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Original article

MitoQ improves mitochondrial dysfunction in heart failure induced by pressure overload

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ABSTRACT

Heart failure remains a major public-health problem with an increase in the number of patients worsening from this disease. Despite current medical therapy, the condition still has a poor prognosis. Heart failure is complex but mitochondrial dysfunction seems to be an important target to improve cardiac function directly.

Our goal was to analyze the effects of MitoQ (100 μM in drinking water) on the development and progression of heart failure induced by pressure overload after 14 weeks. The main findings are that pressure overload-induced heart failure in rats decreased cardiac function *in vivo* that was not altered by MitoQ. However, we observed a reduction in right ventricular hypertrophy and lung congestion in heart failure animals treated with MitoQ. Heart failure also decreased total mitochondrial protein content, mitochondrial membrane potential in the intermyofibrillar mitochondria. MitoQ restored membrane potential in IFM but did not restore mitochondrial protein content. These alterations are associated with the impairment of basal and stimulated mitochondrial respiration in IFM and SSM induced by heart failure. Moreover, MitoQ restored mitochondrial respiration in heart failure induced by pressure overload. We also detected higher levels of hydrogen peroxide production in heart failure and MitoQ restored the increase in ROS production. MitoQ was also able to improve mitochondrial calcium retention capacity, mainly in the SSM whereas in the IFM we observed a small alteration. In summary, MitoQ improves mitochondrial dysfunction in heart failure induced by pressure overload, by decreasing hydrogen peroxide formation, improving mitochondrial respiration and improving mPTP opening.

1. Introduction

Despite diagnosis and treatment, heart failure remains a major clinical problem and a huge economic burden on the health care system. The incidence and costs are projected to double in the next twenty years. The disease is highly prevalent in elderly patients and is associated with high mortality rates within five years of diagnosis despite current optimal medical therapy. Current medical therapy can prevent new onset and slow the progression once heart failure is established but prognosis is still poor even for most favorable treated patients, and new therapeutic approaches are needed. Heart failure rehospitalizations rates remain high (35%) within 90 days after discharge and this event has not changed over the last 15 years [1–5].

Commonly prescribed therapies although beneficial in improving some symptoms often do not approach the underlying causes of progressive left ventricular dysfunction presented in heart failure [6].

Left ventricular dysfunction is accompanied by derangements in myocardial fuel metabolism and bioenergetics that contribute to the development and progression of the disease. The energy supply must match the energy demand in the adult heart due to its high requirement of energy to sustain contractile function. In the adult heart, almost all energy production comes from mitochondrial oxidative phosphorylation continually generated by oxidation of carbon fuels [7–11]. Growing evidence provides information that alterations in mitochondrial ATP production are causally linked to the development of heart failure. Notably, it has been shown that alterations in fatty acid oxidation and oxidative phosphorylation can cause cardiomyopathy in humans and decreased levels of phosphocreatine accelerates the decline during the transition from left ventricular hypertrophy to heart failure [12–15].

In addition, studies in animals and humans have revealed reprogramming of myocardial fuel utilization in the failing heart: a shift from

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the fatty acid oxidation (FAO) to increased reliance on glucose utilization. The mechanisms through which the failing heart shifts substrate utilization are still poorly understood.

Moreover, mitochondria are recognized as the main source of reactive oxygen species (ROS) within the cell. ROS production is usually low under physiological conditions, however, when pathological ROS production outpaces endogenous scavenging capacity; it can alter proteins and lipid function, triggering cell death, which typically occurs in heart failure, therefore, decreasing mitochondrial quality control and energy supply [16].

To decrease mitochondrial oxidative damage, many mitochondria-targeted antioxidants have been developed in the last few years and a promising mitochondrial antioxidant that has emerged is MitoQ, which consists of a quinone moiety linked to a triphenylphosphonium moiety by a 10-carbon alkyl chain [17].

MitoQ has been shown to protect against oxidative damage in many animal models of pathology, including cardiac ischemia–reperfusion (IR) injury [18], hypertension [19] but the effects on the development of heart failure remains understood. Therefore, our goal was to analyze the effects of MitoQ on the development and progression of heart failure induced by pressure overload.

2. Material and methods

2.1. Experimental design and aortic constriction surgery

The animal protocol was conducted according to the Guideline for the Care and Use of Laboratory Animals (NIH publication 85–23) and was approved by the University of Maryland School of Medicine Institutional Animal Care and Use Committee (Protocol number 1009011).

Animals were anesthetized with 2.5% isoflurane in oxygen, intubated and ventilated. A partial median sternotomy was performed and the thymus resected. After dissection of the aortic arch, a tantalum clip (.45 mm internal diameter-Hemoclip) was placed on the aorta between the brachiocephalic trunk and the left common carotid artery. The sternotomy was closed with interrupted sutures and the skin closed with running sutures. After their vital signs were reestablished, rats were extubated. Rats were kept in 100% oxygen and on warming pads during the surgery and recovery periods. Age-matched Sham-operated animals underwent the same procedure without clip application. Three days after surgery, the animals were assigned to receive MitoQ (100 μM in drinking water).

