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Effect of sodium-glucose cotransporter 2 (SGLT2) inhibition on weight loss is partly mediated by liver-brain-adipose neurocircuitry

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ABSTRACT

Sodium-glucose cotransporter 2 (SGLT2) inhibitors have both anti-diabetic and anti-obesity effects. However, the precise mechanism of the anti-obesity effect remains unclear. We previously demonstrated that the glycogen depletion signal triggers lipolysis in adipose tissue via liver-brain-adipose neurocircuitry. In this study, therefore, we investigated whether the anti-obesity mechanism of SGLT2 inhibitor is mediated by this mechanism. Diet-induced obese mice were subjected to hepatic vagotomy (HVx) or sham operation and loaded with high fat diet containing 0.015% tofogliflozin (TOFO), a highly selective SGLT2 inhibitor, for 3 weeks. TOFO-treated mice showed a decrease in fat mass and the effect of TOFO was attenuated in HVx group. Although both HVx and sham mice showed a similar level of reduction in hepatic glycogen by TOFO treatment, HVx mice exhibited an attenuated response in protein phosphorylation by protein kinase A (PKA) in white adipose tissue compared with the sham group. As PKA pathway is known to act as an effector of the liver-brain-adipose axis and activate triglyceride lipases in adipocytes, these results indicated that SGLT2 inhibition triggered glycogen depletion signal and actuated liver-brain-adipose axis, resulting in PKA activation in adipocytes. Taken together, it was concluded that the effect of SGLT2 inhibition on weight loss is in part mediated via the liver-brain-adipose neurocircuitry.

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1. Introduction

Type 2 diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia due to insulin resistance and relative insulin deficiency [1]. The prevalence of type 2 diabetes has been increasing dramatically worldwide in recent years, and more than 380 million people worldwide have diabetes mellitus [2]. Thus, diabetes is now a major threat to global public health.

Recently, sodium-glucose cotransporter 2 (SGLT2) inhibitors have attracted much attention as they exert both anti-diabetic and anti-obesity effects [3,4]. SGLT2 is a glucose transporter that was cloned in 1994 by Kanai et al. [5], and is known to play an important role in the renal reabsorption of glucose, which is dependent on the sodium concentration gradient. SGLT2 is mainly present in the apical aspect of the S1 segment of the proximal renal tubules and is responsible for approximately 90% of the total renal glucose reabsorption. Recent clinical studies have indicated that oral administration of SGLT2 inhibitors induces urinary glucose excretion (UGE), improves hyperglycemia and reduces body weight of patients with type 2 diabetes [6–12]. In animal studies, SGLT2 inhibitors also improve glucose and lipid metabolism in diabetic animal model

Abbreviations: HVx, hepatic vagotomy; SGLT2, sodium-glucose cotransporter 2; TOFO, tofogliflozin; PKA, protein kinase A.

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[13–15]. However, mechanisms of anti-obesity effect of SGLT2 inhibitors were not fully understood.

Recently, a new paradigm of inter-organ networks via the autonomic nervous system has emerged as a potential regulatory mechanism of metabolic homeostasis [16,17]. In fact, a number of studies are being conducted on the roles of the neural signal from the liver [18–21]. Especially, we previously documented that liver glycogen shortage directly facilitates lipolysis in white adipose tissue by activating a liver-brain-adipose neurocircuitry independently of the blood glucose and insulin/glucagon levels, demonstrating the presence of "glycogen depletion signal" [20]. Because liver glycogen reduction is reported with tofogliflozin (TOFO) and dapagliflozin [22,23], it is possible that SGLT2 inhibition can activate this neural circuit by depleting hepatic glycogen.

These situations prompted us to hypothesize that SGLT2 inhibition triggers the glycogen depletion signal in the liver and activates lipolysis in adipocytes through a liver-brain-adipose neurocircuitry, leading to fat mass reduction and body weight loss. Therefore, we investigated the effect of SGLT2 inhibitor on the neural pathway using hepatic vagotomy (HVx) mice model in the current study.

2. Materials and methods

2.1. Materials

Tofogliflozin (TOFO) was provided by KOWA Co., Ltd. Anti-PKA substrate and anti-α-tubulin antibodies were purchased from Cell Signaling Technology. Mouse Insulin ELISA KIT was purchased from Shibayagi Co., Ltd. Ketone test B liquid was purchased from Sanwa Co., Ltd. Glucose CII test Wako, Triglycerides E-test Wako and Cholesterol E-test Wako were purchased from Wako chemicals.

