Available online at www.sciencedirect.com
ScienceDirect
Chinese Journal of Natural Medicines 2016, **14**(7): 0518–0526
Chinese Journal of Natural Medicines

Berberine enhances antidiabetic effects and attenuates untoward effects of canagliflozin in streptozotocin-induced diabetic mice

TIAN Cai-Ming [1, 2], JIANG Xin [2, 3], OUYANG Xiao-Xi [2], ZHANG Ya-Ou [2], XIE Wei-Dong [2*]

[1] *Department of chemistry, Tsinghua University, Beijing 100084, China;*
[2] *Shenzhen Key Lab of Health Science and Technology, Division of Life Science & Health, Graduate School at Shenzhen, Tsinghua University, Shenzhen 518055, China;*
[3] *School of Life Sciences, Tsinghua University, Beijing 100084, China*

Available online 20 Jul., 2016

[ABSTRACT] The present study aimed at determining whether berberine can enhance the antidiabetic effects and alleviate the adverse effects of canagliflozin in diabetes mellitus. Streptozotocin-induced diabetic mice were introduced, and the combined effects of berberine and canagliflozin on glucose metabolism and kidney functions were investigated. Our results showed that berberine combined with canagliflozin (BC) increased reduction of fasting and postprandial blood glucose, diet, and water intake compared with berberine or canagliflozin alone. Interestingly, BC showed greater decrease in blood urea nitrogen and creatinine levels and lower total urine glucose excretion than canagliflozin alone. In addition, BC showed increased phosphorylated 5' AMP-activated protein kinase (pAMPK) expression and decreased tumor necrosis factor alpha (TNFα) levels in kidneys, compared with berberine or canagliflozin alone. These results indicated that BC was a stronger antidiabetic than berberine or canagliflozin alone with less negative side effects on the kidneys in the diabetic mice. The antidiabetic effect was likely to be mediated by synergically promoting the expression of pAMPK and reducing the expression of TNFα in kidneys. The present study represented the first report that canagliflozin combined with berberine was a promising treatment for diabetes mellitus. The exact underlying mechanisms of action should be investigated in future studies.

[KEY WORDS] Canagliflozin; Berberine; Diabetes mellitus; AMP-activated protein kinase; Sodium-glucose cotransporter-2

[CLC Number] R965 **[Document code]** A **[Article ID]** 2095-6975(2016)07-0518-09

Introduction

The incidence of hyperglycemia and diabetes increases globally, driven by population growth, aging, and increasing age-specific prevalence [1]. Diabetes mellitus is associated with classic diabetic syndromes and concomitant complications, such as cardiopathy, retinopathy, and nephropathy. These syndromes do not only cause severe physical pains and psychological distresses in patients but also bring about huge economic burdens to the patients, family and community. Hyperglycemia remains a main detriment and is the first complication required to be strictly controlled in the management of diabetes. Insulin supplement is one of the most effective methods to reduce hyperglycemic effects and normalize other syndromes of diabetes [2]. However, some patients with diabetes cannot tolerate this treatment [3], and long-term injection of exogenous insulin may cause inconvenience to some patients.

Canagliflozin (Pubchem CID: 24812758) is a newly developed treatment for type 2 diabetes whose mechanism of action is independent of insulin; instead, it involves the inhibition of sodium-glucose cotransporter-2 (SGLT2) in

[Received on] 17-Dec.-2015
[Research funding] This work was supported by National Natural Science Foundation of China (No. 81373460), Natural Science Foundation of Guangdong Province (No. 2014A030313744), Shenzhen Science and Technology R&D Foundation (SGLH20121008144756945), and China Scholarship Council (201308440130).
[*Corresponding author] Tel/Fax: 86-755-26036086, E-mail: xiewd@sz.tsinghua.edu.cn.
These authors have no conflict of interest to declare.

kidneys, blocking the reabsorption of renal glucose [4]. Although canagliflozin alone shows effective hypoglycemic effects, the frequently reported adverse events still occur, such as female genital mycotic infections, urinary tract infections, increased urination, and severe renal impairment [5]. Therefore, the independent use of canagliflozin may complicate the treatment of diabetic nephropathy, a frequently occurring complication. Canagliflozin combined with metformin [6], sulfonylurea [7], insulin [8], and pioglitazone [9] is used to treat diabetes, demonstrating good tolerances and compatibilities. However, these combinations cannot completely attenuate the adverse effects of canagliflozin.

Berberine (Pubchem CID: CID 2353) is one of the isoquinoline alkaloids from a traditional Chinese medicine with broad-spectrum antimicrobial properties and antidiabetic activities [10-11]. Berberine seems to be able to improve kidney functions [12]. Therefore, we suspected that the combination of berberine and canagliflozin (BC) could enhance the antidiabetic properties with reduced adverse effects in diabetic individuals. In the present study, we investigated the synergic effects of BC on streptozotocin (STZ)-induced diabetic mice.

