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Oxidative state and oxidative metabolism of the heart from rats with adjuvant-induced arthritis

Amanda Caroline Schubert [a], Mariana Marques Nogueira Wendt [a], Anacharis Babeto de Sá-Nakanishi [a], Ciomar Aparecida Bersani Amado [b], Rosane Marina Peralta [a], Jurandir Fernando Comar [a], Adelar Bracht [a,*]

[a] Department of Biochemistry, University of Maringá, 87020900 Maringá, Brazil
[b] Department of Pharmacology and Therapeutics, University of Maringá, 87020900 Maringá, Brazil

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ABSTRACT

The aim of the present work was to investigate, in a more extensive way, the oxidative state and parameters related to energy metabolism of the heart tissue of rats using the model of adjuvant-induced arthritis. The latter is a model for the human arthritic disease. Measurements were done in the total tissue homogenate, isolated mitochondria and cytosolic fraction. The adjuvant-induced arthritis caused several modifications in the oxidative state of the heart which, in general, indicate an increased oxidative stress (+80% reactive oxygen species), protein damage (+53% protein carbonyls) and lipid damage (+63% peroxidation) in the whole tissue. The distribution of these changes over the various cell compartments was frequently unequal. For example, protein carbonyls were increased in the whole tissue and in the cytosol, but not in the mitochondria. No changes in GSH content of the whole tissue were found, but it was increased in the mitochondria (+33%) and decreased in the cytosol (−19%). The activity of succinate dehydrogenase was 77% stimulated by arthritis; the activities of glutamate dehydrogenase, isocitrate dehydrogenase and cytochrome c oxidase were diminished by 31, 25 and 35.3%, respectively. In spite of these alterations, no changes in the mitochondrial respiratory activity and in the efficiency of energy transduction were found. It can be concluded that the adjuvant-induced arthritis in rats causes oxidative damage to the heart with an unequal intracellular distribution. Compared to the liver and brain the modifications caused by arthritis in the heart are less pronounced on variables such as GSH levels and protein integrity. Possibly this occurs because the antioxidant system of the heart is less impaired by arthritis than that reported for the former tissues. Even so, the modifications caused by arthritis represent an imbalanced situation that probably contributes to the cardiac symptoms of the arthritis disease.

1. Introduction

Rheumatoid arthritis is a chronic autoimmune disease characterized by systemic inflammation that affects mainly the synovial membranes, articular cartilages and bones. Rheumatoid arthritis occurs in 0.5–1.0% of the adult population worldwide and is associated with an increased mortality rate, mainly due to cardiovascular complications (Gabriel and Michaud, 2009; Kitas and Gabriel, 2011). The pathophysiology of arthritis involves an intense hyperplasia of the articular cartilage with intense participation of proinflammatory cytokines, particularly interleukin-1 (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) (McInnes and Schett, 2011). The overproduction of pro-inflammatory cytokines stimulates phagocytes to secrete reactive species in the synovium, which act as mediators of tissue injury (Halliwell and Gutteridge, 2007; Kundu et al., 2012). Cytokines released into the synovium may also reach the systemic circulation and act in other tissues. In accordance, the oxidative stress biomarkers are increased in both articular inflammation sites and serum of patients with rheumatoid arthritis (Sarban et al., 2005; Vasanthi et al., 2009; Stamp et al., 2012).

Rheumatoid arthritis also evokes inflammatory responses and immunological alterations in other organs, such as vascular tissue, brain, liver and skeletal muscles (Sattar et al., 2003; Haruna et al., 2006; McInnes and Schett, 2011). Metabolic alterations are equally prominent, as for example, the muscle wasting condition known as rheumatoid cachexia, which is mediated by TNF-α and IL-1β and occurs in approximately two-thirds of all patients with rheumatoid arthritis (Roubenoff et al., 1994). The liver of rats with adjuvant-induced arthritis presents higher rates of oxygen uptake, reduced gluconeogenesis, increased glycolysis, modifications in the urea cycle, and modifications in calcium homeostasis (Fedatto- et al., 2000; Yassuda-Filho et al., 2003; Utsunomiya et al., 2013). The systemic inflammation in rheumatoid arthritis also affects the heart, where it causes pericarditis, myocarditis, cardiomyopathy, increased left ventricular mass, congestive heart failure and ischemic heart disease (Voskuyl, 2006; Rudominer et al., 2009; Davis et al.,

* Corresponding author.
E-mail address: adebracht@uol.com.br (A. Bracht).

