European Journal of Pharmacology 741 (2014) 186–194

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Cardiovascular pharmacology

Contribution of receptor for advanced glycation end products to vasculature-protecting effects of exercise training in aged rats

Qi Gu [a,*], Bing Wang [a], Xiao-Feng Zhang [b], Yan-Ping Ma [c], Jian-Dong Liu [d], Xiao-Ze Wang [e]

[a] School of Physical Education, Xi'an Technological University, 4 Jinhua Road, Xi'an 710032, Shaanxi Province, China
[b] Department of Gynecology, Chengyang People's Hospital, Qingdao, Shandong Province, China
[c] Department of Neurology, Chengyang People's Hospital, Qingdao, Shandong Province, China
[d] Department of Gastroenterology, The Third People's Hospital of Datong, Datong, Shanxi Province, China
[e] Department of General Urgery, Yuquan Hospital of Tsinghua University, Beijing, China

ARTICLE INFO

Article history:
Received 12 June 2014
Received in revised form
15 August 2014
Accepted 18 August 2014
Available online 24 August 2014

Keywords:
Exercise training
Aging
Vascular dysfunction
Advanced glycation end products

ABSTRACT

The aim of present work was to investigate the underlying mechanism of vasculature-protecting effects of exercise training in aged rats. Experiment 1: aged rats were given moderate-intensity exercise for 12 weeks. Exercise training suppressed advanced glycation evidenced by reduced activity of aldose reductase, increased activity of glyoxalase 1, reduced levels of methylglyoxal and N^{ε}-(carboxymethyl) lysine, and decreased expression of receptor for advanced glycation end products (RAGE) in aged aortas. Experiment 2: aged rats were given moderate-intensity exercise for 12 weeks or treated with FPS-ZM1, an inhibitor of RAGE. Exercise training attenuated aortic stiffening with age marked by reduced collagen levels, increased elastin levels and reduced pulse wave velocity (PWV), and prevented aging-related endothelial dysfunction marked by restored endothelium-mediated vascular relaxation of aortas in response to acetylcholine. Exercise training in aging aortas reduced formation of malondialdehyde, 3-nitrotyrosin and reactive oxygen species, increased GSH/GSSG ratio, suppressed activation of NFκB, and reduced levels of IL-6 and chemokine (C-C motif) ligand 2. Similar effects were demonstrated in aged rats treated with FPS-ZM1. Collectively, exercise suppressed advanced glycation in the aortas of aged rats, which, at least in part, explained the vasculature-protecting effects of exercise training in aged population.

1. Introduction

Cardiovascular diseases are the most common cause of death among the elderly patients in modern societies (Lloyd-Jones et al., 2010). Aging is an independent cardiovascular risk factor associated to large artery stiffness and impairment of endothelial function (Herrera et al., 2010; Seals et al., 2011; Fleenor, 2012). Vascular aging, formerly being considered an immutable and inexorable risk factor, is now viewed as a target process for intervention in order to achieve a healthier old age. Several studies have found that physical activity enhanced cardiovascular fitness during the course of the lifecycle, and attenuated age-related arterial stiffening (Vaitkevicius et al., 1993; Shibata and Levine, 2012) and endothelial dysfunction (DeVan et al., 2013; Kitzman et al., 2013) in older adults and aged rodents. Although the mechanisms underlying this beneficial effect probably include favorable changes in plasma lipids and lipoproteins, blood pressure, and insulin resistance (Shephard and Balady, 1999), little is known about the molecular mechanisms by which aerobic exercise exerts its protection in vasculature against aging.

The phenomenon of nonenzymatic glycation – by which the carbonyl group of glucose can directly condense with a free amino group – may be relevant for the process of aging. Advancing age promoted the accumulation of advanced glycation end products (AGEs), a non-enzymatic glycosylation of proteins that, in turn, acted with their chief cell surface receptor–receptor for advanced glycation end products (RAGE) to promote large elastic artery stiffness and endothelial dysfunction (Verbeke et al., 1997; Li et al., 2005; Hallam et al., 2010). Compounds that break AGEs cross-links, such as Alagebrium, have been shown to attenuate arterial

Abbreviations: AR, aldose reductase; GLO-1, glyoxalase 1; MG, methylglyoxal; CML, N^{ε}-(carboxymethyl) lysine; AGEs, advanced glycation end products; RAGE, receptor for advanced glycation end products; PWV, pulse wave velocity; ACh, acetylcholine; SNP, sodium nitroprusside; MDA, malondialdehyde; GSH, glutathione; GSSG, glutathione disulfide; CCL2, chemokine (C-C motif) ligand 2; O_2^-, superoxide; $OONO^-$, peroxynitrite; CSA, citrate synthase activity; MAP, mean arterial blood pressure

* Corresponding author. Tel./fax: +86 2983209312.
E-mail address: guqi20123@hotmail.com (Q. Gu).

http://dx.doi.org/10.1016/j.ejphar.2014.08.017
0014-2999/

stiffening in older adults (Kass et al., 2001) and rodents (Steppan et al., 2012). Moreover, treatment of biologically active AGEs *ex vivo* induced greater mechanical stiffness in cultured aortic rings isolated from young mice, supporting an important role of AGEs in vascular dysfunction (Fleenor et al., 2012). Aldose reductase (AR) is the first enzyme of the polyol pathway. A critical consequence of flux via the AR pathway is the generation of a precursor of AGE- methylglyoxal (MG) (Lal et al., 1995), which could be detoxified by Glo-1 (Thornalley, 2003). Delbin et al. 2012 found that exercise training reduced N(ε)-(carboxymethyl) lysine (CML, AGE biomarker) levels in femoral and coronary arteries from diabetic rats.

