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Effect of canagliflozin and metformin on cortical neurotransmitters in a diabetic rat model

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ABSTRACT

Background: The rapid economic development in the Arabian Gulf has resulted in lifestyle changes that have increased the prevalence of obesity and type 2 diabetes, with the greatest increases observed in Kuwait. Dyslipidemia and diabetes are risk factors for disruptions in cortical neurotransmitter homeostasis. This study investigated the effect of the antidiabetic medications canagliflozin (CAN) and metformin (MET) on the levels of cortical neurotransmitters in a diabetic rat model.

Materials and methods: The rats were assigned to the control (C) group, the diabetic group that did not receive treatment (D) or the diabetic group treated with either CAN (10 mg/kg) or MET (100 mg/kg) for 2 or 4 weeks. Blood and urine glucose levels and cortical acetylcholinesterase (AChE) activity were assayed, and amino acid and monoamine levels were measured using HPLC.

Results: The diabetic group exhibited a significant increase in AChE activity and a decrease in monoamine and amino acid neurotransmitter levels. In the CAN group, AChE was significantly lower than that in the D and D + MET groups after 2 weeks of treatment. In addition, a significant increase in some cortical monoamines and amino acids was observed in the D + MET and D + CAN groups compared with the D group. Histopathological analysis revealed the presence of severe focal hemorrhage, neuronal degeneration, and cerebral blood vessel congestion, with gliosis in the cerebrum of rats in the D group. The CAN-treated group exhibited severe cerebral blood vessel congestion after 2 weeks of treatment and focal gliosis in the cerebrum after 4 weeks of treatment. Focal gliosis in the cerebrum of rats in the MET-treated group was observed after 2 and 4 weeks of treatment.

Conclusions: We conclude that the effect of CAN and MET on neurotransmitters is potentially mediated by their antihyperglycemic and antihyperlipidemic effects. In addition, the effects of CAN on neurotransmitters might be associated with its receptor activity, and the effect of MET on neurotransmitters might be associated with cerebral metabolism.

1. Introduction

Diabetes mellitus (DM) is a disease characterized by defects in insulin function and insulin secretion that results in glucose intolerance and chronic hyperglycemia [1]. Arabic-speaking countries have some of the highest prevalence rates of type 2 diabetes mellitus (T2DM) in the world, with the highest prevalence observed in the Arabian Gulf. The rapid economic development of Arabic-speaking countries has resulted in significant changes in socioeconomic status and lifestyle, and it has promoted an obesogenic environment. The prevalence rates of T2DM among adults of Arabic-speaking countries are between 4% and 21%, with the highest prevalence of T2DM observed in Kuwait [2,3]. Obesity is a

Abbreviations: CAN, canagliflozin; MET, metformin; C, control; D, diabetic group that did not receive treatment; DM, Diabetes mellitus; T2DM, type 2 diabetes mellitus; BBB, blood-brain barrier; SGLT, sodium-glucose co-transporter; FDA, The Food and Drug Administration; STZ, streptozotocin; HFD, high-fat diet; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; HPLC, high performance liquid chromatography; AChE, acetylcholinesterase; Glu, glutamic acid; Asp, aspartic acid; Asn, asparagine; GABA, γ-aminobutyric acid; Gly, glycine; Ser, serine; Tau, taurine; His, histidine; NE, norepinephrine; DA, dopamine; 5-HT, serotonin; H&E, Hematoxylin and eosin; LSD, Least significant difference; SPSS, Statistical Package for Social Science; ATP, Adenosine triphosphate; Acetyl CoA, acetyl coenzyme A; Na/K pump, sodium/potassium pump; GAD, Glutamate decarboxylase; NMDARs, *N*-methyl-D-aspartate receptors; pMCAO, permanent middle cerebral artery occlusion; AMPK, 5′ adenosine monophosphate-activated protein kinase.

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major risk factor for developing T2DM and insulin resistance in both males and females [4,5]. The clinical complications of obesity and the increased consumption of foods rich in saturated fats have been implicated in defects in brain physiology and function. Previous studies have reported that deficits in learning, memory, and executive function are more commonly observed in obese patients than in non-obese individuals [6–8]. In addition, dyslipidemia contributes to diabetic neuropathy by increasing plasma-oxidized low-density lipoprotein levels, an effect that directly causes oxidative stress [9]. Diabetes also increases the risk and severity of stroke and cognitive impairment [10]. Several studies have reported an association between diabetes and impaired neurotransmitter homeostasis and brain function [11–13]. Changes in brain glucose metabolism might also occur in diabetes. Glucose enters the brain via glucose transporters in endothelial cells at the blood-brain barrier (BBB), including through sodium-glucose co-transporter 2 (SGLT2) [14]. Changes in glucose transporter function and expression dramatically disrupt brain glucose homeostasis and function. The cerebral cortex is the outermost layer of the brain and is responsible for higher brain functions, including language, memory, and consciousness. Patients with diabetes exhibit changes in glucose transporter function and expression. Previous studies demonstrated that cerebral blood flow is impaired in people with diabetes and that these patients exhibit signs of cortical and subcortical atrophy [15]. The cerebral metabolic capacity varies depending on the region, which may account for the increased sensitivity to hyperglycemia in the cerebral cortex compared with other regions of the brain [16].

