Gene 570 (2015) 213–220

Contents lists available at ScienceDirect

Gene

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

Research paper

Resveratrol improves hepatic insulin signaling and reduces the inflammatory response in streptozotocin-induced diabetes

Gökhan Sadi [a,*], Mehmet Bilgehan Pektaş [b], Halit Bugra Koca [c], Murat Tosun [d], Tulay Koca [e]

[a] Department of Biology, K.Ö.Science Faculty, Karamanoglu Mehmetbey University, Karaman, Turkey
[b] Department of Medical Pharmacology, Faculty of Medicine, Afyon Kocatepe University, Afyonkarahisar, Turkey
[c] Department of Medical Biochemistry, Faculty of Medicine, Afyon Kocatepe University, Afyonkarahisar, Turkey
[d] Department of Histology Embryology, Faculty of Medicine, Afyon Kocatepe University, Afyonkarahisar, Turkey
[e] Department of Medical Laboratory, Ataturk Vocational School of Health Services, Afyon Kocatepe University, Afyonkarahisar, Turkey

ARTICLE INFO

Article history:
Received 20 March 2015
Received in revised form 23 May 2015
Accepted 6 June 2015
Available online 10 June 2015

Keywords:
Diabetes
Resveratrol
Liver
Resistin
Insulin signaling
PI3K/Akt

ABSTRACT

Diabetes mellitus is a heterogeneous metabolic disorder essentially characterized by deficiency of insulin secretion, insulin receptor or post-receptor events. This study aims to investigate the effects of resveratrol administration on the metabolic characteristics, hepatic functions, histopathological features and insulin signaling pathway components in streptozotocin induced diabetes.

Male Wistar rats were randomly divided into four groups: (1) control/vehicle; (2) control/20 mg/kg resveratrol; (3) diabetic/vehicle; and (4) diabetic/20 mg/kg resveratrol. Histopathological examinations were carried out to reveal hepatic tissue damage and inflammation. In addition to hepatic glucose, lipid, insulin, ALT, AST, resistin and XOD contents, gene and protein expressions of insulin signaling pathway components such as insulin Rβ, IRS-1, IRS-2, eNOS, PI3K, Akt, and FOXO3a were analyzed by qRT-PCR and Western blot. The rats in the diabetes group had significantly lower terminal body weight and hepatic insulin level, but significantly higher hepatic glucose, total cholesterol, triglyceride and resistin concentrations. Diabetes triggered the inflammatory process in the liver tissues that was evidenced by histopathological deformations and increase in the hepatic ALT and AST levels. Hepatic inflammation was considerably associated with insulin signaling pathway ever since a significant down-regulation of insulin signaling components; IRS-1, IRS-2, PI3K, Akt and mTOR have been identified in the diabetic group. To some extent, resveratrol treatment reversed the diabetes-induced changes in the liver tissues. Taken together, resveratrol partly improved hepatic dysfunction induced by diabetes. This may be due to the healing activity of resveratrol on insulin signaling pathway, resistin levels and hepatic glucose-lipid contents.

1. Introduction

Diabetes mellitus is a systemic disorder which affects carbohydrate, protein and lipid metabolism due to decreased insulin levels in blood or its reduced action on cells. Decreased insulin secretion and/or insulin resistance, which are the hallmarks of diabetes, lead to hyperglycemia and dysfunction in a variety of tissues including the liver (Skov et al., 2012).

Insulin exerts its effects through the key proteins acting at cellular levels. These proteins start from insulin receptor (insulin Rβ) and continue up to several transcription factors regulating the cellular activities. Binding of insulin to its receptor activates several adaptor proteins, such as the members of the insulin receptor substrate family proteins (IRS) playing a key role in transmitting signals from insulin to intracellular pathways one of which is a highly conserved and tightly controlled PI3K/Akt pathway. Among the pathway components, phosphatidylinositol 3-kinase (PI3K) has a major role in insulin function, mainly *via* the activation of a serine–threonine kinase known as Akt or protein kinase B (PK-B). Akt is recruited to the plasma membrane (Lizcano and Alessi, 2002) and once active, it enters into the cytoplasm and leads to the phosphorylation of several substrates acting on gluconeogenesis and glycogenolysis. Activated Akt also induces glycogen synthesis through the inhibition of glycogen synthase kinase-3 (GSK-3);

Abbreviations: Akt, serine/threonine–protein kinase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Diab, diabetic; eNOS, endothelial nitric oxide synthase; FOXO3a, forkhead box O3a; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GLUT4, glucose transporter, type 4; H&E, hematoxylin and eosin; HRP, horseradish peroxidase; IR, insulin receptor; IRS-1, IRS-2, insulin receptor substrate-1/2; mTOR, mammalian target of rapamycin; PAGE, polyacrylamide gel electrophoresis; PI3K, phosphatidylinositol 3-kinase; PMSF, phenylmethanesulfonylfluoride; PVDF, polyvinylidene fluoride; qRT-PCR, quantitative real-time PCR; Res, resveratrol; SDS, sodium dodecyl sulfate; STZ, streptozotocin; XOD, xanthine oxidase.

