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Research Paper

Melatonin improves cardiac function in a mouse model of heart failure with preserved ejection fraction

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ABSTRACT

Melatonin has been shown to inhibit myocardial infarction-induced apoptosis, its function in heart failure with preserved ejection fraction (HFpEF) has not been investigated. This study aimed to investigate whether melatonin attenuates obesity-related HFpEF. Male mice were fed a high-fat diet (HFD) from weaning to 6 months of age to induce HFpEF. The mice were orally administered melatonin (50 mg/kg) by 3 weeks. Diastolic function was significantly improved by melatonin supplementation in mice fed an HFD. Melatonin attenuated obesity-induced myocardial oxidative stress and apoptosis and promoted the secretion of C1q/tumour necrosis factor-related protein 3 (CTRP3) by adipose tissue. And depletion of circulating CTRP3 largely abolished melatonin-mediated cardio-protection. Melatonin-mediated secretion of adipocyte-derived CTRP3 activated NF-E2-related factor 2 (Nrf2), which were largely abrogated by knocking down CTRP3 in adipocytes or Nrf2 in cardiomyocytes. Nrf2 activation was mediated by miR-200a, and a miR-200a antagomir offset the effects of melatonin-conditioned medium on Nrf2 expression. Our results indicate that melatonin can be used to treat and prevent obesity-related HFpEF.

1. Introduction

Epidemiological studies have reported that heart failure with preserved ejection fraction (HFpEF) accounts for approximately half of all heart failure (HF) cases [1]. More importantly, there is no standard therapy for HFpEF [2]. Therefore, it is greatly important that researchers find a drug that prevents HFpEF development. Obesity is an independent risk factor for HFpEF [3]. Obesity-induced cardiac dysfunction is coordinated by an orchestrated network responsible for inducing numerous pathological phenomena, including oxidative stress and cell apoptosis [4,5]. Theoretically, finding molecules that suppress obesity-induced oxidative stress and cell apoptosis would be of great benefit with respect to the management of HFpEF.

Adipose is now recognised as an endocrine organ. Adipose tissue could secrete a number of adipokines and inflammatory factors, acting on many other metabolically active tissues [6]. Adipose tissue is involved in the pathogenesis of pathological cardiac hypertrophy, systolic dysfunction and HFpEF [6,7], which suggests that functional crosstalk occurs between adipose tissue and the heart. C1q/tumour necrosis factor-related protein 3 (CTRP3), a newly identified factor secreted by adipocytes [8], plays a key role in cardiometabolic diseases [9]. CTRP3 deficiency in cardiomyocytes increased oxidative stress and cell apoptosis and that CTRP3 overexpression prevented diabetic cardiomyopathy [10]. However, the pathophysiological role of CTRP3 in HFpEF is unknown.

Melatonin is a hormone produced at night by the pineal gland. Melatonin is also a free-radical scavenger and a potent antioxidant [11–13]. Although melatonin has been shown to protect against myocardial infarction-induced apoptosis [14], its function in HFpEF has not been investigated. Previous studies reported that melatonin treatment attenuated obesity in rodents [15,16], and a recent study showed that melatonin induced adipogenesis in adipocytes and alleviated pyroptosis in mouse adipose tissue [17,18]. Given the close interplay between adipose tissue and the heart, we postulated that melatonin causes adipocyte tissue to secrete CTRP3, which improves high-fat diet (HFD)-induced HFpEF. As expected, we found that increases in the secretion of CTRP3 by adipose tissue after melatonin treatment resulted in the specific activation of miR-200a-NF-E2-related factor 2 (Nrf2) in cardiomyocytes to prevent the development of HFpEF.

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2. Materials and methods

2.1. Reagents

Melatonin and palmitic acid (PA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Recombinant human CTRP3 (rhCTRP3) was purchased from Aviscera Bioscience (Santa Clara, CA, USA). Anti-B-cell lymphoma-2 (Bcl-2, 1:1000) was obtained from Cell Signaling Technology (Danvers, Massachusetts). Anti-Nrf2 (1:1000), anti-heme oxygenase-1 (HO-1, 1:1000), anti-superoxide dismutase 2 (SOD2, 1:1000), anti-tumour necrosis factor-α (TNF-α, 1:200 for staining), anti-CD68 (1:200 for staining) and anti-CTRP3 (1:500 for western blotting, 1:200 for staining) were purchased from Abcam (Cambridge, UK). Anti-adiponectin (1:200 for staining) was purchased from Proteintech (Chicago, IL, USA). The secondary antibody used in the study was purchased from LI-COR Biosciences (1:10,000 dilution). Dihydroethidium (DHE) was purchased from Invitrogen (Carlsbad, CA, USA). 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA), total SOD assay kit, catalase (CAT) assay kit, and glutathione (GSH) assay kit were purchased from the Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The mini-osmotic pump was purchased from Alzet (DURECT Corp, Cupertino, CA). Cell counting kit-8 (CCK-8) was obtained from Dōjindo Laboratories (Kumamoto, Japan). All other chemicals were of analytical grade.

