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 ± 0.23-fold and 2.31 ± 0.46-fold greater levels in actin and tropomyosin carbonylation, respectively, and 1.77 ± 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 ± 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.

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, mechanoeenergetic 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.

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).
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 carboxylation; 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 micro-particle 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 dodecyl sulphate–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. Carboxylated 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).

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% betamercaptoethanol. This conventional procedure, referred to as “reducing condition,” was compared with the “nonreducing conditions” obtained by using the same sample buffer devoid of betamercaptoethanol. 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-nitrosothiolamine (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,
Michigan). This method is aimed at converting nitrosylated cysteines into biotinylated cysteines. Briefly, free thiols of proteins were blocked with the thiol-specific agent methymethanethiosulfonate. Then S-nitrosothiols were selectively reduced by ascorbate to form thiols, which were reacted with N-[6-(biotinamido)hexyl]-3′-(2′-pyridyldithio)-propionamide, a sulfhydryl-specific biotinylating reagent. The addition of ascorbate is essential to provide this method with specificity. In fact, ascorbate generates a thiol from S-nitrosothiols but not from alternatively S-oxidized thiols (e.g., disulfides, sulfenic acids) (16). Both preparative procedures were performed in the dark to prevent light-induced cleavage of S-nitrosothiols. Before immunoblot analysis, the biotinylated/S-nitrosylated Tm was precipitated with avidin agarose beads and eluted with 1 mM dithiothreitol. Finally, the resuspended Tm was subjected to: 1) anti-Tm immunoblotting; and 2) mass spectrometric analysis.

**Protein identification by mass spectrometric analysis.** Tropomyosin was subjected to SDS-PAGE and stained by Coomassie Brilliant Blue G-250 (Bio-Rad). The identity of the protein was determined by mass spectrometry of the protein material purified by reverse-phase HPLC. Mass determination was obtained with an electrospray-ionization mass spectrometer with a Q-Tof analyzer micro from Micromass (Manchester, United Kingdom). The measurements were conducted at a capillary voltage of 2.5 to 3 kV and a cone voltage of 30 to 35 V. The molecular masses of protein samples were estimated using Mass-Lynx software 4.1 (Micromass).

**Detection of protein S-nitrosylation in LV biopsies.** The procedure used for the purified protein was slightly modified to assess protein S-nitrosylation in human biopsies. Briefly, samples were homogenized in ice-cold phosphate-buffered saline (pH 7.2) containing 1 mM EDTA and 0.1 mM neocuproine and centrifuged at 12,000 g for 10 min. The resulting pellet underwent the biotin-switch assay according to the S-nitrosylation detection kit. Finally, the biotinylated/S-nitrosylated samples were immunoprecipitated with anti-Tm antibodies and analyzed by nonreducing SDS-PAGE, followed by immunoblotting with anti-biotin or anti-Tm antibodies. Densitometry was performed on scanned immunoblots using ImageJ software (National Institutes of Health).

**Statistics.** Results are presented as mean ± SD. Contrasts between NF and HF groups were performed via 2-tailed unpaired Student t test and Fisher exact tests when indicated. Multiple linear regression analyses (SPSS version 17.0, SPSS, Inc., Chicago, Illinois) were performed to determine correlation between oxidative measures and sample parameters such as LV ejection fraction (LVEF) and prior cTnI release. For all tests, p < 0.05 was considered significant.

**Results**

The oxidative modification of MPs was assessed in heart samples from patients with HF undergoing heart transplantation (New York Heart Association functional class IV) as compared with NF donors. Initially we measured the most widely studied protein modification induced by ROS, namely carboxylation. This alteration, resulting from severe oxidative stress, was assessed by means of Oxyblot. Figure 1A shows that several proteins were carboxylated. We measured the degree of oxidation of 2 bands, whose migration corresponded to that of Tm and actin. Their identity was confirmed by immunoblot (Fig. 1A) and by 2-dimensional Oxyblots (Online Fig. 1). Figure 1B shows that Tm and actin carboxylation was significantly higher in patients with HF than in NF donors. The total amount of actin and tropomyosin was not different between failing and nonfailing hearts (data not shown).