2.1.1. Echocardiography

Cardiac function was assessed using a Vevo 2100 High-Resolution Imaging Systems (Visual Sonics) with a 15-MHz linear array transducer (MS200) at 13 weeks of treatment as previously described [20]. Rats were anesthetized with 2.5% isoflurane in oxygen, shaved and placed on a warming pad. Two-dimensional cine loops and guided M-mode frames were recorded from the parasternal short and long axes. At the end of the study, all data were analyzed offline with software resident on the ultrasound system, and calculations were made to determine left ventricular volumes. Ejection fraction was calculated as: (end-diastolic diameter − end-systolic diameter)/end-diastolic diameter. Relative wall thickness was calculated as the sum of the posterior and anterior wall thickness at end diastole divided by the end diastolic diameter. Mean wall thickness was taken as the average of the posterior and anterior wall thickness at end diastole. All calculations were made from parasternal short axis measurements.

2.1.2. Tissue harvest

After 14 weeks of treatment, the animals were anaesthetized with 5.0% isoflurane between 3 and 6 h after initiation of the light phase while given free access to food. The thorax was opened and blood was collected from the left ventricle and immediately placed on ice tubes containing or not EDTA, and centrifuged at 1500 g for 15 min at 4 °C to obtain serum and plasma, respectively. The heart was removed, and three sections of the left ventricle free wall were taken for biochemical analysis and stored at −80 °C, and the remainder was used for mitochondrial isolation as described below.

2.1.3. Mitochondrial isolation

Subsarcolemmal mitochondria (SSM) and interfibrillar mitochondria (IFM) were isolated as previously described [20–22]. Briefly, the LV was rinsed in ice cold Chappel-Perry buffer (100 mM KCl, 50 mM MOPS, 5 mM MgSO4, 1 mM ATP, 1 mM EGTA, 2 mg/mL BSA), blotted dry and then weighed. The ventricles were minced and homogenized in 1:10 (wt/vol) ice cold Chappel-Perry buffer. The homogenates were centrifuged at 580 × *g* for 10 min. The supernatant containing SSM was extracted and centrifuged again at 5000 × *g* to isolate SSM. The remaining pellet from the 580 × *g* spin was resuspended in KCl-MOPS-EGTA buffer containing 100 mM KCl, 50 mM MOPS, and 0.5 mM EGTA at pH 7.4, and treated with trypsin (5 mg/g) for 10 min at 4 °C. The samples were incubated with Chappel-Perry buffer (albumin 2 mg/mL) to inhibit trypsin and spun down at 580 × *g* for 10 min. The IFM-containing supernatant was spun down at 5000 × *g* for 10 min. The pellets were washed in ice cold Chappel-Perry buffer and spun down at 5000 × *g* for 10 min and washed in KME (100 mM KCl, 50 mM MOPS, and 0.5 mM EGTA at pH 7.4) and then resuspended in KME. The concentration of mitochondrial protein was measured by the Lowry method using bovine serum albumin as standard.

2.1.4. Mitochondrial respiration

Mitochondrial respiration was assessed in both IFM and SSM as described previously [21,23]. Isolated mitochondria (0.5 mg/mL) were respired in respiration buffer containing 100 mM KCl, 50 mM MOPS, 5 mM KH_2PO_4, 1 mM EGTA and 1 mg/mL BSA/Fraction V, using a polarographic oxygen sensing systems for measurements of dissolved oxygen consumption in liquid suspension (Qubit System, Kingston, ON, Canada). States 3 and 4 were measured with glutamate + malate (10 and 5 mM, respectively), palmitoylcarnitine (40 μM) and Succinate (20 mM) with rotenone (7.5 μM) was used to assess respiration through Complex II of the ETC exclusively. State 3 respiration (ADP-stimulated) was measured in the presence of 200 μM ADP. State 4 respiration (ADP-limited) was assessed after ADP consumption. Respiratory Control Ratio, the ratio of State 3 to State 4 was calculated to assess the control of oxygen consumption by phosphorylation. The ratio of ADP added in the chamber to the total amount of oxygen consumed in state 3 (ADP:O ratio) was calculated as an index of the efficiency of oxidative phosphorylation.

2.1.5. Mitochondrial membrane potential (ΔΨm), size and complexity

Membrane potential, size and internal complexity were determined by flow cytometry analyses using Facscan (Becton Dickinson) as previously described [23]. Distinct mitochondrial subpopulations, IFM and SSM were incubated with the ratiometric dye 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazol carbocyanine iodide (JC-1; Invitrogen) for 15 min at 37 °C (final concentration 300 nM), which is a lipophilic cation that enters selectively into mitochondria, or MitoTracker Deep Red 633 (Invitrogen), which passively diffuses into intact mitochondria due to membrane potential and selectively stain intact mitochondria. Each individual parameter (gating, size, and complexity) was performed using specific light sources, detectors and 100,000 gated events were analyzed per sample. Measurements were performed on freshly isolated mitochondria and values are expressed as the mean orange fluorescence divided by the mean green fluorescence for membrane potential analyses (JC-1). For size and internal complexity analyses (MitoTracker), geometric mean representing FSC (forward scatter-logarithmic scale) was used as an indicator of size, whereas values from SSC (side scatter-logarithmic scale) were used to indicate complexity.