Archives of Biochemistry and Biophysics 645 (2018) 12–18

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

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

Exercise training protects against cancer-induced cardiac remodeling in an animal model of urothelial carcinoma

Ana Isabel Padrão[a,b,1], Rita Nogueira-Ferreira[c,d,*,1], Rui Vitorino[c,d], Dulce Carvalho[a], Catarina Correia[a], Maria João Neuparth[b], Maria João Pires[e], Ana Isabel Faustino-Rocha[e], Lúcio Lara Santos[f], Paula Alexandra Oliveira[e], José Alberto Duarte[b], Daniel Moreira-Gonçalves[b,d,**], Rita Ferreira[a]

[a] QOPNA, Department of Chemistry, University of Aveiro, Campus Universitário de Santiago, 3810-193, Aveiro, Portugal
[b] CIAFEL, Faculty of Sports, University of Porto, R. Dr. Plácido da Costa 91, 4200-450, Porto, Portugal
[c] iBiMED, Department of Medical Sciences, University of Aveiro, Agra do Crasto, 3810-193, Aveiro, Portugal
[d] Departamento de Cirurgia e Fisiologia, Faculdade de Medicina, Universidade do Porto, Alameda Professor Hernâni Monteiro, 4200-319 Porto, Portugal
[e] CITAB, Department of Veterinary Sciences, School of Agrarian and Veterinary Sciences, University of Trás-os-Montes and Alto Douro (UTAD), Quinta de Prados, 5001-911 Vila Real, Portugal
[f] Experimental Pathology and Therapeutics Group, Research Center of Instituto Português de Oncologia, R. Dr. António Bernardino de Almeida, 4200-072, Porto, Portugal

ARTICLE INFO

Keywords:
Bladder cancer
Exercise training
Heart remodeling
Fibrosis
Therapeutics

ABSTRACT

Limiting cancer-induced cardiac damage has become an increasingly important issue to improve survival rates and quality of life. Exercise training has been shown to reduce cardiovascular complications in several diseases; however, its therapeutic role against cardiovascular consequences of cancer is in its infancy. In order to add new insights on the potential therapeutic effect of exercise training on cancer-related cardiac dysfunction, we used an animal model of urothelial carcinoma submitted to 13 weeks of treadmill exercise after 20 weeks of exposure to the carcinogenic *N*-butyl-*N*-(4-hydroxybutyl)-nitrosamine (BBN). Data showed that 13 weeks of treadmill exercise reverted cancer-induced cardiomyocytes atrophy and fibrosis, improved cardiac oxidative capacity given by citrate synthase activity and MnSOD content, and increased the levels of the mitochondrial biogenesis markers PGC-1α and mtTFA. Moreover, exercise training reverted cancer-induced decrease of cardiac c-kit levels suggesting enhanced regenerative ability of heart. These cardiac adaptations to exercise were related to a lower incidence of malignant urothelial lesions and less signs of inflammation. Taken together, data from the present study support the beneficial effect of exercise training when started after cancer diagnosis, envisioning the improvement of the cardiovascular function.

1. Introduction

A growing body of evidence is pointing for a direct negative impact of cancer itself on heart, independently of chemo- and/or radiotherapy cardiotoxicity. For instance, cardiac atrophy and left ventricle remodeling, including wall thinning and fibrosis, were found in patients with gastrointestinal, pancreatic, and nonsmall cell lung cancer who died from cardiac cachexia [1]. In addition, circulating levels of cardiovascular peptides like NT-proBNP, MR-proANP, MR-proADM, CT-pro-ET-1 and hsTnT were elevated in patients with cancer prior the induction of any cardiotoxic anticancer therapy, suggesting the presence of subclinical functional and morphological myocardial damage [2]. Several pre-clinical models further support this cross-talk between cancer and cardiac dysfunction, with the most severe cases presenting with heart failure [3–5]. The molecular pathways underlying these cardiac changes are still being disclosed but seem to involve a tumor-related pro-inflammatory state. Pro-inflammatory cytokines such as IL-6, TNF-α and TWEAK were found to be increased in tumor-bearing animals and may activate specific signaling pathways in the heart, including NF-κB and apoptosis [5,6]. Increased activity of the ubiquitin-proteasome system and autophagy, as well as deregulation of metalloproteinases activity were also shown to contribute to changes of extracellular matrix in cardiac muscle resulting in cardiac fibrosis [7,8]. The catabolic phenotype presented by cardiomyocytes is also

