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Original Research

Gallic acid ameliorates hyperglycemia and improves hepatic carbohydrate metabolism in rats fed a high-fructose diet

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ARTICLE INFO

Article history:
Received 27 May 2015
Revised 2 October 2015
Accepted 8 October 2015

Keywords:
Gallic acid
Rat
Hyperglycemia
Insulin resistance
Carbohydrate metabolism
High-fructose diet

ABSTRACT

Herein, we investigated the hypoglycemic effect of plant gallic acid (GA) on glucose uptake in an insulin-resistant cell culture model and on hepatic carbohydrate metabolism in rats with a high-fructose diet (HFD)–induced diabetes. Our hypothesis is that GA ameliorates hyperglycemia via alleviating hepatic insulin resistance by suppressing hepatic inflammation and improves abnormal hepatic carbohydrate metabolism by suppressing hepatic gluconeogenesis and enhancing the hepatic glycogenesis and glycolysis pathways in HFD-induced diabetic rats. Gallic acid increased glucose uptake activity by 19.2% at a concentration of 6.25 μg/mL in insulin-resistant FL83B mouse hepatocytes. In HFD-induced diabetic rats, GA significantly alleviated hyperglycemia, reduced the values of the area under the curve for glucose in an oral glucose tolerance test, and reduced the scores of the homeostasis model assessment of insulin resistance index. The levels of serum C-peptide and fructosamine and cardiovascular risk index scores were also significantly decreased in HFD rats treated with GA. Moreover, GA up-regulated the expression of hepatic insulin signal transduction–related proteins, including insulin receptor, insulin receptor substrate 1, phosphatidylinositol-3 kinase, Akt/protein kinase B, and glucose transporter 2, in HFD rats. Gallic acid also down-regulated the expression of hepatic gluconeogenesis–related proteins, such as fructose-1,6-bisphosphatase, and up-regulated expression of hepatic glycogen synthase and glycolysis-related proteins, including hexokinase, phosphofructokinase, and aldolase, in HFD rats. Our findings indicate that GA has potential as a health food ingredient to prevent diabetes mellitus.

Abbreviations: 2-NBDG, 2-(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-2-deoxyglucose; A/G, albumin/globulin; Akt/PKB, Akt/protein kinase B; Alk-P, alkaline phosphatase; $AUC_{glucose}$, area under the curve for glucose; Bili-total, total bilirubin; BUN, blood urea nitrogen; CAT, catalase; DM, diabetes mellitus; F-1,6-BP, fructose-1,6-bisphosphatase; GA, gallic acid; GLUT2, glucose transporter 2; GSH-Px, glutathione peroxidase; GS, glycogen synthase; HDL-C, high-density lipoprotein cholesterol; HFD, high-fructose diet; HOMA-IR, homeostasis model assessment of insulin resistance; HXK, hexokinase; IR, insulin receptor; IRS-1, insulin receptor substrate 1; OGTT, oral glucose tolerance test; PBS, phosphate-buffered saline; PFK, phosphofructokinase; PI3K, phosphatidylinositol-3 kinase; Pio, pioglitazone hydrochloride; SOD, superoxide dismutase; STZ, streptozotocin; T2DM, type 2 diabetes mellitus; TG, triglyceride; TNF-α, tumor necrosis factor α.

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http://dx.doi.org/10.1016/j.nutres.2015.10.001
0271-5317/

1. Introduction

Diabetes mellitus (DM) causes dysfunction of carbohydrate, protein, and lipid metabolism, which eventually leads to a number of secondary complications. The World Health Organization estimates that more than 346 million people worldwide have DM and that this number is likely to double by 2030 without intervention [1]. More than 95% of all patients with DM have type 2 DM (T2DM) and exhibit characteristic insulin resistance. Fructose-induced insulin resistance is commonly characterized by profound metabolic dyslipidemia that results from hepatic and intestinal overproduction of atherogenic lipoprotein particles [2]. Rats fed a high-fructose diet (HFD) are widely used as a model for investigating T2DM-associated insulin resistance [3]. After long-term fructose feeding, the rats develop insulin resistance, reduced liver antioxidant status, and increased lipid peroxidation [4].

The consumption of fresh fruits, vegetables, and plants that are rich in natural antioxidants is associated with the prevention of DM [5]. Plant-derived polyphenols possess a wide range of pharmacological properties, and their mechanism of action has been the subject of considerable interest in recent years. The endogenous plant phenol gallic acid (GA) (3,4,5-trihydroxybenzoic acid) has received much attention because of its potent free radical scavenging and antihyperglycemic activity [6]. Gallic acid is abundant in tea, grapes, berries, fruits, and wine. The anti-inflammatory, anticancer, and antioxidant effects of GA have been reported [7]. Gallic acid exhibits antihyperglycemic, antilipid peroxidative, and antioxidant activities in streptozotocin (STZ)–induced diabetic rats [8]. Punithavathi et al [6] demonstrated that oral treatment with GA (10 and 20 mg/kg) significantly diminishes the levels of blood glucose and glycosylated hemoglobin and improves the levels of plasma insulin in STZ-induced diabetic rats.

