



Dexmedetomidine restores autophagy and cardiac dysfunction in rats with streptozotocin-induced diabetes mellitus

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Abstract

Aims Dexmedetomidine (DEX), a highly selective and potent α_2 -adrenergic receptor agonist, has anti-apoptotic, anti-inflammatory, and anti-oxidative stress effects in diabetes mellitus (DM) rats. The underlying molecular mechanisms and signaling pathways of diabetic cardiomyopathy remain poorly understood. This study aimed to elucidate the effect of DEX on cardiac function in DM rats.

Methods Eight-week-old male Sprague Dawley rats were divided into three groups: control ($n=5$), diabetes (DM, $n=7$), and diabetes + DEX (DM + DEX, $n=10$). DM was induced via intraperitoneal injection of streptozotocin (70 mg/kg); at 3 days later, DEX (1 $\mu\text{g}/\text{kg}/\text{h}$) was administered for 4 weeks. Cardiac function was evaluated using pressure–volume loop analysis and echocardiography. Left ventricular (LV) histological sections were used to analyze the interstitial collagen fraction. Using the LV samples, we performed a western blot analysis to evaluate signaling pathways and autophagic markers.

Results The DM group had lower body weight and higher blood glucose level and heart weight/body weight ratio than the control group. However, metabolic changes did not differ between the DM and DM + DEX groups. Pressure–volume loop analysis and echocardiography showed impaired cardiac function, evidenced by a decrease in systolic and diastolic function, in both DM groups. DEX treatment in DM rats was associated with increased LV end-systolic pressure, LV contractility, cardiac output, and relaxed LV function compared with that in non-treated DM rats. LC3B and autophagy-related gene (ATG) proteins increased in the hearts of DM rats compared with the hearts of control rats. However, DEX reduced the expression of LC3B and ATG proteins in the hearts of DM rats. Increased p-ERK and decreased p-AKT were reduced in the hearts of DEX-treated DM rats.

Conclusions DEX reduces cardiac dysfunction and impaired autophagy in DM rats. This study reinforces our understanding of the potential anti-autophagic effect of DEX in patients with diabetic cardiomyopathy.

Keywords Autophagy · Cardiac function · Diabetic cardiomyopathy · Dexmedetomidine · Type 1 diabetes

Abbreviations

DM Diabetes mellitus
DCM Diabetic cardiomyopathy

LV Left ventricular
DEX Dexmedetomidine
STZ Streptozotocin
ECL Enhanced chemiluminescence
ERK Extracellular signal-regulated kinase

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PV loop	Pressure–volume loop
HR	Heart rate
LVESP	LV end-systolic pressure
dP/dt_{\max}	Maximal slope of the systolic pressure increment
dP/dt_{\min}	Maximal slope of the diastolic pressure decrement
E_{\max}	Slope of end-systolic pressure–volume relationship
SW	Stroke work
EF	Ejection fraction
SV	Stroke volume
CO	Cardiac output
PRSW	Preload recruitable stroke work
EDPVR	End-diastolic pressure–volume relationship
LVEDD	LV end-diastolic dimension
LVESD	LV end-systolic dimension
ATG	Autophagy-related gene
TDI E/E'	Ratio of transmitral Doppler early filling velocity to tissue Doppler early diastolic mitral annular velocity
E/A	Ratio between early (E)-to-late (A) diastolic mitral inflow
MAPK	Mitogen-activated protein kinase

Introduction

Diabetes mellitus (DM) is one of the most important risk factors for heart failure and is associated with increased morbidity and mortality. Diabetic cardiomyopathy (DCM) has become a major cause of DM-related mortality and occurs independently of coronary artery disease, hypertension, and other cardiovascular diseases [1, 2]. DM impairs cardiac structure and function, and is characterized by myocyte hypertrophy, myocardial interstitial fibrosis, increased apoptosis, and suppressed autophagy, eventually leading to left ventricular (LV) systolic and diastolic dysfunction [3].

Dexmedetomidine (DEX), a highly selective and potent α_2 adrenergic receptor agonist, has sedative, analgesic, and anxiolytic properties, as well as anesthetic-sparing effects [4]. Moreover, recent animal and clinical studies showed that DEX provided protection against ischemia reperfusion injury in the kidney, heart, and brain through its anti-apoptotic, anti-inflammatory, and anti-oxidative stress effects in DM rats [5–7]. To date, there are few published data addressing the effects of DEX in animal models of DCM.

Autophagy is the physiologic process whereby cytoplasmic components, including long-lived proteins and organelles, are engulfed by a double-membrane structure and targeted for destruction in lysosomes [8]. It selectively removes damaged mitochondria as a cytoprotective mechanism to limit mitochondria-derived oxidative stress and

prevent apoptosis [9]. A low level of constitutive autophagy is important for the maintenance of normal cellular function and quality of cardiac proteins and organelles. Defects in this process result in cardiac dysfunction and heart failure, particularly during increased cellular stress [10]. Although autophagy is implicated in various pathologic conditions, including cardiac hypertrophy, cardiomyopathy, and heart failure, little information is available about its pathophysiologic roles in the pathogenesis of DCM. Despite the importance of this complication, the underlying molecular mechanisms and signaling pathways of DCM remain poorly understood. In this study, we focused on the effects of DEX treatment on DCM, especially the molecular signaling pathways. We aimed to elucidate the potential effect of DEX on alterations in cardiac dysfunction in streptozotocin (STZ)-induced DM rats and the mechanisms involved in autophagy.