* Corresponding author at: Department of Biology, K.Ö.Science Faculty, Karamanoglu Mehmetbey University, 70100 Karaman, Turkey.
E-mail addresses: sadi@kmu.edu.tr, sadi.gokhan@gmail.com (G. Sadi), mbpektas@hotmail.com (M.B. Pektaş), bugrakoca@yahoo.com (H.B. Koca), drmtosun@yahoo.com (M. Tosun), tulay_akan@yahoo.com (T. Koca).

http://dx.doi.org/10.1016/j.gene.2015.06.019
0378-1119/

protein synthesis *via* mammalian target of rapamycin (mTOR); and cell survival through the inhibition of several pro-apoptotic agents such as forkhead transcription factors (FOXO), thereby regulates the intracellular metabolism. PI3K and Akt are also known to play a role in type 4 glucose transporter (GLUT4) translocation (Lizcano and Alessi, 2002). Being one of the substrates of Akt, a serine–threonine kinase mTOR integrates multiple signals to promote either cellular growth or catabolic processes during stress conditions. Furthermore, FOXO proteins, which are excluded from the nucleus following phosphorylation by Akt, play a role in the regulation gene expression induced by insulin (Barthel et al., 2001; Schmoll et al., 2000).

There is a growing body of evidence for the roles of both oxidative stress and inflammatory activity in the pathogenesis of diabetes mellitus. Resveratrol (RSV), a naturally occurring polyphenol found in grapes and red wine, has recently been shown to exert potent anti-diabetic, anti-oxidative and anti-inflammatory actions (Chang et al., 2012; Hamadi et al., 2012). It was also shown to regulate the activities of IRS-1, IRS-2, sirtuin-1 and FOXO3a pathways in tissues (Babacanoglu et al., 2013; Yu et al., 2013). Engagement of resveratrol with the insulin receptor has been found to induce IRS-1 and IRS-2 phosphorylation (Liu et al., 2015). Phosphorylated IRS-1 and IRS-2 may act through two different pathways. In the first pathway, phosphorylated IRS-1 induces PI3K and Akt phosphorylation and increases endothelial nitric oxide synthase (eNOS) phosphorylation to regulate tissue functions (Symons et al., 2009); while in the second pathway, endothelin-1 release is triggered by providing mitogen activated protein kinase activation (Deng et al., 2008).

Recently, we have publicized that impaired glucose metabolism in the liver tissues leads to adverse effects on the insulin signaling pathway by affecting IRS-1, IRS-2, eNOS and sirtuin-1 protein contents (Babacanoglu et al., 2013; Sadi et al., 2014). Based on these findings, to understand tissue-specific molecular alterations in diabetes and *in vivo* effects of resveratrol, we hypothesized that diabetes-related alterations in hepatic dysfunction and insulin signaling pathway in the liver tissues could be returned to normal values with resveratrol. To make track for the concrete molecular action mechanism of the resveratrol through the regulation of the liver function, the present study was designed to investigate the effects of resveratrol on the metabolic characteristics, hepatic functions, histopathological features and insulin signaling pathway components in an animal model of streptozotocin (STZ)-induced diabetes.

2. Materials and methods

2.1. Chemicals

Trans-resveratrol was purchased from Molekula (Gillingham, Dorset, UK) and STZ was obtained from Sigma (St. Louis, MO, USA). Total RNA isolation kits were obtained from Qiagen (Venlo, Netherlands) and reagents for cDNA synthesis were from Thermo Scientific (Burlington, Canada). SYBR Green I Master Mix was procured from Roche (Foster City, CA, USA). Antibodies were supplied from Abcam (Cambridge, MA, USA) and Santa Cruz (Santa Cruz, CA, USA). PVDF (polyvinylidene fluoride) membranes were acquired from Bio-Rad (Hercules, California, USA). All other chemicals used in this study were of the highest analytical grade available, and the buffers were prepared using sterile ultra pure water.

