Full paper

Characterization and comparison of sodium-glucose cotransporter 2 inhibitors in pharmacokinetics, pharmacodynamics, and pharmacologic effects

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A B S T R A C T

The sodium-glucose cotransporter (SGLT) 2 offer a novel approach to treating type 2 diabetes by reducing hyperglycaemia via increased urinary glucose excretion. In the present study, the pharmacokinetic, pharmacodynamic, and pharmacologic properties of all six SGLT2 inhibitors commercially available in Japan were investigated and compared. Based on findings in normal and diabetic mice, the six drugs were classified into two categories, long-acting: ipragliflozin and dapagliflozin, and intermediate-acting: tofogliflozin, canagliflozin, empagliflozin, and luseogliflozin. Long-acting SGLT2 inhibitors exerted an antihyperglycemic effect with lower variability of blood glucose level via a long-lasting increase in urinary glucose excretion. In addition, ipragliflozin and luseogliflozin exhibited superiority over the others with respect to fast onset of pharmacological effect. Duration and onset of the pharmacologic effects seemed to be closely correlated with the pharmacokinetic properties of each SGLT2 inhibitor, particularly with respect to high distribution and long retention in the target organ, the kidney. While all six SGLT2 inhibitors were significantly effective in increasing urinary glucose excretion and reducing hyperglycemia, our findings suggest that variation in the quality of daily blood glucose control associated with duration and onset of pharmacologic effects of each SGLT2 inhibitor might cause slight differences in rates of improvement in type 2 diabetes.

1. Introduction

Due to increasing prevalence of obesity and physical inactivity, the number of patients with diabetes is dramatically increasing and is expected to rise to 366 million worldwide by 2030 (1). Approximately 90% of all patients with diabetes have type 2 diabetes, a progressive metabolic disease characterized by hyperglycemia and relative insulin deficiency as a result of impaired insulin secretion from pancreatic β-cells or insulin resistance. Chronic hyperglycemia leads to progressive impairment of insulin secretion, exacerbates insulin resistance, and worsens diabetes (2). However, while many antidiabetic drugs have been developed and used for treatment, most type 2 diabetic patients' therapeutic goals are still not

achieved (3), highlighting the need for efficient new therapeutic strategies for treating type 2 diabetes, including combination therapy.

In recent years, inhibitors of sodium-glucose cotransporter (SGLT) 2, which can inhibit reabsorption of glucose by blocking SGLT2 and stimulate glucose excretion in the urine, have been proposed as novel drugs for treating type 2 diabetes (4), with several shown to improve hyperglycemia in this patient population (5). Although many studies have focused on nonclinical and clinical pharmacologic effects of SGLT2 inhibitors (6,7), most have examined these compounds on an individual basis, with only one study comparing several SGLT2 inhibitors in terms of *in vitro* inhibitory activity and selectivity for SGLT2 (8) and none comparing their *in vivo* effects.

Here, to clarify and compare the pharmacological properties of all six SGLT2 inhibitors commercially available in Japan (ipragliflozin, dapagliflozin, tofogliflozin, canagliflozin, empagliflozin, and luseogliflozin), we conducted pharmacokinetic, pharmacodynamic, and pharmacologic experiments in normal and type 2 diabetic mice.

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2. Materials and methods

2.1. Materials

Ipragliflozin (9), dapagliflozin (10), tofogliflozin (11), canagliflozin (12), empagliflozin (8), and luseogliflozin (13) were synthesized at Astellas Pharma Inc. (Ibaraki, Japan) and suspended in 0.5% methylcellulose solution for oral administration. Drugs were administered around the beginning (19:00) of the active period of mice, which corresponds to the administration timing in clinical practice. Doses of drugs were expressed as the free base form.

2.2. Animals

Male ICR (normal) mice for investigating pharmacokinetic and pharmacodynamic properties were purchased from Japan SLC, Inc. (Shizuoka, Japan). Male C57BL/6 (normal) and KK/A^y type 2 diabetic mice for investigating pharmacologic effects were purchased from CLEA Japan (Kanagawa, Japan). The diabetic mice were uniformly grouped by blood glucose levels. 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 *ad libitum*. All animal experimental procedures were approved by the Institutional Animal Care and Use Committee of Astellas Pharma Inc. Astellas Pharma Inc., Tsukuba Research Center was awarded Accreditation Status by the AAALAC International.

