Available online at www.sciencedirect.com
SCIENCE DIRECT
Biochimica et Biophysica Acta 1741 (2005) 173 – 182
BIOCHIMICA ET BIOPHYSICA ACTA
BBA
http://www.elsevier.com/locate/bba

Cardiac volume overload rapidly induces oxidative stress-mediated myocyte apoptosis and hypertrophy

C. Fiorillo[a,*,1], C. Nediani[a,1], V. Ponziani[a], L. Giannini[a], A. Celli[a], N. Nassi[a], L. Formigli[b], A.M. Perna[c], P. Nassi[a]

[a]*Department of Biochemical Sciences, Viale Morgagni 50, 50134 Florence, Italy*
[b]*Department of Human Anatomy and Histology, Viale Morgagni 85, 50134 Florence, Italy*
[c]*Department of Experimental Surgery, Viale Morgagni 85, 50134 Florence, Italy*

Received 14 October 2004; received in revised form 17 February 2005; accepted 21 March 2005
Available online 28 April 2005

Abstract

Oxidative stress stimulates both growth and apoptosis in cardiac myocytes in vitro. We investigated the role of oxidative stress in the initial phases of cardiac remodeling induced in an animal model by volume overload. As plausible candidates for a connection between oxidative stress and cardiomyocyte apoptosis or hypertrophy, we explored the behaviour of two MAPKs, specifically JNK and ERK. At 48 h of overload, the greatest increase in oxidative stress coincided with a peak of cardiomyocyte apoptosis. This was possibly induced through the mitochondrial metabolism, as evidenced by the release of cytochrome *c* and a significant increase in the active forms of caspase-9 and -3, but not caspase-8. Oxidative stress markers significantly decreased at 96 h of overload, combined with a marked attenuation of apoptosis and the appearance of hypertrophy. The highest levels of JNK and the lowest levels of ERK phosphorylation were observed at 48 h of overload. Conversely, a sharp increase in ERK phosphorylation was detected at 96 h of overload coinciding with the hypertrophic response. Together these results show that oxidative stress is an early and transient event in myocardial volume overload. They suggest that oxidative stress mediates amplitude dependent apoptotic and hypertrophic responses in cardiomyocytes through the selective activation of, respectively, JNK and ERK.

Keywords: Oxidative stress; Cardiac volume overload; Apoptosis; Hypertrophy; MAPKs

1. Introduction

Volume overload is a major cause of myocardial remodeling (MR), a process linked closely to the development of heart failure. MR is characterized by progressive morphological and functional alterations, specifically changes in chamber geometry and impairment of pump function. At the cellular level, MR involves modifications of interstitial matrix turnover by cardiac fibroblasts, myocyte hypertrophy, and myocyte loss, chiefly by apoptosis. Although many factors, including neurohormones, cytokines, and growth factors, have been proposed as connecting these cellular events to the mechanical strain induced by volume overload [1], experimental evidence increasingly indicates a central role of oxidative stress. Several recent reports demonstrate that reactive oxygen species (ROS) regulate the phenotype of cardiomyocytes and that oxidative stress, due to an increased generation of ROS and eventually to an impairment of antioxidant defences, is a possible mediator of both cell death and growth [2,3]. A plausible explanation for such different effects is the magnitude of the increase in ROS concentration [4]. Siwik et al. [5] found that graded increases in cardiomyocyte oxidative stress, induced through the inhibition of (Cu, Zn) superoxide dismutase by the copper chelator diethyldithiocarbamic acid

* Corresponding author. Tel.: +39 55 413765; fax: +39 55 4222725.
E-mail address: claudia.fiorillo@unifi.it (C. Fiorillo).
[1] C. Fiorillo and C. Nediani contributed equally to this work.

0925-4439/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbadis.2005.03.015

(DDC), resulted in two distinct responses. Low DDC concentrations, which induced moderate oxidative stress (with 40% increase in ROS production over control), stimulated protein synthesis and myocyte hypertrophy. Conversely, high DDC concentrations caused larger increases in ROS production and led to myocyte apoptosis. Other studies have shown a relationship between myocardial mechanical strain (as occurs in volume overload) and oxidative stress. Pimentel et al. [6] reported that mechanical stretching of myocytes causes an amplitude-dependent increase in ROS production, which is associated with different phenotypic effects. Low levels of ROS production, observed in cardiomyocytes stretched at low amplitude, correlate with fetal gene expression and hypertrophy, while high amplitude stretch levels induce higher ROS production and apoptosis.

