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Original Research

Melatonin protects against streptozotocin-induced diabetic cardiomyopathy by the phosphorylation of vascular endothelial growth factor-A (VEGF-A)

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Abstract: The aim of the present study is to investigate if the melatonin has any protective effect on diabetic cardiomyopathy and antioxidant enzymes via phosphorylation of vascular endothelial growth factor-A (VEGF-A). A total of 40 male Wistar rats were enrolled in the study. Rats were divided into four groups: group 1 (control, n=10), group 2 (DM, n=10), group 3 (melatonin, n=10), and group 4 (melatonin+DM, n=10). Melatonin was injected intraperitoneally at a dose of 50 mg/kg/day for 56 days to group 3 and group 4. We investigated expression and phosphorylation of the VEGF-A in coronary vessels of all groups. Staining intensities, biochemical, immunohistochemistry analysis, and transthoracic echocardiography were performed. In comparison to the group 1, DM induced a decrease in p-VEGF-A in coronary vessels of group 2. The lower constitutive phosphorylation of VEGF-A in the group 2 was also increased in coronary vessels after melatonin treatment ($p<0.05$). Diabetic rats developed myocardial hypertrophy with preserved cardiac function ($p<0.05$). Cardio-protective effect of melatonin may reduce the damages of diabetes mellitus on the heart muscle fibers and coronary vessels via the phosphorylation of VEGF-A. Melatonin-dependent phosphorylation of VEGF-A in coronary angiogenesis may be associated with the physiological as well as with the pathological cardiac hypertrophy.

Key words: Diabetic cardiomyopathy; Diabetes mellitus; Echocardiography; Melatonin; Vascular endothelial growth factor-A.

Introduction

Hyperglycemia mediated oxidative stress plays an important role in the pathogenesis and development of various complications in diabetes mellitus (DM) (1, 2). Diabetic cardiomyopathy, which is defined as myocardial dysfunction in the absence of coronary artery disease or systemic hypertension, is one of these major DM complications (3). High glucose-induced alterations in the myocardium structure, including cardiac fibrosis, cardiomyocyte hypertrophy, and cardiac microvascular injury play important role in the pathophysiology of diabetic cardiomyopathy and cause loss of systolic and diastolic functions (4-6). Besides that, progressive reductions of the microvasculature and an impaired angiogenic response to chronic ischemia have been demonstrated with the development of DM (7, 8). Further, release of free radicals caused by high glucose levels and consequent oxidative stress accelerate lipid peroxidation in muscle fibers and that results in the damage of the normal structure and disruption of the myocardium (9, 10). These microvascular changes may lead to reduced perfusion of myocardium and contribute to adverse cardiovascular events (11).

VEGF is a pivotal regulator of physiological and pathological neovascularization (12, 13), playing crucial roles in developmental blood vessel formation and regulation of hypoxia-induced tissue angiogenesis (14). Previously it was shown that attenuated augmentation of VEGF expression in response to ischemia was responsible for the impaired recovery observed in diabetic animals (15). There is a possible role of decreased VEGF in the pathogenesis DM mediated impairment of myocardial angiogenesis (16).

Melatonin (N-acetyl-5-methoxytryptamine) is a circadian endocrine molecule that secreted by the pineal gland which has important functions such as powerful antioxidant, anti-apoptotic, free radical purifier (17). As it was shown in previous studies, melatonin is highly effective antioxidant that can prevent the harmful effects of DM on the heart and greatly depresses oxidative damage in myocardial cells (18, 19). In light of the potential protective role of melatonin against myocardial damage, it is particularly noteworthy to explore whether melatonin interrupts the progression of diabetic cardiomyopathy. In a DM setting, the beneficial effect of melatonin on diabetic cardiomyopathy is unknown. Therefore, the present study aims to investigate the effects on melatonin on antioxidant enzymes and on VEGF-A expression in coronary vessels and myocardial tissue of diabetic rats.

Materials and Methods

Number, strain and animal model

A total of 40 male Wistar rats (8 weeks of age wei-

Table 1. Definition of animal groups.

