tended to decrease the ACR compared to diabetic control rats (p < 0.06). A reduction in the ACR, as well as a mild decrease in eGFR, following treatment with the SGLT2 inhibitor empagliflozin was also observed in patients with T2DM, which was not explained by SGLT2 inhibition-related decreases in hemoglobin A1c, SBP or body weight. Such alterations in ACR, as with the reduction in Ccr, may also imply the reduction in intraglomerular pressure as a consequence of the local renal hemodynamic effects of SGLT2 inhibition.

The present study clearly demonstrated the acute and direct effects of the selective SGLT2 inhibitor ipragliflozin on whole-kidney GFR in SDT fatty rats, an animal model of T2DM. These findings are comparable to those of a previous report, which demonstrated an acute reduction in SNGFR following SGLT2 blockade in an animal model of T1DM. Further, these effects may contribute considerably to the renoprotective effects of SGLT2 inhibition observed in SDT fatty rats.

However, several limitations of the study warrant mention. First, to calculate Ccr, we used post-drug 1-hour total urinary creatinine excretion and the plasma creatinine concentration obtained after completion of urine collection. As plasma creatinine levels were assumed to gradually increase after administration of ipragliflozin, post-drug Ccr may have been underestimated. Second, Ccr is a rough estimate of GFR. Creatinine is known to be produced in muscles at a fairly constant rate, and reabsorbed and secreted at the renal tubules. Given that we showed that treatment with ipragliflozin increased serum creatinine levels, it may be important to determine whether ipragliflozin increases the creatinine production rate in muscles and affects the tubular reabsorption and/or secretion of creatinine. More precise measurements of GFR, such as inulin clearance, should be performed to confirm our findings and interpretations. Third, we did not evaluate the effect of glycemic control on Ccr. Our results showed that ipragliflozin acutely reduced Ccr and decreased plasma glucose levels. Future studies should examine the acute effect of insulin on Ccr in the same rat strain to determine whether the acute glycemic control affects Ccr. Further studies are also needed to clarify the precise mechanism of action of SGLT2 inhibition on local renal hemodynamics to elucidate its clinical value outside of its anti-hyperglycemic effect.

In conclusion, a single intravenous administration of the SGLT2 inhibitor ipragliflozin acutely reduced Ccr, an index of GFR, in SDT fatty rats, a model of T2DM. This finding suggests that treatment with an SGLT2 inhibitor directly reduces the intraglomerular pressure in T2DM patients. Such glomerular hemodynamic effects of SGLT2 inhibitors, in cooperation with their anti-hyperglycemic effect, may contribute to preventing the progression of DN in patients with T2DM.

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Conflict of Interest S. Takakura and T. Takasu are employees of Astellas Pharma Inc. The study was funded by Astellas Pharma Inc.

REFERENCES