In the HF group, LVEF was significantly lower (Table 1) (24.2% ± 8.7% vs. 64.2% ± 12.9% in the NF group; p < 0.0001). Age, sex, and cardiac ischemic time (from aortic cross-clamp to freezing of biopsy) did not differ significantly between the NF and HF groups and had no detectable impact on any of the molecular analyses as determined by multiple linear regression analyses (coefficient, standard error, and p value, respectively: age −0.00377, 0.0023, 0.108; sex −0.129, 0.0852, 0.136; cardiac ischemia −0.0005, 0.00048, 0.259).

Notably, the degree of Tm and actin carboxylation significantly correlated with the contractile impairment, as shown by a reduction of LVEF (Fig. 2A). The within-group correlation between LVEF and Tm oxidation in HF was significant (NF y = −0.0365x + 3.7962, r² = 0.27, p = 0.02; HF y = −0.0747x + 5.6563, r² = 0.14, p = 0.04). The within-group correlation between LVEF and actin oxidation in HF was also significant (NF y = −0.032x + 4.5437, r² = 0.17, p = 0.04; HF y = −0.1068x + 8.302, r² = 0.39, p = 0.0004). This is the first demonstration that HF is related to oxidative changes of specific MPs in humans.

Oxidative stress is well established in causing loss of cellular viability, and cTnI release into plasma is a useful clinical diagnostic marker of myocardial cell injury. Plasma cTnI levels were significantly higher in the HF than in the NF group (4.2 ± 2.3 μg/l vs. 1.3 ± 2.1 μg/l; p < 0.0001) (Table 1), and importantly, correlated with the extent of Tm and actin carboxylation (Fig. 2B). Significant within-group correlations were evident between cTnI and Tm oxidation in HF (NF y = 0.3718x + 1.352, r² = 0.07, p = 0.28; HF y = 0.2755x + 2.39, r² = 0.12, p = 0.048) and between cTnI and Actin oxidation in HF (NF y = 0.5816x + 2.19, r² = 0.14, p = 0.11; HF y = 0.3156x + 4.06, r² = 0.22, p = 0.012).

Based upon these findings that provided evidence of the correlation between contractile impairment and MP carboxylation in failing myocardium, we characterized the oxidative modifications occurring at the level of cysteine residues. In particular, we analyzed Tm oxidation because we previously demonstrated that this protein modification is linearly related to contractile impairment in experimental models (12).
As shown by the typical example in Figure 3A, anti-Tm immunoblots displayed additional bands in samples obtained from the HF group. Because the appearance of high-molecular-weight bands could also result from other cross-linking processes, such as the activation of transglutaminase, the attribution to DCB formation was determined by comparing the immunoblots obtained after SDS-PAGE carried out under reducing and nonreducing conditions. The appearance of these bands reflected DCB formation because they were visible only in nonreducing electrophoresis. Densitometric analysis (Fig. 3B) shows that the amount of DCB in Tm was significantly increased in the HF group.

### Table 1 NF Donor and Severe Chronic HF Patient Group Details

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<th>NF</th>
<th>All HF</th>
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<th>ICM</th>
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<tr>
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<td>Weight (kg)</td>
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<td>Height (cm)</td>
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<td>LVEF (%)</td>
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<td>1/46</td>
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NF donors died of subarachnoid hemorrhage-related brain death and were free of overt cardiovascular disease. Plasma Tnl was measured prior to cardiac explantation (mean ± SD). *p < 0.0001 vs. NF. DCM = dilated cardiomyopathy; HF = heart failure; ICM = ischemic cardiomyopathy; LV = left ventricular; LVEF = left ventricular ejection fraction; NF = nonfailing; Tnl = troponin I.
Interestingly, Tm appeared especially susceptible to oxidation because we could not detect DCB in actin and desmin (Fig. 4), although these proteins undergo oxidation both in vitro and in experimental models (11). Similarly, troponin C, cTnI, and myosin light chain did not show DCB formation.