Materials and methods

Ethical approval

All animal procedures were approved by the Committee for the Care and Use of Laboratory Animals at Yonsei University College of Medicine (no. 2015-0129) and were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health. All animals were housed in a temperature-controlled room (25 ± 2 °C) with a 12-h light/12-h dark cycle and humidity of 60–65% and were provided with free access to normal chow and tap water. Except for drinking water, rats were fasted for 8 h before surgery and were allowed free access to food and water after surgery.

STZ models and study groups

Sprague Dawley rats are widely used in animal studies on type 1 DM induced by STZ. Male Sprague Dawley rats aged 8–10 weeks received a single intraperitoneal injection of STZ (Sigma-Aldrich, Saint Louis, MO, USA) to induce severe DM. DM rats were intraperitoneally injected with STZ at a dose of 70 mg/kg ($n = 20$), whereas control animals received an equivalent volume of citric acid buffer ($n = 5$) [11, 12]. Glucose and insulin levels were determined on the third day following STZ injection. In this study, 80% of rats became diabetic. Rats with a fasting blood glucose level > 200 mg/dL were considered diabetic and randomized into two groups: non-treated DM group ($n = 7$) or DEX-treated DM group (1 μ g/kg/h, $n = 10$) [13]. A group of randomly selected STZ-induced DM rats was treated with DEX (1 μ g/kg/h), which was administered via an osmotic pump (ALZET model 2ML4 2002, DURECT Corp., Cupertino, CA, USA) for 28 days. After anesthesia induction with

isoflurane, the pump was subcutaneously implanted between the scapulae of DM rats. The adequacy of anesthesia was monitored based on the lack of pedal withdrawal reflex, slow constant breathing, and absence of response to surgical manipulation.

Chemical reagents and antibodies

The following chemical agents were used: STZ and radioimmunoprecipitation assay with protease and phosphatase inhibitors were obtained from Sigma-Aldrich (St. Louis, MO, USA). Citric acid was purchased from the Shanghai Chemical Reagent Co., Ltd. (Shanghai, China), whereas DEX was obtained from Hospira (Rocky Mount, NC, USA). Moreover, 0.45% sodium chloride injections and a Bradford protein assay kit were obtained from Thermo Fisher Scientific (Rockford, IL, USA), and an Amersham enhanced chemiluminescence (ECL) western blotting detection reagent was acquired from GE Healthcare (Little Chalfont, UK). Anti-LC3 was obtained from Novus (San Diego, CA, USA); anti-Beclin1, anti-Atg3, anti-Atg5, anti-Atg7, anti-Atg12, anti-Atg16L1, anti-p-AKT and total-AKT, anti-p-ERK and total-ERK, and anti-p-p70S6 and anti-total-p70S6 were acquired from Cell Signaling Technology (Beverly, MA, USA); and anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was acquired from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

Biochemical analysis

Blood samples were obtained from the femoral artery of rats after overnight fasting. Fasting blood glucose level was measured using a blood glucose monitoring system (CareSens II, Wonju, Korea). Insulin level was determined using a Mercodia rat insulin enzyme-linked immunosorbent assay kit [oxidase method, with a biochemical auto-analyzer (Beck-man CX-7 Biochemical Auto-analyzer, Brea, CA, USA)]. Data on heart weight and body weight were collected and measured. Heart tissues were snap-frozen in liquid nitrogen and stored at -80°C to obtain protein.

Pressure–volume (PV) loop analysis

Pressure and volume were calibrated using the MPVS-Ultra system (Millar Inc., Houston, TX, USA). Anesthetized rats were placed on a heating pad in the supine position, and an incision was created in the anterior midline of the neck to expose the trachea. The right carotid artery was dissected and exposed, and a Millar catheter was introduced into the artery and advanced into the LV via the aortic valve. After stabilization for 5–10 min, PV loop signals were continuously recorded at a sampling rate of 1000 Hz using an MPVS-Ultra Single Segment Pressure–volume unit (Millar

Inc., Houston, TX, USA). Using a special PV loop analysis software (Millar Inc., Houston, TX, USA), we computed and calculated the heart rate (HR), LV end-systolic pressure (LVESP), LV end-diastolic pressure, pressure increment (dP/dt_{\max}) and pressure decrement (dP/dt_{\min}), stroke work (SW), and ejection fraction (EF). Stroke volume (SV) and cardiac output (CO) were calculated and corrected according to in vitro and in vivo volume calibrations using the PV loop analysis software. In addition, the relationship between LV and PV was evaluated based on the occlusion of the inferior cava. The slope of the LV end-systolic PV relationship (E_{\max}), preload recruitable stroke work (PRSW), and slope of the end-diastolic PV relationship (EDPVR) were calculated. At the end of each experiment, 50 mL of 30% saline was intravenously injected to establish a parallel conductance volume from the shift of PV loop relations, and this was used to correct the volume of the cardiac mass. The volume was calibrated using a Millar volume calibration cuvette. All measurements were evaluated using LabChart8 (ADInstruments, Colorado Springs, CO, USA).

