Journal of the American College of Cardiology
© 2011 by the American College of Cardiology Foundation
Published by Elsevier Inc.

Vol. 57, No. 3, 2011
ISSN 0735-1097/$36.00
doi:10.1016/j.jacc.2010.06.058

Oxidation of Myofibrillar Proteins in Human Heart Failure

Marcella Canton, PhD,* Sara Menazza, MSc,* Freya L. Sheeran, PhD,†
Patrizia Polverino de Laureto, PhD,‡ Fabio Di Lisa, MD,* Salvatore Pepe, PhD†

Padova, Italy; and Melbourne, Australia

Objectives We investigated the incidence and contribution of the oxidation/nitrosylation of tropomyosin and actin to the contractile impairment and cardiomyocyte injury occurring in human end-stage heart failure (HF) as compared with nonfailing donor hearts.

Background Although there is growing evidence that augmented intracellular accumulation of reactive oxygen/nitrogen species may play a key role in causing contractile dysfunction, there is a dearth of data regarding their contractile protein targets in human HF.

Methods In left ventricular (LV) biopsies from explanted failing hearts (New York Heart Association functional class IV; HF group) and nonfailing donor hearts (NF group), carbonylation of actin and tropomyosin, disulphide cross-bridge (DCB) formation, and S-nitrosylation in tropomyosin were assessed, along with plasma troponin I and LV ejection fraction (LVEF).

Results The LV biopsies from the HF group had 2.14 $\pm$ 0.23-fold and 2.31 $\pm$ 0.46-fold greater levels in actin and tropomyosin carbonylation, respectively, and 1.77 $\pm$ 0.45-fold greater levels of high-molecular-weight complexes of tropomyosin due to DCB formation, compared with the NF group. Tropomyosin also underwent S-nitrosylation that was 1.3 $\pm$ 0.15-fold higher in the HF group. Notably, actin and tropomyosin carbonylation was significantly correlated with both loss of viability indicated by plasma troponin I and contractile impairment as shown by reduced LVEF.

Conclusions This study demonstrated that oxidative/nitrosylative changes of actin and tropomyosin are largely increased in human failing hearts. Because these changes are inversely correlated to LVEF, actin and tropomyosin oxidation are likely to contribute to the contractile impairment evident in end-stage HF. (J Am Coll Cardiol 2011;57: 300–9) © 2011 by the American College of Cardiology Foundation

Heart failure (HF) is a common clinical disorder characterized by complex pathophysiology with substantial morbidity and mortality. Increased production of reactive oxygen species (ROS) has been suggested to contribute to HF (1–3) because oxidative stress appears to be involved in cardiac remodeling, mechanoenergetic uncoupling, and altered calcium sensitivity (4–6). The key signaling role of ROS in myocyte hypertrophy, apoptosis, and interstitial fibrosis has been demonstrated in genetic models, such as the manganese superoxide dismutase knockout mice (7), and by pharmacologic studies in experimental animal models (8,9). Although formation of ROS can be directly measured in experimental models (10), oxidative stress can only be assessed by measuring lipid peroxidation and DNA and protein oxidation in clinical settings. Nevertheless, the identity of individual myocardial proteins and the nature of structural modifications arising from their reactivity with ROS have been investigated infrequently in experimental models of HF, and virtually no data are available in human HF.

See page 310

Actin and tropomyosin (Tm) have been shown to be major targets of oxidation during ischemia-reperfusion of the isolated rat heart (11). We previously demonstrated in experimental models of coronary microembolization that the resultant contractile dysfunction was related to disulfide cross-bridge (DCB) formation in Tm, at the level of its single cysteinyl residue (12).

From the *Department of Biomedical Sciences, University of Padova, Padova, Italy; †Department of Surgery, Monash University, Alfred Hospital and Heart Research CCN, Murdoch Children's Research Institute, Melbourne, Australia; and the ‡CRIBI Biotechnology Centre, Padova, Italy. The study was supported by the University of Padova (CPDA068417/06 PA to Dr. Canton), MIUR, CNR (to Dr. Di Lisa), and the National Health & Medical Research Council of Australia (project grants to Dr. Pepe and Dora Lush postgraduate scholarship to Dr. Sheeran). The authors have reported that they have no relationships to disclose.

Manuscript received April 2, 2010; revised manuscript received June 10, 2010, accepted June 16, 2010.

The present study was designed to extend these findings to human HF, with specific aims to: 1) investigate the occurrence of myofibrillar protein (MP) oxidation in biopsies from explanted end-stage failing and nonfailing (NF) donor hearts; 2) quantify the extent of these modifications; and 3) evaluate the correlation of these parameters with markers of myocardial viability and contractile impairment. The results indicated a significant increase of: 1) actin and Tm carbonylation; and 2) Tm DCB formation in left ventricular (LV) biopsies from the HF group compared with the NF group. Strikingly, MP oxidation correlated significantly with both contractile impairment and loss of myocardial viability. In addition, besides changes due to ROS, we found that Tm is modified by S-nitrosylation and this modification occurs to a larger extent in failing hearts.

