Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 358 (2007) 189–195
BBRC
www.elsevier.com/locate/ybbrc

Transgenic MMP-2 expression induces latent cardiac mitochondrial dysfunction

Hui-Zhong Zhou [a], Xiaokui Ma [a], Mary O. Gray [e], Bo-qing Zhu [a], Anita P. Nguyen [b], Anthony J. Baker [a], Ursula Simonis [d], Gary Cecchini [c], David H. Lovett [b], Joel S. Karliner [a,*]

[a] *Cardiology Section, Department of Medicine, UCSF and VA Medical Center, 4150 Clement Street, 111C-5, San Francisco, CA 94121, USA*
[b] *Nephrology Section, Department of Medicine, UCSF and VA Medical Center, San Francisco, CA, USA*
[c] *Molecular Biology Division, Department of Biochemistry and Biophysics, UCSF and VA Medical Center, San Francisco, CA, USA*
[d] *Department of Chemistry and Biochemistry, SF State University, San Francisco, CA, USA*
[e] *Cardiology Division, Department of Medicine, UCSF and SF General Hospital, San Francisco, CA, USA*

Received 12 April 2007
Available online 23 April 2007

Abstract

Matrix metalloproteinases (MMPs) are central to the development and progression of dysfunctional ventricular remodeling after tissue injury. We studied 6 month old heterozygous mice with cardiac-specific transgenic expression of active MMP-2 (MMP-2 Tg). MMP-2 Tg hearts showed no substantial gross alteration of cardiac phenotype compared to age-matched wild-type littermates. However, buffer perfused MMP-2 Tg hearts subjected to 30 min of global ischemia followed by 30 min of reperfusion had a larger infarct size and greater depression in contractile performance compared to wild-type hearts. Importantly, cardioprotection mediated by ischemic preconditioning (IPC) was completely abolished in MMP-2 Tg hearts, as shown by abnormalities in mitochondrial ultrastructure and impaired respiration, increased lipid peroxidation, cell necrosis and persistently reduced recovery of contractile performance during post-ischemic reperfusion. We conclude that MMP-2 functions not only as a proteolytic enzyme but also as a previously unrecognized active negative regulator of mitochondrial function during superimposed oxidative stress.

Keywords: Matrix metalloproteinase-2; Ischemia; Reperfusion; Mitochondria; Preconditioning

Matrix metalloproteinases (MMPs) are a family of zinc-dependent proteolytic enzymes encompassing more than 20 members. Under normal physiological conditions, MMPs are regulated by circulating protease inhibitors and a series of endogenous tissue inhibitors [1]. MMP-2 (EC 3.4.24.24, gelatinase A) and MMP-9 (EC 3.4.24.35, gelatinase B) have been implicated in the response to acute and chronic cellular injury and in chronic ventricular remodeling after myocardial infarction [2–6]. Activated MMP-2 degrades many major components of the myocardial extracellular matrix including types I–V collagen, gelatins, laminin, fibronectin and elastin, thereby impairing the normal structural support of cardiomyocytes [1]. MMP-2 is also synthesized by both cardiac myocytes and fibroblasts, and is colocalized with troponin I within myofilaments [5], sarcomeres [6], and nuclei [7]. Acute activation of MMP-2 leads to a reduction of contractile performance following ischemia/reperfusion (I/R) injury [8].

We have generated a transgenic mouse model expressing constitutively active MMP-2 promoter that is governed by hypoxia [9–11], and have shown that acute I/R in isolated murine heart activates both the MMP-2 promoter and MMP-2 protein synthesis [12]. In the present study, we used the heterozygous MMP-2 Tg mice to ask several questions concerning the effects of chronic activation of MMP-2 in the pathogenesis of acute myocardial injury: (1) Are MMP-2 Tg hearts more vulnerable to I/R injury? (2) Is ische-

* Corresponding author. Fax: +1 415 750 6959.
E-mail address: joel.karliner@va.gov (J.S. Karliner).

