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Aging related functional and structural changes in the heart and aorta: MitoTEMPO improves aged-cardiovascular performance

Yusuf Olgar[a], Sinan Degirmenci[a], Aysegul Durak[a], Deniz Billur[b], Belgin Can[b], Gizem Kayki Mutlu[c], Ebru Arioglu Inan[c], Belma Turan[a,*]

[a] Department of Biophysics, Ankara University, Faculty of Medicine, Ankara, Turkey
[b] Department of Histology-Embriology, Ankara University, Faculty of Medicine, Ankara, Turkey
[c] Department of Pharmacology, Ankara University, Faculty of Pharmacy, Ankara, Turkey

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ABSTRACT

Aging in humans represents declining in cardio-protective systems, however its mechanisms are not known yet. We aimed to analyse how aging affects key mechanisms responsible for contractile dysfunction *via* comparing the improperly synchrony between electrical and mechanical activities in male aged-rats (24-month old) comparison to those of adult-rats (6-month old). We determined significantly increased systemic oxidative stress with decreased antioxidant capacity, clear insulin resistance and hypertrophy in aged-rats with normal fasting blood glucose. We also determined significantly high level of reactive oxygen species, ROS production in fluorescent dye chloromethyl-2′,7′-dichlorodihydrofluoroscein diacetate (DCFDA) loaded isolated cardiomyocytes from aged-rats, confirming the increased oxidative stress in these hearts. *In situ* electrocardiograms, ECGs presented significant prolongations in RR- and QT-intervals in the aged-rats. Invasive hemodynamic measurements demonstrated marked increases in the heart rate and mean arterial pressure and decreases in the ejection-fraction and preload-recruitable stroke-work, together with depressed contraction and relaxation activities in aortic rings. In light and electron microscopy examinations in aged-rats, significant increases in muscle fibre radius and amount of collagen fibres were detected in the heart as well as markedly flattened and partial local splitting in elastic lamellas in the aorta, besides irregularly clustered mitochondria and lysosomes around the myofilaments in cardiomyocytes. MitoTEMPO treatment of tissue samples and cardiomyocytes from aged-rats for 1-h induced significant structural improvements. In the second part of our study, we have shown that mitochondria-targeted antioxidant MitoTEMPO antagonized all alterations in the heart samples as well as penylephrine-induced contractile and acetylcholine-induced relaxation responses of aged-rat aortic rings. Overall, the present data strongly support the important role of mitochondrial oxidative stress in the development of aged-related insufficiencies and that antioxidant strategies specifically targeting this organelle could have therapeutic benefit in aging-associated complications.

1. Introduction

Aging, being a complex process, is the most important risk factor for most diseases in humans giving a dominant risk factor for *cardiovascular* changes, and, as latter consequence, contributes to cardiac morbidity and mortality in the aged-humans (Lakatta et al., 2001; Yang et al., 2005). A number of parameters contribute to the pathogenesis of cardiac aging including action potential prolongation, changes in ionic exchanges across sarcolemma, and dysregulation in Ca^{2+} homeostasis, increased fibrosis, mitochondrial defects and increased oxidative stress in cardiomyocytes (Anversa et al., 1990; Bhashyam et al., 2007; Hacker et al., 2006; Lakatta et al., 2001; Preston et al., 2008; Yang et al., 2005). One of components of aging heart *via* dysfunction development, is due to altered cardiac energy metabolism (Bhashyam et al., 2007; Mcmillin et al., 1993; Sample et al., 2006) including development of insulin resistance together with a reduction in oxygen consumption and ATP formation (Kumaran et al., 2005; Preston et al., 2008). However, the precise role of all these changes in cardiac function in the aging heart has not been clearly presented.

Furthermore, not only experimental studies but also theoretical approaches on the role of metabolic or mitochondrial alterations as negative impact on myocardial contractile performance, point out the role mitochondrial changes as the main contributor to the aged-associated cardiac complications. Supporting these statements, recent data

* Corresponding author.
E-mail address: belma.turan@medicine.ankara.edu.tr (B. Turan).

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well-documented the impaired metabolic flexibility with a decreased capacity to oxidize fatty acids, a link between metabolic and contractile dysfunction in aged cells, and enhanced dependence on glucose metabolism as well as impairment in mitochondrial oxidative phosphorylation (Barton et al., 2017; Lesnefsky et al., 2016). Furthermore, supporting these studies, it has been demonstrated that cardiac oxidative stress and lipid overload together with mitochondrial oxidative stress could cause increased sarcoplasmic reticulum Ca^{2+}-leak by hyperphosphorylation/oxidization of RyR2-channels and affecting ventricular function *via* reduction in a mitochondrial-targeted anti-oxidant capacity under different pathophysiological conditions (Joseph et al., 2017; Okatan et al., 2016; Tuncay et al., 2013; Yaras et al., 2008; Yaras et al., 2005). These results suggest a potential role for mitochondrial-targeted anti-oxidants to prevent cardiac dysfunction in aging humans. Moreover, studies imply that any disconnection between the electrical and mechanical activation of the left ventricle is a good candidate for aging heart dysfunction (Fujiwara et al., 2014).