In spite of progress in understanding the cellular mechanisms of MR, the exact order of events between myocyte stimulation, generation of ROS, and induction of specific phenotypes remains to be elucidated [4]. One possible means to distinguish between the two different responses to oxidative stress (hypertrophy and apoptosis) may be attributed to the redox-sensitive, mitogen activated protein kinases (MAPKs) [6,7]: c-Jun NH_2 terminal protein kinase (JNK) is a leading candidate for the transduction mechanisms that transmit and convert stress signaling into apoptosis, and extracellular signal-regulated kinase (ERK) seems to have an antiapoptotic role and be involved in cell growth processes. Different ROS levels might selectively activate these kinases leading to apoptosis or, alternatively, to myocyte hypertrophy.

Considering that most studies on MR have focused on late changes, the present investigation examines the initial phases of MR in an animal model of cardiac acute volume overload. We explored the occurrence of oxidative stress, its relationship with cardiomyocyte apoptosis and hypertrophy, and possible molecular mechanisms to account for these processes.

2. Materials and methods

2.1. Experimental model

The experimental protocol described in the study conforms with 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). Twenty farm pigs (Landrace), aged 2–3 months, weighing 30–45 kg were randomly divided in four groups: a group of sham-operated controls ($n=5$) and three groups undergoing aortacaval shunt for respectively 24, 48, 96 h ($n=5$ for each group). Animals, fasting overnight, were premedicated with intramuscular ketamine (15 mg/kg) and diazepam (1 mg/kg). Anesthesia was induced with ketamine (0.5 mg/kg) and atropine (0.5 mg/kg) given intravenously through an ear vein. Pigs were subsequently oro-tracheally intubated and ventilated with oxygen supplemented with 50% N_2O and fluothane at 1–1.5%. Pancuronium bromide (0.1 mg/kg) was given at the beginning of the surgical procedure. The abdomen was opened via a midline incision, and the inferior part of the vena cava and abdominal aorta distal to the renal arteries were cleaned of fat and adventitia. The shunt was performed using a Dacron prosthesis (8-mm diameter) which was sutured latero-laterally to the abdominal aorta and to the inferior vena cava using partial occluding clamps. When the anastomosis was completed, the clamps were released, hemostasis was obtained and the abdomen was closed. At 24, 48, and 96 h after surgery, the animals were again anesthetized, subjected to hemodynamic measurements and then sacrificed using humanitarian methods. After the sacrifice the heart was removed by a midline sternotomy and specimens of left ventricle (LV) tissue were taken for biochemical and histological studies. Sham operated animals underwent an identical procedure (laparotomic incision followed by the hemodynamic and echocardiographic evaluations) except for aortacaval shunt.

2.2. Hemodynamic and echocardiographic measurements

Hemodynamic and echocardiographic measurements were performed with the animal under general anesthesia. A 6F pigtail catheter was introduced into the left femoral artery and advanced to monitor left ventricular and descending aortic pressure. A Swan–Ganz catheter was advanced from an external jugular vein to the pulmonary artery to measure pulmonary capillary wedge pressure and cardiac output (thermodilution). Two dimensional and M-mode echocardiographic studies (2.25/3.5 MHz transducer, SIM 5000) and relative measurements were performed on the right parasternal area and recorded on videotape [8]; wall thicknesses were measured according to the recommendations of the American Society for Echocardiography [9]. Left ventricular mass (LVM) was calculated using a validated formula [10]. Measurements were analyzed independently by two experienced echocardiographers. Inter-observer and intra-observer variabilities were $4.1\pm0.5\%$ and $2.5\pm0.3\%$ for cavity size and $3.7\pm0.4\%$ and $2.1\pm0.3\%$ for wall thickness, respectively.

2.3. Preparation of homogenates

100 mg of myocardium samples were incubated for 1 h at 37 °C in phosphate buffered saline containing 0.1% collagenase type 1. The mixture was centrifuged at $100\times g$ for 15 min and the pellet was homogenized (20% wt/vol with a glass-glass Potter-Elvejhem homogenizer) in ice-cold 20 mM Tris–HCl (pH 8) buffer containing 2 mM EDTA, 1% Triton X-100, 10% glycerol, 137 mM NaCl, 6 M Urea, 0.2 mM PMSF and 10 μg/ml of aprotinin and leupeptin. The homogenate was then sonicated and centrifuged at $10{,}000\times g$ for 10 min. The resulting supernatant