* Corresponding author. Departamento de Cirurgia e Fisiologia, Faculdade de Medicina, Universidade do Porto, Alameda Professor Hernâni Monteiro, 4200-319, Porto, Portugal.
** Corresponding author. Departamento de Cirurgia e Fisiologia, Faculdade de Medicina, Universidade do Porto, Alameda Professor Hernâni Monteiro, 4200-319, Porto, Portugal.
E-mail addresses: rmferreira@ua.pt (R. Nogueira-Ferreira), danielmgon@gmail.com (D. Moreira-Gonçalves).
[1] Equally contributors.

https://doi.org/10.1016/j.abb.2018.03.013
Received 10 October 2017; Received in revised form 20 February 2018; Accepted 12 March 2018
Available online 13 March 2018
0003-9861/ © 2018 Elsevier Inc. All rights reserved.

characterized by impaired mitochondria evidenced by distorted morphology [7].

Despite the advances in the treatment of cancer, little progress has been made in the treatment of cancer-related cardiac maladaptation. There are unquestionable evidences of the cardiac protection conferred by exercise training; however, the exact molecular mechanisms by which exercise training protects the heart remain poorly understood [9], and even less known in the set of cancer. The limited number of studies on the cross-tolerance effect of exercise training on cancer-related cardiac dysfunction supports its preventive effect through the modulation of tumor burden and inflammation [5,10]. It was recently shown that 35 weeks of treadmill exercise prevented the cancer-induced increase in cardiac levels of TWEAK, TRAF-6, atrogin-1, and NF-κB p50, which was related to a lower incidence of mammary lesions in a preclinical model of breast cancer [5]. However, exercise training was initiated early after the first contact with the carcinogenic. Thus, it remains to be clarified if similar benefits can be observed when exercise training is started in latter stages, when carcinoma is already present. So, in the present study we explored the potential therapeutic effect of exercise training on cancer-related cardiac dysfunction in an animal model of urothelial carcinoma submitted to 13 weeks of treadmill exercise after 20 weeks of exposure to the carcinogenic *N*-butyl-*N*-(4-hydroxybutyl)-nitrosamine (BBN).

2. Material and methods

2.1. Animals and experimental design

The animal protocol was approved by the Portuguese Ethics Committee for Animal Experimentation, *Direção Geral de Alimentação e Veterinária* and was performed in accordance to European Commission Recommendation 2007/526/CE. Forty-four male Wistar rats were obtained at the age of 5 weeks from Harlan (Barcelona, Spain). During the experimental protocol, animals were housed in groups of 4 rats/cage under controlled conditions of 22 ± 2 °C and 60 ± 5% of relative humidity with 12/12 h dark-light cycle, with free access to food (standard laboratory diet 4RF21® (Mucedola, Italy)) and water. After a week of acclimatization, the animals were randomly divided into two experimental groups: exposed to 0.05% N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN) in the drinking water over the course of 20 weeks (BBN group, n = 24) and with access to tap water (CONT group, n = 20). After this 20 week-period, half of the animals from each group started an exercise training program in a treadmill during 13 weeks (subgroups BBN + EX (n = 12) and CONT + EX (n = 10)). The activity of the animals from the sedentary groups (subgroups BBN + SED (n = 12) and CONT + SED (n = 10)) was confined to the cage's space. The number of animals *per* group was planned taking in consideration the potential biological variability to BBN exposure and to exercise training, mortality rate and 3Rs policy of animal experimentation [11].

Animals from the EX group were submitted to a treadmill exercise training program on an electric treadmill (Treadmill Control® LE 8710, Panlab, Harvard Apparatus, USA) for 13 weeks during 5 days/week. In the first two weeks, exercise duration and treadmill speed was gradually increased until reaching 60 min/day at 20 m/min and maintained thereafter for 11 weeks. At the end of the experimental protocol, all animals were weighed and sacrificed with ketamine/xylazine (Imalgen® and Rompun®, respectively). Urinary bladder, heart and *gastrocnemius* muscles were collected. The urinary bladders were inflated *in situ* by intravesical instillation of 100 μL of buffered 10% phosphate formalin solution for 12 h. Urinary bladders were processed for histology. Heart and *gastrocnemius* muscles were weighed. Heart muscle was divided and one piece (the apex of the heart) was immediately prepared for histological analysis and the remaining tissue was stored at −80 °C for subsequent biochemical analysis.