Echocardiography

Murine transthoracic echocardiography was performed using the GE Vivid 7 ultrasound system (General Electric, Atlanta, GA, USA) with a 10-MHz transducer. The rats were lightly anesthetized using 2% sevoflurane and restrained on a heated imaging table. Images were obtained using M-mode echocardiography in the parasternal short-axis view, pulse-wave Doppler imaging through the mitral valve in an apical four-chamber view, and tissue Doppler imaging at the level of the septal mitral annulus in an apical four-chamber view to calculate the cardiac diastolic and systolic functions.

Western blotting

Proteins were extracted from the heart using a radioimmunoprecipitation assay buffer with protease and phosphatase inhibitors for western blot analysis. We loaded 50 mg of protein in each well to perform sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Membranes were blocked with 5% skim milk for 1 h and incubated overnight with the first antibodies. Signals were measured using the Amersham ECL western blotting detection reagent (GE Healthcare, Little Chalfont, UK), scanned using ImageQuant LAS 4000 Mini (GE Healthcare, Little Chalfont, UK), and quantified using the ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Statistical analysis

All data are expressed as means \pm standard deviations. Statistical analysis was performed using *t* test or one-way

analysis of variance (Bonferroni test), and differences were determined to be statistically significant when p values were <0.05 . All experiments were performed at least three times.

Results

Metabolic changes in DM rats

Blood glucose levels (mg/dL), insulin levels ($\mu\text{g/L}$), body weight (g), heart weight (mg), and heart weight/body weight (mg/g) were measured to estimate the effects of DEX on metabolic changes in DM rats. We observed that DEX treatment failed to improve the metabolic changes in DM rats including the blood glucose level, insulin level, and heart weight. However, DEX significantly restored the body weight of DM rats ($p < 0.05$) (Fig. 1).

DEX treatment improved cardiac performance in DM rats

The baseline hemodynamic data of rats are summarized in Table 1. Most parameters in DM rats showed significant differences from those in control rats. Non-treated DM rats exhibited decreased HR, LVESP, SV, SW, CO, EF, dP/dt_{max} , and dP/dt_{min} and increased time constant of LV pressure decay (τ) compared with control rats. Similarly, DEX-treated DM rats showed a decrease in HR, LVESP, SV, SW, CO, EF, dP/dt_{max} , and dP/dt_{min} and an increase in tau compared with control rats. Further, SV, SW, CO, dP/dt_{max} , and dP/dt_{min}

were higher and tau was lower in DEX-treated DM rats than in non-treated DM rats. Functional indices were derived using the PV loop analysis at different preloads during the transient occlusion of the inferior vena cava. E_{max} was steeper in control rats than in DM rats. In contrast, the EDPVR tended to be increased in DM rats, albeit without statistical significance. The PRSW values were significantly lower in DM rats than in control rats. DEX treatment in DM rats was associated with increased E_{max} and PRSW compared with that in non-treated DM rats.

LV systolic function by echocardiography was lower in DM rats than in control rats; moreover, both LV end-diastolic dimension (LVEDD) and LV end-systolic dimension (LVESD) increased in DM rats compared with those in control rats. In DM rats treated with DEX, LV systolic function significantly improved, and both LVEDD and LVESD decreased compared with those in non-treated DM rats. Pulse-wave Doppler and tissue Doppler imaging indicated a significant increase in the E/E' ratio and a significant decrease in the E/A ratio in DM rats, suggestive of diastolic dysfunction. Conversely, no significant changes in these diastolic dysfunction measures were observed in DM rats treated with DEX compared with those in control rats (Fig. 2).

DEX regulates the expression of LC3 in the hearts of DM rats

To investigate the role of DEX in autophagic regulation in DM rats, we examined the expression of critical autophagic

Fig. 1 DEX attenuates metabolic changes in DM rats. **a** A schematic timeline of treatment, surgery, and evaluation. **b** Blood glucose levels (mg/dL). **c** and **d** insulin levels ($\mu\text{g/L}$). **e** Body weight (g). **f** Heart weight (mg). **g** Heart weight/body weight (mg/g). Results represent triplicate experiments. Data are expressed as mean \pm standard deviation ($n=6$). * $p < 0.05$ vs. the control group; # $p < 0.05$ vs. the DM group. DM diabetes mellitus, DEX dexmedetomidine, Con control

