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The Protective Role of Resveratrol on Diabetic Cardiomyopathy in Streptozocin Induced Diabetic Rats

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Abstract

Objectives: We aimed to investigate the effect of resveratrol on diabetic cardiomyopathy in streptozocin-induced diabetic rats.

Materials and Methods: Rats were injected with streptozocin to establish diabetes model. After four weeks, heart tissues were collected for histopathological examination and immunoexpression of nitric oxide synthases-2 (NOS-2) and transforming growth factor-β1 (TGF-β1). Lipid peroxidation was evaluated.

Results: In diabetic rats, cardiac muscle cell thickness (hypertrophy), TGF-β1 and NOS-2 expression were increased significantly when compared to control group. Administration of resveratrol in diabetic rats causes a significant reduction both in cardiac muscle cell thickness, TGF-β1 and NOS-2 expression in these rats.

Blood glucose levels were significantly increased in diabetic rats expectedly, but there was no important difference between diabetic rats and resveratrol administrated diabetic rats in terms of blood glucose levels.

Conclusion: We showed protective effects of resveratrol on dilated cardiomyopathy on diabetic rats by reducing oxidative stress. As the prevalence of diabetes mellitus is increasing, resveratrol supplementation could help preventing diabetic cardiomyopathy.

Keywords: Resveratrol, diabetic cardiomyopathy, type 2 diabetes mellitus

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Introduction

Diabetic cardiomyopathy (DCM) is a clinical entity diagnosed when ventricular dysfunction occurs after excluding coronary atherosclerosis and hypertension[1]. The mechanism leading to DCM is not clear yet but myocardial hypertrophy and fibrosis have been shown to be the major pathogenesis of DCM[2]. Activation of reactive oxygen species (ROS) linked pathways play a major role in the pathogenesis of myocardial hypertrophy and fibrosis[2].

Malondialdehyde (MDA) is a three-carbon low molecular weight aldehyde produced from free radical species of poly unsaturated fatty acids reflecting the degree of lipid peroxidation[3]. Transforming growth factor-β1 (TGF-β1) is a multifunctional cytokine regulating cell proliferation and extracellular matrix production which leads to fibrosis in tissues and organs when produced excessively[4]. Nitric oxide synthases-2 (NOS-2) is absent in healthy states, rather it's expressed under inflammatory conditions[5].

Resveratrol is a polyphenol mainly found in grape skin and seeds[6]. Resveratrol has a wide range of protective effect on ageing, inflammation and glycation[7]. In this study, we evaluated the protective role of resveratrol on streptozocin-induced diabetic rats.

Materials and Methods

Animals

Male Sprague Dawley albino mature rats at eight weeks, weighing 200-220 g, were used in the experiments. The animals had access to food and water ad libitum. The animals were housed under a temperature-controlled environment (22-24°C) with light/dark cycles of 12:12 hours. Experimental procedures were approved by the Committee for Animal Research of Celal Bayar University. All animal studies are strictly conformed to the animal experiment guidelines of the Committee for Human Care.

Experimental protocol

Diabetes was induced by intraperitoneal (i.p.) injection of streptozocin (STZ, Sigma-Aldrich, Inc.; Saint Louis, MO, USA) (60 mg/kg in 0.9% NaCl, adjusted to a pH 4.0 with 0.2 M sodium citrate) for 14 rats. Remaining rats without streptozocin injection were selected as control group (n=6). Diabetes was verified as blood glucose levels of 250 mg/dL and higher after 24 hours by evaluating blood glucose levels with the use of glucose oxidase reagent strips (Boehringer-Mannheim, Indianapolis). Diabetic rats were randomly divided into two groups; diabetes group treated with 1 mL/kg saline (Diabetes) (n=6), and diabetes group treated with 10 mg/kg/day resveratrol (Sigma Aldrich), (Diabetes + resveratrol) (n=6) was administrated by i.p. for four weeks.

The animals were euthanized and blood samples were collected by cardiac puncture. Removal of the heart was performed for histopathological examination.

Histopathological examination of heart tissue

All animals were anesthetized by an i.p. of ketamin (40 mg/kg, (40 mg/kg, Alfamine®, Ege Vet, Alfasan International B.V., Holland)/xylazine (4 mg/kg, Alfazyne®, Ege Vet, Alfasan International B.V., Holland) and formaldehyde was used for histological and immunohistochemical studies. Formalin-fixed hearts cut into 5 µm sections were stained with hematoxylin and eosin (H&E). All sections were photographed with Olympus C-5050 digital camera mounted on Olympus BX51 microscope.

