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Matrix metalloproteinase-2 mediates cytokine-induced myocardial contractile dysfunction

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Abstract

Objective: Pro-inflammatory cytokines depress myocardial contractile function by enhancing peroxynitrite production, yet the mechanism by which peroxynitrite does this is unknown. As matrix metalloproteinases (MMPs) can be activated by peroxynitrite and can proteolytically cleave troponin I in hearts, we determined whether this occurs in cytokine-induced myocardial dysfunction. **Methods:** Isolated working rat hearts were perfused with buffer containing interleukin-1β, interferon-γ, and tumor necrosis factor-α. **Results:** Cytokines induced a marked decline in mechanical function during 60–120 min of perfusion. This decline was accompanied by increased myocardial inducible NO synthase activity and perfusate dityrosine (a marker of peroxynitrite), compared to control hearts. Before the decline in mechanical function there was enhanced MMP-2 activity in the perfusate. This was accompanied by decreased tissue levels of MMP-2, tissue inhibitor of matrix metalloproteinases-4 and troponin I in cytokine-treated hearts. The collagen content of the heart was not affected by cytokine treatment. A neutralizing anti-MMP-2 antibody or the MMP inhibitors Ro31-9790 or PD166793 attenuated the decline in myocardial function. Moreover, the MMP-2 antibody prevented the decline in myocardial MMP-2 and troponin I levels. **Conclusions:** Myocardial contractile dysfunction caused by pro-inflammatory cytokines results in MMP-2 activation and a decline in tissue inhibitor of matrix metalloproteinases-4 in the heart. Troponin I is also a target for the proteolytic action of MMP-2 during acute heart failure triggered by pro-inflammatory cytokines. Inhibition of MMPs may be a novel pharmacological strategy for the treatment of acute inflammatory heart disease.

Keywords: Contractile function; Cytokines

1. Introduction

Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases involved in remodeling the extracellular matrix during various physiological and pathological conditions, such as embryonic development, wound healing, inflammation and tumor invasion. MMPs are synthesized in a latent form (zymogen or pro-MMP), and are activated by proteolytic cleavage of an amino-terminal domain or by conformational changes induced by denaturing agents or oxidative stress molecules such as peroxynitrite.

NO and superoxide can react at a diffusion-limit rate to form peroxynitrite [1]. Myocardial generation of perox-

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ynitrite is acutely enhanced during reperfusion of the ischemic heart and contributes to myocardial stunning injury [2,3]. More recently, we demonstrated that peroxynitrite is a major contributor to pro-inflammatory cytokine-induced myocardial dysfunction by concerted enhancement in both superoxide and NO generating activities in the heart [4]. Peroxynitrite can react and modify various biological molecules such as proteins, thiols, lipids, and nucleic acids [5]. Indeed, peroxynitrite was shown to be a potent activator of pro-MMPs without proteolytic removal of the propeptide domain, including pro-MMP-1 [6], pro-MMP-8 [6], pro-MMP-9 [6] and pro-MMP-2 [7]. Meanwhile, peroxynitrite may also alter the structural and binding characteristics of endogenous tissue inhibitors of MMPs (TIMPs), thus inhibiting their ability to prevent MMP activation [8]. This imbalance between MMPs and TIMPs favors the activation of MMPs and a proteolytic environment within the cell. The upregulation of MMP activity and downregulation of TIMP action has been shown to occur in pathological alterations of myocardial ischemia [9], infarct [10] and dilated cardiomyopathies [11,12]. The mechanism by which these changes occur during these processes are, however, not well known.

Changes in MMP activity or expression that affect matrix remodeling are normally thought to occur on the time scale of hours to days. However, there is increasing evidence that MMPs can also very rapidly regulate diverse cellular functions within minutes, including platelet aggregation [13], vascular tone [14] and modulation of the inflammatory response [15], independent of their effects on the extracellular matrix. Recently we discovered that in cardiac myocytes MMP-2 co-localizes with troponin I (TnI), a regulatory element of the actin–myosin contractile apparatus, and that it is susceptible to TnI proteolytic degradation by MMP-2 [16]. During acute ischemia–reperfusion injury of the heart the proteolytic degradation of TnI by MMP-2 and mechanical dysfunction were prevented by inhibiting MMP activity [16]. Therefore, this study was designed to investigate whether there is an acute role of MMPs in the heart in the setting of pro-inflammatory cytokine-induced myocardial dysfunction.

2. Methods

This investigation conforms with *The Guide to the Care and Use of Experimental Animals* published by the Canadian Council on Animal Care (revised 1993).