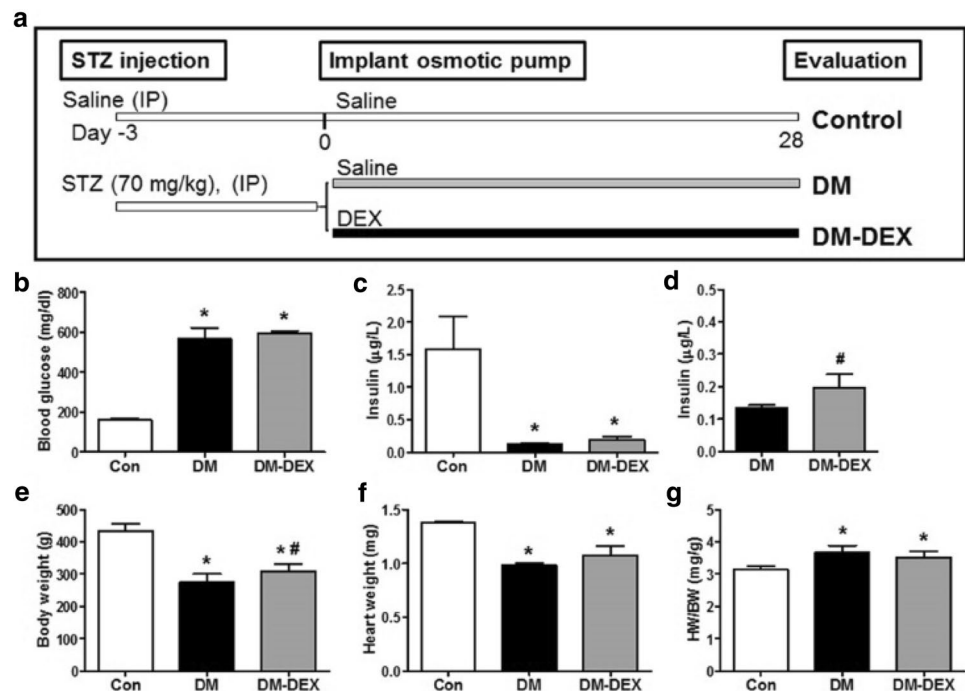


Table 1 Steady-state hemodynamic parameters

Parameters	Con	DM	DM-DEX
HR (beats/min)	274.6 ± 10.8	206.1 ± 10.1*	220.3 ± 12.6*#
LVESP (mmHg)	110.1 ± 5.9	84.0 ± 5.1*	91.8 ± 4.2*#
LVEDP (mmHg)	8.8 ± 0.5	9.1 ± 0.5	8.9 ± 0.7
SV (μL)	146.7 ± 11.4	106.9 ± 13.6*	130.1 ± 12.5*#
SW (mmHg μL)	12,805.5 ± 1018.4	8021.1 ± 1084.3*	9990.2 ± 1046.4*#
CO (μL/min)	42,435.8 ± 2286.4	24,048.2 ± 2487.1*	34,590.0 ± 4082.9*#
EF (%)	63.0 ± 3.6	51.1 ± 3.4*	55.2 ± 5.6
dP/dt_{max} (mmHg/s)	7397.0 ± 233.4	5176.5 ± 189.9*	5792.5 ± 212.6*#
dP/dt_{min} (mmHg/s)	6954.5 ± 232.2	4782.8 ± 164.0*	5236.7 ± 202.2*#
τ (ms)	11.1 ± 1.1	15.0 ± 1.31*	12.9 ± 1.7*#
E_{max} (mmHg/μL)	1.92 ± 0.47	0.68 ± 0.13*	1.16 ± 0.23*#
Slope of EDPVR (mmHg/μL)	0.049 ± 0.014	0.065 ± 0.011	0.054 ± 0.008
PRSW (mmHg)	69.6 ± 7.8	40.5 ± 4.1*	55.6 ± 8.5*#

Con control, DM diabetes mellitus, DEX dexmedetomidine, HR heart rate, LVESP left ventricular end-systolic pressure, LVEDP left ventricular end-diastolic pressure, SV stroke volume, SW stroke work, CO cardiac output, EF ejection fraction, dP/dt_{max} maximal slope of the systolic pressure increment, dP/dt_{min} maximal slope of the diastolic pressure decrement, τ time constant of left ventricular pressure decay, E_{max} slope of end-systolic pressure–volume relationship, EDPVR end-diastolic pressure–volume relationship, PRSW preload recruitable stroke work. Values are expressed as mean ± standard deviation

Significance indicated by * $p < 0.05$ vs. the control group and by # $p < 0.05$ vs. the DM group

markers such as LC3 and beclin-1. The LC3-II protein level was significantly increased in DM rats compared with that in control rats ($p < 0.05$). However, DEX treatment in DM rats significantly suppressed the LC3-II protein level compared with that in non-treated DM rats (Fig. 3, $p < 0.05$).

DEX treatment affected AKT and ERK signaling in DM rats

We confirmed the association of signaling pathway with representative western blot data for LC3 and beclin-1 proteins. AKT and p70S6 activation was significantly increased in DM rats ($p < 0.05$), and DEX treatment reversed the phosphorylation of AKT (Fig. 4a), but not of p70S6 (Fig. 4b). Moreover, ERK activation was significantly decreased in DM rats, and DEX treatment reversed the phosphorylation of ERK (Fig. 4c).