Computerized image analysis system was used to assess morphological analysis. Heart muscle cell hypertrophy degree was examined by light microscopy. Thickness of muscle cells was calculated from the cross-sectional image. Muscle fiber was measured by image analysis software (Image- Pro Express 1.4.5, Media Cybernetics, Inc. USA). Average of 50 cardiac muscle cell from each animal was used for analysis.

NOS-2, TGF-β1 immunoexpression

For immunohistochemistry, sections were incubated in primary antibodies (TGF-β1, NOS-2 Bioss, Inc.; 1/100) for 24 h at 4°C. Histostain-Plus Bulk kit (Bioss, Inc) against rabbit IgG was used to detect

antibody, and the final product was visualized by 3.3' diaminobenzidine (DAB). All sections were washed in PBS and photographed with an Olympus C-5050 digital camera mounted on Olympus BX51 microscope.

Measurement of plasma TGF-β

Plasma TGF-β were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kit (Biosciences). TGF-β levels were expressed as pg/mL.

Evaluation of lipid peroxidation

Malondialdehyde (MDA) levels as thiobarbituric acid reactive substance (TBARS) was measured for the evaluation of lipid peroxidation. MDA levels were expressed as nM and tetraethoxypropane was used for calibration.

Statistical Analysis

Non-parametric (Mann-Whitney U) test was used to assess all quantitative data. Between-group differences were assessed by Student's t-test. All data are shown as mean values ± standard error of the mean. P values of <0.05 were regarded as statistically significant. All analyses were performed using SPSS v.21.0 for Windows (SPSS, Inc., Chicago, Illinois, USA).

Results

In diabetic rats, cardiac muscle cell thickness (hypertrophy), TGF-β1 and NOS-2 expression were increased significantly when compared to control group (Figure 1 and 2). Administration of resveratrol in diabetic rats causes a significant reduction both in cardiac muscle cell thickness, TGF-β1 and NOS-2 expression in these rats (Table 1).

Diabetic rats had significantly higher levels of blood glucose levels but there was no significant difference between diabetic rats and resveratrol administrated diabetic rats in terms of blood glucose levels (Table 1). Plasma levels of TGF-β1 and MDA were significantly elevated in diabetic rats and resveratrol administration caused significant reduction (Table 2).

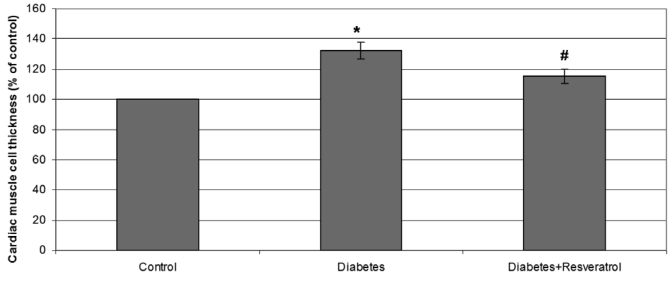

Figure 1. Cardiac muscle cell thickness in rats
**p<0.001 Control group compared diabetes*
#p<0.05 Diabetes group compared diabetes+resveratrol

Discussion

In this present study, resveratrol reduced oxidative stress and myocardial hypertrophy in streptozocin-induced diabetic rats. Diabetes mellitus is a condition defined by elevations on blood glucose levels leading to deaths mostly from cardiovascular causes[8]. Hyperglycemia and insulin resistance contributes to impaired mitochondrial calcium handling and oxidative stress leading to cardiac hypertrophy and fibrosis[9].

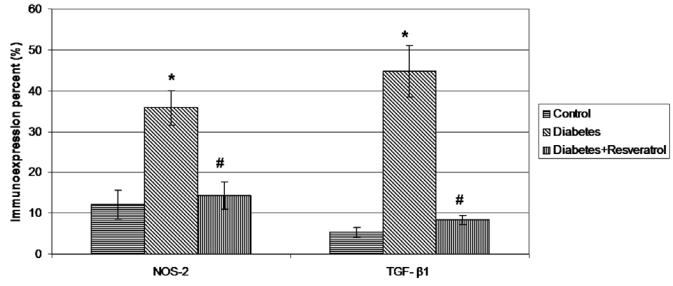

Figure 2. Immunoexpression percent of NOS-2 and TGF-β1
**p<0.05 Control group compared diabetes*
#p<0.01 Diabetes group compared diabetes+resveratrol

Table 1. Cardiac muscle cell thickness, TGF- β1 and NOS-2 immunoexpression and blood glucose levels of rats