Groups	Injection	Dose	Period	n
Group 1 (Control)	Ethanol	10 mg/kg	For 56 days	10
Group 2 (DM)	Streptozotocin	130 mg/kg	First day	10
Group 3 (Melatonin)	Melatonin	50 mg/kg/day	For 56 days	10
Group 4 (Melatonin+DM)	Melatonin+ Streptozotocin	50 mg/kg/day + 130 mg/kg	For 56 days + First day	10

ghing 200-250 g) were used for experimental study. The experimental design was controlled by our university Institutional Animal Care and Use Committee Policies for Animal Use under an approved animal. As it was shown in Table 1, rats were randomly allocated into the following four groups; group 1 was control group, group 2 was DM group (Streptozotocin induced DM group), group 3 was melatonin treatment group (only melatonin administrated group), and group 4: melatonin treatment plus DM group (melatonin administrated group after DM induction). For induction of experimental DM (pancreatic insulin release was abolished iatrogenically by affecting Langerhans islet cells), rats were injected intraperitoneally with a single dose of Streptozotocin (STZ, 130 mg/kg, Sigma Chemical Co, St. Louis, MO, USA) in 0.1 mol/L citrate buffer with a pH 4.5. As described previously, successful induction of DM was defined as a constant blood glucose >300 mg/dL (20, 21).

Transthoracic echocardiography

Transthoracic echocardiography was performed in rats of all groups using the ultrasound system (iE33, Philips Medical Systems, Andover, MA, USA) with a 1-3 MHz broadband linear array transducer at initially and 8 weeks after induction of DM or injection of citrate buffer, respectively. After rats were anesthetized, heart was imaged in the 2-dimensional parasternal short-axis view three times over at least 20 cardiac cycles to assess reproducibility. Mid ventricle diameters were recorded at the level of the papillary muscle by the M-mode echocardiography, and interventricular septal and posterior wall thickness (IVS and LVPW), left ventricular end-diastolic and end-systolic internal diameters (LVEDD, LVESD) were determined. According to well established method, left ventricular mass (LVM) was calculated by the M-mode (cubic) using the formula: $LVM=1.04[(IVS+LVID+LVPW)^3-(LVID)^3]$, where 1.04 is the specific gravity of muscle (22). Left ventricle fractional shortening (FS) was used as an index of cardiac contractile function and calculated from the inner diameters according to the formula: FS (%)=(LVEDD-LVESD)/LVEDD*100.

Melatonin administration

Melatonin (Sigma Chemical Co, St. Louis, MO, USA) was dissolved in the 10% ethanol and diluted to normal saline, and final concentration was arranged as 50 mg/kg. Melatonin was daily injected intraperitoneally to the melatonin groups (group 3 and 4) for 56 days.

Tissue preparation and Immunohistochemistry analysis

After 8 weeks of the diabetic status, animals were sacrificed; the hearts were prepared with immersion-fixed in a fixative containing 4% paraformaldehyde and 0.2% picric acid for 24 hrs at 4 °C. After washing in 0.1 M PBS, pH: 7.4, 24 hrs, at 4 °C, samples were cryoprotected with 30% saccharose and frozen-embedded in Tissue-Tek. The 10 µm thick cryo-sections were incubated for 24 hrs at 4 °C with rabbit antibodies against total vascular endothelial growth factor A (t-VEGF-A) (1:500; Cell Signaling Tech., Beverly, MA, USA) and phosphorylated (1:3000; Santa Cruz Biotech., Dallas, Texas, USA) followed by incubation with biotin-conjugated goat anti-rabbit IgG (1:300 and 1:1000; 114 Vector Lab., Peterborough, UK). Then, the sections were treated with avidin-biotin peroxidase complex (1:100; Elite ABC Kit, Vector Lab., Peterborough, UK) for 1 hr. The reaction was visualized with 0.05% 3.3-daminobenzidine tetrahydrochloride (Sigma Immunochemicals, St. Louis, MO, USA) in 0.05 M Tris-HCl buffer with a pH: 7.6, containing 0.01% H_2O_2 and 0.01% nickel sulfate as described previously (10). Incubations without the primary antisera served as controls.