We then analyzed the relationship between Tm oxidation and cTnI released prior to explantation. Plasma cTnI levels and LVEF correlated with the extent of DCB formation in Tm when all of the samples were analyzed (y = 0.0743x + 0.6785, r² = 0.18, p = 0.003 and y = −0.0107x + 1.3364, r² = 0.20, p = 0.0002, respectively). However, when the within-group correlation was considered, DCB formation in the HF group was not significantly correlated with cTnI (NF y = 0.0214x + 0.5768, r² = 0.03, p = 0.53; HF y = 0.0214x + 1, r² = 0.01, p = 0.657) nor with LVEF (NF y = 0.0172x − 0.5196, r² = 0.31, p = 0.01; HF y = −0.0087x + 1.28724, r² = 0.015, p = 0.52).

Considering the large body of evidence related to the involvement of nitric oxide in HF, we investigated whether Tm is also a target of reactive nitrogen species. Because no information was available on nitric oxide–induced changes in MPs, initially we analyzed the occurrence of S-nitrosylation (i.e., the covalent attachment of nitric oxide to cysteine thiol) by incubating Tm purified from rat myocardium with increasing concentrations of S-nitrosogluthathione, a donor of nitric oxide. The purity of the protein was checked by reverse-phase HPLC, and the identity was confirmed by mass spectrometry (Fig. 5A). A mass of 32722.28 ± 0.12 Da was measured, which matches the theoretic value. Figure 5B shows that isolated Tm undergoes S-nitrosylation.

Based upon this in vitro evidence, the occurrence and extent of MP S-nitrosylation were assessed in biopsies from the HF and NF groups. Figure 6A shows that several MPs underwent S-nitrosylation. Most of these proteins were similarly S-nitrosylated in NF and HF samples. The major exception was a band at 37 kDa, which corresponded to Tm, as shown by immunoblot analysis (Fig. 6A). To provide additional evidence of the identity of the band corresponding to Tm, samples from the NF and HF groups were immunoprecipitated after biotinylation with anti-Tm antibodies (Fig. 6B). The upper panel of Figure 6B shows increased biotinylation/S-nitrosylation of Tm in HF samples. The densitometric analysis of the Tm band in anti-biotin immunoblots, normalized to the corresponding bands in the Ponceau red–stained blots, displayed a significant increase in the HF group (Fig. 6C) (154 ± 47.4 AU vs. 100 ± 28.2 AU in the NF group; p < 0.01). This finding provides the first evidence of S-nitrosylation of a specific MP, namely Tm, in human failing hearts. However, Tm nitrosylation did not correlate with cTnI
or LVEF ($R^2 = 0.1006$, $p = 0.028$ and $R^2 = 0.0901$, $p = 0.038$, respectively).

**Discussion**

The present results demonstrate a significant increase in oxidative modifications of MPs in end-stage human HF compared with NF donor hearts. In particular, we found that actin and Tm are carbonylated and Tm undergoes DCB formation as well as S-nitrosylation. In addition, this study for the first time to our knowledge related MP oxidation with both contractile impairment and loss of myocardial viability. This study was not only aimed at providing qualitative modifications of covalent changes, but also at establishing a quantitative correlation between biochemical changes and functional alterations not previously investigated in clinical settings. Therefore, the present findings extend to human HF the correlation between MP oxidation and contractile impairment that was previously demonstrated in an experimental model of coronary microembolization (12).