DEX reduced the autophagosomal markers that were increased in the hearts of DM rats

To further explore underlying autophagic molecular markers for the effects of DEX on STZ-induced autophagy, we performed western blotting for autophagy-related gene (ATG) proteins in the hearts of STZ-induced DM rats treated with or without DEX. The expressions of ATG5, ATG7, and ATG12 were significantly increased in DM rats, but were markedly decreased after DEX administration (Fig. 5, $p < 0.05$). Autophagy is a conserved, genetically controlled process that leads to the degradation of cytoplasmic

components within lysosomes and has recently gained much attention because of its paradoxical relationship with apoptosis [14]. We observed an increase in the expression of autophagosomal markers such as LC3B and ATG proteins.

Discussion

To the best of our knowledge, this is the first study that evaluated the cardiac effects of DEX in STZ-induced DM rats with respect to autophagy. We showed that DEX restores the cardiac function in STZ-induced DM rats by maintaining the homeostasis of autophagic markers, including LC3B, ATG5, ATG7, and ATG12. Additionally, DEX regulates the impaired activation of ERK and AKT signaling pathways. Additionally, we showed that STZ-induced autophagy was suppressed by DEX treatment via the phosphorylation of AKT and dephosphorylation of ERK. AKT and ERK signaling pathways are important for the regulation of the effect of DEX on cardiac dysfunction in STZ-induced DM rats. Our findings suggest that DEX could be an effective therapeutic target to protect diabetic patients from experiencing cardiac dysfunction.

DEX is clinically well known to exert pharmacological effects such as sedation and analgesia [4, 15]. Recent studies on DEX, including clinical reports, have indicated its protective effects in the kidney, heart, lung, and brain of DM rats [5, 7, 16, 17]. In the present study, DEX treatment did not attenuate metabolic changes such as the blood glucose level, insulin level, and heart weight/body weight ratio

Fig. 2 DEX improves cardiac function in DM rats. **a** Representative M-mode images from rats in each group. **b** Representative pulsed-wave Doppler image of mitral valve inflow. **c** Representative tissue Doppler image of E/E' ratio. **d** HR. **e** LVEDD. **f** LVESD. **g** FS (%). **h** E/A ratio. **i** E/E' ratio. * $p < 0.05$ vs. the control group; # $p < 0.05$ vs. the DM group. HR heart rate, LVEDD LV end-diastolic dimension, LVESD LV end-systolic dimension, FS fractional shortening, DM diabetes mellitus, DEX dexmedetomidine, Con control