In this study, we tested the hypothesis that chronic moderate-intensity exercise training suppresses AR activity and upregulates Glo-1 activity to reduce formation of MG and AGEs, and suppresses activation of RAGE in aortas of aged rats. The suppression of AR–MG–AGEs–RAGE axis might explain the vascular protection of exercise training in aged rats, at least in part.

2. Materials and methods

2.1. Materials, animals and study design

Unless otherwise specified, regents were purchased from Sigma-Aldrich (St Louis, MO, USA).

The Young (2-month old) and Old (23-month old) male Fisher 344 × Brown Norway rats were provided by Vital River Laboratory Animal Technology Company (Beijing, China). All the rats were entrained to controlled temperature (22–24 °C), 12-h light and 12-h dark cycles (light, 08:00–20:00 h; darkness, 20:00–08:00 h), and free access to food and tap water. The 'Old' group was 23 months old when the exercise and RAGE antagonism treatment were finished. The 'Young' group is 2 months old when they are killed.

Experiment 1: rats were divided into three groups (n=50–52 in each group) as follows: (1) sedentary young group (Young); (2) sedentary old group (Old); (3) exercised-trained old group (Old+EX). Chronic aerobic exercise training on treadmill (Table 1S, Supplementary data) was performed as indicated in the published protocol (Husain, 2004). The intensity of the exercise training was moderate. After exercise, enzyme activities of AR (n=10 in each group) and GLO-1 (n=10 in each group), aortic levels of sorbitol and fructose (n=10 in each group) and aortic MG levels (n=10 in each group), and CML content in plasma and aortas (n=10 in each group) were determined.

Experiment 2: rats were divided into four groups (n=60–63 in each group) as follows: (1) sedentary young group (Young); (2) sedentary old group (Old); (3) exercised-trained old group (Old+EX); (4) old group treated with FPS-ZM1 (Old+ FPS-ZM1). The RAGE inhibitor FPS-ZM1 (EMD Millipore Chemicals, Billerica, MA, USA) was given to rats via daily oral gavages at 1 mg/kg of body weight for 12 weeks. After exercise or treatment, hemodynamic parameters, citrate synthase activity of soleus muscles (n=10 in each group), histology (n=10 in each group), aortic remodeling (n=10 in each group), PWV and endothelial function (n=10 in each group) were determined. Indices of oxidative stress (n=10 in each group) and inflammation (n=10 in each group) were determined.

2.2. Ethical approval

All the animals used in this work received humane care in compliance with institutional animal care guidelines, and were approved by the Local Institutional Committee. All the surgical and experimental procedures were in accordance with institutional animal care guidelines.

2.3. Anesthesia

24 h after the last session of exercise or treatment, animals (not the animals used in measurement of pulse wave velocity) were anesthetized with sodium pentobarbital (50 mg/kg) administrated intraperitoneally. The thoracic aortas and soleus muscles were gently removed for subsequent analyses.

2.4. Assessment of citrate synthase activity

Citrate synthase (a respiratory enzyme which underwent adaptive increases due to exercise in skeletal muscle fibers) was used as a marker of training efficacy. Soleus muscles from each rat were collected for determination of citrate synthase activity (CSA) to determine the efficacy of the training protocol (Husain, 2004). CSA was measured from whole muscle homogenate by using a citrate synthase activity assay kit (Sigma, St. Louis, MO, USA).

2.5. Measurement of blood pressure, heart rate, and plasma glucose

After the induction of anesthesia with a combination of diazepam (6 mg/kg, i.p.) and ketamine (40 mg/kg, i.p.), the rat was placed in a supine position on a heated operating table to maintain the temperature of the body temperature at 37 °C. One of the femoral arteries was cannulated for measurement of mean arterial pressure (MAP) and heart rate (HR) as previously described (Ogihara et al., 2010). Both BP and HR were monitored continuously on a computer running the Chart 5 software (AD Instruments, Lasec CPT, SA) through a BP transducer linking the arterial cannula to a PowerLab® via a BP amplifier.

The plasma fasting glucose level was determined by a colorimetric method using a glucosemeter (Advantage. Boehringer Mannheim, USA). Plasma was separated by centrifugation (1500 × g) for 15 min at 4 °C and stored at −80 °C until assayed.

2.6. Measurement of AR/MG/AGEs/RAGE pathway

Aortic aldose reductase (AR) activities were measured by using spectrophotometric techniques as described previously (Hwang et al., 2003).

Aortic glyoxalase 1 (GLO-1) activity was assayed by spectrophotometry according to the method of McLellan and Thornalley, monitoring the increase in absorbance at 240 nm due to the formation of S-D-lactoylglutathione for 10 min at 25 °C.

Levels of sorbitol and fructose were measured as described previously (Hwang et al., 2003). Briefly, tissues were homogenized with water, extracted with methanol and centrifuged (4 °C, 10,000 rpm, 1 min). The supernatant was applied to an InertSep SAX/SCX (50 mg/50 mg/1 ml) cartridge (GL Sciences, Inc., Tokyo, Japan). The eluate was evaporated to dryness under a stream of nitrogen at 40 °C. The residues were dissolved in 200 μl of the mixture of acetonitrile/water (9:1, v/v). Then sorbitol and fructose contents were determined with the LC/MS/MS system which consisted of an SIL-HTC and LC-10A (Shimadzu Corp., Kyoto, Japan) and the API4000 tandem mass spectrometer (Applied Biosystems/MDX SCIEX, MA, USA) with atmospheric pressure chemical ionization.

Aortic methylglyoxal (MG, a precursor in the formation of AGE) was measured in the neutralized perchloric acid extracts of aortas by HPLC methods according to previously published procedures (Hwang et al., 2004).