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Research Article

The 8-oxo-deoxyguanosine glycosylase increases its migration to mitochondria in compensated cardiac hypertrophy

Jorge E. Vela-Guajardo, MD, Perla Pérez-Treviño, MSc, Irais Rivera-Álvarez, MSc, Fabio A. González-Mondellini, MD, Julio Altamirano, PhD, and Noemí García, MSc*

Medicina Cardiovascular y Metabolómica, Tecnologico de Monterrey, Escuela de Medicina y Ciencias de la Salud, San Pedro Garza García, Nuevo León, México

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Abstract

Cardiac hypertrophy is a compensatory mechanism maladapted because it presents an increase in the oxidative stress which could be associated with the development of the heart failure. A mechanism proposed is by mitochondrial DNA (mtDNA) oxidation, which evolved to a vicious cycle because of the synthesis of proteins encoded in the genome is committed. Therefore, the aim of the present work was to evaluate the mtDNA damage and enzyme repairing the 8-oxo-deoxyguanosine glycosylase mitochondrial isoform 1-2a (OGG1-2a) in the early stage of compensated cardiac hypertrophy induced by abdominal aortic constriction (AAC). Results showed that after 6 weeks of AAC, hearts presented a compensated hypertrophy (22%), with an increase in the cell volume (35%), mitochondrial mass (12%), and mitochondrial membrane potential (94%). However, the increase of oxidative stress did not affect mtDNA most probably because OGG1-2a was found to increase 3.2 times in the mitochondrial fraction. Besides, mitochondrial function was not altered by the cardiac hypertrophy condition but in vitro mitochondria from AAC heart showed an increased sensibility to stress induced by the high Ca^{2+} concentration. The increase in the oxidative stress in compensated cardiac hypertrophy induced the OGG1-2a migration to mitochondria to repair mtDNA oxidation, as a mechanism that allows maintaining the cardiac function in the compensatory stage. J Am Soc Hypertens 2017;11(10):660–672.

Keywords: DNA oxidation; heart failure; oxidative stress.

Introduction

Cardiac hypertrophy is an adaptive or compensatory mechanism, characterized by an increase in cell volume and extracellular matrix which results in enlargement and thickening of the heart muscle and increased functionality. These changes occur in response to a variety of stimuli which can be of physiological or pathologic origin, such as hemodynamic overload, neurohormonal activation, or ischemia. However, under chronic conditions, cardiac hypertrophy turns into uncompensated and can lead to heart failure.[1,2] The mechanism implicated in this progress is varied. However, various insults that cause all forms of heart failure show a collection of unifying common changes. These include oxidative stress, adenosine triphosphate (ATP) depletion, interstitial fibrosis, inflammation, Ca^{2+} signaling alterations, and cell death.[3–5] Regarding oxidative stress, it has been reported that hypertrophic cardiomyocytes present an increase in the reactive oxygen species (ROS) and mitochondrial dysfunction.[6] It is known that ROS can act as a second messenger; however, the abnormal production and accumulation favor the oxidation of lipids, proteins, and DNA. Being the mtDNA more susceptible than nuclear DNA (nDNA) because it lacks histones and is located near to electron transport chain (ETC).[7]

Conflict of interest: The authors declare that there are no conflicts of interest.

*Corresponding author: Noemí García, MSc, Medicina Cardiovascular y Metabolómica, Tecnológico de Monterrey, Escuela de Medicina y Ciencias de la Salud, Hospital Zambrano Hellión, Batallón de San Patricio, 112, San Pedro Garza García, Nuevo León CP 66278, México. Tel: +52 (81) 8888-0466; Fax: +52 (81) 8888-2223.

E-mails: garcianr@itesm.mx, ramgarnoe@gmail.com

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In mtDNA, all nitrogenous bases can be oxidized. The most susceptible are guanine, whose oxidation product is 8-hydroxy-2′-deoxyguanosine (8-OHdG).[8,9] Several pathologies have been associated with the mtDNA oxidation including the developing of heart failure.[6] However, it is still unclear how mtDNA oxidation leads to heart failure. Nevertheless, one of the proposed mechanisms is the localization of oxidized mtDNA fragments in the cytosol, which probably escaped by autophagy[10] or possibly leaked on activation of the permeability transition pore.[11] These cytosolic oxidized mtDNA fragments would activate the inflammasome and nuclear factor kappa B, which exacerbate the inflammatory process, leading to heart failure.[10,12] Other of the proposed mechanisms is the level expression of the proteins codified in the mitochondrial genome. Alterations in the expression of proteins led to low ATP synthesis and increased ROS production, both present in the heart failure.[13,14]