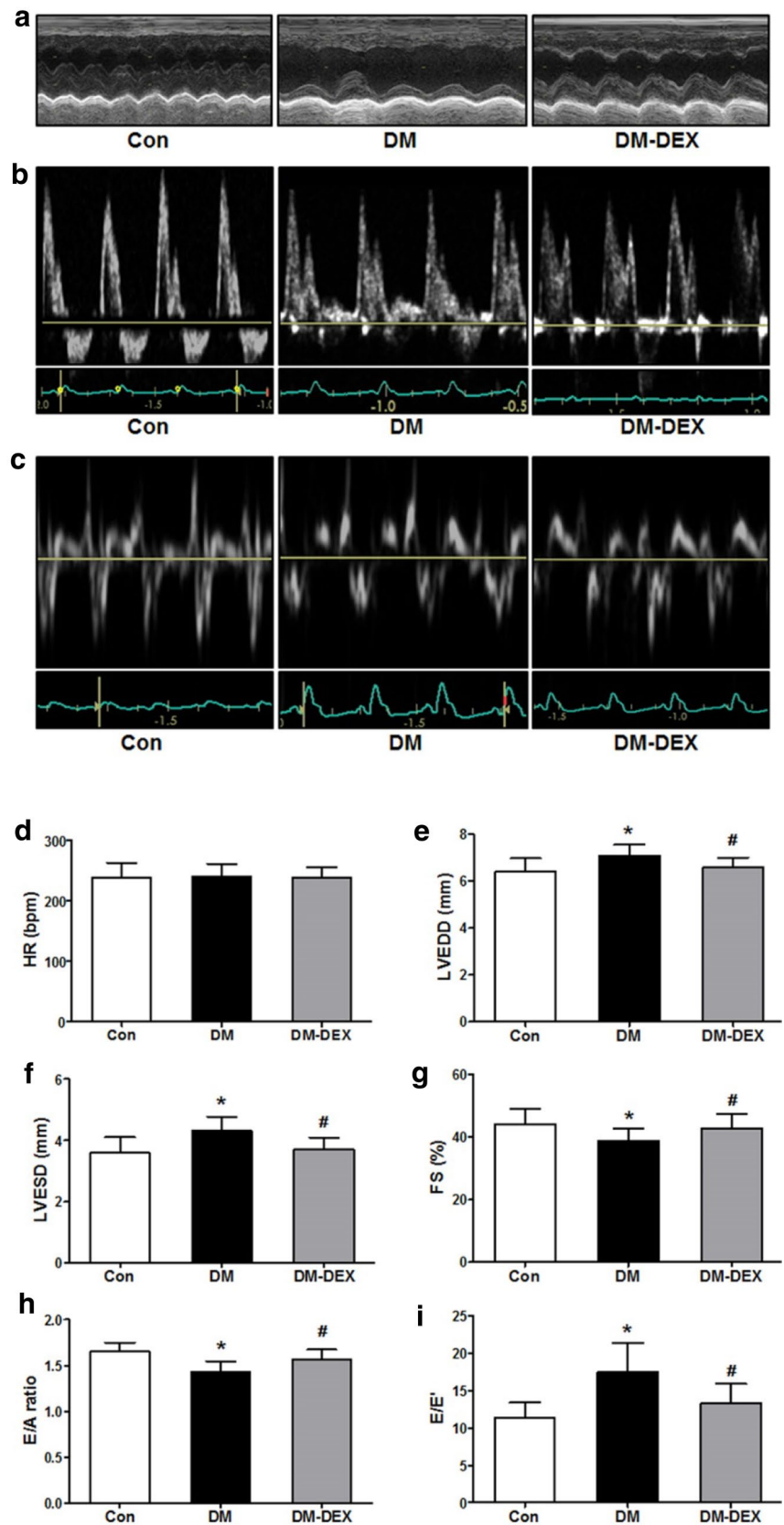


Fig. 3 DEX regulates the level of LC3 protein in the hearts of DM rats. Representative western blot data for LC3 and beclin-1 proteins. **a** Graph for the beclin-1 level. **b** Graph for the LC3-II level. **c** Graph for the LC3-II/LC3-I ratio. GAPDH was used as the protein loading control. Results represent triplicate experiments. Data are expressed as mean \pm standard deviation ($n=5$). * $p < 0.05$ vs. the control group; # $p < 0.05$ vs. the DM group. *GAPDH* glyceraldehyde 3-phosphate dehydrogenase, *DM* diabetes mellitus, *Con* control, *DEX* dexmedetomidine

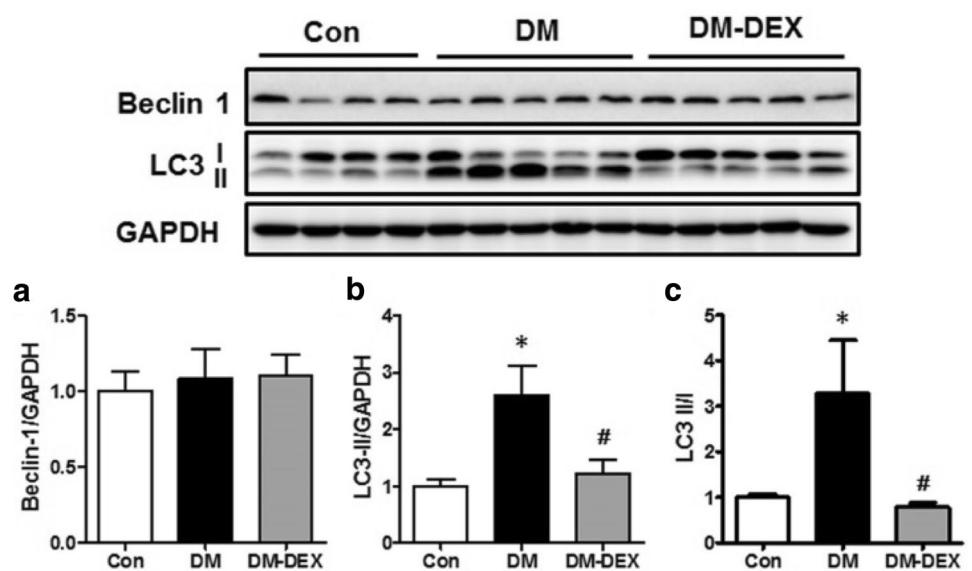
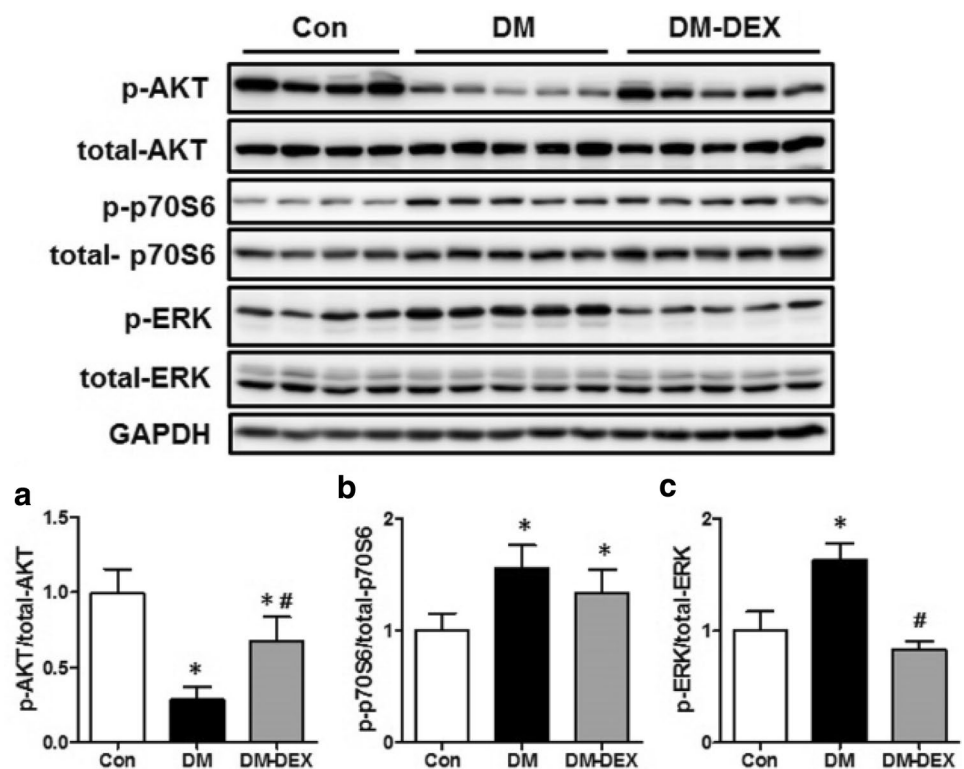