2.3. Pharmacokinetics

After oral administration of each SGLT2 inhibitor (3 mg/kg) to nonfasting normal mice in the evening (19:00), blood was withdrawn from the abdominal vena cava, and tissues (kidney, liver, and brain) were isolated under isoflurane anesthesia at designated time points for up to 24 h. Isolated tissues were homogenized with phosphate-buffered saline. The plasma and tissue concentrations of the test drug were measured using high-performance liquid chromatography (HPLC). Acetonitrile (100 μL) and methyl tert-butyl ether (100 μL) were added to the plasma or tissue homogenate (100 μL), mixed, and centrifuged (15,000 rpm, 10 min). The supernatant was transferred to a tube and evaporated in a vacuum centrifugal concentrator, and the residue was dissolved in the mobile phase for use as the assay sample. Concentrations of drug in assay sample were analyzed using HPLC with an ultraviolet detector (ipragliflozin and tofogliflozin: 265 nm, dapagliflozin, canagliflozin, empagliflozin, and luseogliflozin: 280 nm) and a 4.6×250-mm reversed-phase ODS-80Ts column (Tosoh, Tokyo, Japan). The column temperature was maintained at 60 °C, the mobile phase used was acetonitrile/20 mM ammonium acetate solution (60/40 [v/v]), and the flow rate was 1 mL/min. The pharmacokinetic parameters, maximum plasma/tissue concentration (C_{max}), time to C_{max} (T_{max}), elimination half-life ($t_{1/2}$), and area under the plasma/tissue drug concentration–time curve (AUC) for 24 h, were calculated.

2.4. Pharmacodynamics

Each SGLT2 inhibitor (ipragliflozin and dapagliflozin: 0.3–3 mg/kg, tofogliflozin, canagliflozin, empagliflozin, and luseogliflozin: 1–10 mg/kg) was administered orally in the evening (19:00) to the normal mice under nonfasting conditions. Spontaneously voided urine was collected every 6 h throughout the first 24 h after dosing while the animals were kept in metabolic cages under nonfasting conditions. After the urine volume was measured, the glucose concentration in the urine was measured using the Glucose CII test reagent (Wako Pure Chemical Industries, Ltd., Osaka, Japan).

2.5. Effects of SGLT2 inhibitors on blood glucose and plasma insulin levels

Each SGLT2 inhibitor (ipragliflozin and dapagliflozin: 0.01–3 mg/kg, tofogliflozin, canagliflozin, empagliflozin, and luseogliflozin: 0.03–10 mg/kg) was administered orally in the evening (19:00) to diabetic mice, and blood samples were obtained from a tail vein at each sampling point for up to 24 h under nonfasting conditions. Blood sampling during nighttime (dark period) was performed using a spotlight to minimize lighting, taking special care not to affect food consumption or related parameters. Blood glucose concentrations were measured as described above. Plasma insulin levels were measured using an ultra-high sensitive mouse insulin enzyme-linked immunosorbent assay (ELISA) kit (Morinaga Institute of Biological Science, Inc., Kanagawa, Japan).

2.6. Effects of SGLT2 inhibitors on glucose tolerance during the oral glucose tolerance tests

Durability experiment: Each SGLT2 inhibitor (3 mg/kg) was administered orally in the evening (19:00) to diabetic mice fasted for half a day, and glucose solution (2 g/kg) was orally loaded 0.5, 6, and 12 h after drug administration. Blood samples were obtained at each sampling point.

Rapid-onset experiment: Each SGLT2 inhibitor (3 mg/kg) was administered orally in the evening (19:00) to diabetic mice fasted for half a day at 30 min before, 5 min before, or 10 min after oral loading of glucose solution (2 g/kg). Blood samples were obtained at each sampling point.

2.7. Statistical analysis

The experimental results are expressed as the mean, mean ± standard deviation (SD), or mean ± standard error of means (SEM). The AUCs were calculated from blood glucose and plasma insulin concentrations measured over time. 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 Dunnett's multiple comparison test. 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

3.1. Pharmacokinetics

Following oral administration of each SGLT2 inhibitor (3 mg/kg) to normal mice, the maximum plasma concentration was reached at 0.5–1 h, followed by time-dependent elimination (Fig. 1 and Table 1). The drug concentrations in the kidney, liver, and brain also peaked at 0.5–1 h, followed by time-dependent elimination. Although distribution in the brain was low for all SGLT2 inhibitors, distribution in the kidney, the SGLT2-expressing site, varied widely among drugs. T_{max}, as determined from drug concentrations in plasma and kidney, was 0.5 h for ipragliflozin and luseogliflozin and 1 h for the other four drugs. The $t_{1/2}$ was longest in plasma in the descending order of canagliflozin > dapagliflozin > ipragliflozin > empagliflozin > tofogliflozin > luseogliflozin and longest in kidney in the descending order of dapagliflozin > ipragliflozin > canagliflozin > empagliflozin = tofogliflozin > luseogliflozin.