Experimental Gerontology 58 (2014) 78–82

Contents lists available at ScienceDirect

Experimental Gerontology

journal homepage: www.elsevier.com/locate/expgero

Mitochondrial quality control and age-associated arterial stiffening

Thomas J. LaRocca *, Christopher M. Hearon Jr., Grant D. Henson, Douglas R. Seals

Department of Integrative Physiology, University of Colorado Boulder, Boulder, CO 80309, USA

ARTICLE INFO

Article history:
Received 23 December 2013
Received in revised form 13 June 2014
Accepted 13 July 2014
Available online 14 July 2014

Section Editor: Christiaan Leeuwenburgh

Keywords:
Collagen
Mitophagy
Mitochondrial biogenesis

ABSTRACT

Stiffening of large elastic arteries with age increases the risk of cardiovascular diseases (CVD), but the underlying mechanisms are incompletely understood. We investigated the role of mitochondrial quality control (QC, i.e., mitophagy and biogenesis) in arterial stiffening with aging. In C57BL6 mice, aging was associated with impaired aortic expression of mitochondrial QC mediators, greater activation of the mitochondrial redox/stress sensor p66shc, elevated superoxide production and increased arterial stiffness—as indicated by ~25% higher aortic pulse wave velocity (aPWV). In old mice, supplementation with trehalose, a nutraceutical reported to enhance mitophagy, normalized mitochondrial QC markers, p66shc activation and superoxide production, and reduced aPWV and aortic collagen I (a structural protein that confers stiffness). In vitro experiments suggested that mitochondrial QC processes were enhanced in the aortas from old trehalose-treated mice, and in aortic rings studied ex vivo, both aging and treatment with the mitochondrial stressor rotenone were associated with increases in p66shc activation and intrinsic mechanical stiffness, whereas co-incubation with trehalose prevented these effects. Taken together, these findings suggest that mitochondrial stress/dysfunction as a result of impaired mitochondrial QC contributes to large elastic artery stiffening with age. Enhancing mitochondrial QC with agents such as trehalose may be a novel strategy for reducing age-associated arterial stiffness and CVD.

1. Introduction

Advancing age is the primary risk factor for cardiovascular diseases (CVD) (Lloyd-Jones et al., 2010). A major cause of age-associated CVD is stiffening of large elastic arteries, including the aorta (Lakatta and Levy, 2003). Indeed, aortic pulse wave velocity (aPWV), the benchmark clinical measure of large elastic artery stiffness, is a powerful predictor of incident CVD in older adults (Mitchell et al., 2010). Age-related arterial stiffening results from structural changes in the arterial wall including increases in collagen deposition and degradation of elastin (Zieman, 2005), but the mechanisms by which these changes develop with aging are poorly understood.

A compelling but uninvestigated hypothesis is that dysregulation of mitochondrial quality control (QC) contributes to arterial stiffening with age. Mitochondrial QC requires a balance between the production of healthy mitochondria (mitochondrial biogenesis) and the degradation of damaged mitochondria (mitophagy). Impaired mitochondrial QC results in mitochondrial dysfunction that plays a central role in aging and disease (Nunnari and Suomalainen, 2012) and could explain certain events associated with structural remodeling in the arterial wall (e.g., adverse redox/stress signaling and increased production of reactive oxygen species, such as superoxide) (Marzetti et al., 2013). However, the role of mitochondrial QC in large elastic artery stiffening with aging is unknown.

We recently reported that the nutraceutical trehalose enhances autophagy, the cellular process of degrading damaged proteins and organelles, in the aortas of old mice (LaRocca et al., 2012). Although we did not examine specific targets of trehalose-mediated autophagy, evidence indicates that trehalose may protect against mitochondrial stress/dysfunction by inducing mitophagy (Rodriguez-Navarro et al., 2010; Sarkar et al., 2007). If so, trehalose may have the potential to improve mitochondrial QC and prevent or reverse arterial stiffening with age. Accordingly, the aims of the present study were to determine the associations between aging, markers of mitochondrial QC and arterial stiffness (aPWV), and to evaluate the efficacy of trehalose for enhancing mitochondrial QC and reducing arterial stiffness in a mouse model of arterial aging. A series of in vitro experiments was also conducted to investigate mechanisms underlying these processes.