Fig. 4 Effects of DEX on signaling pathway in the hearts of DM rats. Representative western blot data for AKT, p70S6, and ERK activation. **a** Data are expressed as the ratio of p-AKT to total AKT. **b** Ratio of p-p70S6 to total p70S6. **c** Ratio of p-ERK to total-ERK. GAPDH was used as the protein loading control. Results are representative of triplicate experiments. Data are expressed as mean \pm standard deviation ($n=5$). * $p < 0.05$ vs. the control group; # $p < 0.05$ vs. the DM group. *GAPDH* glyceraldehyde 3-phosphate dehydrogenase, *STZ* streptozotocin, *DM* diabetes mellitus, *Con* control

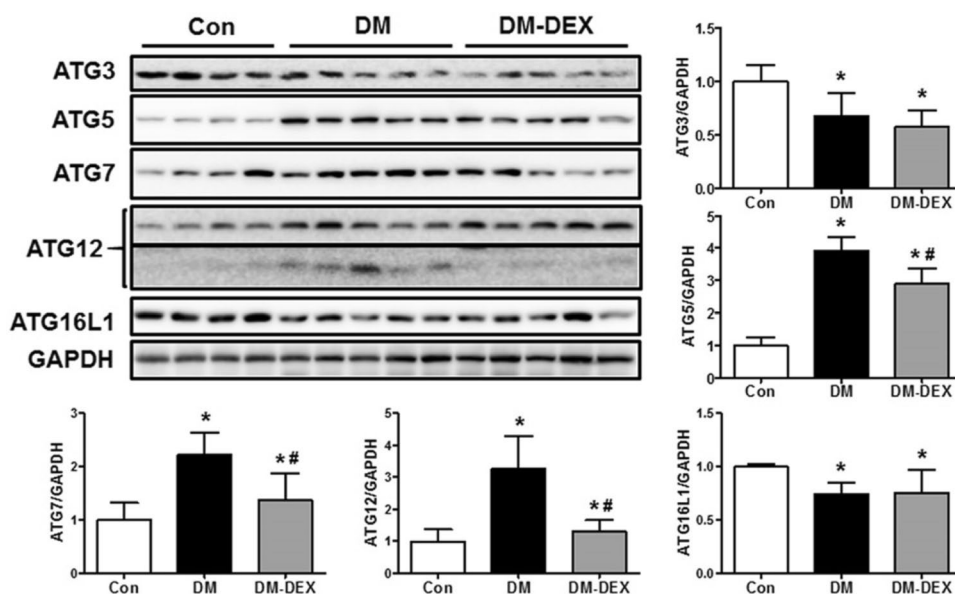


in DM rats. Using PV loop analysis and echocardiography, we determined that DEX improved the cardiac function in STZ-induced DM rats. DEX treatment suppressed the cardiac autophagic activity enhanced in STZ-induced DM rats, as evidenced by the decrease in LC3-II/LC3-I ratio, which is an autophagic marker, and the expression of autophagy-related proteins.

DEX was reported to possess organ-protective properties in several injury models, including models of

ischemia–reperfusion, inflammation, and trauma to multiple organs. The cardioprotective effects of DEX have been extensively shown in ischemic hearts, both regionally and globally [18–20]. Although the precise mechanisms of its protective effects in these organs are not well elucidated, several possible mechanisms have been reported, including modulation of cell death by apoptosis (inhibition of apoptosis and activation of the anti-apoptotic signaling pathway), activation of cell survival kinases, and modulation

