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Multivessel analysis of progressive vascular aging in the rat: Asynchronous vulnerability among vascular territories

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ABSTRACT

Aging induces vascular dysfunction, representing the major risk factor for cardiovascular disease. Our aim was to ascertain specific vulnerability of vascular territories to aging by evaluating the progressive impact of aging on vascular function in four different vascular beds: aorta, mesenteric artery (MA), coronary artery (CA), and penile corpus cavernosum (CC) from 3, 6, 9, 12, 20 or 24 months-old male rats. Contractile/relaxant responses were evaluated in organ chambers (A/CC) and wire myographs (MA/CA). Relationships of systemic biomarkers with endothelial function impairment were also determined. Although all vessels manifested aging-related impairment in endothelial vasodilation, CA was the most impacted by aging considering the onset (at 6 months) and magnitude of endothelial dysfunction (reduction by 1.5 log units in the concentration required for 50% of maximal relaxation for acetylcholine). H_2O_2-induced vasodilations were progressively reduced by aging in aorta, CC and CA while NO-donor-induced vasodilation was impaired by aging only in CA. Serum asymmetric dimethylarginine significantly correlated to endothelial decline in aorta, MA, and CC, while HOMA-IR was significantly associated with endothelial dysfunction in CA and MA. CA are especially vulnerable to aging-related vascular dysfunction. Correlations of vascular dysfunction with systemic biomarkers differ among vessels, further suggesting heterogeneity in aging-induced vascular impact.

1. Introduction

Aging is considered to be the major risk factor for cardiovascular disease (CVD). In fact, the incidence and severity of subclinical and clinical manifestations of CVD increase with age (Lakatta and Levy, 2003; Paneni et al., 2015) even in the absence of traditional risk factors (Wu et al., 2014). This is due, in part, to the presence of altered endothelial function, manifested by reduced endothelium-dependent vasodilation. In fact, endothelial dysfunction is considered to be the primary antecedent for atherosclerotic diseases. In this sense, arterial stiffness, which is a key feature of aging-related vascular alterations (Kotsis et al., 2011; Wen et al., 2015) is always preceded by an impaired endothelial vasodilation suggesting that this arterial alteration is also linked to endothelial dysfunction (Scuteri et al., 2008). Furthermore, the presence of an altered endothelial function may, in turn, aggravate media thickness and fibrosis (Paneni et al., 2015). Thus, endothelial dysfunction represents a key step in the initiation and maintenance of atherosclerosis and is an independent predictor of cardiovascular events (Steyers and Miller et al., 2014).

The aging process is associated with reduction of the endothelium-dependent vasodilation, both in the micro and the macrovasculature derived from animal models (Lakatta and Levy, 2003; Dal-Ros et al., 2012; Gano et al., 2014) and humans (Rodríguez-Mañas et al., 2009; Angulo et al., 2012; Walker et al., 2014). Although inflammation and oxidative stress outstand as most probable candidate processes leading to vascular impairment in aging (Wadley et al., 2013; El Assar et al., 2013; 2016a), the mechanism(s) responsible for aging-related vascular dysfunction has(have) not been completely elucidated. The maintenance of a correct function of the vascular bed seems to be an essential determinant of healthy ageing (Virdis et al., 2010). Supporting the role of endothelial function in determining the outcome of aging, it has been reported that a variant of the protein bactericidal/

Abbreviations: ADMA, asymmetric dimethylarginine; CVD, cardiovascular disease; DDAH, dimethylarginine dimethylaminohydrolase; HOMA, homeostasis model assessment of insulin resistance; A, aorta; MA, mesenteric artery; CA, coronary artery; CC, penile corpus cavernosum; SNP, sodium nitroprusside; TNF-α, tumor necrosis factor-α

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permeability-increasing fold-containing-family-B-member-4 (BPIFB4) which promotes endothelial function and repair is associated with exceptional longevity in humans (Villa et al., 2015).

However, the concept of a general unimodal impact of aging on the whole vasculature could be not completely right and specific vascular beds could be differentially affected by aging process. Although a heterogeneous impact of aging between micro- and macrovasculature with respect to the prostanoids influencing vasodilation has been reported (Matz et al., 2000), there is scarce evidence of studies determining the vulnerability of specific vascular beds to the impact of aging.

The aim of this work was to evaluate the progressive impact of aging on vascular function, with special emphasis on endothelial vasodilation and reactive oxygen species (ROS)-induced responses, in four very different vascular beds of the rat, the large conductance vessel, aorta, the resistance mesenteric small vessels, the coronary arteries, and the highly specialized vascular tissue, penile corpus cavernosum. Endothelial function in the different vascular territories was correlated with systemic biomarkers of oxidative stress, inflammation, insulin resistance and NO pathway.

2. Methods

2.1. Experimental animals

Male Sprague-Dawley rats were obtained from the Animal Facilities of the Hospital Universitario de Getafe. Animals were maintained in 12 h light/dark cycles with free access to food and water until experimental procedures. Rats were bred and let to age in the facilities. Animal studies were performed in accordance with the Declaration of Helsinki and with the Guide for the Care and Use of Laboratory Animals, as adopted and promulgated by National Institutes of Health, following European regulations and were approved by the Ethics Committees for Animal Experimentation of the Hospital Universitario de Getafe and the Hospital Universitario Ramón y Cajal (PROEX 005/15).