2.2. Histological analysis of urinary bladder and cardiac muscle

Cubic pieces from cardiac muscle and urinary bladders were fixed [4% (v/v) buffered paraformaldehyde] by diffusion during 24 h and subsequently dehydrated with graded ethanol and included in paraffin blocks. Serial sections (5 μm of thickness) of paraffin blocks were cut by using a microtome and mounted on silane-coated slides. The slides were dewaxed in xylene and hydrated through graded alcohols finishing in phosphate buffered saline solution. Deparaffinized sections of cardiac tissue were stained for hematoxylin-eosin and Picrosirius red staining for the analysis of cardiomyocyte cross-sectional area (CSA) and cardiac fibrosis, respectively, as previously described by us [12]. Urinary bladder histology was performed by an experienced researcher (Oliveira PA) to confirm the spectrum of urothelial lesions previously observed [13].

2.3. Cardiac muscle preparation for biochemical analysis

A portion (~50 mg) of cardiac muscle was homogenized in 100 mM phosphate buffer, pH 7.4, supplemented with protease (200 mM PMSF, 1:1000) and phosphatase inhibitors (P0044 and P5726, Sigma 1:1000), using a Teflon pestle on a motor-driven Potter-Elvehjem glass homogenizer at 0–4 °C (3–5 times for 5 s at low speed, with a final burst at a higher speed). The protein content of the cardiac muscle homogenates was assayed with the Bio-Rad RC-DC method, following the instructions of the manufacturer, using bovine serum albumin as a standard.

2.4. ATP synthase activity

ATP synthase activity was measured in cardiac muscle fractions using a spectrophotometric assay as previously described [14]. The phosphate produced by hydrolysis of ATP reacts with ammonium molybdate in the presence of reducing agents to form a blue-color complex, the intensity of which is proportional to the concentration of phosphate in solution. Optical densities were measured at 610 nm. Oligomycin was used as an inhibitor of mitochondrial ATPase activity.

2.5. Citrate synthase activity

Citrate synthase activity was measured in cardiac muscle homogenates using the method described by Coore et al. [15]. In brief, the CoASH released from the reaction of acetyl-CoA with oxaloacetate was measured by its reaction with 5, 5′-dithiobis-(2-nitrobenzoic acid) (DTNB) at 412 nm (molar extinction coefficient of 13.6 mM-1cm-1).

2.6. Immunoblotting analysis

Equivalent amounts of cardiac muscle protein of each experimental group (30 μg) were electrophoresed on a 12.5% SDS-PAGE as described by Laemmli [16]. Gels were blotted onto a nitrocellulose membrane (Whatman®, Protan) in transfer buffer (25 mM Tris, 192 mM glycine, pH 8.3 and 20% methanol) during 2 h (200 mA). Then, nonspecific binding was blocked with 5% (w/v) nonfat dry milk in TBS-T (100 mM Tris, 1.5 mM NaCl, pH 8.0 and 0.5% Tween 20). Membranes were incubated with primary antibody diluted 1:1000 with 5% nonfat dry milk in TBS-T for 2 h at room temperature (rabbit anti-TWEAK, ab37170, Abcam; rabbit anti-NF-κB p105/p50, ab32360, Abcam; rabbit anti-NF-κB p65, ab16502, Abcam; mouse anti-ATP synthase subunit beta, ab14730, Abcam; rabbit anti-GAPDH, ab9485, Abcam; rabbit anti-MnSOD, ab13533, Abcam; rabbit anti-ETFDH, ab91508, Abcam; rabbit anti-PGC1 alpha, ab54481, Abcam; rabbit anti-mtTFA, sc-28200, Santa Cruz; rabbit anti-connexin 43, ab63851, Abcam; rabbit anti-c-kit, sc-168, Santa Cruz). After washed with TBS-T, the membranes were incubated with secondary peroxidase-conjugated anti-mouse or anti-rabbit (GE Healthcare) diluted 1:10000 with 5% nonfat dry milk in TBS-T, for 1 h at room temperature. Immunoreactive bands were

Download English Version:

https://daneshyari.com/en/article/8288639

Download Persian Version:

https://daneshyari.com/article/8288639

Daneshyari.com