2. Materials and methods

2.1. Animals

Young (4–5 months) and old (27–28 months; ~50% survival rate) male C57BL6 mice were obtained from the National Institute on Aging rodent colony. Controls received regular drinking water, and treated animals received 2% trehalose (Sigma-Aldrich) supplemented water for 4 weeks. Mice were kept on a 12:12 h light–dark cycle with ad libitum access to water and normal chow. All procedures conformed to the

* Corresponding author at: Department of Integrative Physiology, University of Colorado at Boulder, 354 UCB, Boulder, CO 80309, USA.
E-mail addresses: thomas.larocca@colorado.edu (T.J. LaRocca), seals@colorado.edu (D.R. Seals).

http://dx.doi.org/10.1016/j.exger.2014.07.008
0531-5565/

Guide for the Care and Use of Laboratory Animals (NIH publication no. 85-23) and were approved by the University of Colorado Boulder Institutional Animal Care and Use Committee.

2.2. Aortic pulse wave velocity and arterial blood pressure

aPWV was measured as previously described (Fleenor et al., 2012a, 2012b). Mice were anesthetized with 2% isoflurane and positioned supine on a heating board with limbs secured to ECG electrodes. Pulse waves were detected at the transverse aortic arch and the abdominal aorta using Doppler probes (MouseDoppler data acquisition system; Indus Instruments). Time elapsed between the ECG R-wave and the foot of the Doppler signal was determined for each site, and aPWV was calculated as: aPWV = (distance between probes) / ($\Delta time_{abdominal} - \Delta time_{transverse}$). To examine the potential contribution of changes in arterial blood pressure to any treatment-related differences in aPWV, systolic and diastolic blood pressure were assessed using a CODA noninvasive tail-cuff system (Kent Scientific) as previously described Fleenor et al. (2012b). Five acclimation cycles followed by 20 data collection cycles of blood pressure measurements were recorded on three consecutive days and averaged.

2.3. Aortic protein expression and superoxide production

Protein mediators of mitochondrial QC and arterial stiffening were analyzed in excised and cleaned thoracic aortas by standard Western blotting techniques (Criterion System; Bio-Rad) (Fleenor et al., 2012a; LaRocca et al., 2012). Primary antibodies: Parkin (1:1000 dilution; Cell Signaling), sirtuin 3 (SIRT3, 1:1000; Abcam), BCL2/adenovirus E1B 19 kDa protein-interacting protein 3 (BNIP3, 1:1000; Novus Biologicals), PPARγ co-activator-1α (PGC-1α, 1:1000; Novus Biologicals), Ser36 phosphorylated p66shc adaptor protein (1:500; Cell Signaling), collagen I (1:1000; Abcam), glyceraldehyde-3-phosphate dehydrogenase (GAPDH, 1:1000; Cell Signaling), and voltage dependent anion channel (VDAC, 1:500; Cell Signaling). Proteins were detected using HRP-conjugated secondary antibodies (Jackson ImmunoResearch) and ECL chemiluminescent substrate (Pierce Biotechnology).

Aortic superoxide production was assessed by electron paramagnetic resonance (EPR) spectroscopy using the superoxide-specific spin probe 1-hydroxy-3-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine (Enzo Life Sciences) as previously described (Fleenor et al., 2012b; LaRocca et al., 2012). Briefly, freshly isolated 2 mm aortic segments were incubated for 60 min at 37 °C in Krebs buffer containing 0.5 mM spin probe, and EPR signal amplitude was analyzed immediately on an MS300 X-band EPR spectrometer (Magnettech).

2.4. Dynamic mitochondrial QC

To estimate flux through mitochondrial QC pathways, changes in the mitochondrial marker VDAC were monitored using a modified Western blot protocol (Zhu et al., 2011). Equal length segments of thoracic aorta were snap-frozen immediately or after 8 h incubation at 37 °C in either nutrient-complete Dulbecco's Modified Eagle Medium (DMEM) or Hank's Balanced Salt Solution (HBSS, without glucose or amino acids)—a stimulus for starvation-induced autophagy/mitophagy (Ding and Yin, 2012; Kim and Lemasters, 2011; Mizushima et al., 2010)—with or without the mitophagy inhibitor chloroquine (100 μM) (Ding and Yin, 2012; Webster et al., 2013; Zhu et al., 2011). Nutrient deprivation was used as a stimulus in order to avoid off-target effects associated with pharmacological mitophagy inducers. Samples were lysed and analyzed via Western blotting as described above, and the difference between VDAC levels in the presence vs. absence of chloroquine was interpreted as a rough indicator of mitochondrial QC (degradation/synthesis of mitochondria) (Ding and Yin, 2012; Mizushima et al., 2010; Webster et al., 2013; Zhu et al., 2011).