Biochemical analysis of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) activity and malondialdehyde (MDA) levels

Superoxide dismutase (SOD) activity was determined using a SAD activity assay kit (Cayman Chemical, Ann Arbor, MI, USA). The enzyme activity was evaluated by measuring the dismutation of superoxide radicals generated by xanthine oxidase and hypoxanthine in a convenient well format. One unit of enzyme activity was defined as the amount of enzyme that caused 50% dismutation of superoxide radical. Catalase (CAT) activity was estimated using a kit (Cayman Chemical, Ann Arbor, MI, USA according to manufacturer's instructions. One unit of CAT activity was determined as the amount of enzyme to exhibit the formation of 1 mmol formaldehyde per minute at 25 °C. Glutathione peroxidase (GPX) activity was measured using the commercially available GPX assay kit (Sigma-Aldrich Chemie, Germany) according to manufacturer's instructions. The enzyme activity assay kit determines enzyme activity indirectly by a coupled with glutathione reductase. Oxidized glutathione was recycled to its reduced state by glutathione reductase and nicotinamide adenine dinucleotide phosphate (NADPH). The oxidation of NADPH to NADP was accompanied by a decrease in absorbance at 340 nm. One unit of GPX activity was defined as the amount of enzyme that caused the oxidation of 1 mmol NADPH to NADP per minute at 25 °C. Malondialdehyde (MDA) levels were measured by a fluorometric method as described in a previous paper using 1.1.3.3-tetraethoxypropane as a standard (23). MDA levels were determined fluorometrically with excitation and emission wavelengths of 532 and 547 nm, respectively (Perkin Elmerm Luminescence spectrometer, LS50B, Waltham, Massa-chusetts, USA). The protein concentrations were estimated spectrophotometrically (Shimadzu RF-5500, Kyoto, Japan) by a protein

assay reagent kit (Pierce, Rock-ford, Illinois, USA), which was based on a modified Bradford method using bovine albumin as a standard.

Quantification of staining intensities and statistical analysis

The densitometry was performed with a Zeiss Axioscop-2 Plus microscope at 40x magnification coupled with Image System Analysis, Axiovision Ver. 4.7 150 (Carl Zeiss, Jena, Germany). The background grey value was measured from four selected regions at a section free area. The coronary vessels grey values were measured from four selected areas of the across sectioned blood vessels wall. Immunostaining intensity was presented as the mean of measured blood vessels grey value minus mean of measured background grey value. Continuous variables were presented as mean plus standard deviation (SD) for normal distributions. Normality of distribution was verified using the Shapiro-Wilks normality test. All of the continuous variables were distributing normally. Thus, One Way Anova was performed after assessment of distributions for more than two independent groups and a post hoc Dunnet test for unpaired data were applied to detect any differences between animal groups. SPSS software 21.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for all statistical analysis. A p value of less than 0.05 was considered to indicate statistical significance.

Results

Blood glucose levels

Glucose levels were significantly increased to 413±17 mg/dL in the DM group (group 2, $p<0.05$) and to 418±16 mg/dL in the melatonin+DM group (group 4, $p<0.05$) at the end of the in vivo study (Table 2). The blood glucose levels were found to be close to normal in group 1 and 3 at the end of the study. STZ injection resulted in an exchangeable robust increase in blood glucose in group 2 and group 4 with significant difference throughout the observational period (Table 2). Additionally, at the end of the study (day 56), there was statistically significant weight loss in group 2 compared to first day (Table 3).

Effect of hyperglycemia on cardiac phenotype

Hearts of animals in group 2 were enlarged and the ratio of the heart weight and length as a measure of myocardial hypertrophy was significantly increased [W/L (mg/cm), from 71±3 to 92±8; $p<0.05$]. Echocardiography confirmed myocardial hypertrophy with preserved systolic function in group 2 whereas echocardiographic variables were unaltered in group 4 (Table 3).

Expression of total vascular endothelial growth factor-A (t-VEGF-A) in coronary vessels

When compared with staining intensities of total vascular endothelial growth factor-A (t-VEGF-A), a significant decrease in the staining intensities of t-VEGF-A was observed in coronary vessels of group 2 (140±30.1 densitometry units (DU) compare with in group 1 [400±32.8 DU). In the expression of t-VEGF-A in coronary vessels, there was also a significant difference between group 1 and group 2 ($p<0.05$). The immunohistochemical expression of t-VEGF-A in coronary vessels of group 3 showed a higher staining intensity in comparison of those of group 1 (600±36.8 DU vs. 400±31.9 DU, $p<0.05$). While 62.5% decrease was

Table 2. Blood glucose levels of group 2 and 4.

