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Bendavia restores mitochondrial energy metabolism gene expression and suppresses cardiac fibrosis in the border zone of the infarcted heart ☆

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ABSTRACT

Aims: We have observed that Bendavia, a mitochondrial-targeting peptide that binds the phospholipid cardiolipin and stabilizes the components of electron transport and ATP generation, improves cardiac function and prevents left ventricular remodeling in a 6 week rat myocardial infarction (MI) model. We hypothesized that Bendavia restores mitochondrial biogenesis and gene expression, suppresses cardiac fibrosis, and preserves sarco/endoplasmic reticulum (SERCA2a) level in the noninfarcted border zone of infarcted hearts.
Main methods: Starting 2 h after left coronary artery ligation, rats were randomized to receive Bendavia (3 mg/kg/day), water or sham operation. At 6 weeks, PCR array and qRT-PCR was performed to detect gene expression. Picrosirius red staining was used to analyze collagen deposition.
Key findings: There was decreased expression of 70 out of 84 genes related to mitochondrial energy metabolism in the border zone of untreated hearts. This down-regulation was largely reversed by Bendavia treatment. Down-regulated mitochondrial biogenesis and glucose & fatty acid (FA) oxidation related genes were restored by administration of Bendavia. Matrix metalloproteinase (MMP9) and tissue inhibitor of metalloproteinase (TIMP1) gene expression were significantly increased in the border zone of untreated hearts. Bendavia completely prevented up-regulation of MMP9, but maintained TIMP1 gene expression. Picrosirius red staining demonstrated that Bendavia suppressed collagen deposition within border zone. In addition, Bendavia showed a trend toward restoring SERCA2a expression.
Significance: Bendavia restored expression of mitochondrial energy metabolism related genes, prevented myocardial matrix remodeling and preserved SERCA2a expression in the noninfarcted border, which may have contributed to the preservation of cardiac structure and function.

1. Introduction

Myocardial infarction (MI) results in substantial left ventricular damage, which may be followed by heart failure. Despite the considerable improvement in the treatment of acute MI in the past 20 years, MI mortality at 1 year still remains about 15%, and approximately 20% of patients with a first MI at ≥65 years of age will develop heart failure in 5 years [1]. The search for better therapies is one of the major challenges in cardiovascular disease. Emerging evidence shows that following MI, the heart becomes an energy-starved pump with decreased adenosine triphosphate (ATP) concentrations [2,3], reduced fatty acid (FA) oxidation rates [4] and impaired mitochondrial biogenesis [5]. The modulation of mitochondrial function may be a promising new approach for the treatment of heart failure related to MI.

Mitochondria are the powerhouse of the cells and are responsible for transforming chemical energy into ATP in order to supply energy for the demands of cardiac muscle contraction. ATP is synthesized primarily by mitochondrial oxidative phosphorylation (OXPHOS) at the electron transport chain. Mitochondrial OXPHOS is composed of four complexes: I, II, III, and IV that are embedded in the inner mitochondrial membrane and the ATP synthase (complex V). The reductions in the expression level and activity of the respiratory chain complexes have been reported in animal models of heart failure post MI and in failing human hearts [6,7].

Bendavia, a cell-permeable peptide, is an analog of Szeto–Schiller (SS)-peptides SS-31 and is also referred to as MTP-131 in the literature

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[8]. Bendavia selectively targets the inner mitochondrial membrane where it reduces levels of reactive oxygen species (ROS) and improves energetics through a cardiolipin-dependent mechanism [9–14]. Unlike mitochondrial-targeted antioxidant such as MitoQ, the uptake of Bendavia is independent of the mitochondrial membrane potential [15]. In a series of studies [9,10], we reported that Bendavia showed cardioprotective properties in the setting of acute ischemia/reperfusion injury models by enhancing mitochondrial energetics and reducing the production of cellular ROS levels. Recently, our laboratory has further demonstrated that chronic Bendavia therapy started 2 h after myocardial infarction improves cardiac function and limits left ventricular remodeling in a 6 week rat MI model, without altering blood pressure or heart rate [16].

In the present study, to further characterize the mitochondrial directed mechanism of Bendavia as it relates to improved cardiac function, we determined whether treatment with Bendavia restores mitochondrial function, suppresses cardiac fibrosis, preserves SERCA2a expression in a model of chronic left ventricular dysfunction following MI.

2. Methods

All experimental protocols were approved by the Institutional Animal Care and Use Committee, and performed in accordance with the "Guide for the Care and Use of Laboratory Animals" (National Academy Press, Washington DC, revised 2011, Eighth Edition). The Heart Institute at Good Samaritan Hospital was accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

2.1. Experimental groups

Myocardial infarction was induced in female Sprague–Dawley rats as described previously [16]. The artery that we occlude is the proximal left coronary artery right below the level of the left atrial appendage. Starting 2 h after left coronary artery ligation, rats were randomized to receive Bendavia (3 mg/kg/day) or water delivered by Alzet Osmotic Pump at 0.15 μl/h (model 2006; 200 μl). Noninfarcted rats served as shams. At 6 weeks, hearts were rapidly removed and snap frozen in liquid nitrogen. Before freezing, left ventricles (LV) with MI were surgically separated into border zone (a strip of non-infarcted heart tissue about 2 mm in width surrounding the scar) and the remote nonischemic area (the segment from LV septum which is opposite of the infarct area). There are 5 groups that were studied: Sham = noninfarcted normal hearts, MI/BZ = noninfarcted border zone of water-treated infarcted hearts, MI/BZ + Bendavia = noninfarcted border zone of Bendavia-treated hearts, MI/R = remote noninfarcted area of water-treated hearts, and MI/R + Bendavia = remote noninfarcted area of Bendavia-treated hearts. Data on cardiac function and LV remodeling using this model were previously reported [16].

