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ORIGINAL ARTICLE

Functional and Structural Alterations of Cardiac and Skeletal Muscle Mitochondria in Heart Failure Patients

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Background and Aims. The fundamental mechanisms involved in the genesis and progression of heart failure are not clearly understood. The present study was conducted to analyze the cardiac mitochondrial involvement in heart failure, the possible parallelism between cardiac and skeletal muscle and if there is a link between clinical symptoms and mitochondrial damage.

Methods. Left ventricle and pectoral biopsies were obtained from patients with heart failure (*n*: 21) and patients with inter-auricular communication as the unique diagnosis for surgery (*n*: 6). Mitochondria were isolated from these tissues and studied through electron microscopy, spectrophotometry to measure the activity of respiratory complex III and immunohistochemistry to determine the presence of reactive oxygen species.

Results. More than 90% of cardiac and skeletal muscle mitochondria presented structural and functional alterations in relation to an increment in the reactive oxygen species production, even in patients without the presence of any clinical Framingham criteria.

Conclusions. We demonstrated some parallelism between cardiac and skeletal muscle mitochondrial alterations in patients with heart failure and that these alterations begin before the major clinical Framingham criteria are installed, pointing to mitochondria as one of the possibly responsible factors for the evolution of cardiac disease.

Key Words: Heart failure, Cardiac and skeletal muscle, Mitochondria.

Introduction

Heart failure is a very important cause of morbidity and mortality throughout the world (1,2). Even though there have been several studies performed on the subject, the fundamental mechanisms involved in the genesis and progression of left ventricular failure are not clearly understood (3,4). The development of heart failure involves abnormalities at tissue and cellular levels such as reduction in myocardial contractility as a consequence of cardiac ischemia, deficit in the capacity to respond to reactive oxygen species (ROS), changes in ionic fluxes, electrophysiological alterations and fibrosis and cardiac remodeling (4,5), leading to cardiomyocyte loss and modifications in the ability to produce and metabolize energy (5). Several studies have proposed that cardiac cell response to oxidative stress causes progressive cellular changes that target mitochondria (3,6–8). It has been demonstrated that oxidative stress triggers the mitochondrial apoptotic pathway, which would be involved in the pathophysiology of ischemic heart disease and heart failure (6).

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The heart is an organ with great energy demand; mitochondria represent 30% of the total volume of the myocytes and provide 90% of the cardiac energy, fatty acids being the primary energy substrates for cardiac muscle ATP generation through the mitochondrial oxidative phosphorylation pathway.

Observations carried out in patients and animal models of heart failure propose that mitochondria would be the key to understand the beginning and progression of several heart diseases, such as dilated and hypertrophic cardiac disease, ischemic and alcoholic cardiopathy, electrical disturbances and myocarditis, among others (5,9).

Taking into consideration that heart failure represents a major public health problem, largely preventable through blood pressure controls and the reduction of other vascular risk factors that affect the heart, we conducted this study in order to establish the mitochondrial involvement in heart failure, the possible parallelism between cardiac and skeletal muscle and if there is a link between clinical symptoms and mitochondrial damage.

Improved understanding of the impact of cardiac and skeletal muscle mitochondrial alterations in cardiac diseases will improve and facilitate their diagnosis and prognosis, and increase the possibility of modifying the natural evolution of heart disease.

Subjects and Methods

Patients

Twenty seven patients who underwent cardiovascular surgery for different reasons and accepted to participate in this study were included.

Inclusion criteria for control group (*n*: 6) were patients from either gender with normal left ventricular ejection fraction (LVEF) (>60%), without rheumatologic disease, diabetes, hypertension, dyslipidemia, artery obstruction and with inter-auricular communication as the unique diagnosis for surgery.

Inclusion criteria for the group with chronic heart failure (*n*: 21) were patients with chronic heart failure as a result of dilated cardiomyopathy, with left ventricular ejection fraction <60%, without rheumatologic or immunologic disease. They all belonged to functional classification II of the New York Heart Association because their cardiac compromise was compensated before surgery.

This study complies with the Declaration of Helsinki and was approved by the ethics committee of the Allende Clinic, Córdoba, and INCOR, La Rioja, Argentina.

A detailed clinical analysis was obtained from each patient recording age, heart failure etiology, risk factors, electrocardiography, echocardiography, coronary catheterization and medication.

Muscle Biopsy

Cardiac and skeletal muscle biopsies of about 1 mm^2 (from the left ventricle and from the pectoral muscle, which contains predominantly type I fibers because individuals included in this study were sedentary) were obtained from patients during surgery. Each sample was divided into two sections: one was examined under a Zeiss electron microscope and the other was used to study the functional activity of the mitochondrial respiratory chain enzymes or to carry out the immunohistochemical analysis.

Electron Microscopy Studies

A 0.5-mm^2 section from either cardiac or skeletal muscle samples (*n*: 10 from heart failure group and three from control group) was fixed immediately after extraction in a Karnovsky solution (4% formaldehyde and 1.5% glutaraldehyde in 0.1 M cacodylate buffer) for at least 2 h at room temperature. The tissues were then washed three times in cacodylate buffer and postfixed in 1% osmium tetroxide for 1–2 h. After dehydration in a graded acetone solution (50, 70 and 90%), the samples were included in a mixture of araldite 506 epoxy composite (48.5%), dodecenylsuccinic anhydride (48.5%), dibutylphthalate (0.5%) and dimethylaminobenzene accelerator (2.5%). Ultrathin cuts were stained with uranyl acetate and lead citrate and examined under a Zeiss electron microscope.

Mitochondrial diameter and area were analyzed using Axiovision 4.8. In order to evaluate the changes on mitochondrial morphology observed in either group of patients (five micrographs for each patient as done previously) (10–12), a 4 degree classification was used:

Grade 0: normal structure
Grade I: normal size, dilated cristae
Grade II: normal size and/or altered shape; intact membrane with few cristae
Grade III: mitochondrial swelling

Three-dimensional studies were carried out using the Femtoscan program.

Mitochondria Isolation

Cardiac and skeletal muscle sections were washed and suspended in ice-cold isolation buffer (5 mM HEPES, pH 7.2 containing 210 mM mannitol, 70 mM sucrose, 1 mmol EGTA, and 0.5% BSA [fatty acid-free], tissue/buffer ratio, 1:10 w/v) and immediately homogenized. Homogenates were centrifuged at 1500 *g*, 4°C for 20 min and the supernatant transferred to a new tube and resuspended in isolation buffer, homogenized, and centrifuged again at 10,000 *g*, 4°C for 5 min. The supernatant was rejected and the pellet resuspended in buffer and centrifuged at