Materials and methods

Animals

Male 4-week-old NIH mice were purchased from Guangdong Medical Laboratory Animal Center, Foshan, China. The animals were housed in an environmentally controlled breeding room (temperature, 20 ± 2 °C; humidity, 60% ± 5%; dark/light cycle, 12 h/12h). The mice were fed with standard laboratory chow and water *ad libitum*. The experiment was performed in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the Institutional Animal Care and Use Committee of Tsinghua University, Beijing, China. The protocol was approved by the Animal Welfare and Ethics Committee of Tsinghua University. All animals were fasted from 09 : 00 h to 15 : 00 h before the experiments.

Animal experimental procedures

Male NIH mice (weighing 18 ± 2 g) that were fasted for 24 h were used as diabetic models with intraperitoneal injection of 100 mg·kg^{-1} STZ (Sigma-Aldrich, St. Louis, MO, USA). One week after STZ injection, the diabetic animals were divided into five groups (n =10/group): normal and diabetic control mice, berberine (100 mg·kg^{-1}·d^{-1}, ShangHai Biochempartner Co., Ltd., Shanghai, China), canagliflozin (100 mg·kg^{-1}·d^{-1}, ShangHai Biochempartner Co., Ltd., Shanghai, China), and beberine combined with canagliflozin (BC, 100 mg·kg^{-1}·d^{-1} of canagliflozin plus 100 mg·kg^{-1}·d^{-1} of berberine). The drugs were freshly prepared and orally administered at 0.1 mL/10 g body weight twice a day. Normal and diabetic control mice were treated with identical volumes of distilled water. Diet and water intakes were periodically measured in metabolic cages within 24 h. Urinal excretions were periodically collected in metabolic cages overnight and then stored at −80 °C for further analysis. Blood glucose was assayed once a week. After 4 weeks of drug administration, the animals were fasted for 6 h and then subjected to slight anesthesia by intraperitoneal injection of 10% urethane (10 g·100 mL^{-1} in phosphate-buffered saline, PBS) at a dosage of 0.1 mL/10 g body weight. Blood samples were collected from the orbital venous plexus, and sera were instantly isolated for further biochemical assays. The animals were terminated by cervical dislocation, and kidneys were collected and weighed. The left kidneys were frozen transiently in liquid nitrogen and stored at −80 °C for Western blot and biochemical assays. The right kidneys were fixed in 10% formalin-containing PBS for immunohistochemistry (IHC) assays.

Oral glucose tolerance test (OGTT)

OGTT was conducted as previously described with slight modifications [13]. After 4 weeks of drug administration, six mice from each group were randomly selected and fasted for 6 h. Blood samples were collected from the tail veins to determine the blood glucose levels at 0, 0.5, 1, and 2 h after glucose (2.5 g·kg^{-1}) administration. Blood glucose concentration was analyzed using a blood glucose meter (ACCU-CHEK®, Roche, German). The area under the curve (*AUC*) of blood glucose and time was calculated as $AUC_{0\text{-}2h} = [(G_{0h} + G_{0.5h}) \times 0.5\ h + (G_{0.5h} + G_{1h}) \times 0.5\ h + (G_{1h} + G_{2h}) \times 1\ h]/2$, where G is the blood glucose value.

Biochemical analysis

Blood glucose, triglycerides, total cholesterol, low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c), urea nitrogen (BUN), and creatinine (Cre) levels were analyzed using common clinical diagnostic kits (Biosino Bio-technology and Science Inc., Beijing, China). Serum insulin level was assayed according to the method of ELISA (Westang Biotechnology Co., Ltd., Shanghai, China). Malondialdehyde (MDA) level and superoxide dismutase (SOD) activity in kidneys were measured using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Western blot and IHC analyses

For Western blot analysis, the left kidneys of six randomly selected mice in each group were obtained. Briefly, 10% of the whole left kidney homogenates in PBS were centrifuged at 10 000 r·min^{-1} at 4 °C for 10 min. The supernatants were separated by 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene fluoride (PVDF) membranes. The membranes were blocked with 5% nonfat dry milk in PBS containing 0.1% Tween-20 (*V*/*V*, PBST) for 1–2 h and incubated with primary antibody (5' AMP-activated protein kinase *α*, AMPK; phosphorylates AMPK, pAMPK; Glyceraldehyde 3-phosphate dehydrogenase, GAPDH; Cell Signaling TECHNOLOGY®) in 2.5% nonfat dry milk/PBST at 4 °C overnight. After rinsing thrice with PBST, the membranes