Attenuation of Oxidative Stress and Remodeling by Cardiac Inhibitor of Metalloproteinase Protein Transfer

Michael J. Cox, MD; Urseline A. Hawkins, MD; Brian D. Hoit, MD; Suresh C. Tyagi, PhD

Background—Matrix metalloproteinase (MMP) and cardiac inhibitor of metalloproteinase (CIMP) are coexpressed in the heart. Although it is known that oxidative stress activates MMP and CIMP inhibits MMP, it is unclear whether CIMP administration attenuates oxidative stress and MMP-mediated cardiac dilatation.

Methods and Results—Arteriovenous fistula (AVF) was created in C57BL/J6 mice, and CIMP was administered to AVF and sham mice by protein transfer into peritoneal cavity by minipump for 4 weeks. Mice were grouped as follows: sham; sham+CIMP; AVF; and AVF+CIMP (n=6). In vivo left ventricular (LV) pressure was measured. Plasma and LV tissue levels of CIMP were measured by Western analysis. LV levels of NADPH oxidase activity, marker of oxidative stress, were increased in AVF mice and decreased in AVF mice treated with CIMP. Compared with sham, CIMP was decreased in AVF mice, and CIMP protein transfer increased plasma and LV tissue levels of CIMP in AVF mice; there was no increase in sham animals. In situ zymography demonstrated a robust increase in MMP activity in the hearts from AVF mice compared with sham, and treatment with CIMP decreased MMP activity. In AVF mice, the cardiac pressure-length relationship was similar to that observed in sham mice after administration of CIMP. Contractile responses of normal LV rings were measured in the presence and absence of CIMP. CIMP shifted the pressure-length relationship to the left, attenuated LV dilatation, and had no effect on CaCl₂-mediated contraction.

Conclusions—Treatment of AVF mice with CIMP significantly abrogated the contractile dysfunction and decreased the oxidative stress in volume overload–induced heart failure. (Circulation. 2004;109:2123-2128.)

Key Words: proteomics ■ nitric oxide ■ NADPH oxidase ■ relaxation ■ heart failure

Additional text (not part of the main content):

Extracellular matrix (ECM) surrounds and tethers individual myocytes and myofibrils, enabling optimal transduction of coordinated force generated by cardiac contraction. Indeed, the structure and function of cardiomyocytes are altered by neutral matrix metalloproteinase (MMP)—mediated degradation of ECM. Although MMPs were activated and the levels of cardiac inhibitor of metalloproteinase (CIMP) were decreased in congestive heart failure (CHF), the mechanism(s) responsible for these changes is unsubstantiated. Studies have shown that chronic volume overload increases oxidative stress. Superoxide (O₂⁻) (generated, 2O₂+2H₂O ≡ 2H₂O₂ + O₂⁻), dependent and independent of inflammatory or mitochondrial NADPH oxidase activities, was increased in CHF, but there is little information about oxidative stress in volume overload–induced heart failure.

Method

Animals

Wild-type, C57BL/6J, male mice aged 8 to 12 weeks were obtained from Jackson Laboratories (Bar Harbor, Maine). An arteriovenous fistula (AVF) was created in tribromoethanol-anesthetized mice (100 mg/kg IP, n=24) between the aorta and the caudal vena cava approximately 1 cm below the kidney with the use of a 30-gauge needle. Mice were divided into 4 groups: (1) sham; (2) sham plus CIMP; (3) AVF; and (4) AVF plus CIMP (n=6 in each group). CIMP was purified from mouse hearts in milligram quantity and infused by an ALZET micro-osmotic pump model 1002 at 0.25 μL/h, 10 μg/d in the intraperitoneal cavity. Because the binding constant between MMP and TIMP is in the nanomolar range, this amount saturates all the binding sites in MMPs. Oxidative stress produced by the fistula and proteolytic activation track together; therefore, to avoid early activation of MMP, CIMP was administered 1 hour before creation of fistula. Because the heart was well compensated after 4 weeks of chronic volume overload due to AVF, mice were anesthetized at 4 weeks. A PE-10 catheter was inserted into common carotid artery and advanced to the left ventricle (LV), and pressure was measured with a Micro-Med pressure transducer.

Received February 3, 2003; de novo received October 16, 2003; revision received January 20, 2004; accepted January 22, 2004.

From the Department of Physiology and Biophysics, School of Medicine, University of Louisville, Louisville, Ky (M.J.C., U.A.H., S.C.T.); and Department of Medicine, Division of Cardiology, Case Western Reserve University, Cleveland, Ohio (B.D.H.).

Correspondence to S.C. Tyagi, PhD, University of Louisville, School of Medicine, A-1115, Department of Physiology and Biophysics, 500 S Preston St, Louisville, KY 40202. E-mail styagi01@louisville.edu

© 2004 American Heart Association, Inc.