0006-291X/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2007.04.094

mic preconditioning (IPC)-mediated cardioprotection against I/R injury preserved in MMP-2 Tg mice? and (3) Does chronic MMP-2 overexpression disrupt normal mitochondrial function? Our study demonstrates that compared to their WT littermates, MMP-2 Tg hearts are more vulnerable to I/R injury during oxidative stress. These hearts exhibit disturbed mitochondrial respiration and excessive lipid peroxidation that may contribute to the deleterious effects of myocardial injury and to the abolition of IPC-mediated cardioprotection during post-ischemic reperfusion.

Methods

Materials. Generation of cardiac-specific expression MMP-2 transgenic mice has been described in detail previously [9,10]. Transgenic animals were maintained as heterozygotes within the outbred CD-1 background. In this study, 6 month old, male transgenic mice and age-matched wild-type littermates (WT) were used. At this time, transgenic mice exhibited significant elevation of MMP-2 expression whereas MMP-9, MMP-13 and MMP-14 were unchanged [9,10]. Animals were acclimated in a quiet quarantine room for at least 3 days before starting experiments. The study was approved by the Animal Care Subcommittee of the SF VA Medical Center. All protocols conformed to the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and the "Guiding Principles in the Care and Use of Animals" of the American Physiological Society. All chemicals and reagents were purchased from Sigma Chemical (St. Louis, MO) unless otherwise stated.

In vivo hemodynamics. Transthoracic echocardiography was performed on conscious mice using a 15-MHz linear array transducer coupled to an Acuson Sequoia c256 echocardiograph [14]. Systolic blood pressure was measured in trained, awake mice using a non-invasive computerized tail cuff system. Results were the means of two-independent daily measurements [14].

Ex vivo hemodynamics. Mice were anesthetized with sodium pentobarbital (60 mg/kg, IP) and anticoagulated with heparin sodium (5000 USP U/kg, IP). Excised hearts were cannulated via the aorta and perfused in retrograde fashion at constant pressure (70 mmHg) and temperature (37 °C) with Krebs–Henseleit buffer containing (mmol/L): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 24, glucose 11, and EDTA 0.5. Hearts were paced at 6 Hz, and hemodynamic measurements were recorded throughout the experiment using a micromanometer (Millar Instruments, Houston, TX) passed into a polyvinylchloride balloon in the left ventricular cavity. Coronary sinus effluent (CF) was collected as a measure of coronary flow and expressed as ml/min.

Ischemia–reperfusion protocol. After baseline hemodynamics were recorded during a 20 min equilibration period, all mouse hearts were subjected to 30 min of global ischemia and 30 min of reperfusion. Ischemic preconditioning was performed using 2 min of single cycle no-flow global ischemia followed by a 5 min washout prior to index ischemia.

Creatine kinase assays. Creatine kinase (CK) release was measured from the coronary effluent collected during reperfusion using a commercially available kit (Stanbio CK, Stanbio Laboratory, Boerne, TX) and corrected for flow rate and wet heart weight. The results are expressed as U/min/mL/g wet heart weight.

Infarct size measurement. Upon completion of reperfusion, hearts were perfused with 1% 2,3,5-triphenyltetrazolium chloride solution and fixed in 10% neutral buffered formalin [15]. The left ventricle was sliced into transverse sections and each section was weighed. Both sides of each section were imaged with a color digital videocamera (Leica, COHU Y/C 460 HTYL, 768 × 494 array, San Diego CA) connected to a microscope (Leica, Stereo Zoom 6 photo, San Diego CA). Images of the viable areas (red-stained) and necrotic areas (unstained) were analyzed using NIH Scion Image software in a blinded fashion. Infarct size was adjusted to the weight of each section and expressed as a percentage of total left ventricular mass [14].

