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Prevention of progression of diabetic nephropathy by the SGLT2 inhibitor ipragliflozin in uninephrectomized type 2 diabetic mice

Atsuo Tahara*, Toshiyuki Takasu

Candidate Discovery Science Laboratories, Astellas Pharma Inc., Ibaraki, Japan

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ABSTRACT

Diabetic nephropathy is the leading cause of end-stage renal disease in the world. Although recent development of sodium-glucose cotransporter (SGLT) 2 inhibitors offers a new antidiabetic therapeutic strategy, it remains unclear whether such treatments are beneficial for limiting the progression of type 2 diabetic overt nephropathy. This study examined the effect of the SGLT2 inhibitor ipragliflozin on the progression of nephropathy in uninephrectomized KK/A^{y} type 2 diabetic mice, which exhibit not only typical diabetic symptoms such as hyperglycemia, hyperinsuemia, glucose intolerance, insulin resistance, hyperlipidemia, inflammation, and obesity, but also moderate hypertension and overt nephropathy with decline in renal function. Four-week repeated administration of ipragliflozin improved various diabetic symptoms, including hyperglycemia, insulin resistance, and inflammation by increasing urinary glucose excretion. In addition, ipragliflozin ameliorated albuminuria/proteinuria; decline in renal function, as measured by creatinine clearance; hypertension; and renal injury, including glomerulosclerosis and interstitial fibrosis. These effects were significant at doses of 1 mg/kg or higher and were similar to those observed following administration of losartan (30 mg/kg). These results suggest that the SGLT2 inhibitor ipragliflozin prevents progression to diabetic overt nephropathy in uninephrectomized type 2 diabetic mice. SGLT2 inhibitors may therefore represent a promising therapeutic option for the management of type 2 diabetes to slow the progression of diabetic nephropathy.

1. Introduction

Diabetic nephropathy is one of the most common complications of diabetes and the leading cause of end-stage renal disease. It is also associated with high cardiovascular risk and significant morbidity and mortality worldwide (Pálsson and Patel, 2014). In the past, several mechanisms have been implicated in the initiation and deterioration of diabetic nephropathy, including hyperglycemia, hypertension, dyslipidemia, and obesity, as well as ethnic and genetic factors (Tavafi, 2013). Mounting evidence suggests that proper control of blood glucose and blood pressure can reduce the risk of developing this complication. While current antidiabetic therapies to control blood glucose and administration of renin-angiotensin-aldosterone system (RAAS) inhibitors, angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor antagonists (ARBs) to control blood pressure can slow the progression of diabetic nephropathy, they do not sufficiently prevent it (Fioretto et al., 2010). Therefore, there is a strong need to explore additional therapies for limiting the progression of diabetic nephropathy.

In recent years, sodium-glucose cotransporter (SGLT) 2 inhibitors, which improve hyperglycemia via the stimulation of glucose excretion into the urine, have been proposed as novel drugs for treating type 2 diabetes (Chao, 2014). As chronic hyperglycemia is thought to contribute to the progression of diabetic nephropathy, the potent antihyperglycemic effects of SGLT2 inhibition may be promising for treating diabetic nephropathy. Several SGLT2 inhibitors including ipragliflozin have been shown to reduce urinary albumin excretion in type 2 diabetic animals with incipient nephropathy, which, at least in part, was attributed to inflammation, oxidative stress, and insulin resistance (Škrtić and Cherney, 2015; Tahara et al., 2013). However, the potential effects of SGLT2 inhibitors on the progression of overt nephropathy in type 2 diabetic animal models have not been determined in detail.

Here, we investigated the effects of the SGLT2 inhibitor ipragliflozin on the progression of nephropathy in uninephrectomized type 2 diabetic mice, which exhibit not only typical diabetic symptoms such as hyperglycemia, insulin resistance, hyperlipidemia, hepatic steatosis, inflammation and obesity, but also moderate hypertension and overt nephropathy with decline in renal function. In addition, we compared the effects of ipragliflozin with those of the ARB losartan.