Groups	3rd day post-injection	14th day Post-injection	28th day Post-injection	42th day Post-injection	56th day Post-injection
Group 1	136±12	139±13	141±16	138±14	142±18
Group 2	348±13	389±22	409±27	413±30	413±17*
Group 3	143±15	152±17	150±11	147±16	140±14
Group 4	363±14	387±19	413±25	416± 29	418±16*

Blood glucose levels of the DM (group 2) and Melatonin+DM (group 4) groups in day post injection. * Indicates a p value<0.5 compared with 3rd day injection.

Table 3. Systolic and diastolic echocardiographic findings of the groups.

	Variables	Group 1 (n=10)	Group 2 (n=10)	Group 3 (n=10)	Group 4 (n=10)
Systolic	**Septum (mm)**	1.53±0.02	1.91±0.06 *	1.55±0.08	1.44±0.02
	LVESD (mm)	1.7±0.076	1.33±0.18 *	1.73±0.14	1.62±0.11
	LVPW (mm)	1.15±0.30	1.69±0.03 *	1.14±0.07	1.33±0.04
Diastolic	**Septum (mm)**	0.70±0.02	1.08±0.02 *	0.74±0.03	0.86±0.04
	LVEDD (mm)	3.77±0.09	3.16±0.14*	3.64±0.14	3.59±0.12
	LVPW (mm)	0.71±0.05	1.18±0.02*	0.80±0.04	0.84±0.05
	LVM (mg)	95±11	127±10*	107±90	106±11
	FS (%)	0.55±0.02	0.56±0.06	0.54±0.02	0.53±0.01
	Heart rate (bpm)	482±22	472±22	473±16	480±23
Body weight	**First day (g)**	214.7±8.42	227.8±8.49	231.6±10.3	228.9±7.93
	56th day (g)	217.6±7.32	192.4±11.8*	228.4±9.7	222.6±9.75

The myocardial dimensions determined by two-dimensional echocardiography included the thickness of the septum and the left ventricular posterior wall (LVPW), left ventricular end- diastolic diameters (LVEDD), and left ventricular end systolic diameters (LVESD). Data are shown as means±SD. FS: fractional shortening; LVM: left ventricular mass; bpm: beats per min. * Indicates a p value <0.05 compared with control group.

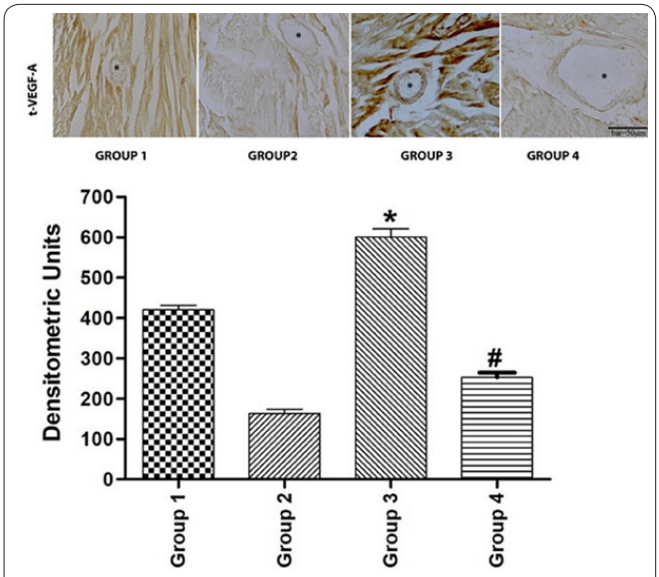

Figure 1. Expression and densitometric analysis of total form of VEGF-A (t-VEGF-A) in coronary vessels of rats. The endogenously t-VEGF-A was detected in the coronary vessels of the control (group 1) (A), DM (group 2) (B), melatonin (group 3) (C) and melatonin+DM (group 4) (D). Data were presented as mean±SD; n=10 for each group. "*" indicates statistical significance melatonin (group 3) vs. control group (group 1), "#" indicates statistical significance DM (group 2) vs. control group (group 1), $p<0.05$. Bar: 50 µm.