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2011). In addition, patients with rheumatoid arthritis have twice the risk of developing congestive heart failure when compared to the general population (Nicola et al., 2005).

The metabolic changes observed in the skeletal muscle and liver are accompanied by accentuated alterations in the oxidative state of these tissues (Vijayalakshmi et al., 1997; Comar et al., 2013). The liver of rats with adjuvant-induced arthritis presents higher levels of ROS, protein carbonyl groups and lipoperoxides in several subcellular fractions (cytosol, mitochondria and peroxisomes). These alterations are accompanied by decreased activities of antioxidant enzymes and diminished levels of reduced glutathione (Comar et al., 2013). Similar alterations in the oxidative status were also observed in the subcellular fractions (mitochondria and cytosol) of the brain from arthritic rats (Wendt et al., 2015). However, no similar study has yet been done about the oxidative state and the cellular compartmentation of the possible alterations in the heart of arthritic rats. The latter presents several myocardial abnormalities, including tissue fibrosis, inflammation and alterations in cell metabolism (Shi et al., 2012). With an essentially aerobic metabolism, the heart muscle presents high rates of oxygen consumption even under resting conditions. This makes it susceptible to develop oxidative stress, especially because the antioxidant defenses of the heart, particularly the activities of superoxide dismutase (SOD), catalase, and glutathione peroxidase, are relatively modest when compared to other organs such as the liver and kidney (Chen et al., 1994). Corroborating this view it has been shown that oxidative stress is indeed generated by arthritis as revealed by the overproduction of ROS in the aorta (Haruna et al., 2006), the increased lipid peroxidation and the increased GSSG/(GSH + GSSG) ratio in the heart of arthritic rats (Shi et al., 2012). The present work was, thus, planned to investigate in a more extensive way the oxidative state and parameters related to energy metabolism of the heart tissue of rats using the model of adjuvant-induced arthritis. The latter is an inducible experimental immunopathology in rats which shares many features of human rheumatoid arthritis and is often used as a model for studying autoimmune chronic inflammation (Pearson and Wood, 1963; Szekanecz et al., 2000). Besides examining the whole tissue, the oxidative state of two intracellular compartments, mitochondria and cytosol, was also investigated in the present study. In principle at least, the data that were obtained should allow to infer about the oxidative state of the heart of patients with rheumatoid arthritis.

2. Materials and methods

2.1. Chemicals

Dinitrophenylhydrazine (DNPH), 5,5-dithiobis 2-nitrobenzoic acid (DTNB), 2′-7′-dichlorofluorescein-diacetate (DCFH-DA), oxidized dichlorofluorescein (DCF), 1,1′,3,3′tetraethoxypropane, horse-radish peroxidase (HRP), *o*-phtalaldehyde (OPT), reduced glutathione (GSH) and oxidized glutathione (GSSG), glutathione reductase (GR) and nitrate reductase (NR) were purchased from Sigma Chemical Co (St Louis, MO, USA). All other chemicals were of analytical grade.

2.2. Animals and treatments

Male *Holtzman* rats were fed ad libitum with a standard laboratory diet (Nuvilab®, Colombo, Brazil) and maintained on a regulated light–dark cycle. For the induction of arthritis, animals weighing 180–210 g were injected in the left hind paw with 0.1 ml of Freund's adjuvant (heat inactivated *Mycobacterium tuberculosis*, derived from the human strain H37Rv), suspended in mineral oil at a concentration of 0.5% (w/v) (Pearson and Wood, 1963). Animals showing the characteristic lesions at 18 days after adjuvant injection were selected for the experiments. Rats of similar ages served as controls. All experiments of adjuvant arthritis induction were done in accordance with the accepted ethical guidelines for animal experimentation and previously approved by the Ethics Committee for Animal Experimentation of the University of Maringá (Protocol 3127110215-CEEA).

