Life Sciences 197 (2018) 80–90

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Antidiabetic effects of SGLT2 inhibitor ipragliflozin in type 2 diabetic mice fed diets containing different carbohydrate contents

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ARTICLE INFO

Keywords:
Ipragliflozin
SGLT2
Hyperglycemia
Obese
Diabetes

ABSTRACT

Aims: Daily intake of carbohydrates differs among individual patients with type 2 diabetes. Here, we investigated whether or not dietary carbohydrate content affects the efficacy of the sodium-glucose cotransporter 2 (SGLT2) inhibitor ipragliflozin in type 2 diabetic mice.

Main methods: Diabetic mice were fed a regular (50% kcal), high (75% kcal)-, or low (25% kcal)-carbohydrate diet. Ipragliflozin was orally administered once a day for 4 weeks.

Key findings: In all groups, mice exhibited characteristics of type 2 diabetes, including hyperglycemia, hyperinsulinemia, and obesity. Hyperglycemia was more severe in the high-carbohydrate diet group and milder in the low-carbohydrate diet group than in the regular diet group. In all diabetic mice, ipragliflozin significantly increased urinary glucose excretion and improved hyperglycemia, hyperinsulinemia, glucose tolerance, insulin resistance, obesity, and nephropathy. Although these antidiabetic effects of ipragliflozin were more marked in the high-carbohydrate diet group (which showed more severe hyperglycemia) than in the other two groups, no significant differences in effective dose or degree of response were observed among the three groups.

Significance: The antidiabetic effects of ipragliflozin were not greatly affected by dietary carbohydrate content, suggesting that ipragliflozin may have similar efficacy for patients with type 2 diabetes regardless of carbohydrate intake.

1. Introduction

The major biochemical alteration in type 2 diabetes is hyperglycemia, caused by varying combinations of insulin resistance in peripheral tissues—including the liver, muscle, and fat—and impaired glucose-stimulated insulin secretion from pancreatic β-cells. The onset and progression of type 2 diabetes is strongly associated with obesity caused by either or both dietary or a hypokinetic lifestyle, as well as genetic factors [1]. Basic management of type 2 diabetes therefore involves lifestyle interventions such as dietary instruction and implementation of an exercise regimen [2]. A number of studies have investigated the relationships between dietary lifestyle, particularly carbohydrate intake, and risk of onset and progression of type 2 diabetes, with findings suggesting that excessive intake of carbohydrates is strongly associated with hyperglycemia and insulin resistance [3,4]. Refraining from such excessive intake is therefore considered an effective means of treating diabetes, with reduced intake of carbohydrates reported to improve diabetic conditions such as hyperglycemia [5,6]. Failure to maintain such a restricted lifestyle can lead to the development of chronic hyperglycemia, directly impairing both insulin secretion and sensitivity in a phenomenon known as “glucose toxicity”, which contributes to the progressive worsening of hyperglycemia [7]. Many diabetes patients are therefore treated using a combination of lifestyle improvement and drug therapies. To date, several drugs that either or both increase insulin secretion or improve insulin sensitivity have been developed for the treatment of diabetes. However, type 2 diabetes patients present with various pathologies depending on their lifestyle, including dietary habits, such that antidiabetic drugs vary in terms of the degree and nature of their effectiveness. An antidiabetic drug that exerts equivalent efficacy regardless of lifestyle habits or diabetic pathology is therefore required.

Inhibitors of sodium-glucose cotransporter 2 (SGLT2), which stimulate glucose excretion in urine, have recently been proposed as novel drugs for treating diabetes, and several SGLT2 inhibitors have been shown to ameliorate hyperglycemia in type 2 diabetic patients, including ipragliflozin [8]. We previously investigated the efficacy of ipragliflozin in models of type 1 and type 2 diabetes with various degrees of hyperglycemia, impaired insulin secretion, insulin resistance, and obesity, and confirmed that this drug exhibited efficacy in all models [9–12]. Other studies have also reported the therapeutic effects

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https://doi.org/10.1016/j.lfs.2018.02.009
Received 19 October 2017; Received in revised form 26 January 2018; Accepted 5 February 2018
Available online 06 February 2018
0024-3205/

Table 1
Parameters of normal and type 2 diabetic mice receiving regular, high-carbohydrate, or low-carbohydrate diets.