Recently, it has been presented that protective effects of an antioxidant targeting mitochondria, MitoTEMPO against doxorubicin cardiotoxicity (Rocha et al., 2016). Moreover, a therapeutic inhibition of mitochondrial ROS with MitoTEMPO reduces diabetic cardiomyopathy (Ni et al., 2016), while resolution of oxidative stress in mitochondria by MitoTEMPO in the old mice restored cardiac function and the capacity of coronary vasodilation to the same magnitude observed in the young mice (Owada et al., 2017). Overall, one can hypothetize that a direct mitochondria targeting antioxidant enhancement strategy may have promissive therapeutic benefits in aging-heart complications. Therefore, this study first aimed to analyse how aging affects key mechanisms that are responsible for contractile dysfunction *via* comparing the improperly synchrony between electrical and mechanical activities. Second, taking into consideration a possible existence of mitochondrial oxidative stress in aging myocardium, we examined whether a mitochondria-targeted antioxidant MitoTEMPO, acts as a mitochondrial manganese superoxide dismutase (SOD) mimetic, could prevent cardiac dysfunction in an aged-rat model.

2. Material and methods

2.1. Animals

All experimental procedures were performed in accordance with the standards of the European Community guidelines on the care and use of laboratory animals and approved by the Ankara University with a reference number of 2016-18-165 in accordance with the guide for the care and use of laboratory animals. Male 6-month-old and 24-month-old Wistar rats were used. All animals were exposed to a 12-h light–dark cycle in standard animal housing rooms and were given free access to tap water. They were freely fed with standard chow *ad libitum* daily.

2.2. Oral glucose tolerance test

Rats fasted overnight with free access to tap water, then they were received 1 g/kg glucose in double-distilled water by orogastric gavage, as described previously (Okatan et al., 2015). After measuring starved blood glucose 15th, 30th, 60th, and 120th minute's measure were performed following glucose administration. Blood glucose levels were assessed using standard glucose test strips (GlucoCheck Analyzer).

2.3. Measurement of arterial pressure

Systolic and diastolic blood pressures as well as heart rates were measured by an indirect tail-cuff method *via* an NIBP200-A non-invasive blood pressure meter (BIOPAC Systems Inc., USA), as described previously (Krege et al., 1995).

2.4. In situ electrocardiogram measurement

Continuous electrocardiograms (ECGs) during 10 min were recorded *in vivo* through the animals' paws by using two custom-made electrodes (MP150, BIOPAC Systems, Inc.) placed to paws with a third one on the tail as reference electrode under light ether anesthesia. ECGs were band-pass filtered (50–500 Hz) and two bipolar limb leads (20-gauge needles) were analyzed. The heart rate, peak-to peak amplitude of ECG traces (QRS value) and their durations such as PR-, RR- and QT-intervals were determined.

2.5. Total antioxidant status (TAS) and total oxidant status (TOS) measurement in serum

TAS levels were measured using commercially available kit (RL0024, *Rel Assay Diagnostics*, Turkey) as described, previously (Erel, 2004). Shortly, the novel automated method is based on the bleaching of characteristic color of a more stable ABTS (2,2′ - Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical cation by antioxidants. The results were expressed as mmol Trolox equivalent/L.

TOS levels were measured using commercially available kits (RL0024, *Rel Assay Diagnostics*, Turkey) as described, previously (Erel, 2004). Shortly, the oxidation reaction is enhanced by glycerol molecules abundantly present in the reaction medium. The ferric ion produced a colored complex with xylenol orange in an acidic medium. The color intensity, which could be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with H_2O_2 and the results were expressed in terms of μM H_2O_2 equivalent/L.

2.6. Assessment of in vivo cardiac function

Pressure volume analysis (P-V loop analysis) is made under isofluorane inhalation. Body temperature of rats is stabilized at 37 °C during the experiment. After incision of right carotid artery, the pressure-volume catheter (Transonic, NY, USA) was inserted to record arterial pressure. Then the catheter was moved to the left ventricle of the heart in order to measure different cardiac parameters. Preload-recruitable stroke-work, PRSW, was calculated by inferior vena cava occlusion. The measured parameters were normalized to body weights to eliminate body weight differences between animals. All parameters were calculated by using Labscribe 2 acqusition software (Arioglu-Inan et al., 2013).

2.7. Experiments with Langendorff-perfused hearts

The rats were anesthetized with pentobarbital sodium (30 mg/kg by intraperitoneal injection) and hearts were prepared for Langendorff-perfusion apparatus, as described previously (Okatan et al., 2015). The hearts were electrically stimulated (DCS, Harward) at 300 beats/min with 1.5 ms square waves (at twice the threshold voltage). Changes in the left ventricular developed pressure (LVDP) were measured with a water-filled latex balloon inserted into the left ventricle and all data were recorded online then stored and processed (Model 1050BP; BIOPAC Systems, Goleta, California, USA). The experiments were repeated after 1-h with 0.1 μM MitoTEMPO perfusion for all groups.

2.8. Contractile activity of aortic rings

Rats were anesthetized with pentobarbital sodium (30 mg/kg by intraperitoneal injection) and the thoracic aorta was removed and placed in cold Krebs-Henseleit solution (mM): NaCl, 119; KCl, 4.8; $MgSO_4$, 1.2; $CaCl_2$, 1.8; $NaHCO_3$, 25; KH_2PO_4, glucose 10 (bubbled with 95% O_2–5% CO_2). The segments were carefully cleaned from fat and loose connective tissue sectioned into 3-mm long rings. Aortic rings were stretched to a 1-g initial tension and were equilibrated for 60 min.