At an age of 3 (n = 18), 6 (n = 15), 9 (n = 10), 12 (n = 14), 20 (n = 16) and 24 months (n = 6), rats were weighed and anesthetized with diazepam (5 mg/kg) and ketamine (90 mg/kg). Blood samples were obtained via cardiac puncture and collected in anticoagulant-free and potassium EDTA-containing tubes for biochemical measurements. Blood collection was always carried out at 9:00–9:30 h. Sera and plasma were obtained by centrifugation and stored at −80 °C until determinations of circulating biomarkers were made. Always under deep anaesthesia, exsanguination caused the humane death of the animals. Right after blood collection, heart, thoracic aorta, omentum (for isolation of mesenteric small vessels), and penis were carefully excised for functional evaluations. Experiments with rats from all ages were intercalated to avoid possible sequence-dependent bias.

2.2. Blood pressure measurements

An additional set of animals from each age group (3 M, n = 7; 6 M, n = 7; 9 M, n = 8; 12 M, n = 8; and 20 M, n = 6) were anesthetized as described above and left carotid artery was catheterized with a PE50 tube connected to a pressure transducer and a MacLab data acquisition system for monitoring blood pressure. Blood pressure was recorded for, at least, ten minutes and mean arterial pressure (MAP) and heart rate (HR) were determined from the last five minutes of the record. These animals were not utilized for evaluation of vascular function or blood collection.

2.3. Functional evaluation of aortic segments

The thoracic aorta was carefully excised, cleaned of surrounding fat and connective tissue and placed in a Petri dish with Krebs-Henseleit solution (KHS) at 4 °C. Composition of KHS was (in mM): NaCl 119, KCl 4.6, $CaCl_2$ 2.5, $MgCl_2$ 1.2, $NaHCO_3$ 24.9, glucose 11, KH_2PO_4 1.2 and EDTA 0.027. Aortae were cut into 4–5 mm-long cylindrical segments. For circular isometric tension recording, each vascular cylinder was set up in an organ bath containing KHS at 37 °C continuously bubbled with 95% O_2/ 5% CO_2 mixture, which gave a pH of 7.4, according to the method described elsewhere (El Assar et al., 2015; 2016b). Tension was continuously recorded in a data acquisition system (MP100A BIOPAC System, Santa Barbara, CA, USA). To assess vessel viability preparations were then exposed to 75 mM K^+ and the contractile response was measured. Aortic segments were contracted with norepinephrine (NE; 10–30 nM; 80% of K^+-induced contraction, approximately) and, when a stable plateau was reached, increasing concentrations of acetylcholine (ACh; 0.01–10 μM), hydrogen peroxide (H_2O_2; 0.1 μM to 0.1 mM) or sodium nitroprusside (SNP; 1 nM to 1 μM) were added and vasodilatory responses were determined. Contraction responses were evaluated by cumulatively adding NE (1 nM to 10 μM) to non-pre-contracted vascular segments. Each preparation was exposed to just one pharmacological agent. This also applies for the other vascular preparations.

2.4. Vascular reactivity of rat mesenteric arteries

Second to third order branches of mesenteric arterial tree (lumen diameter 200–400 μm) were obtained from omentum specimens and dissected by carefully removing the adhering fat tissue. Arterial ring segments (~2 mm long) were subsequently mounted on microvascular wire myographs (J.P. Trading, Aarhus, Denmark) for circular isometric tension recordings, as described elsewhere (El Assar et al., 2015; 2016b). The vessels were allowed to equilibrate for 30 min in KHS continuously bubbled with 95% O_2/5% CO_2 mixture to maintain a pH of 7.4. The passive tension and internal circumference of vascular segments when relaxed in situ under a transmural pressure of 100 mmHg (L_{100}), were determined. The arteries were then set to an internal circumference equivalent to 90% of L_{100}, at which the force development is close to maximal. To assess vessel viability, preparations were then exposed to 125 mM K^+ (KKHS, equimolar substitution of NaCl for KCl in KHS) and the contractile response was measured. After a stabilization period, rat arteries were contracted with 1–3 μM NE (80% of KKHS-induced contraction, approximately) and relaxation responses were evaluated by cumulative additions of ACh (1 nM to 10 μM), H_2O_2 (1 μM to 1 mM), or SNP (1 nM to 100 μM) to the chambers. Contraction responses were evaluated by cumulatively adding NE (1 nM–30 μM) to non-pre-contracted vascular segments.

2.5. Functional evaluation of coronary arteries

Left and right coronary arteries (average diameter: 376 ± 6 μm) were isolated from excised hearts and cleaned from surrounding cardiac tissue. Segments of coronary arteries (~2 mm long) were set in wire myographs for isometric tension recording in the same way as above described for mesenteric arteries. For relaxation experiments, coronary arterial segments were contracted with 1–3 μM serotonin (5-HT; 80% of KKHS-induced contraction, approximately) and relaxation responses were evaluated by cumulative additions of ACh (1 nM to 10 μM), H_2O_2 (1 μM to 1 mM), or SNP (1 nM to 10 μM) to the chambers. Contraction responses were evaluated by cumulatively adding 5-HT (1 nM to 30 μM) to non-pre-contracted arterial rings. There were no differences in vascular responses between right and left coronary arteries (data not shown).

Where indicated, endothelium was removed by repeatedly passing a human hair through the lumen of mounted arterial segments. After that, viability of the arterial preparation and success in endothelium removal were confirmed by exposure to 120 mM K + and 10 μM ACh, respectively.