Circulation is available at http://www.circulationaha.org DOI: 10.1161/01.CIR.0000127429.53391.78

2123
After 10 minutes of stabilization, maximum LV pressure (LVP<sub>max</sub>), its peak time derivatives (dP/dt<sub>max</sub>), and LV end-diastolic pressure (EDP) were recorded. The heart was excised under deep anesthesia, lung and body weights were measured, LV and right ventricle (RV) were separated and weighed, and LV rings were prepared. To measure NO, ROS, and NADPH oxidase, fresh LV tissue was homogenized.

Ex Vivo Study
Cardiac muscle function determined in isolated papillary muscle preparation does not represent function in the entire transmural myocardial wall, and Langendorff preparations do not differentiate the specific contributions of regional ischemia, hypertrophy, stunning, and/or in situ hypertrophy and RV function. To address these limitations, we examined function in cardiac rings. Preparations from hypertensive rats<sup>23</sup> produced pressure-volume curves similar to those obtained in the Langendorff preparation.<sup>24</sup> Rings can be prepared to include or exclude the homogeneous or inhomogeneous regions of the transmural myocardial wall<sup>22,23,25</sup> and to distinguish regional differences in contractile function. The “donut”-shaped LV rings were mounted by 2 wires in a tissue myobath<sup>23,25</sup> containing different doses of CIMP. One of the 2 wires was connected to a force transducer. The ring was stretched and brought to resting tension (measured in grams), and 20 mmol/L CaCl<sub>2</sub> was added to the bath. To keep the spherical shape of the ring, a pediatric esophagus balloon was placed inside the ring. The tension in grams was converted to dynes per square centimeter of ring tissue, and the tension generated in grams was converted to atmospheric pressure (mm Hg). For cardiac dilation, after contraction to 20 mmol/L CaCl<sub>2</sub>, active MMP-2 was added. The percent dilatation was based on 100% CaCl<sub>2</sub> contraction. To minimize differences due to geometric alignment of the different muscle layers in the endomyocardium, midmyocardium, and epicardium, the rings were rotated 90°; the contractile data were repeated, and the average of the 2 was recorded. The bubbling of oxygen at 20 psi was sufficient to avoid ischemia up to 40 minutes as measured by release of creatine phosphokinase–MB, a cardiac muscle–specific enzyme released during injury.<sup>25</sup> The drug was also diffusible at this pressure.<sup>25</sup> To minimize differences due to the weight of the ring, the tension in grams was normalized to the weight of the ring in grams.

LV Levels of NO, ROS, and NADPH/NAD Oxidase
Concentration of total LV NO was estimated by measurements of total nitrate/nitrite. Oxidative stress was assessed by measuring LV ROS by incubating LV tissue homogenates with 2,7'-dichlorofluorescein (DCFH); although generation of O<sub>2</sub>· is transient, O<sub>2</sub>· and H<sub>2</sub>O<sub>2</sub> (2OH<sup>·</sup>) are stable. DCFH acquires fluorescence properties on reaction with ROS and yields fluorescent product dichlorofluorescein. This product was detected by a 530-nm emission when excited at 485 nm. Levels of NADPH oxidase were measured.<sup>26</sup>

Western Blot Analysis of CIMP
Levels of CIMP in plasma and LV tissue homogenates were measured by Western blot analysis under reducing conditions.

In Situ MMP Activity
To determine cardiac hypertrophy, fibrosis, and dilation, LV tissue sections were stained with trichrome blue. Myocyte size was measured by a micrometer. Fibrosis was measured by estimating blue stain (arbitrary unit per centimeter). Because MMP/CIMP complex is dissociated in SDS-PAGE zymography, in situ zymography was performed to measure total MMP activity.<sup>27</sup> Collagen degradation was measured by incubation with 5 μmol/L MMP-2 for 0.5 and 5 minutes.<sup>23</sup>

Statistical Analysis
Values are given as mean±SEM (n=6 in each group). Differences between groups were evaluated by ANOVA, followed by the Bonferroni post hoc test, focusing on the effects of volume overload (sham mice compared with AVF mice [asterisk] and treatment (AVF+CIMP-treated mice compared with AVF mice [double asterisk]). P<0.05 was considered significant.

Results

Oxidative Stress in CHF
Levels of NO were decreased and levels of ROS were increased in AVF hearts compared with sham controls. Treatment with CIMP increased NO and decreased ROS. Levels of NADPH oxidase were substantially increased in AVF mice compared with sham and were decreased with CIMP treatment (Table).

In Vivo Protective Role of CIMP in LV Stress
The slope of the pressure-length relationship was decreased in AVF mice compared with sham controls. Treatment with CIMP increased the slope in AVF mice (Figure 5). The force generated (in dynes per square centimeter of tissue) was reduced in LV of AVF hearts and ameliorated by CIMP.