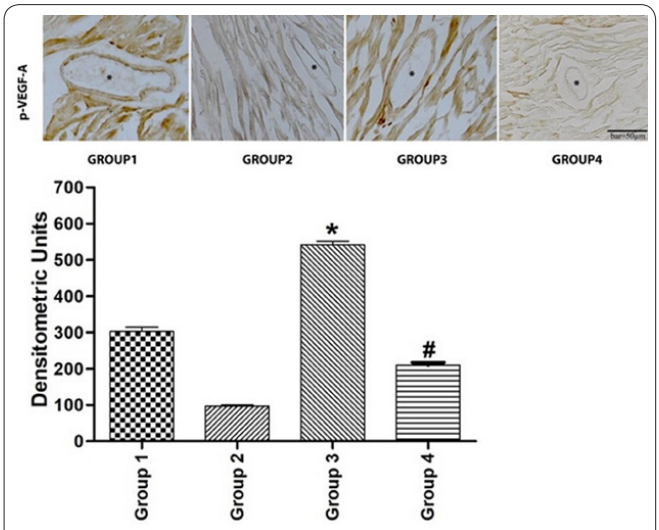

Figure 2. Localization and densitometric analysis of phosphorylated form of VEGF-A (p-VEGF-A) in coronary vessels of rats. The p-VEGF-A was detected in the coronary vessels of the control (group 1) (A), DM (group 2) (B), melatonin (group 3) (C) and melatonin+DM (group 4) (D). Data were presented as mean±SD; n=10 for each group. "*" indicates statistical significance melatonin (group 3) vs. control group (group 1), "#" indicates statistical significance DM (group 2) vs. control group (group 1), $p<0.05$. Bar: 50 µm.

found after induction of DM, 50% increase was found after melatonin treatment in group 3 and 25% decrease in group 4 (300±32.4 DU) (Figure 1).

Localization of phosphorylated vascular endothelial growth factor-A (p-188 VEGF-A) in coronary vessels

When compared with the group 2, higher staining intensities of p-VEGF-A were detected in coronary vessels of group 1 (300±33.1 DU vs. 100±34.5 DU, $p<0.05$). These results are compatible with a negative

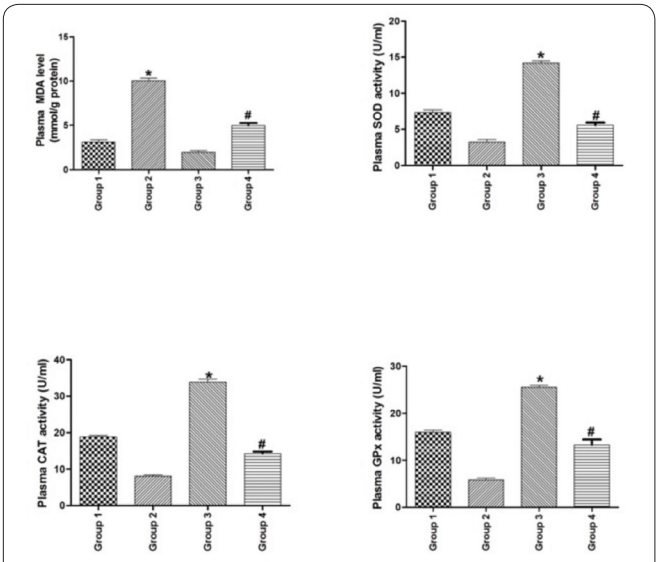

Figure 3. Malondialdehyde (MDA) levels and antioxidant enzyme (GPX, CAT, and SOD) activities. Diagram showing GPX, CAT, and SOD enzyme activities and MDA levels in plasma samples of animal groups. "*" indicates significant difference between group 1 and group 2 for MDA levels; group 1 and group 3 for GPX, CAT, and SOD enzyme activities. "#" indicates significant difference between group 3 and group 4. Note that GPX, CAT, and SOD activities and MDA levels were significantly elevated after melatonin administration in both experimental paradigms.

effect of DM on the expression of p-VEGF-A in coronary vessels of the group 2 (Figure 2). Immunohistochemical expression of p-VEGF-A in coronary vessels of group 3 showed a significantly higher staining intensity in comparison to those of group 4 (550±34.8 DU vs. 200±33.6 DU, $p<0.05$) (Figure 2). There was also a significant difference between group 4 and group 2 (200±33.3 DU vs. 100±35.6 DU, $p<0.05$). Percentages of decrease after induction of the DM were 66.6% in the non-diabetic group and 63.6% in melatonin treatment group (Figure 2).