	Normal	Diabetes		
	Regular	Regular	High-carbohydrate	Low-carbohydrate
Body weight (g)	28.9 ± 0.4	43.8 ± 0.6*	42.9 ± 0.7*	44.6 ± 0.7*
Food intake (g/day)	3.73 ± 0.05	5.70 ± 0.13*	5.91 ± 0.11*	5.51 ± 0.06*
Calorie intake (kcal/day)	14.9 ± 0.2	22.2 ± 0.5*	22.5 ± 0.4*	22.0 ± 0.2*
HbA_{1c} (%)	4.02 ± 0.03	9.23 ± 0.32*	10.05 ± 0.28*	8.28 ± 0.30*
Blood glucose (mg/dL)	152 ± 10	412 ± 19*	518 ± 42*,#	313 ± 19*,#
Plasma insulin (ng/mL)	1.7 ± 0.1	52.7 ± 4.5*	63.3 ± 8.7*	44.8 ± 3.9*
Plasma triglycerides (mg/dL)	72 ± 7	396 ± 40*	243 ± 30*,#	486 ± 44*
Plasma NEFAs (mEq/L)	0.58 ± 0.03	1.32 ± 0.06*	1.22 ± 0.07*	1.43 ± 0.09*
Plasma cholesterol (mg/dL)	68 ± 2	160 ± 3*	153 ± 7*	173 ± 14*
Plasma BCAA (mM)	0.98 ± 0.04	1.19 ± 0.07*	0.70 ± 0.08*,#	1.34 ± 0.07*
Plasma urea nitrogen (mg/dL)	25.8 ± 1.3	41.3 ± 1.5*	32.2 ± 0.5*,#	44.6 ± 2.4*
Plasma total ketone bodies (μmol/L)	22.9 ± 0.7	38.3 ± 1.9*	32.0 ± 1.2*,#	42.7 ± 2.0*
Liver weight (g)	1.56 ± 0.03	3.42 ± 0.16*	3.38 ± 0.30*	3.00 ± 0.11*
Hepatic triglycerides content (mg/g liver)	8.6 ± 0.3	60.3 ± 6.0*	51.9 ± 2.8*	65.6 ± 2.6*
Hepatic cholesterol content (mg/g liver)	1.2 ± 0.1	17.3 ± 1.5*	16.7 ± 0.8*	19.2 ± 0.8*
Epididymal adipose tissue weight (g)	0.43 ± 0.02	1.96 ± 0.11*	1.71 ± 0.10*	2.13 ± 0.07*
Urine volume (mL/day)	1.9 ± 0.2	11.4 ± 1.0*	11.5 ± 1.0*	9.6 ± 1.2*
Urinary glucose excretion (mg/day)	3.1 ± 0.6	725 ± 52*	1133 ± 57*,#	461 ± 70*,#
Urinary urea nitrogen excretion (mg/day)	130 ± 22	358 ± 34*	283 ± 30*	406 ± 30*
Urinary albumin excretion (μg/day)	540 ± 96	4832 ± 660*	5414 ± 683*	3957 ± 367*

NEFAs, non-esterified fatty acids; BCAA, branched-chain amino acid.
Values are mean ± S.E.M. for six animals per group.
* $P < 0.05$ vs. normal group.
# $P < 0.05$ vs. diabetic regular feeding group.