Effect of CIMP on In Vivo Systolic and Diastolic Function
LV systolic force, ie, LVP<sub>max</sub>/LV weight, was decreased in AVF hearts compared with sham, and the levels of EDP were increased. Treatment with CIMP ameliorated the abnormal pressure–derived indices of cardiac systolic and diastolic dysfunction in AVF mice (Table). LV chamber size was enlarged in AVF mice. Treatment with CIMP decreased this enlargement (Table).

Ex Vivo CIMP Effect on Cardiac Stress and Contraction in Normal Hearts
In normal cardiac rings, CIMP shifted the stretch-strain relationship to the left (Figure 6). The dose-response curves of CaCl<sub>2</sub>-mediated contraction demonstrated no difference in contraction in the presence versus absence of CIMP.
Ex Vivo MMP-2–Induced Cardiac Dilatation Was Inhibited by CIMP

To determine whether MMP-2 induces cardiac dilatation, active myocardial MMP-2 (5 μmol/L) was added to normal rings in a myobath. Within 0.5 minute, there was significant collagen disruption (Figure 7) and cardiac dilation. Moreover, the increases in cardiac dilatation by MMP-2 were dose dependent, and the addition of CIMP ameliorated cardiac dilation (Figure 7).

Discussion

Results of the study show that administration of CIMP by protein transfer ameliorates cardiac dysfunction secondary to a decrease in oxidative and proteolytic stresses after chronic volume overload–induced heart failure. Several mechanisms...
may be responsible for the reduction of oxidative stress by CIMP. First, CIMP inhibited MMP and consequently preserved ECM connections and increased cardiac muscle synchronized strength. Second, CIMP induced NO in endocardial endothelial cells and increased cardiac muscle relaxation. In addition, increased NO decreased MMP activity. Third, CIMP decreased NADPH oxidase in endocardial endothelial cells, and, finally, CIMP behaved like an oxidative sink by generating nitrotyrosine.

Differential cellular functional roles of the TIMPs have been suggested. For example, TIMP-1 has been shown to demonstrate antimitogenic activity. We previously have shown that TIMP-1 has proliferative activity in endothelial cells. In addition, TIMP-2 has been shown to be a growth-stimulatory protein for transformed fibroblasts. Baker et al demonstrated that TIMP-3 induced apoptosis in vascular smooth muscle cells and regression of neointimal growth. CIMP (TIMP-4) induced apoptosis in transformed cardiac fibroblast cells and did not affect normal cells. Inhibitors of neutral proteinases are sensitive to oxidative inactivation. In normal physiological conditions, a critical balance between neutral serine proteinase inhibitor and metalloproteinase inhibitor is maintained. During inflammatory pathogenesis, oxidants disrupt this balance. Serine proteinases degrade the inhibitors of metalloproteinase, and metalloproteinases degrade the inhibitors of serine proteinase. Therefore, a
cardiac tensile strength. To determine whether the activation of MMP played a significant role in the development of LV hypertrophy and wall stress, and especially to determine whether LV hypertrophy will normalize wall stress and cardiac dilatation will increase wall stress, it was important to inhibit oxidative stress and myocardial MMP. In this regard, CIMP inhibited ECM disruption and preserved myocardial contractility. CIMP may also increase NO and improve LV relaxation, resulting in a decrease in EDP. To determine whether LV hypertrophy will normalize wall stress and cardiac dilatation will increase wall stress, it was important to inhibit oxidative stress and myocardial MMP. In this regard, CIMP inhibited ECM disruption and preserved myocardial contractility. CIMP may also increase NO and improve LV relaxation, resulting in a decrease in EDP.

Potential Limitations

In AVF, increased or nearly normal collagen content has been demonstrated. The seeming paradox that fibrosis, increased MMP activity, and decreased TIMP-4 track together can be explained by the following scenario. During increases in load, particularly with reductions of endothelial NO synthase, latent MMPs are activated and dilate the heart. The LV compensates by developing hypertrophy and rearranging the ECM. The media of the vessel and basement membrane of capillary endothelium contains substantial amounts of elastin and ultrastructural collagen are responsible for interstitial connections. Constitutively expressed MMP-2 and inducible MMP-9 degrade elastin as well as ultrastructural (i.e., newly synthesized) collagen efficiently. Because turnover of ultrastructural collagen and elastin is remarkably lower than that of oxidized collagen, degraded ultrastructure collagen and elastin are replaced by oxidatively modified stiffen collagen.

Acknowledgments

This work was supported in part by National Institutes of Health grants HL-71010 and HL-74815.

References

Attenuation of Oxidative Stress and Remodeling by Cardiac Inhibitor of Metalloproteinase Protein Transfer
Michael J. Cox, Urseline A. Hawkins, Brian D. Hoit and Suresh C. Tyagi

_Circulation_. 2004;109:2123-2128; originally published online April 26, 2004;
doi: 10.1161/01.CIR.0000127429.53391.78
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/109/17/2123