2.2. Animals and treatments

All animal experiments were performed according to the guidelines for the Care and Use of Laboratory Animals, which was published by the United States National Institutes of Health (NIH Publication, revised 2011), and were approved by the Animal Care and Use Committee of Zhengzhou University. Male C57/B6 mice (weaning age; body weight: 10–12 g) were purchased from the Institute of Laboratory Animal Science, Chinese Academy of Medical Sciences (Beijing, China). All the mice were housed at a controlled temperature (20–25 °C) and humidity (50 ± 5%) under a 12-h light-dark cycle (light from 6:00 a.m. to 6:00 p.m.) and were allowed free access to food and water. All the mice were fed an HFD (45% kilocalories from fat) or a normal diet (ND, 10% kilocalories from fat) from weaning to 6 months of age. To explore the protective effects of melatonin, 3 weeks before the end of the study, we orally administered the mice melatonin (50 mg/kg) by gavage once daily. The melatonin treatment continued for 3 weeks. One day before the end of the study, we measured the fasting blood glucose levels of the mice. At the end of treatment, all the mice were anaesthetized with isoflurane (1.5%) and subjected to echocardiographic and haemodynamic analyses. All the mice were subsequently euthanized with an overdose of sodium pentobarbital (200 mg/kg; i.p.), and the hearts were dissected to calculate the heart weight (HW)-to-tibia length (TL) ratio. Wet lung weight (LW) and dry LW were also determined. The left ventricles of the hearts were stored at − 80 °C for further study. The mesenteric white adipose tissue (Mes WAT) and subcutaneous white adipose tissue (SC WAT) were also weighed.

2.3. Mini-osmotic pump implantation

To verify that the protective effects of melatonin are mediated by adipose-derived CTRP3, we depleted circulatory CTRP3 using a mini-osmotic pump carrying a CTRP3 antibody. Briefly, all the mice were subcutaneously infused with CTRP3 antibodies (2.5 μg/g/day) or the same volume of IgG by an Alzet osmotic minipump for 4 weeks beginning 1 week before melatonin treatment. To ensure sustained antibody release, we rubbed daily the location at which the minipump was implanted. At the end of the study, we evaluated cardiac diastolic function.

2.4. Echocardiography and pressure-volume analysis

Transthoracic echocardiography was performed as described in the previous studies [10,19–23]. Briefly, the left hemithorax of each mouse was shaved under isoflurane (1.5%) anaesthesia, and echocardiography was performed by a MyLab 30CV ultrasound machine (Esaote SpA, Genoa, Italy) with a 10-MHz linear array ultrasound transducer. M-mode images of the left ventricle were recorded at the level of the papillary muscle. Heart rate and the systolic intraventricular septum (IVSs), left ventricle (LV) end-diastolic dimension (LVIDd) and ejection fraction (EF) were measured. All measurements represent the average of five cardiac cycles and were performed by the same observer.

Pressure-volume analysis was performed as described previously [10,19–23]. The mouse carotid artery was isolated, and a 1.4 French Millar catheter transducer (SPR-839; Millar Instruments, Houston, TX, USA) was advanced from the right carotid artery into the LV under deep anaesthesia. The data were then analysed by PVAN data analysis software.

2.5. Histological analysis

The hearts were fixed with 4% formaldehyde overnight. After dehydration, the hearts were embedded in paraffin and cut into sections. To observe morphometric alterations, we stained the sections with haematoxylin and eosin (H&E) to outline the cross-sectional area of the cardiomyocytes and picrosirius red (PSR) to evaluate cardiac fibrosis, as described in the previous studies [10,19–23]. The cross-sectional area and fibrotic area were measured with a digital analysis system (Image-Pro Plus 6.0, Media Cybernetics, Bethesda, MD, USA) using light-microscopic images of the heart sections. To outline the cardiomyocyte area, we analysed a total of 200 cells in 5 mice. Adipose tissues were also fixed in 4% formaldehyde overnight and embedded in paraffin. These sections were then stained with H&E. The diameter of the adipocytes (Mes WAT) was measured using Image-Pro Plus 6.0. A total of 200 cells in 5 mice were analysed. All the sections were examined by two authors without knowledge of the treatments to which the tissues had been subjected.

2.6. Quantitative real-time PCR and western blotting

Total RNA was prepared using TRIzol reagent (Invitrogen) from heart samples and Mes WAT. Two micrograms of RNA was reverse-transcribed into cDNA with random primers using a Transcriptor First Strand cDNA Synthesis Kit [Roche (Basel, Switzerland), 04896866001]. Real-time PCR was performed using LightCycler 480 SYBR Green 1 Master Mix (Roche, 04707516001). The PCR reaction conditions were as follows: 95 °C for 10 min, followed by 40 cycles of 5 °C for 10 s, 60 °C for 10 s and 72 °C for 20 s and a final step at 72 °C for 10 min *Gapdh* was used as an internal reference. All the primers used for the experiment are provided in Table S1. miRNA levels were detected using a Bulge-Loop™ miRNA qRT-PCR System, which was obtained from Guangzhou RiboBio Co., Ltd. (Guangzhou, China). *U6* was used as a miRNA internal reference.

Western blotting was performed as previously described [20]. Frozen heart samples and cells were harvested in RIPA lysis buffer, and then the proteins were separated on 10% SDS-PAGE and transferred onto PVDF membranes (Merck Millipore), which were blocked with nonfat milk for 1 h at room temperature before probed with antibodies to CTRP3 (1:500), Nrf2 (1:1000), HO-1 (1:1000), SOD-2 (1:1000) and GAPDH (1:1000) for 12 h at 4 °C. The membranes were then incubated with the appropriate secondary antibody for 1 h at room temperature. The membranes were scanned, and the relative expression levels of the proteins were normalized to that of GAPDH.