2.2. PCR gene array

Total RNA was extracted using a TRIzol reagent (Invitrogen). RNA was treated with RNase-free DNase and purified using RNase mini kit (Qiagen). Reverse transcription reaction was performed with 500 ng of total RNA using RT^2-first strand kit (SABioscience). Rat mitochondrial energy metabolism PCR array was performed to measure gene expression of the electron transport chain and oxidative phosphorylation complexes (Rat mitochondrial energy metabolism, PARN-008ZD. SABioscience) by using Bio-Rad CFX 96 touch real-time PCR detection system.

2.3. qRT-PCR

Total RNA was extracted using a TRIzol reagent. RNA was treated with RNase-free DNase and purified using RNase mini kit (Qiagen). iScriptTM cDNA Synthesis Kit (Bio-Rad) was used for cDNA synthesis and quantitative RT-PCR was performed using a CFX96 touch real-time PCR system (Bio-Rad). Primers used for qPCR include: PGC1α forward GACCCTCCTCACACCAAAC, reverse GCGACTGCGGTTGTGTATG; β-actin forward CTGTGTGGATTGGTGGCTCT, reverse GCTCAGTAACAGTCCGCCTA; NRF1 forward CGCTCATCCAGGTTGGTACT, reverse TTCACCGCCCTGTAATGTGG; Tfam forward AGGGGGCTAAGGATGAGTC, reverse ATCACTTCGCCCAACTTCAG; ERRα forward AACGCCCTGGTGTCTCATC, reverse CTGATGGTGACCACTATCTC; PPARα forward CTCGGGGATCTTAGAGGCGA, reverse GCACCAATCTGTGATGACAACG; CD36 forward CTCACACAACTCAGATACTGCTG, reverse GCACTTGCTTCTTGCCAACT; GLUT4 forward TACCGTCTTCACGTTGGTCTC, reverse TAACTCATGGATGGAACCCGC; MMP9 forward GATCCCCAGAGCGTTACTCG, reverse GTTGTGGAAACTCACACGCC; Timp1 forward ACAGCTTTCTGCAACTCGGA, reverse AGCGTCGAATCCTTTGAGCA; and SERCA2a forward TTGTGGCCCGAAACTACCTG, reverse GGGCTGGAAGATGTGTTGCT.

2.4. Picrosirius red staining

After 6 weeks of treatment, the hearts were arrested in diastole by injecting intravenous potassium chloride and were pressure-fixed (13 cm water column) in 10% formalin. The formalin-fixed hearts were embedded in paraffin and 5 μm thickness slides were staining with picrosirius red to estimate the extent of interstitial collagen using Image J. The collagen volume fraction was determined as the percentage of picrosirius red positive-stained area relative to total area.

2.5. Electron microscopy

All rats were assigned randomly to treatment with Bendavia or water for 6 weeks.

Rats were anesthetized with an intraperitoneal injection of xylazine and ketamine. A catheter was inserted into the abdominal aorta toward the heart for perfusion fixation and a small nick was placed in the inferior vena cava to drain blood. Phosphate buffer solution was infused for 3 min to remove blood (pressure equal to the mean blood pressure at 122 cm H_2O, 90 mm Hg); thereafter the heart was arrested in diastole by potassium chloride injection followed by 15 min perfusion with modified Karnovsky solution. The heart was excised and ~1 mm × 1 mm × 2 mm thick slices were cut from the border zone and remote area, immersed in modified Karnovsky's fixative overnight at 4 °C for further fixation and then processed for ultrastructural analysis using a transmission electron microscope (JEOL JEM-2100 at 100 kV). Quantitative analysis was performed blindly from 10 images per sample (3000× magnification). The following quantitative measurements were obtained using Image J: mitochondrial size (μM^2), mitochondrial number/10 μM^2, width (μM), length (μM) and ratio of width to length. At least 300 mitochondria in the border zone and 200 mitochondria in the remote area of each rat were measured for quantitative assessment.

2.6. Cardiolipin analysis

Cardiolipin analysis was carried out by Miao Wang at Sanford-Burnham Medical Research Institute, Orlando, FL. Heart tissue was pulverized into a fine powder by a stainless steel biopulverizer at the temperature of liquid nitrogen. The tissue powders of 10 to 20 mg were weighed and homogenized in 0.5 mL 10× diluted PBS in 2.0 ml cryogenic vials (Corning Life Sciences, Tewksbury, MA) by using a digital sonifier (Branson 450, Danbury, CT). Protein assay on the homogenates was performed by using a bicinchoninic acid protein assay kit (Thermo Scientific, Rockford, IL) with bovine serum albumin as standards. The determined cardiolipin levels were normalized to the protein content of individual samples. Individual homogenate of the heart samples (equal ~0.8 mg protein amount) was accurately transferred into a disposable glass culture test tube. Cardiolipin internal standard was added prior to lipid extraction. Lipid extraction was performed by using a modified Bligh and Dyer