MP oxidation and cardiomyocyte dysfunction. Actin is known to be especially susceptible to carbonylation. This modification has been shown to occur in isolated rat hearts subjected to post-ischemic reperfusion (11). Notably, carbonylation has been demonstrated to result in progressive disruption of actin filaments in vitro (17). Therefore, it is likely that actin oxidation affects contractile performance. This concept is strongly supported by the present findings. In fact, the significant increase in actin carbonyl content detected in HF hearts was significantly correlated with both contractile derangement and loss of viability. Actin carbonylation appears to reflect reliably the oxidative degradation of MPs. Our study also detected a similar alteration in Tm. Tropomyosin carbonylation correlated with both the decrease in LVEF and increase in plasma cTnI. The increase of plasma cTnI levels has been documented in several cardiovascular diseases, such as acute coronary syndromes, acute decompensated heart failure (18), and chronic HF (19). Indeed, increased circulating cTnI levels are associated with poor clinical outcome in patients with cardiomyopathy (20) and are inversely correlated with LVEF in patients with septic shock (21). These relationships might explain the reduced calcium responsiveness of myofilaments that has been proposed to underlie HF (22).

Although elevation of serum cTnI is a well-validated marker of necrotic myocyte injury during myocardial infarc-
tion, the specific pathophysiology behind serum cTnI elevation in HF is probably distinctly different than that caused by acute ischemia (23). However, the chronic release of cTnI occurring in HF is still associated with progressive worsening of ventricular pump function and poor clinical outcome (20).

Carbonylation is likely to result from severe oxidative stress. Under milder stress, proteins can be oxidatively modified at the level of Cys residues (i.e., DCB formation). Tropomyosin oxidation was detected as the appearance of high-molecular-weight peptides in immunoelectrophoreses performed under nonreducing conditions. The band at 82 kDa was attributed to a dimer of Tm (12), whereas the bands with an apparent molecular mass $>200$ kDa are likely to reflect high-molecular-weight complexes among several monomers of Tm or between Tm and other proteins. It is worth pointing out that in rodents, cardiac Tm contains only the alpha isoform, whereas Tm from human cardiac muscle contains up to 20% of the beta isoform. Different than the alpha isoform by the presence of a single cysteine (Cys190), beta-Tm contains 2 Cys residues that might result in the covalent aggregation of more than 2 proteins by means of DCB formation.

The present results showed that DCB content in Tm was significantly higher in failing hearts. However, unlike carbonylation, DCB formation correlated significantly with the decrease in LVEF and the increase in plasma cTnI only when the HF and NF groups were considered in combination (LVEF: $r^2 = 0.20$, $p = 0.0002$; cTnI: $r^2 = 0.17$, $p = 0.003$), whereas within-group regression analysis (i.e., considering the HF and NF groups separately) did not result in significant correlations. This might be because our study analyzed samples collected at end-stage failure, whereas DCB formation is likely to occur at an earlier stage. In fact, in an acute experimental setting of HF, DCB formation correlated with contractile dysfunction (12).

We also considered nitrosative stress, which has been demonstrated to occur in failing hearts (1,24). Indeed, we found that, besides DCB formation, Tm underwent S-nitrosylation. Because no information was available on nitrosative modifications of MPs, initially we investigated the occurrence of S-nitrosylation in purified Tm upon incubation with S-nitrosoglutathione, a nitric oxide donor. This finding might have been anticipated by analyzing the primary structure of Tm. Indeed, Cys190 in Tm is flanked by an “acid-base motif” that is crucial for the occurrence of S-nitrosylation. This process is further favored by local pH, redox tone, or presence of magnesium or calcium, which can all function as allosteric effectors to control thiol accessibility or reactivity (25).
After obtaining the first evidence of S-nitrosylation in Tm, we analyzed its occurrence in human hearts. Interestingly, S-nitrosylation of Tm was significantly greater in the HF than in the NF group. Nitric oxide synthase–dependent and nitric oxide synthase–independent (26) nitric oxide formation has been reported to contribute to contractile dysfunction induced by chronic ischemia, eventually resulting in myocardial hibernation (27,28). Although the underlying mechanisms have not yet been elucidated, it may be hypothesized that a contribution to these contractile dysfunctions may arise from MP nitrosylation, along with ROS-induced covalent changes and nitrosylative modifications of proteins involved in Ca\(^{2+}\)/H\(^{+}\) homeostasis (29).