2.2. Animals and treatment procedure

The animal protocols were confirmed by the Ethical Animal Research Committee of Karamanoglu Mehmetbey University (K.M.U. ET-11/01-02). Eight week old male Wistar rats were housed under temperature-controlled rooms (20–22 °C) with a 12-h light–dark cycle. The animals were fed with standard rodent diet composed of 62% starch, 23% protein, 4% fat, 7% cellulose, standard vitamins and salt mixture (chow pellet). After acclimation for 1 week, animals were randomly assigned to 4 groups. The control group included 12 rats which were injected only vehicle; 10% dimethyl sulfoxide (DMSO) for 4-weeks. The resveratrol group consisted of 12 rats which were given a daily intraperitoneal dose of 20 mg/kg/day resveratrol in vehicle throughout the 4-week period. The diabetes group included 12 rats which received a single dose of STZ (55 mg/kg) dissolved in 0.05 M citrate buffer (pH: 4.5) and daily vehicle for 4-weeks. The diabetes + resveratrol group consisted of 9 rats which received a daily intraperitoneal dose of 20 mg/kg/day resveratrol throughout the 4-week period, starting from 2 days after STZ administration. Blood glucose concentrations were determined by Accu-check-go (Roche, Germany) glucometer weekly from the blood of the tail veins. The criteria for the diabetes were the blood glucose concentration higher than 200 mg/dl. At the end of the study period, all rats were decapitated and the liver tissues were blotted dry, frozen in liquid nitrogen, and stored at −85 °C for further use.

2.3. Measurement of hepatic glucose, lipids, insulin, ALT, AST, resistin and XOD levels

Tissue samples were homogenized with phosphate buffer 1:10 (w/v), 0.1 M, pH 7.4, centrifuged at 10,000 *g* for 10 min and then supernatants were collected. Total protein contents were measured with the Lowry method (Lowry et al., 1951). The levels of hepatic glucose, total cholesterol, triglycerides (Spinreact, Santa Coloma, Spain), alanine transaminase (ALT), aspartate transaminase (AST) (IFCC, SingleVial, Biolabo SA, Maizy, France) and xanthine oxidase (XOD) (Cusabio, Wuhan Hi-tech Medical Devices Park, P.R. China) were determined by standard enzymatic techniques. Insulin (DRG Instruments GmbH, Germany) and resistin (BioVendor-Laboratornimedicina, Czech Republic) concentrations were measured using commercially available specific Rat ELISA kits according to the manufacturer's protocols.

2.4. Determination of gene expressions of insulin Rβ, irs-1, irs-2, enos, pi3k, akt, mtor and foxo3a with real time polymerase chain reaction

Total RNAs were isolated from the liver tissues using the RNeasy total RNA isolation kit (Qiagen, Venlo, Netherlands) as described according to the manufacturer's protocol. After isolation, the amount and quality of the total RNA were determined using spectrophotometry at 260/280 nm and the Agilent 2100 bioanalyzer (Santa Clara, USA). 1 μg of total RNA was reverse transcribed to cDNA using the commercial first strand cDNA synthesis kit (Thermo Scientific, USA). Gene expressions were determined by mixing 1 μl cDNA, 5 μl SYBR Green Master mix (Roche FastStart Universal SYBR Green Master Mix) and primer pairs (Table 1) at 0.5 μM final concentrations in a total volume of 10 μl as described in detail elsewhere (Sadi et al., 2014). The relative expression of genes with respect to the internal control, *gapdh* (glyceraldehyde 3-phosphate dehydrogenase) were calculated with the efficiency corrected advance relative quantification tool provided by the LightCycler® 480 SW 1.5.1 software.

2.5. Immunoblot analysis of insulin Rβ, IRS-1, eNOS, PI3K, Akt and p-Akt

After homogenization in appropriate medium (50 mM Tris, 150 mM NaCl, 5 mM EDTA, 1% (w/w) Triton X-100, 0.26% (w/v) sodium deoxycholate, 50 mM sodium fluoride, 0.1 mM sodium orthovanadate and 0.2 mM phenylmethanesulfonylfluoride; PMSF), protein concentrations were determined (Lowry et al., 1951). Insulin Rβ, IRS-1, eNOS, PI3K, Akt and p-Akt protein contents were determined by immunoblot analysis as described previously (Sadi et al., 2014). Briefly, homogenates containing 50 μg of proteins were separated by SDS-PAGE and electroblotted onto PVDF membranes. After incubation with primary and secondary antibodies, protein bands corresponding to interested proteins were visualized by Clarity™ Western ECL (Bio-Rad Laboratories,