Biochemical analysis

The serum antioxidant enzymes activities; SOD, CAT, and GPX were significantly lower in DM group (group 2) than in non-DM groups (for all, $p<0.05$), whereas lipid peroxidation was significantly higher (for all, $p<0.05$). Comparison of the MDA levels of the plasma samples revealed a beneficial effect of melatonin treatment. Injection of melatonin (group 4) significantly decreased the levels of MDA compared with that in the DM group (group 2). On the contrary, antioxidant enzyme activities were found to be significantly increased as a result of melatonin administration in group 3 and group 4 ($p<0.05$). Induction of DM significantly decreased antioxidant enzyme activities compared with that in the non-DM groups ($p<0.05$). Antioxidant enzyme activities (SOD, CAT, and GPX) and MDA levels are shown in Figure 3.

Discussion

The present study found that streptozotocin-induced DM caused cardiac enlargement and hypertrophy. The phosphorylation of VEGF-A in the coronary vessels was decreased in diabetic conditions by higher glucose

levels and it contributes to the development of diabetic cardiomyopathy. A significant increase in the phosphorylation of VEGF-A in coronary vessels was observed with melatonin treatment. Melatonin treatment prevented the development of cardiac hypertrophy in hyperglycemia conditions. Additionally, melatonin treatment increased the activity of the antioxidant enzyme, which was reduced by the effect of the DM.

It is known that phosphorylation of VEGF-A is required for physiological angiogenesis (24-26). The regulation of the endothelial cell migration by phosphorylation of VEGF is also essential for angiogenesis and it was found that the phosphorylation residue of VEGF receptor 2 (VEGFR2) and VEGF-A in endothelial cells regulates endothelial cell migration during angiogenesis (27). In our incubations, we used a specific antibody against p-VEGF-A and we detected a higher phosphorylation of VEGF-A in coronary vessels of the rats. It is possible that migration of endothelial cells in coronary vessels during physiological angiogenesis may be regulated by constitutive activation of VEGF-A. As a result of our study, the development of hypertrophy in coronary capillaries and myocardial tissue supports that constitutive activation of VEGF-A in capillaries may be associated with cardiac hypertrophy.

As it was previously shown, the diabetic cardiomyopathy observed in insulin-resistant or hyperinsulinemic states is characterized by impaired myocardial insulin signaling, mitochondrial dysfunction, endoplasmic reticulum stress, impaired calcium homeostasis, abnormal coronary microcirculation, activation of renin-angiotensin-aldosterone system, sympathetic nervous system, and maladaptive immune responses (28, 29). These pathophysiological changes lead to multiple toxic effects on cardiomyocytes. In our study, too much decrease in the phosphorylation of VEGF-A was observed in coronary vessels of the streptozotocin-induced DM rats. According to the results of the present study, we can remark that in the diabetic conditions the phosphorylation of VEGF-A may be reduced in the coronary angiogenesis and it may be associated with pathological cardiac hypertrophy.

In comparison to the non-diabetic rats, staining intensity of p-VEGF-A was significantly increased in coronary vessels of melatonin treatment non-diabetic rats. This finding may suggest that melatonin treatment may lead to excessive increase in phosphorylation of the VEGF-A in coronary vessels of the rats. These results indicated that for a constitutive activation of VEGF-A in coronary vessels, melatonin may have a protective effect. The phosphorylation of VEGF-A in coronary vessels of diabetic rats is weakly decreased in comparison to the phosphorylation of VEGF-A in coronary vessels of melatonin treatment diabetic rats. Therefore, it may be possible that melatonin might prevent the pathological effects of DM on the activation of VEGF-A in coronary vessels.