	Control	Diabetes	Diabetes and resveratrol
Cardiac muscle cell thickness (% of control)	100	132.3±5.6	115.2±4.8
Immunoexpression NOS-2 percent (%)	12.1±3.5	35.8±4.2	14.3±3.4
Immunoexpression TGF-β1 percent (%)	5.3±1.2	44.7±6.3	8.4±1.1
Blood glucose (mg/dL)	103.2±7.2	482.3±30.4	468.4±18.5

NOS-2: Nitric oxide synthases -2, TGF- β1: Transforming growth factor-b1

The role of resveratrol on blood glucose levels is conflicting. Lekli et al. showed that resveratrol reduces the blood glucose levels on diabetic rats[10]. However, some other studies suggested that resveratrol has no effect on blood glucose levels[11,12]. In our study, blood glucose levels were decreased, but this change was non-significant compared to diabetic rats who did not receive resveratrol treatment. These conflicting results in studies may suggest that the protective role of resveratrol on DCM is not related to improved hyperglycemia.

Cardiac fibrosis has a major role in the pathogenesis of DCM. TGF-β1 is a regulator cytokine for cell proliferation and excessive production of TGF-β1 leads to tissue fibrosis[13]. TGF-β1 has been showed to be overexpressed in DCM[14,15]. Zhang et al. investigated the role of microRNA-155 on myocardial fibrosis induced by diabetes in mice and showed that myocardial fibrosis was regulated via TGF-β1-Smad 2 signaling pathway[16]. Resveratrol had been shown to reduce pulmonary fibrosis in rats[17]. In our study, the level of TGF-β1 was upregulated in diabetic rats as shown in previous studies, and administration of resveratrol reduced the level of TGF-β1.

Oxidative stress is defined as an imbalance between generation of ROS and the antioxidant defense. Oxidative stress plays an important role in the process of diabetic complications[18]. Mitochondrial oxidative stress and ROS generation had been shown to be elevated in diabetic rats contributing to myocardial hypertrophy and fibrosis[19].

Table 2. Plasma levels of TGF- β1 and MDA

	TGF-Beta (pg/mL)	MDA (nM)
Normal Control (group 1)	10.2±2.8	94.2±11.4
Diabetes (saline treatment) (group 2)	43.5±5.1*	402.8±32.5*
Diabetic rat (resveratrol treatment) (group 3)	19.7±2.9#	286.1±22.3##

TGF- β1: Transforming growth factor-b1, MDA: Malondialdehyde
**p<0.000, saline tretment diabetic rats compared control group*
#p<0.001, resveratrol treatment diabetic rats compared saline tretment diabetic rats
##p<0.0001, resveratrol treatment diabetic rats compared saline tretment diabetic rats

Resveratrol is a well-confirmed antioxidant inhibiting excessive ROS production and lipid peroxidation[20]. MDA, final product of polyunsaturated fatty acid peroxidation, is indirectly reflecting the degree of cell damage. Fang et al. had shown increased expression of antioxidant enzymes and reduced MDA levels in diabetic mice treated with resveratrol[11]. In our study, the level of MDA was significantly increased as shown in previous studies, but administration of resveratrol reduced the level of MDA.

Nitric oxide synthases (NOS) mediates the synthesis of nitric oxide (NO), a short-living free radical that is an important mediator for cardiac contractility and vasodilatation[21]. NOS-2 isoform, known as induced NOS, is expressed only under unhealthy conditions contributing to endothelial dysfunction[21]. NOS-2 level was found to be elevated in cardiomyocytes in myocarditis, dilated cardiomyopathy[22]. Mungrue et al. showed that cardiomyocyte overexpression of NOS-2 resulted in peroxynitrite generation, heart block and sudden death[5]. In our study, the level of NOS-2 was significantly increased. Administration of resveratrol reduced the level of NOS-2.

Study Limitations

As a limitation of our study, streptozocin induced diameters mellitus may cause atherosclerotic heart disease, we didn't perform coronary artery histopathological examination. We may have missed atherosclerosis.

Conclusion

In conclusion, we demonstrated protective effects of resveratrol on dilated cardiomyopathy on diabetic rats by reducing oxidative stress. As the prevalence of DM is increasing, resveratrol supplementation could help preventing diabetic cardiomyopathy.

Ethics

Ethics Committee Approval: Experimental procedures were approved by the Committee for Animal Research of Celal Bayar University. All animal studies are

strictly conformed to the animal experiment guidelines of the Committee for Human Care.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: O.E., T.Ç., G.Y., Concept: İ.P.C., O.E., Design: İ.P.C., O.E., U.A., Data Collection or Processing: İ.P.C., O.E., Analysis or Interpretation: İ.P.C., O.E., Literature Search: İ.P.C., Writing: İ.P.C.

Conflict of Interest: No conflict of interest was declared by the authors.

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