Methods

Patient samples and clinical measures. The use of discarded explanted failing heart tissue biopsies was approved by the Alfred Hospital Human Ethics Committee for Discarded Tissue Research. The NF donor hearts that were excluded from transplantation were approved for research by donor family consent and the Victorian Organ Donation Service, Australian Red Cross. Biopsies were snap-frozen in liquid nitrogen within approximately 3 h of aortic cross-clamp for cardiac explantation. Plasma cardiac troponin I (cTnI) levels were measured within a few days prior to explantation using an automated chemiluminescent microparticle immunoassay as per the assay manufacturer (Architect ci8200 Integrated System, Abbott Laboratories, Abbott Park, Illinois). Ejection fraction was estimated by the Simpson biplane method at echocardiography. Septal and posterior wall thickness, LV end-diastolic diameter, and LV end-systolic diameter were measured in the parasternal long-axis view, whereas left atrial area and right atrial area were measured in the apical 4-chamber view at end-systole (13). When multiple measures per patient were available for cTnI or echocardiography, the last recorded values (closest to cardiac explantation) were used.

Protein carbonylation. Total myocardial protein carbonylation was measured using the Oxyblot protein oxidation detection kit (Chemicon, Temecula, California) according to the manufacturer's protocol. Briefly, carbonyl groups were derivatized by reaction with 2,4-dinitrophenylhydrazine for 15 min. Dinitrophenyl-derivatized proteins were resolved by sodium dodecylsulphate–polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a polyvinylidene fluoride membrane. Membranes were incubated overnight with anti-dinitrophenyl primary antibodies and then with goat anti-rabbit/horseradish peroxidase antibodies. Signals were visualized by chemiluminescence detection using a Kodak X-Omat film processor. Carbonylated actin and Tm were quantified by normalizing the bands of actin and Tm in the Oxyblot for the corresponding band stained with EZBlue-Coomassie brilliant blue G-250 (Sigma-Aldrich, St. Louis, Missouri) using the Quantity One software (Bio-Rad, Hercules, California). The membranes were stripped with Restore Western blot stripping buffer (Thermo Scientific, Waltham, Massachusetts) and reprobed with anti-alpha-sarcomeric actin 5C5 clone and anti-Tm CH1 clone antibodies (Sigma-Aldrich).

Abbreviations and Acronyms

cTnI = cardiac troponin I
DCB = disulphide cross-bridge
HF = heart failure
LV = left ventricular
LVEF = left ventricular ejection fraction
MP = myofibrillar protein
NF = nonfailing
ROS = reactive oxygen species
Tm = tropomyosin

Protein extraction and immunoelectrophoresis. Tropomyosin oxidation was assessed in a blinded manner as reported (12). Briefly, heart biopsies were homogenized in ice-cold phosphate-buffered saline (pH 7.2) containing 5 mM EDTA. Just before use, the solution was stirred under vacuum and bubbled with argon to reduce the oxygen tension. The homogenate was centrifuged, and the resulting pellet was resuspended in sample buffer containing 1% beta-mercaptoethanol. This conventional procedure, referred to as "reducing condition," was compared with the "nonreducing conditions" obtained by using the same sample buffer devoid of beta-mercaptoethanol. To avoid artifacts owing to oxidation of thiol groups in vitro, nonreducing conditions were performed in the presence of 1 mM N-ethylmaleimide. Protein samples were subjected to SDS-PAGE and then transferred overnight to nitrocellulose membranes (Bio-Rad) at 150 mA. The membranes were incubated with monoclonal anti-Tm antibody CH1 clone, as described (12). Tropomyosin oxidation was quantified by assessing the density of high-molecular-weight complexes that appeared only under nonreducing conditions. Densitometry was performed on scanned immunoblots by using ImageJ software (National Institutes of Health, Bethesda, Maryland). The density of immunoblot bands was normalized to Ponceau red staining to take differences in sample loading into account. An identical procedure of comparison between reducing and nonreducing conditions was used to investigate the oxidation of other MPs. To this aim, the following monoclonal antibodies were used: 1) anti–alpha-sarcomeric actin 5C5 clone (Sigma-Aldrich); 2) anti-desmin DE-B-5 clone (Oncogene Science, Cambridge, Massachusetts); 3) anti–myosin light chain MY-21 clone (Sigma-Aldrich); 4) anti-TnI (Mab 8I-7, Spectral Diagnostic, Toronto, Ontario, Canada); and 5) anti-TnC (Biodesign, Saco, Maine).

Detection of S-nitrosylation in purified Tm. Rat cardiac Tm prepared as described (14) was purified by high-pressure liquid chromatography (HPLC) Agilent 1100 series (Agilent Technologies, Santa Clara, California) using a Jupiter-C18 column (Phenomenex, Torrance, California) and incubated with 0.1 or 0.5 mM S-nitrosoglutathione (Sigma-Aldrich) for 1 h at room temperature in the dark. The occurrence of S-nitrosylated Tm was assessed by means of the biotin-switch assay (15,16) using the S-nitrosylation protein detection kit (Cayman Chemical, Ann Arbor,

Download English Version:

https://daneshyari.com/en/article/2949818

Download Persian Version:

https://daneshyari.com/article/2949818

Daneshyari.com