Anti-oxidative enzymes (SOD, CAT, GPx) which metabolize radicals or reactive oxygen species (ROS) into non-radical products play a crucial role in antioxidant defense (30). In different studies, it has been established that melatonin treatment increases the activity of antioxidant enzymes, such as SOD, CAT, and GPx in different tissues in which oxidative stress was induced by various agents (31, 32). It was proposed by several studies that over levels of oxidative stress are related to apoptosis of myocardial cells and the pathological process that causes cardiac hypertrophy (17). It was shown in the current study that MDA levels increased with DM, indicating that over stress from ROS is involved in the pathological development of diabetic cardiomyopathy. Additionally, the melatonin treatment group had remarkably increased antioxidant enzymes activities and significantly decreased MDA levels, which is a marker of oxidative stress compared to the diabetic group.

In the streptozotocin-induced DM conditions, the phosphorylation of VEGF-A was significantly decreased. But, melatonin treatment prevented the reduction of this phosphorylation. This is indicating that the cardio-protective effect of melatonin by regulating phosphorylation of VEGF-A may prevent cardiac hypertrophy. Demonstrating the underlying mechanism behind VEGF-A-mediated pathophysiological features in the heart will be important in improving influential therapies against diabetic cardiomyopathy in terms of melatonin.

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Declaration of interests
None declared.

References

1. Kowluru, R. A., & Chan, P. S. Oxidative stress and diabetic retinopathy. Experimental diabetes research. 2007; 2007:43603.
2. Salido, E.M., Bordone, M., De Laurentiis, A., Chianelli, M., Keller Sarmiento, M.I., Dorfman, D.,Rosenstein, R.E. Therapeutic efficacy of melatonin in reducing retinal damage in an experimental model of early type 2 diabetes in rats. J Pineal Res. 2013; 54:179–189.
3. Hayat SA, Patel B, Khattar RS, Malik RA: Diabetic cardiomyopathy: mechanisms, diagnosis and treatment. ClinSci (Lond). 2004; 107:539-557.
4. Lin XJ, Mei GP, Liu J, et al. Therapeutic effects of melatonin on heatstroke-induced multiple organ dysfunction syndrome in rats. J Pineal Res. 2011; 50:436-444.
5. Liu LF, Qin Q, Qian ZH, et al. Protective effects of melatonin on ischemia-reperfusion induced myocardial damage and hemodynamic recovery in rats. Eur Rev Med Pharmacol Sci. 2014; 18: 3681-3686.
6. Zhang P, Xu X, Hu X, Van Deel ED, Zhu G, Chen Y. Inducible Nitric Oxide Synthase deficiency protects the heart from systolic overload–induced ventricular hypertrophy and congestive heart failure. Circ Res. 2007; 100:1089-1098.
7. Hinkel R, Howe A, Renner S, Ng J, Lee S, Klett K, Kaczmarek V, Moretti A, Lauqwitz KL, Skroblin P, Mayr M, Milting H, Dendorfer A, Reichart B, Wolf E, Kupatt C: Diabetes mellitus-induced microvascular destabilization in the myocardium. J Am Coll Cardiol. 2017; 69:131-143.
8. Boodhwani M, Sodha NR, Mieno S, Xu SH, Feng J, Ramlawi B, Clements RT, Sellke FW: Functional, cellular, and molecular characterization of the angiogenic response to chronic myocardial ischemia in diabetes. Circulation. 2007; 116:131-137.
9. Shende P, Plaisance I, Morandi C, et al. Cardiac raptor ablation impairs adaptive hypertrophy, alters metabolic gene expression, and