* Correspondence to: Candidate Discovery Science Laboratories, Astellas Pharma Inc., 21 Miyukigaoka, Tsukuba, Ibaraki 305-8585, Japan.
E-mail address: atsuo.tahara@jp.astellas.com (A. Tahara).

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2. Materials and methods

2.1. Materials

Ipragliflozin was synthesized at Astellas Pharma Inc. (Ibaraki, Japan) and losartan was purchased from LKT Laboratories, Inc. (St. Paul, MN, USA). These drugs were suspended or dissolved in 0.5% methylcellulose solution and administered orally via a stomach tube. Doses of these drugs were expressed as the free base form.

2.2. Animals

Male C57BL/6 (normal) and KK/A^{y} type 2 diabetic mice were purchased from CLEA Japan (Kanagawa, Japan) at age 6 weeks and used at age 14 weeks. The left kidney of diabetic mice was removed under isoflurane anesthesia. Two weeks after uninephrectomy, diabetic mice were grouped such as to attain uniform mean blood glucose levels among the groups. All animals were housed under standard conditions of controlled temperature, humidity, and light (12-h light-dark cycle), and were given free access to standard commercial chow and water. All animal experimental procedures were approved by the Institutional Animal Care and Use Committee of Astellas Pharma Inc. Astellas Pharma Inc., Tsukuba Research Center has been awarded Accreditation Status by the AAALAC International.

2.3. Repeated administration study

Groups comprised the following: (1) normal mice: normal (vehicle); (2) non-nephrectomized (non-Nx) diabetic mice: control (vehicle); (3) nephrectomized (Nx) diabetic mice: (i) vehicle, (ii) ipragliflozin (0.1 mg/kg), (iii) ipragliflozin (0.3 mg/kg), (iv) ipragliflozin (1 mg/kg), (v) ipragliflozin (3 mg/kg), (vi) losartan (30 mg/kg). Six mice were used in each group/subgroup. To measure pretreatment creatinine clearance values, mice were transferred to metabolic cages where spontaneously voided urine was collected for 24 h, and blood samples were obtained from the tail vein. Subsequently, each drug was orally administered to Nx diabetic mice once daily (at night) for 4 weeks. Body weight and food intake were measured weekly. An oral glucose tolerance test (OGTT) was performed at Week 3. Mice were fasted over the inactive period (from 7:00–19:00) prior to drug administration. Two hours later, blood was sampled from a tail vein for the evaluation of fasting blood glucose and plasma insulin levels. A glucose solution (2 g/kg) was then orally administered, and blood sampling was conducted for 2 h. After drug administration on day 25, spontaneously voided urine was collected for 24 h. The morning after the final drug administration on day 28, blood samples were collected from the abdominal vena cava and tissues (kidneys, pancreas, liver, and epididymal adipose tissue) were isolated under isoflurane anesthesia. To measure blood pressure, a second set of animals was subjected to the same group composition and drug administration schedule. The morning after the final drug administration on day 29, systolic blood pressure and heart rate were measured using a photoelectric tail cuff pulse detection system (BP-98A-L; Softron, Tokyo, Japan).

2.4. Biochemical measurements

Blood and urinary glucose concentrations were measured using Glucose CII test reagent (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Hemoglobin A_{1c} (HbA_{1c}) levels were measured using a DCA2000 System (Bayer Medical, Tokyo, Japan). Plasma insulin levels were measured using an ultra-high-sensitivity mouse insulin enzyme-linked immunosorbent assay (ELISA) kit (Morinaga Institute of Biological Science, Inc., Kanagawa, Japan). Pancreatic insulin content was measured using an insulin ELISA kit according to a previously reported method (Tahara et al., 2013). Plasma lipid [triglycerides, non-esterified fatty acids (NEFAs), and cholesterol] levels were measured