Mitochondrial respiratory activity. Intact cardiac mitochondria were isolated as previously described in our laboratory [13]. Mitochondrial oxygen consumption rate (OCR) was estimated polarographically at 25 °C using a Clark-type oxygen electrode connected to a mitochondrial respiration chamber (YSI Incorporated, Yellow Springs, OH) and a linear chart recorder. State 3 respiration was measured by the addition of ADP (0.5 mM) to respiration buffer (20 mM Hepes, 5 mM KH_2PO_4, 0.2 mM EDTA, 2.5 mM $MgCl_2$, 10 mM KCl, 0.25 M sucrose, and 1 mg/mL fatty acid free BSA, pH 7.4) with NADH-linked substrates (glutamate/malate). Mitochondrial state 4 respiration was measured in the absence of ADP. The result was expressed as ng-atoms of oxygen per min per mg of protein. The respiratory control ratio (RCR) was calculated as the ratio of the state 3 to state 4 respiration [13].

Lipid peroxidation. Lipid peroxidation in individual hearts was determined by the appearance of thiobarbituric acid reactive substances measured spectrophotometrically at an absorbance of 532 nm as previously described [16]. The concentration of malondialdehyde (MDA) was calculated using an extinction coefficient of 1.56×10^5/M cm and was expressed as nmol/g wet weight heart.

Transmission electron microscopy (EM). EM examinations of tissue and mitochondrial ultrastructure were performed as previously described [17]. Briefly, heart tissue was fixed in cold 2.5% glutaraldehyde with 2% paraformaldehyde in 0.1 mol/L cacodylate buffer (pH 7.4), post-fixed in 1% osmium tetroxide, dehydrated, and embedded in Epon resin. Ultrathin sections were mounted on copper grids, stained with uranyl acetate and lead citrate, and examined by EM (Philips Tecna I, Holland). Micrographs were taken systematically at 6700× magnification and were analyzed for specific ischemic alterations as described in Results.

Statistics. Values are mean ± SEM. Measurements during reperfusion were compared with the baseline of each heart by Student's *t*-test. Comparisons among groups were by one-way analysis of variance (ANOVA), followed by the Student–Newman–Keuls post hoc test. $P < 0.05$ was considered significant.

Results

Morphology and baseline cardiac function

MMP-2 Tg mice showed normal behavior and general appearance at 6 months of age. As shown in Table 1, body weight, the ratio of heart weight to body weight, systolic

Table 1
Morphometry and baseline hemodynamics in wild-type littermate (WT) and MMP-2 transgenic mice (MMP-2 Tg) at age 6 months

	WT	MMP-2 Tg
In vivo (no anesthesia, $n = 6$) BW (g)	44.5 ± 2.2	43.8 ± 1.6
BP (systolic, mmHg)	104 ± 5	101 ± 8
HR (beat/min)	600 ± 10	595 ± 12
ECHO EF (%)	86.4 ± 1.2	80.7 ± 2.2
FS (%)	61 ± 1.7	54.8 ± 2.7
LV mass (mg)	110.9 ± 2.9	125.3 ± 7.2
Ex vivo ($n = 13$) BW (g)	42.9 ± 0.8	43.5 ± 1
PP (mmHg)	70 ± 0.5	70 ± 0.1
CF (ml/min)	3.8 ± 0.3	3.9 ± 0.2
HW (mg)	214 ± 8	211 ± 7
HW/BW ($\times 10^{-3}$)	4.8 ± 0.13	4.9 ± 0.15
LVDP (mmHg)	98 ± 3.9	95 ± 4.2
LVEDP (mmHg)	6 ± 0.7	7 ± 0.5

All values are mean ± SEM. BW, body weight; HW, heart weight; HW/BW, heart weight/body weight ratio; CF, coronary flow; PP, perfusion pressure; LVDP, left ventricular developed pressure (LVDP = LV systolic pressure−diastolic pressure); LVEDP, LV end-diastolic pressure; ECHO, echocardiography; EF, ejection fraction; FS, fractional shortening. There were no significant differences in any parameters between WT and MMP-2 Tg mice.