2.5. Intrinsic mechanical properties

To simulate age-related mitochondrial dysfunction and trehalose treatment, equal length segments of thoracic aorta were incubated in DMEM with or without the mitochondrial stressor rotenone (0.5 μM, Sigma-Aldrich) and/or trehalose (100 mM) for 48 h at 37 °C. Half of each segment was used for Western blotting, and the remaining half was divided into two rings ~1 mm in length for mechanical and histological analyses as previously described (Fleenor et al., 2012a). Rings were loaded into a pre-heated (37 °C) wire myograph chamber (DMT Inc.) in calcium-free phosphate buffered saline, pre-stretched for 3 min to 1 mm luminal diameter displacement and then returned to baseline (zero force). After 3 pre-stretch cycles, diameter was increased until 1 mN force and then increased by 10% every 3 min until tissue failure. Force was recorded after each interval, and stress and strain were calculated, with stress defined as: $t = (\lambda L) / (2HD)$ [t = one-dimensional stress; λ = strain; L = one-dimensional load; H = wall thickness, determined by histology; D = vessel length]. Strain was defined as: $\lambda = \Delta d / d(i)$ [λ = strain, Δd = change in diameter, d(i) = initial diameter]. Slope of the stress–strain curve was used to determine elastic modulus (intrinsic mechanical stiffness).

2.6. Statistical analyses

Statistical analysis was performed using SPSS 19.0 software. Comparisons between groups/treatment conditions were made by ANOVA with post-hoc tests as appropriate, and significance was determined as $P < 0.05$. All results are presented as means ± SEM.

3. Results

3.1. Effects of aging and trehalose on mitochondrial QC

The critical mitophagy mediator Parkin (Youle and van der Bliek, 2012) and the key mitochondrial fitness/biogenesis modulator SIRT3 (Bell and Guarente, 2011) were reduced in the aorta of old compared with young control mice (Fig. 1A and B). Impaired expression of these mitochondrial QC mediators was associated with significantly greater activation (phosphorylation) of the mitochondrial redox/stress sensor p66shc (Fig. 1C) (Gertz and Steegborn, 2010) and elevated superoxide production (Fig. 1D). In old mice, trehalose supplementation restored Parkin and SIRT3, and also enhanced expression of the mitophagy mediator BNIP3 (Gottlieb and Carreira, 2010) and the mitochondrial biogenesis regulator PGC-1α (Nunnari and Suomalainen, 2012), while markedly reducing p66shc activation and superoxide production (Fig. 1A, B, C and D).

3.2. Effects of aging and trehalose on arterial stiffness

Old mice with reduced expression of mitochondrial QC mediators also had ~25% greater aPWV (Fig. 2A), increased expression of the load-bearing protein collagen I (Zieman, 2005) and a modest reduction in elastin compared with young controls (Fig. 2B). Supplementation with trehalose normalized aPWV and aortic collagen I in old mice, but had no effect on young animals and did not influence elastin levels. In a separate cohort of old mice (n = 4), trehalose had no effect on arterial blood pressure (pre-treatment: 91 ± 2 mm Hg systolic, 66 ± 2 diastolic vs. post-treatment: 90 ± 2 systolic, 64 ± 3 diastolic).

3.3. Trehalose, mitochondrial QC and intrinsic mechanical properties

In the aortas from old control and old trehalose-treated mice studied ex vivo, incubation in complete medium had no effect on markers of mitochondrial mass, whereas nutrient deprivation, a stimulus for mitophagy/autophagy (Gottlieb and Carreira, 2010; Kim and Lemasters, 2011; Zhu et al., 2011), caused reductions in the mitochondrial marker