2.2. Animals

C57BL/6 J mice (6-week-old) were purchased from CLEA. All animals were housed in a temperature-controlled environment with 14 h light/10 h dark cycle and given free access to high fat diet (HFD32 Oriental Yeast, 507.6 kcal/100 g) and water. 2 weeks after HFD feeding, mice were subjected to sham operation (Sham) or to selective hepatic vagotomy (HVx) as described below. Following 2 weeks of recovery period, 0.015% TOFO was administered with HFD for 3 weeks. For tissue sampling, mice were sacrificed after 8 h fasting and samples were strored at −80 °C. All animals studied were anesthetized and euthanized according to a protocol approved by the Tsukuba University Animal Care and Use Committee.

2.3. Selective hepatic vagotomy (HVx)

8-week-old male mice were subjected to dissection of hepatic branch of the vagus [20]. A laparotomy incision was made on the ventral midline and the abdominal muscle wall was opened with a second incision. The gastrohepatic ligament was severed using fine forceps, and the stomach was gently retracted, revealing the descending ventral oesophagus and the ventral subdiaphragmatic vagal trunk. The hepatic branch of this vagal trunk was then transected using fine forceps.

2.4. DEXA analysis

PIXImus2 DEXA (GE Medical Systems) was used to measure weight and composition of lean mass and fat mass.

2.5. mRNA quantification

mRNA measurement was performed as described previously [24,25]. Total RNA from tissues was prepared using Sepasol-RNA I (Nacalai Tesque) and was used for cDNA synthesis (Invitrogen). Real-time PCR was performed using the ABI Prism 7300 System (Applied Biosystems) with SYBR Green master mix (Roche Diagnostics).

The primer sequences are as follows:

Pck1 5′-TGTCATCCGCAAGCTGAAGA-3′ 5′-TTCGATCCTGGCCACATCTC-3′

G6pc 5′-CGGCGCAGCAGGTGTATACTAT-3′ 5′-CAGAATCCCAACCACAAGATGA-3′

Cpt1a 5′−CCTGGGCATGATTGCAAAG -3′ 5′-GGACGCCACTCACGATGTT -3′

Pparα 5′−CCTCAGGGTACCACTACGGAGT -3′ 5′-GCCGAATGTTCGCCGAA -3′

Fgf21 5′−CCTCTAGGTTTCTTTGCCAACAG -3′ 5′-AAGCTGCAGGCCTCAGGAT -3′

Rplp0 5′-GAAGACAGGGCGACCTGGAA -3′ 5′-TTGTCTGCTCCCACAATGAAGC -3′

Data were analyzed using the comparative cycle threshold method, and mRNA expression was normalized to *Rplp0* expression.

2.6. Immunoblot analysis

Immunoblotting was performed as described previously [26,27]. Briefly, tissue extracts were fractionated by SDS−PAGE and transferred to a polyvinylidene difluoride (PVDF) membrane. After blocking with 5% skim milk in TBS-T (10 mM Tris at pH 8.0, 150 mM NaCl, 0.1% Tween 20) for 30 min, the membrane was incubated with anti-PKA substrate and anti-α-tubulin antibodies at 4 °C for 12 h. Membranes were washed three times for 10 min and incubated with rabbit or mouse second antibody at 1:5000 dilution for 1 h. Signals were detected using the ECL western blotting Detection System (GE Healthcare) and exposed to ChemiDocTM XRS+ (Bio-Rad) and IMAGE LAB TM Software (BioRad).

2.7. Measurement of liver glycogen content

Liver glycogen content was measured as described previously [20]. Liver were dissolved in 30% KOH and 66% EtOH was added, then was placed at −20 °C for 1 h. Then liver were centrifuged at 8000 rpm and a supernatant was discarded. Pellet was dissolved with dH_2O, added acetate buffer and amyloglucosidase, and measured with Glucose CII test Wako (Wako).

2.8. Statistical analyses

The results were expressed as the mean ± standard error of the mean (SEM). Statistical differences between groups were analyzed by Mann-Whitney *U* test analysis. $P < 0.05$ was taken as statistical significance.

3. Results

3.1. HVx attenuated weight loss and fat reduction by TOFO

Diet-induced obese mice were subjected to selective HVx and loaded with high fat diet containing 0.015% TOFO for 3 weeks. As shown in Fig. 1A and B, TOFO suppressed body weight and fat mass gain. As expected, the effect of TOFO was significantly attenuated by HVx (Fig. 1A and B). Meanwhile, no change was observed in lean mass among groups (Fig. 1C), indicating that the body weight

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