causes heart failure in mice. Circulation. 2011; 123:1073-1082.
10. Korkmaz Y, Lang H, Beikler T, et al. Irreversible inflammation is associated with decreased levels of the α1-, β1-, and α2-subunits of sGC in human odontoblasts. J Dent Res. 2011; 90:517-522.
11. Chou E, Suzuma I, Way KJ, Opland D, Clermont AC, Naruse K, Suzuma K, Bowling NL, Vlahos CJ, Aiello LP, King GL: Decreased cardiac expression of vascular endothelial growth factor and its receptors in insulinresistant and diabetic States: a possible explanation for impaired collateral formation in cardiac tissue. Circulation. 2002; 105:373-379.
12. Rivera JC, Madaan A, Zhou TE, Chemtob S. Review of the mechanisms and therapeutic avenues for retinal and choroidal vascular dysfunctions in retinopathy of prematurity. Acta paediatrica. 2016; 105:1421-1433.
13. Rubio RG, Adamis AP. Ocular Angiogenesis: Vascular Endothelial Growth Factor and Other Factors. Developments in ophthalmology. 2016; 55:28-37.
14. Shweiki D, Itin A, Soffer D, et al. Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. Nature. 1992; 359:843–845.
15. Rivard A, Silver M, Chen D, et al. Rescue of diabetes-related impairment of angiogenesis by intramuscular gene therapy with adeno-VEGF. Am J Pathol. 1999; 154:355–363.
16. Yoon YS, Uchida S, Masuo O, Cejna M, Park JS, Gwon HC, Kirchmair R, Bahlman F, Walter D, Curry C, Hanley A, Isner JM, Losordo DW: Progressive attenuation of myocardial vascular endothelial growth factor expression is a seminal event in diabetic cardiomyopathy: restoration of microvascular homeostasis and recovery of cardiac function in diabetic cardiomyopathy after replenishment of local vascular endothelial growth factor. Circulation. 2005; 111:2073-2085.
17. Song E, Jahng JW, Chong LP, et al. Lipocalin-2 induces NLRP3 inflammasome activation via HMGB1 induced TLR4 signaling in heart tissue of mice under pressure overload challenge. Am J Transl Res. 2017; 9:2723-2735.
18. Braunwald E. Biomarkers in heart failure. N Engl J Med. 2008; 358:2148-2159.
19. Zhao F, Liu ZQ, Wu D. Antioxidative effect of melatonin on DNA and erythrocytes against free-radical-induced oxidation. Chem Phys Lipids. 2008; 151:77-84.
20. Hoyer DP, Korkmaz Y, Gronke S, Addicks K, Wettschureck N, Offermanns S, et al. Differential expression of protein kinase C isoforms in coronary arteries of diabetic mice lacking the G-protein Galpha11. Cardiovasc Diabetol. 2010; 9:93.
21. Reuter H, Seuthe K, Korkmaz Y, Grönke S, Hoyer DP, Rottlaender D, et al. The G protein Galpha11 is essential for hypertrophic signalling in diabetic myocardium. Int J Cardiol. 2013; 167:1476-1485.
22. Devereux R.B, Reichek N. Echocardiographic determination of left ventricular mass in man. Anatomic validation of the method. Circulation. 1977; 55:613-618.
23. Wasowicz, W., Neve, J., & Peretz, A. Optimized steps in fluorometric determination of thiobarbituric acid-reactive substances in serum: importance of extraction pH and influence of sample preservation and storage. Clinical chemistry. 1993; 39:2522-2526.
24. Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. Nat Med. 2003; 9:669-676.
25. Tammela T, Enholm B, Alitalo K, Paavonen K. The biology of vascular endothelial growth factors. Cardiovasc Res. 2005; 65:550-563.
26. Olsson AK, Dimberg A, Kreuger J, Claesson-Welsh L. VEGF receptor signalling-in control of vascular function. Nat Rev Mol Cell Biol. 2006; 7:359-371.
27. Lamalice L, Le Boeuf F, Huot J. Endothelial cell migration during angiogenesis. Circ Res. 2007; 100:782-794.
28. Reuter H, Seuthe K, Korkmaz Y, Grönke S, Hoyer DP, Rottlaender D, et al. The G protein Galpha11 is essential for hypertrophic signalling in diabetic myocardium. Int J Cardiol. 2013; 167:1476-1485.
29. Jia G, DeMarco VG, Sowers JR. Insulin resistance and hyperinsulinaemia in diabetic cardiomyopathy. Nat Rev Endocrinol. 2016; 12:144-153.
30. Baydas G, Canatan H, Turkoglu A. Comparative analysis of the protective effects of melatonin and vitamin E on streptozocin-induced diabetes mellitus. J Pineal Res. 2002; 32:225-230.
31. Ohta Y, Kongo-Nishimura M, Matsura T, Yamada K, Kitagawa A, Kishikawa T. Melatonin prevents disruption of hepatic reactive oxygen species metabolism in rats treated with carbon tetrachloride. J Pineal Res. 2004; 36:10-17.
32. Sailaja Devi MM, Suresh Y, Das UN. Preservation of the antioxidant status in chemically-induced diabetes mellitus by melatonin. J Pineal Res. 2000; 29:108-115.