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Full paper

Characterization and comparison of sodium-glucose cotransporter 2 inhibitors: Part 2. Antidiabetic effects in type 2 diabetic mice

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ABSTRACT

Previously we investigated the pharmacokinetic, pharmacodynamic, and pharmacologic properties of all six sodium-glucose cotransporter (SGLT) 2 inhibitors commercially available in Japan using normal and diabetic mice. We classified the SGLT2 inhibitors with respect to duration of action as either long-acting (ipragliflozin and dapagliflozin) or intermediate-acting (tofogliflozin, canagliflozin, empagliflozin, and luseogliflozin). In the present study, antidiabetic effects of repeated administration of these SGLT2 inhibitors in type 2 diabetic mice were investigated. When repeatedly administered for 4 weeks, all SGLT2 inhibitors significantly exhibited antihyperglycemic, antihyperinsulinemic, and pancreas-protective effects, as well as insulin resistance-improving effects. When compared at doses producing comparable reduction in hyperglycemia across all drugs, the antidiabetic effects of ipragliflozin and dapagliflozin were more potent than those of the other four drugs, but these differences among the six drugs were not statistically significant. Further, an oral glucose tolerance test performed after repeated administration demonstrated significant improvement in glucose tolerance only with ipragliflozin and dapagliflozin, implying improved insulin resistance and secretion. Taken together, these findings demonstrate that, although all SGLT2 inhibitors exert antidiabetic effects in type 2 diabetic mice, these pharmacologic effects might be slightly superior with the long-acting drugs, which are able to provide favorable blood glucose control throughout the day.

1. Introduction

Type 2 diabetes is characterized by hyperglycemia and relative insulin deficiency as a result of impaired insulin secretion from pancreatic β-cells or insulin resistance, and its incidence has increased dramatically due to increasing prevalence of obesity and physical inactivity (1,2). In addition, substantial evidence suggests that chronic hyperglycemia alone can directly impair both insulin secretion and sensitivity, a phenomenon known as “glucose toxicity” and which contributes to the progressive worsening of hyperglycemia (3). Effective glycemic control is therefore important to prevent both the onset of diabetes and the progressive deterioration of the diabetic disease state. However, while a number of antidiabetic drugs are available, maintaining good long-term glycemic control remains difficult in most type 2 diabetic patients, even when used in combination (4), highlighting the need for efficient new therapeutic strategies for treating type 2 diabetes.

In recent years, sodium-glucose cotransporter (SGLT) 2 inhibitors, which can inhibit reabsorption of filtered glucose in the kidney and increase urinary glucose excretion, have been developed and proposed as novel antihyperglycemic agents for treating type 2 diabetes (5), with several shown to improve hyperglycemia in this patient population (6). However, while many studies have focused on nonclinical and clinical pharmacologic effects of SGLT2 inhibitors (7,8), most have examined these drugs on an individual basis, with only one study comparing several SGLT2 inhibitors in terms of *in vitro* inhibitory activity and selectivity for SGLT2 (9) and none comparing their *in vivo* pharmacologic effects. In our previous experiment, we investigated and compared the pharmacokinetic, pharmacodynamic, and basic pharmacologic properties of all six SGLT2 inhibitors commercially available in Japan using normal and diabetic mice (10). We then classified the SGLT2 inhibitors with

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respect to duration of action as either long-acting (ipragliflozin and dapagliflozin) or intermediate-acting (tofogliflozin, canagliflozin, empagliflozin, and luseogliflozin). That study examined single dosing under free-feeding conditions. In contrast, the present study was planned to investigate the antidiabetic effects of these SGLT2 inhibitors in type 2 diabetic mice after repeated dosing under restricted feeding conditions based on these basic data, as well as parameters including not only hyperglycemia but also pancreatic insulin content and glucose tolerance.

2. Materials and methods

2.1. Materials

Ipragliflozin (11), dapagliflozin (12), tofogliflozin (13), canagliflozin (14), empagliflozin (9), and luseogliflozin (15) were synthesized at Astellas Pharma Inc. (Ibaraki, Japan) and suspended in 0.5% methylcellulose solution for oral administration. Doses of drugs are 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 at age 6 weeks (Kanagawa, Japan) and uniformly grouped by blood glucose levels at age 7 weeks. All animals were housed under conventional conditions with controlled temperature, humidity, and light (12-h light–dark cycle) and were provided with standard commercial diet and water. Starting from the day after grouping, animals received feed only during the active period (i.e. fed for 14-h from 19:00 to 9:00, and fasted for the rest of the 24-h period), and three days after grouping, drug administration was started. 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. Antidiabetic effects of repeated administration of SGLT2 inhibitors