On the other hand, the DNA oxidation can be corrected by repair mechanisms such as the base excision repair[15] pathway constituted by proteins encoded in the nuclear genome. The first step is carried out by a DNA glycosylase that recognizes and removes the damaged nitrogenous base leaving an abasic site, called apurinic site, which is recognized and cleaved by apurinic site endonuclease (APE1) to introduce a DNA strand break 5' to the baseless sugar. Then the DNA polymerase β (Polβ) catalyzes the β-elimination of the 5′-sugar phosphate residue and fills the one nucleotide gap with another nucleotide by DNA ligase action. Considering that the 8-OHdG is the more abundant form of DNA oxidation, the 8-oxoguanine glycosylase (OGG) is the enzyme involved in the repair of this damage in the DNA.[16] The enzyme OGG is codified in the nucleus and transcripted by alternative splicing generating the expression of eight isoforms (OGG1-1a, 1-1b, 1-1c, 1-2a, 1-2b, 1-2c, 1-2d, and 1-2e). The OGG1-1a is the nuclear isoform, and the other seven are mitochondrial,[17,18] of which the OGG1-2a is the most abundant.[19] Recently, it has been described that OGG expression is regulated by ROS through the activation of nuclear factor E2–related factor 2 (Nrf2) which acts as a master regulator of the antioxidant response.[20,21] Besides, also it has been described that ROS induce that mitochondrial OGG isoform migrates to mitochondria in exercised skeletal muscle[22]; nonetheless, the precise mechanism is still unclear, even more in cardiovascular diseases. Therefore, the aim of the present work was to analyze the levels of mtDNA oxidation and the content of OGG1-2a within the cardiac mitochondria in an animal model of compensated cardiac hypertrophy. To assess the enhanced concentration of OGG1-2a in the mitochondria as a mechanism that could diminish mtDNA oxidation and fragmentation, which otherwise might exacerbate heart failure development.

We found that on cardiomyocytes, during compensated cardiac hypertrophy, OGG1-2a migration to mitochondrial augmented in response to increased mitochondrial oxidative stress, which functions as a compensatory mechanism in this stage of hypertrophy that slows heart failure development.

Materials and Methods

Animals

All experiments were performed in accordance with the animal care guidelines of the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). All procedures were approved by the animal use and care committee of the School of Medicine, Tecnologico de Monterrey (Protocol # 2016-008).

Cardiac Hypertrophy Model

Forty male Wistar rats (250–300 g) were anesthetized with pentobarbital sodium (30 mg/kg body weight, intraperitoneally) before the surgical procedure. Twenty-five rats were performed by laparotomy and exposure of the abdominal aorta proximal to the renal arteries, as described by Stanek et al.[23] The abdominal aortic constriction (AAC) was performed between the left and right renal arteries with prolene 6-0 thread and a G-22 needle as a guide to reduce the aortic flow by ~65%.[24] Another group of 15 rats was subjected to the same surgical procedure, but without the arterial constriction, and was used as a control (Sham) group. Animals were fed with standard diet and provided water *ad libitum*. The water of the AAC group was supplemented with NaCl 1%. After 6 weeks of the surgical procedure, the arterial pressure was measured by a noninvasive method, and then animals were sacrificed, and cardiac *ex vivo* and *in vitro* studies were performed. Animals were divided into three groups. (1) Anwere used by evaluated inotropic effect used 10 rats with the addition of isoproterenol. (2) Ten rats were used to isolate cardiac cell for volume cell and mitochondrial mass analysis. (3) Ten rats of each group were used for mitochondria isolated studies (respiration and $\Delta\Psi$) and molecular analysis (mtDNA damage, oxidative stress analysis, and Western blot). Finally, approximately 1% of AAC group were discarded because did not develop hypertension.

Systolic and diastolic blood pressures were measured by a non-invasive method using CODA Monitor (Kent Scientific Corporation), before and after 6 weeks of AAC surgery. Rats were maintained at 30°C for 10 minutes before measured. Ten measured were taken by each animal, the first three measurements were discarded, and the subsequent were averaged and reported.