2.3. Evaluation of the inflammatory response

The weight of the animals and the evaluation of the adjuvant-induced inflammatory response were carried out over an 18-day period. Following adjuvant inoculation, the volume of the right hind paw (non-injected) up to the tibiotarsal joint was measured by plethysmography, as previously described (Bracht et al., 2012). The results were expressed in terms of the paw volume increase (final volume at days 1–18) in relation to the initial volume (volume at day 0).

2.4. Preparation of the heart homogenate and subcellular fractions

Rats fasted for 18 h were anesthetized with intraperitoneal injection of sodium thiopental (50 mg/kg) and the peritoneal cavity was surgically exposed. The heart was then immediately removed, washed with 0.9% saline for removing blood, freeze-clamped and stored in liquid nitrogen. The tissue was homogenized in a van Potter-Elvehjem homogenizer with 7 volumes of ice-cold 0.1 M potassium phosphate buffer (pH 7.4) and an aliquot was separated for use as total homogenate. The remaining homogenate was centrifuged at 11,000 *g* during 15 min and the supernatant separated as the soluble fraction of the homogenate.

The fractions enriched with mitochondria were isolated as previously described (Mela and Seitz, 1979) with some modifications. The fresh heart of the rats was placed in ice-cold buffer containing 200 mM mannitol, 76 mM sucrose, 0.2 mM ethylene glycol tetraacetic acid (EGTA), 0.1 mM PMSF (phenylmethylsulfonyl fluoride), and 2 mM Tris (pH 7.4). PMSF was added to inhibit lysosomal hydrolases. The heart was minced and homogenized by means of a van Potter-Elvehjem homogenizer for lysing the cells. The mitochondrial fraction was isolated by differential centrifugation. Briefly, the homogenate was first centrifuged at 650 *g* for 8 min to remove cell debris and the mitochondria were pelleted at 8000 *g* for 8 min. The pellet was washed twice and resuspended in the buffer described above except that EGTA was omitted. The pellet of the second resuspension was used as intact mitochondria. An aliquot of this preparation was used to prepare disrupted mitochondria. Disruption was done by a repeated freeze-thawing procedure using liquid nitrogen. For this procedure the mitochondria were suspended in 0.1 M potassium phosphate buffer (pH 7.4).

For obtaining the soluble cytosolic fraction the post-mitochondrial supernatant was centrifuged at 105,000 g for 1 h to precipitate the organelles, and the supernatant was collected as the soluble cytosolic fraction. Protein content of the homogenates and subcellular fractions was measured using the Folin phenol reagent (Lowry et al., 1951).

2.5. Mitochondrial respiratory activity

Oxygen consumption by coupled isolated mitochondria was measured polarographically using a Teflon-shielded platinum electrode (Voss et al., 1961). Intact mitochondria (1 mg protein/ml) were incubated in the closed oxygraph chamber in a medium containing 250 mM mannitol, 10 mM KCl, 0.2 mM EGTA, 10 mM TRIS (pH 7.4) and 5 mM potassium phosphate. Succinate (10 mM), α-ketoglutarate (10 mM) and L-glutamate (10 mM) plus L-malate (10 mM) were used as substrates. ADP, for a final concentration of 125 μM, was added to measure phosphorylation. Rates of oxygen consumption were computed from the slopes of the recorded tracings and expressed as $nmol \cdot min^{-1} \cdot (mg\ protein)^{-1}$. These rates were used to compute the respiratory control (RC) and the ADP/O ratios (Voss et al., 1961). The ADP/O ratio is defined as the number ADP phosphorylated moles per atom/g of O_2 consumed (Voss et al., 1961).