Vehicle or each SGLT2 inhibitor (ipragliflozin and dapagliflozin: 0.1–1 mg/kg, tofogliflozin, canagliflozin, empagliflozin, and luseogliflozin: 1–10 mg/kg) was orally administered to diabetic mice (and vehicle was orally administered to normal mice) once daily (just before feeding at night) for 4 weeks. Doses of drugs were set to produce comparable increase in 24-h urinary glucose excretions based on the results of our preliminary study. After administration on Day 1, blood samples (approximately 15 μL) for the evaluation of blood glucose and plasma insulin levels were obtained from the tail vein using capillary glass tubes at every 24-h sampling point. Blood sampling was done during the night (dark period) using a spotlight to minimize lighting, taking special care not to affect food consumption or related parameters. After drug administration on Day 26, mice were transferred to metabolic cages, and spontaneously voided urine was collected for 24 h. The morning (5:00–8:00) after the final drug administration (Day 30), blood samples were collected under nonfasting conditions, and tissues including pancreas were isolated under isoflurane anesthesia.

2.4. Effects of repeated administration of SGLT2 inhibitors on glucose tolerance

Vehicle or each SGLT2 inhibitor (ipragliflozin and dapagliflozin: 0.3 mg/kg, tofogliflozin, canagliflozin, empagliflozin, and luseogliflozin: 3 mg/kg) was orally administered to diabetic mice once daily (just before feeding at night) for 4 weeks. The final administration (Day 29) was followed by a 2-day withdrawal period, and on the day after completion of withdrawal, an oral glucose tolerance test (OGTT) was performed. After mice had fasted during the inactive period (from 9:00 to 19:00), blood was sampled from a tail vein for evaluation of fasting blood glucose and plasma insulin levels. The glucose solution (2 g/kg) was then orally administered, followed by blood sampling for 2 h.

2.5. Biochemical determinations

Blood and urinary glucose concentrations were measured using Glucose CII test reagent (Wako Pure Chemical Industries, Ltd., Osaka, 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). Hemoglobin A_{1c} (HbA_{1c}) levels were measured using a DCA2000 System (Bayer Medical, Tokyo, Japan). Pancreatic hormone levels (insulin, glucagon, and somatostatin) were measured in accordance with the method previously reported (16). Plasma fibroblast growth factor 21 (FGF-21), leptin, and adiponectin concentrations were measured using commercial ELISA kits (R&D Systems Inc., Minneapolis, MN, USA).

2.6. Statistical analysis

The experimental results are expressed as the mean ± standard error of means (SEM). The areas under the curve (AUCs) and standard deviation (SD) were calculated from blood glucose and plasma insulin concentrations measured over time. The Matsuda-DeFronzo index was calculated using the following formula: 10,000/square root of [(fasting blood glucose × fasting plasma insulin) × (mean blood glucose × mean plasma insulin during OGTT)], and disposition index was calculated using the following formula: (plasma insulin AUC/blood glucose AUC during OGTT) × Matsuda-DeFronzo index (17). Significance of differences between normal and diabetic vehicle groups was assessed using Student's t-test, while that between the vehicle- and drug-treated groups was assessed using one-way ANOVA followed by post-hoc Tukey's and Dunnett's multiple comparison tests. A value of $P < 0.05$ was considered to be significant. Statistical and data analyses were conducted using GraphPad Prism 5 (GraphPad Software, La Jolla, CA, USA).

3. Results

The 24-h blood sampling study revealed fasting hyperglycemia in diabetic mice, with increased blood glucose levels after start of feeding which remained high throughout the 14-h feeding (active) period compared with normal mice. After the feeding period, blood glucose levels gradually decreased but still remained high during the fasting (inactive) period. In association with blood glucose levels, plasma insulin levels also remained extremely high across the active and inactive periods. Administration of SGLT2 inhibitors dose-dependently and significantly reduced blood glucose and plasma insulin levels (Fig. 1 and Fig. 2). The effects of ipragliflozin and dapagliflozin on reduction in blood glucose and plasma insulin levels were evident not only during the active period, but also the inactive period after administration, showing 24-h persistence. In contrast, the effects of the other four drugs (tofogliflozin, canagliflozin, empagliflozin, and luseogliflozin) on reduction in blood glucose and plasma insulin levels were potent immediately after administration but were gradually attenuated from 2 h post-dose, leading to obvious hyperglycemia and hyperinsulinemia in the latter half of the active period and throughout the inactive period,