Metformin, a partial insulin-sensitizing agent, is the standard first-line treatment for patients with T2DM. This recommendation is based on data from the UK Prospective Diabetes Study [17]. Metformin can improve cardiovascular outcomes in overweight patients with T2DM and potentially reduces the risk of cancer [18–21]. Increasing renal glucose excretion by inhibiting SGLT2 is a novel approach to reducing hyperglycemia [22].

Canagliflozin is an SGLT2 inhibitor. SGLT2 is expressed in the proximal renal tubules and is responsible for the majority of filtered glucose reabsorption from the tubular lumen. By inhibiting SGLT2, canagliflozin reduces the reabsorption of filtered glucose and lowers the renal threshold for glucose, thereby increasing urinary glucose excretion. The FDA approved Invokana (canagliflozin) for use in patients with T2DM on March 29, 2013.

The present study investigated the effect of canagliflozin and metformin on neurotransmitter levels in the brain cortex of an obese diabetic rat model induced by a high-fat diet (HFD) and a low dose of streptozotocin.

2. Materials and methods

2.1. Experimental animals

This study was conducted using male albino rats (Wistar strain) with an initial body weight of 150 ± 10 g that were obtained from the animal house of the National Organization for Drug Control and Research. The rats were housed in iron mesh cages at a controlled temperature of 21 °C and a 12 h light/12 h dark cycle. Clean sawdust was used to keep the animals dry and clean throughout the experiments. The animals were acclimated to the laboratory conditions for 2 weeks prior to the experiments. They were fed a standard diet of AIN-93G pellets [23]. The food debris, feces, and urine were removed daily to prevent food and water contamination. All of the animal experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996).

2.2. Chemicals

Canagliflozin ($C24H25FO5S \cdot 1/2H_2O$) (Invokana, 300 mg tablets) was manufactured by Janssen Ortho, LLC. (Gurabo, PR 00778) for Janssen Pharmaceuticals, Inc. (Titusville, NJ, 08560). Metformin ($C4H11N5 \cdot HCl$) (Glucophage, 1000 mg tablets) was obtained from Merck Santé S.A.S, and streptozotocin (STZ) was obtained from Sigma-Aldrich Co. (St. Louis, MO, USA; product number: S0130).

2.3. Animal model of diabetes

After the adaptation period, the rats had free access to food pellets. The control group of rats was fed a standard diet. The animals in the diabetic groups were fed a HFD supplemented with a low dose of STZ as previously described [24]; however, melted butter was used in place of lard. This modification was based on the observation that adipose tissue preferentially stores saturated fatty acids (SFA) compared with monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) [25]. According to the USDA National Nutrient Database Release 27, butter consists of 51.368% SFA, 21.021% MUFA, and 3.043% PUFA (Basic Report 01145, Butter, without salt), whereas lard consists of 39% SFA, 45% MUFA, and 11% PUFA (Basic Report 04002, Lard). The SFA of a butter sample were separated into lauric (C12:0), myristic (C14:0), palmitic (C16:0), and stearic (C18:0) acids using gas chromatography and had a composition similar to that reported in a previous study [26]. Animals in the diabetic groups were fed a standard diet supplemented with 1 g of butter administered orally for 10 days, and the amount of butter increased to 2 g over the next 10 days. On day 22, hyperlipidemia was confirmed in rats that had received injections of STZ (35 mg/kg) (i.p.) dissolved in sodium citrate buffer (pH 4.5). The butter-supplemented standard diet was continued for 1 additional week, amounting to a total of 4 weeks of the HFD. One week after the STZ injection, blood samples from the lateral tail vein were obtained and immediately used to measure blood glucose levels using a glucometer (OneTouch, Johnson & Johnson Medical). The device was cleaned with 70% ethyl alcohol after each use. Animals with a blood glucose level >180 mg/dl were classified as diabetic and included in subsequent experiments. Rats in the diabetic groups were fed the butter-supplemented diet throughout the entire experimental period.

2.3.1. Experimental groups

1. Control (C): Normal rats administered 2 ml of water via oral intubation for 4 weeks.
2. Diabetic (D): Untreated diabetic rats.
3. Canagliflozin (D + CAN): Diabetic rats administered canagliflozin (10 mg/kg) daily via oral intubation as described in Liang et al. [27] for 2 or 4 weeks.
4. Metformin (D + MET): Diabetic rats administered metformin (100 mg/kg) via oral intubation for 2 or 4 weeks.

2.3.2. Biochemical assays

At the end of each experimental period (12 h after the administration of the last dose), blood samples from the lateral tail vein were obtained and immediately used to determine blood glucose levels using a glucometer, and urine glucose levels were measured using test strips. The animals were euthanized by rapid decapitation. The animal brains were rapidly resected, weighed, and cleaned. Four brains from each group were fixed in 10% formalin and evaluated using histopathological assays. The cortical area was separated from the whole brain and divided into 2 halves. One half of the brain was used for the acetylcholinesterase activity assay,