Fig. 5 Effects of DEX on autophagic markers in the hearts of DM rats. Representative western blot data for ATG3, ATG5, ATG7, ATG12, and ATG16L1 proteins. GAPDH was used as the protein loading control. Results represent triplicate experiments. Data are expressed as mean \pm standard deviation ($n=5$). * $p<0.05$ vs. the control group; # $p<0.05$ vs. the DM group. GAPDH glyceraldehyde 3-phosphate dehydrogenase, DM diabetes mellitus, DEX dexmedetomidine, Con control



of inflammatory responses and oxidative stress [21–23]. In an incomplete cerebral ischemia–reperfusion model, DEX increased the concentration of anti-apoptotic proteins (B-cell lymphoma 2 and murine double minute 2) and inhibited the increase in the concentration of the proapoptotic protein (Bax) [24]. In a myocardial ischemia–reperfusion injury model, DEX preconditioning attenuated regional injury via the activation of ERK 1/2, AKT, and endothelial isoform of nitric oxide synthase [20]. DEX was reported to inhibit inflammation and autophagy in lipopolysaccharide-induced lung injury via the toll-like receptor 4–nuclear factor- κ B pathway [25]. Furthermore, Shen et al. [26] reported that DEX inhibited neuronal autophagy mediated by the activation of the PI3K/AKT/mTOR pathway in traumatic brain injury. Moreover, it has been reported that DEX protected the mouse brain from ischemia–reperfusion injury via autophagy inhibition through the upregulation of hypoxia-inducible factor 1- α [27].

Autophagy plays an important role in maintaining normal cardiac function and morphology [28, 29]. Recent evidence showed that autophagy at baseline is an important homeostatic mechanism for the maintenance of normal cardiac function and morphology. In contrast, excessive induction of the autophagic process by environmental or intracellular stress plays an important role in several types of cardiomyopathy by functioning as a death pathway [28]. Moreover, autophagy is linked to cardiac dysfunction in diabetic patients, and myocardial fibrosis is related to PI3K/AKT signaling in rats with STZ-induced type 1 diabetes [2, 3, 30]. This suggests that autophagy carries out different functions depending on the cause of autophagy activation, which could be either an adaptor to maintain cardiac function or a contributor to the pathogenesis of heart disease.

Interestingly, autophagic adaptations in DCM differ between type 1 and type 2 diabetes, with cardiac autophagic activity having been reported to be enhanced in type 1 diabetes, but suppressed in type 2 diabetes [31]. In this study, DEX treatment suppressed the cardiac autophagic activity enhanced in STZ-induced type 1 diabetes, which is consistent with the results of previous studies on autophagic activity [31, 32]. In contrast, some studies reported suppression of autophagy in type 1 DM models of STZ-induced mice and genetically engineered mice (diabetic OVE26 mice), proposing it as a response to limit or cause cardiac dysfunction [30, 33, 34]. Further investigation on assay tools or criteria for cardiac autophagic activity in diabetic models is warranted.

Cardiac hypertrophy usually occurs in the late stage of diabetes, which eventually leads to cardiac remodeling, dysfunction, and even heart failure. Consistent changes in hypertrophy and expression of ERK1/2 were also observed in the myocardium of STZ-induced DM rats. It is believed that ERK1/2 activation in response to hyperglycemia results in cardiac hypertrophy [12]. Many researchers have realized the involvement of mitogen-activated protein kinase (MAPK) during DCM development. MAPK signaling pathways (ERK1/2, JNK, and p38) are actively involved in myocardial dysfunction [35], hypertrophy [36, 37], fibrosis [38, 39], and heart failure [40–42]. ERK is a MAPK family member, and ERK signaling is important in cardiac hypertrophy [43, 44]. Recently, several reports have described the important role played by ERK signaling in accelerated DCM development [45, 46]. Additionally, MAPKs are involved in cardiac physiology and various cardiovascular diseases, resulting in further studies that explored pharmacological/genetic blockers/activators and downstream targets of the MAPK pathway in the heart

[47, 48]. In relation to our results, these studies reported that DEX attenuated autophagy via the regulation of ERK and AKT signaling and improved cardiac malfunction. Although DEX improved cardiac function and inhibited autophagy in DM, it may exert an apoptosis-promoting effect at a higher concentration by protecting aberrant cells from metabolic stress and suppressing the cell death pathway. High doses of DEX (5 µg/kg or higher) have recently been reported to activate neuroapoptosis by stimulating the AKT/glycogen synthase kinase-3β signaling pathway [49]. Further studies should be performed to clarify the role of DEX in DM and are required to improve our understanding of the mechanism of DCM.

This study has several possible limitations. First, we did not perform behavioral tests during long-term DEX use; however, we did not observe any odd behavior during grooming. DEX may affect the behavior of rats via its sedative effect. Second, we did not evaluate cardiac function and measure autophagic markers after STZ administration and before DEX treatment owing to the insufficient time interval between them for DCM development. Even with the STZ model, we still do not exactly know the duration or magnitude of hyperglycemia that can lead to the occurrence of cardiac dysfunction and change in autophagic activity. Third, DEX is clinically administered via the intravenous, subcutaneous, or intranasal route. If chronically used in the treatment of DCM, oral or sublingual formulation would be more appropriate in the future.

In conclusion, this study reinforces our understanding of the potential anti-autophagic effect of DEX in patients with DCM. Our findings provide a greater understanding of the protective effects of DEX, with implications for the treatment of DCM.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All animal procedures were approved by the Committee for the Care and Use of Laboratory Animals, Yonsei University College of Medicine (No. 2015–0129), and were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health.

Human and animal rights This article does not contain any studies with human participants performed by any of the authors. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution.

Informed consent For this type of study formal consent is not required.

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