The HFD-induced diabetic rat model has been shown to mimic many symptoms of T2DM in humans, particularly insulin resistance, glucose intolerance, dyslipidemia, renal impairment, and hypertension [9]. The prevalence of obesity, diabetes, and other metabolic syndromes has been linked to increased consumption of fructose-containing foods [3]. However, to the best of our knowledge, no studies have studied the mechanism of GA action on glucose metabolism in HFD-induced T2DM. Therefore, we investigated the effect of GA on glucose uptake in an insulin-resistant cell model and on hepatic insulin signal transduction and glucose metabolism in rats with HFD-induced diabetes.

2. Methods and materials

2.1. Chemicals

Bovine serum albumin, bromophenol blue, D-(+)-glucose, dimethyl sulfoxide, disodium hydrogen phosphate, ethyl ether, ethyl alcohol, fructose, GA (purity >97.5%, wt/wt), Ham's F-12K (Kaighn's) (F12K) medium, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, insulin, pioglitazone hydrochloride (Pio), potassium chloride, potassium dihydrogen phosphate, sodium chloride, sodium phosphate dibasic, thiourea, urea, recombinant mouse tumor necrosis factor α (TNF-α), sulfuric acid, Triton X-100, and N,N,N,N′-tetramethylethylenediamine were all purchased from Sigma (St Louis, MO, USA). Fetal bovine serum was obtained from Gemini Bio-Products (Woodland, CA, USA). The fluorescent dye 2-(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-2-deoxyglucose (2-NBDG) was purchased from Invitrogen (Camarillo, CA, USA). Bio-Rad protein assay dye reagent was obtained from Bio-Rad Laboratories (Richmond, VA, USA). All of the chemicals used in this study were of analytical grade. The antioxidant enzymes, including catalase (CAT), glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD), were purchased from Sigma. Anti-insulin receptor (IR), anti-phosphatidylinositol-3 kinase (PI3K), anti-Akt/protein kinase B (Akt/PKB), and anti-glycogen synthase (GS) antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA). Anti-actin, anti-hexokinase (HXK), anti-phosphofructokinase (PFK), anti-aldolase, anti-fructose-1,6-bisphosphatase (F-1,6-BP), anti-insulin receptor substrate 1 (IRS-1), and anti-glucose transporter 2 (GLUT2) antibodies were purchased from Gene Tex (Irvine, CA, USA).

2.2. Cell culture

Experiments were performed on mouse liver FL83B cells, a hepatocyte cell line derived from a fetal mouse (15-17 days). The cells were incubated in F12K containing 10% fetal bovine serum and 1% penicillin and streptomycin (Invitrogen Corporation, Camarillo, CA, USA) in 10-cm Petri dishes at 37°C and 5% CO_2. The experiments were performed once the cells reached 80% to 90% confluency.

2.3. Uptake of fluorescent 2-NBDG in FL83B mouse hepatocytes

Glucose uptake was assayed referring to the method of Chang and Shen [10]. Briefly, FL83B cells seeded in 10-cm dishes were incubated at 37°C for 48 hours to 80% confluency. Serum-free F12K medium containing 20 ng/mL recombinant mouse TNF-α was added, and the cells were incubated for 5 hours to induce insulin resistance. The effect of GA on the uptake of 2-NBDG in insulin-resistant hepatocytes was then evaluated. FL83B cells were detached with trypsin and suspended in 1200 μL of Krebs-Ringer bicarbonate buffer containing 1 μmol/L insulin. Aliquots of the cell suspension (172 μL) were transferred to Eppendorf tubes and coincubated with 20 μL of 6.25 μg/mL GA and 8 μL of fluorescent 2-NBDG (the final concentration of 2-NBDG was 200 μmol/L) in a 37°C water bath for 1 hour in the dark. The reaction was stopped by placing it on ice. The cell suspension was then centrifuged at 3000*g* (4°C) for 5 minutes to remove the supernatant. The pellet was washed with phosphate-buffered saline (PBS), centrifuged 3 times, and suspended in 1 mL of PBS. The fluorescence intensity of the cell suspension was evaluated by flow cytometry (FACScan; Becton Dickinson, Franklin Lakes, NJ, USA) at an excitation wavelength of 488 nm and an emission wavelength of 542 nm. The fluorescence intensity reflected the cellular uptake of 2-NBDG.

2.4. Animals and diets

Male Wistar rats (5 weeks of age) were obtained from the National Laboratory Animal Center, Taipei, Taiwan. The rats