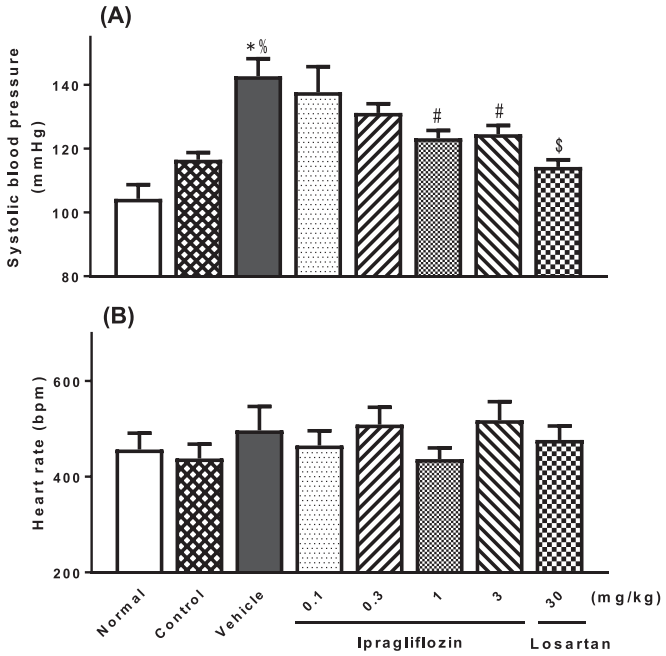

Fig. 1. Effects of 4-week intervention with ipragliflozin or losartan on (A) blood pressure and (B) heart rate in uninephrectomized type 2 diabetic mice. Ipragliflozin and losartan were orally administered to uninephrectomized (Nx) diabetic mice once daily for 4 weeks. Data are expressed as the mean ± S.E.M. for six animals in each group. $^{*}P < 0.05$ vs. normal group, $^{\%}P < 0.05$ vs. non-Nx diabetic (control) group, $^{\#}P < 0.05$ vs. Nx diabetic vehicle group, $^{\$}P < 0.05$ vs. Nx diabetic vehicle group.

using the Triglyceride E-test Wako, NEFA C-test Wako, and Cholesterol E-test Wako kits (Wako Pure Chemical Industries, Ltd., Osaka, Japan), respectively. Hepatic lipid (triglyceride and cholesterol) content levels were measured according to a previously reported method (Tahara et al., 2011). Levels of aminotransferases, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured using the Transaminase CII test reagent (Wako Pure Chemical Industries, Ltd.). Plasma levels of leptin, fibroblast growth factor 21 (FGF-21), adiponectin, interleukin (IL) − 1β, IL-6, tumor necrosis factor α (TNF-α), monocyte chemotactic protein-1 (MCP-1), and C-reactive protein (CRP) were measured using commercial ELISA kits (R&D Systems Inc., Minneapolis, MN, USA). Urinary albumin and protein excretion were measured using mouse albumin ELISA and a protein assay reagent (Bio-Rad, Hercules, CA, USA), respectively. Plasma and urinary creatinine levels were measured using Determiner L CRE (Kyowa Medex, Tokyo, Japan), and creatinine clearance (μl/g body weight/min) was calculated based on urinary and plasma creatinine levels, urine volume, and body weight. Urinary concentrations of nephrin and podocalyxin were measured using ELISA kits (Exocell Inc., PA, USA). Urinary concentrations of kidney injury molecule-1 (KIM-1) were measured using an ELISA kit (R&D Systems Inc.). Urinary N-acetyl-β-D-glucosaminidase (NAG) activity was measured using NAG Test Shionogi (Shionogi Co. Ltd, Osaka, Japan).

2.5. Histopathology

Specimen preparation and histopathological examination were performed at CMIC Bioresearch Center Co., Ltd. (Yamanashi, Japan). Sagittal slices of renal tissue were fixed in 10% neutral buffered formalin, embedded in paraffin, and cut into 2-μm thick sections for morphological study. These sections were stained with hematoxylin and eosin, and periodic acid Schiff. All tissue samples were evaluated by an independent investigator blinded to group information. All glomeruli and the entire microscopic area in each specimen were examined.