**Study limitations.** Our study cannot exclude oxidation or other post-translational modifications, such as (de)phosphorylation and proteolysis, of different MPs that may be critical for the development of contractile impairment. These latter changes, far from being mutually exclusive with ROS–induced alterations, can be favored by oxidation, or in turn, make proteins more susceptible to ROS. Although we did not detect any proteolysis of Tm, actin, and desmin, we cannot rule out that other MPs are proteolyzed. The phosphorylation status of the MPs is important in several models of HF (30). Notably, p38 mitogen-activated protein kinase (p38MAPK) activation is associated with Tm dephosphorylation and reduced sarcomeric function (31). Although we did not assess MP phosphorylation, the activation of p38MAPK favors ROS formation so that any

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**Figure 5** **Tm Is S-Nitrosylated In Vitro by S-Nitrosoglutathione**

(A) Tropomyosin (Tm) purified by high-pressure liquid chromatography from rat myocardium was incubated with S-nitrosoglutathione. (B) As detailed in the Methods section, following the biotin-switch assay, the biotinylated/S-nitrosylated Tm was precipitated with avidin-agarose beads and the pellet was detected by anti-Tm immunoblot.

**Figure 6** **Detection of S-Nitrosylated Proteins in Human Left Ventricles**

(A) MPs were extracted from left ventricular biopsies of the HF (n = 36) and NF (n = 24) groups and subjected to the biotin-switch assay. (B) To ascertain the identity of the band corresponding to Tm in the anti-biotin blot of A, the derived biotinylated proteins were immunoprecipitated with an anti-Tm monoclonal antibody. Immunoblots of the pellets were stained with anti-Tm, to confirm the immunoprecipitation, and anti-biotin antibodies. (C) The density of the band in the HF group was markedly increased in the immunoblot probed with the anti-biotin antibody, indicating major S-nitrosylation (SNO) in this group. Equal loading was checked by RP staining. The amount of S-nitrosylated Tm was given by the ratio between the densitometric values of the S-nitrosylated bands and those of the corresponding bands stained with RP. Values are mean ± SD. *p < 0.05 for the NF versus HF group.
functional changes resulting from Tm dephosphorylation could have been secondary to oxidation or vice versa.

Apart from the general limitation of subject numbers in each group and varying life histories, another limitation involved the availability of “control” human myocardium and sample handling. Donor hearts were obtained following brain death and were subject to the Cushing reflex that may involve acute pacing/ischemia-reperfusion stress on the myocardium, which may have contributed to oxidative modification of MPs. This effect on the heart varies in time and severity among donors and cannot be directly controlled in the clinical setting. Although ischemic time (owing to cardioplic ischemic arrest and storage) can contribute to oxidative modifications of MPs, this was not significantly different between the NF and HF groups (Table 1). Thus, upon sample collection, both failing and NF hearts were exposed to oxidative stress, but this “oxidative background” was probably higher in donors because of the Cushing reflex that is precipitated during brain death. It is likely that, without this background, differences between the NF and HF groups would be greater.

Finally, although the present correlations between MP oxidation and contractile dysfunction appear to confirm evidence similar to that obtained in experimental models, far from emphasizing these interesting findings, we would like to point out that inevitably cardiac function was measured at times different than sample collection. Although it is not likely that major variations in oxidative stress occurred in the narrow time intervals during end-stage HF, our data correlated parameters measured at different times.

Conclusions

The present results demonstrated that human HF, with severe contractile dysfunction as evidenced by poor LVEF that limits cardiac output, involves metabolic stresses that induce specific oxidative and nitrative covalent changes of actin and Tm, thus impeding their molecular function. On the basis of the localization and crucial role of actin and Tm in myofilaments, these molecular changes in the MPs are highly predictive of marked interference with myofibrillar contractile performance. Although changes in gene expression of MPs (i.e., titin, myosin heavy chain) may impact contractile function in HF, the specific oxidative modifications of MPs identified in this study may serve as crucial therapeutic targets because they are likely to occur at earlier stages.

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Key Words: heart failure • myofibrillar proteins • reactive oxygen species.

APPENDIX

For a supplemental Methods section and a supplemental figure, please see the online version of this article.