of SGLT2 inhibitors in various diabetes models [8], suggesting that SGLT2 inhibitors might be useful for treating almost all type 2 diabetic conditions. In clinical practice, however, the fact that SGLT2 inhibitors exert their antidiabetic effects by increasing the excretion of excess blood glucose into urine has raised questions as to whether differences in carbohydrate (glucose) intake may affect the effective dose or efficacy of this class of drugs. More specifically, questions have been raised about whether high-carbohydrate consumers require a higher dose or exhibit a lower response than those consuming regular diets, and whether dose reduction is needed for patients on decreased or low-carbohydrate diets. Concerns have also been raised regarding adverse reactions, such as hypoglycemia and malnutrition, as well as other factors. In addition, the pathologies in most of these diabetes models were induced by congenital gene (ob, A^y, leptin receptor) mutations or chemically (streptozotocin, alloxan)-induced organic injuries of the pancreas. To our knowledge, few studies have evaluated and compared the therapeutic effects of repeated administration of SGLT2 inhibitors in diabetic models that partially reflect chronic a posteriori factors, such as lifestyle habits, on diabetic pathologies.

Here, we examined the effect of repeated administration of the SGLT2 inhibitor ipragliflozin in KK/A^y mice, a genetic animal model of type 2 diabetes, that were fed diets with carbohydrate contents ranging from 25% to 75% kcal to experimentally reflect the diverse dietary styles of diabetic patients.

2. Materials and methods

2.1. Materials and animals

Ipragliflozin was synthesized at Astellas Pharma Inc. (Ibaraki, Japan) and suspended in 0.5% methylcellulose solution for oral administration. Doses of ipragliflozin are expressed as the free base form. Five-week-old male C57BL/6 (normal) and KK/A^y type 2 diabetic mice that exhibit hyperglycemia, insulin resistance, hyperinsulinemia, hyperlipidemia, and obesity were purchased from CLEA Japan (Kanagawa, Japan). Diabetic mice were grouped to attain uniform mean blood glucose levels among groups and fed one of three diets of the following nutrient profiles (calculated as a percentage of total calories [kcal]): regular chow diet consisting of 50% carbohydrate, 39% protein, and 11% fat (total caloric energy value = 3.9 kcal/g: D14050501; Research Diets, Inc., New Brunswick, NJ, USA); high-carbohydrate diet consisting of 75% carbohydrate, 20% protein, and 6% fat (total caloric energy = 3.8 kcal/g: D14050502; Research Diets Inc.); or low-carbohydrate diet consisting of 25% carbohydrate, 58% protein, and 17% fat (total caloric energy = 4.0 kcal/g: D14050503; Research Diets Inc.). After 2 weeks, diabetic mice that exhibited hyperglycemia (regular: 396 ± 8 mg/dL, high-carbohydrate: 391 ± 12 mg/dL, and low-carbohydrate: 334 ± 6 mg/dL) were regrouped to attain uniform mean blood glucose levels among the three feeding groups. All animals were housed under standard conditions with controlled temperature, humidity, and light (12-h light-dark cycle) and provided food 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 has been awarded Accreditation Status by the AAALAC International.

2.2. Repeated administration study

Vehicle or ipragliflozin (0.1, 0.3, 1, 3 mg/kg) was orally administered via a stomach tube to mice once daily at night (19:00–20:00) for 4 weeks, and body weight and food intake were measured once a week. After drug administration on Day 1, blood samples were collected from the tail vein at each sampling point for 24 h to measure blood glucose and plasma insulin levels. An oral glucose tolerance test (OGTT) was performed at Week 3. After drug administration, mice were fasted for 12 h (overnight). 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 26, mice were transferred to metabolic cages to collect spontaneously voided urine for 24 h. To investigate the pharmacological effects of ipragliflozin after repeated administration, blood samples were collected under non-fasting conditions the morning (approximately 12–14 h) after the final drug administration (Day 28), and tissues (liver, kidney, and epididymal adipose tissue) were isolated under isoflurane anesthesia. Blood and urinary glucose concentrations were measured using the Glucose CII