2.1. Isolated heart preparation

Male Sprague–Dawley rats (250–330 g) were anesthetized by intraperitoneal injection of sodium pentobarbital (60 mg/kg). The hearts were isolated and paced at 300 beats per minute during perfusion at 37 °C as working hearts with 110 ml recirculating Krebs–Henseleit solution containing 11 mM glucose, 5 mM pyruvate, 100 μU/ml insulin, 1.75 mM Ca^{2+}, 0.5 mM EDTA, 0.1% bovine serum albumin, and 0.3 mM L-tyrosine as described (without pulmonary artery cannulation) [17]. Cardiac work (cardiac output×peak systolic pressure) was used as an index of mechanical function. After 20 min of equilibration in the working mode, hearts were perfused for 120 min in the absence (Control, $n=7$) or presence of 5 ng/ml human IL-1β, 9 ng/ml rat IFN-γ, and 20 ng/ml human TNF-α (Cytokines, $n=10$) which was added as a bolus to the perfusion buffer at $t=0$ min. This mixture of pro-inflammatory cytokines mimics those measured in the systemic inflammatory response and advanced heart failure syndromes, enhances myocardial peroxynitrite generation and causes a rapid loss in myocardial contractile function within 1–2 h of perfusion [4]. Perfusate samples were taken at −1, 1, 30, 60, 90, and 120 min to determine MMP activities and dityrosine levels. The ventricles were frozen after 120 min of perfusion and processed as described [16]. Additional series of hearts were perfused in the presence of cytokines for 120 min either with MMP inhibitors (Ro31-9790, 3 μM; PD166793, 2 μM), DMSO vehicle (0.3% v:v), a peptide generated, neutralizing MMP-2 antibody (30 μg/ml) or unrelated IgG (30 μg/ml). The specificity and neutralizing activity of the MMP-2 antibody was previously determined [18]. PD166793 has the following reported nM IC_{50} values (in parentheses) for MMP-1 (6100), MMP-2 (47), MMP-3 (12), MMP-7 (8100) and MMP-9 (9900) [19], whereas for Ro31-9790 they are MMP-1 (3), MMP-2 (7) and MMP-9 (12) [20]. Another set of hearts was briefly perfused for 15 min with Krebs–Henseleit buffer at a constant hydrostatic pressure of 70 mmHg to allow for the washout of blood in order to measure TnI content.

2.2. Preparation of heart extracts

For zymography and Western blot experiments, the frozen ventricular tissue was powdered with a pestle and mortar cooled to the temperature of liquid N_2 and then homogenized by sonication in 50 mM Tris–HCl (pH 7.4) containing 3.1 mM sucrose, 1 mM DTT, 10 μg/ml leupeptin, 10 μg/ml soybean trypsin inhibitor, 2 μg/ml aprotinin, and 0.1% Triton X-100. The homogenate was centrifuged at 8500×g at 4 °C for 5 min and the supernatant was collected. For determination of NO synthase activities Triton X-100 was omitted in the homogenization buffer and the samples were centrifuged at 1000×g at 4 °C for 5 min.

2.3. Measurement of NO synthase activities

NO synthase activities in the heart extracts ($n=5$ in each group) were determined from the conversion of L-[^{14}C]arginine to L-[^{14}C]citrulline as described [4,17]. The protein concentration of heart extracts was determined with the use

of a bicinchoninic acid assay utilizing bovine serum albumin as a standard.

2.4. Measurement of cardiac peroxynitrite generation

We have previously determined that the measurement of dityrosine in heart perfusate, formed by the reaction of peroxynitrite with tyrosine can be used as an estimate of peroxynitrite generation [2,4]. Perfusate samples taken at either 1, 30, 60, 90 or 120 min were assayed by spectrofluorometry for dityrosine levels as described [2].

2.5. Measurement of MMPs by zymography

Gelatin zymography was performed as described [18]. Briefly, perfusate samples or heart extracts (20 μg protein) were applied to 7.5% polyacrylamide gel copolymerized with 2 mg/ml gelatin. After electrophoresis, gels were rinsed three times for 20 min each in 2.5% Triton X-100 in order to remove SDS. Then the gels were washed twice in incubation buffer (50 mM Tris–HCl, 5 mM $CaCl_2$, 150 mM NaCl and 0.05% NaN_3) for 20 min each at room temperature and then incubated in incubation buffer at 37 °C for either 18 or 48 h for the perfusate and heart extract samples, respectively. The gels were stained in 2% Coomassie Brilliant blue G, 25% methanol, 10% acetic acid for 2 h and then destained for 1 h in 2% methanol–4% acetic acid. Zymograms were scanned and the band intensities were analyzed using Sigmagel software. MMP activities were expressed as a percentage of the MMP-2 standard (HT 1080 cell conditioned medium).

2.6. Western blot analyses

The heart extract was diluted with protein sample buffer (30% v/v glycerol, 2% w/v SDS, 0.13 M Tris, 0.1 mg/ml bromphenol blue, and 4% v/v mercaptoethanol, pH 6.8), boiled for 5 min and then stored at −20 °C until assay. Then, 1 or 20 μg protein was applied to 12% polyacrylamide gels and electrophoresed under reducing conditions. After electrophoresis, samples were electroblotted onto polyvinylidene membranes (Bio-Rad Laboratories, Hercules, CA, USA) and probed for TIMP-4 (polyclonal anti-rat TIMP-4 antibody, Chemicon, Temecula, CA, USA) or for TnI (anti-human TnI antibody, clone 81-7; Spectral Diagnostics, Toronto, Canada).

2.7. Collagen content

The collagen content in heart tissue was determined by mass spectrometric analysis for 4-hydroxyproline [21]. The frozen, powdered heart tissue was freeze dried and an internal standard (*N*-methyl-L-proline) in 6 M HCL was added to the sample. Each sample was then hydrolyzed overnight at 115 °C. The *o*-butyl ester derivatives were prepared with 10% BF_3-butanol for 30 min at 120 °C after drying the hydrolysate. Liquid chromatography (Eclipse XDB-C_{18} column)/mass spectrometry analysis was performed on a Hewlett-Packard (series 1100, Atlanta, GA, USA) mass selective detector, monitoring the ions with m/z of 188.

2.8. Statistical analysis

Results are expressed as mean±S.E.M. for n hearts. As appropriate, Student's t-test, one-way or two-way (simple or repeated measures) ANOVA were used. Differences were considered significant at $P<0.05$.

3. Results

Fig. 1A shows the time course of changes in cardiac function, measured as cardiac work, in control and cytokine-treated hearts. Cardiac work in control hearts remained stable for the 120 min of perfusion, while cytokine-treated hearts showed a significant loss in cardiac work between 60 and 120 min of perfusion. Neutralizing

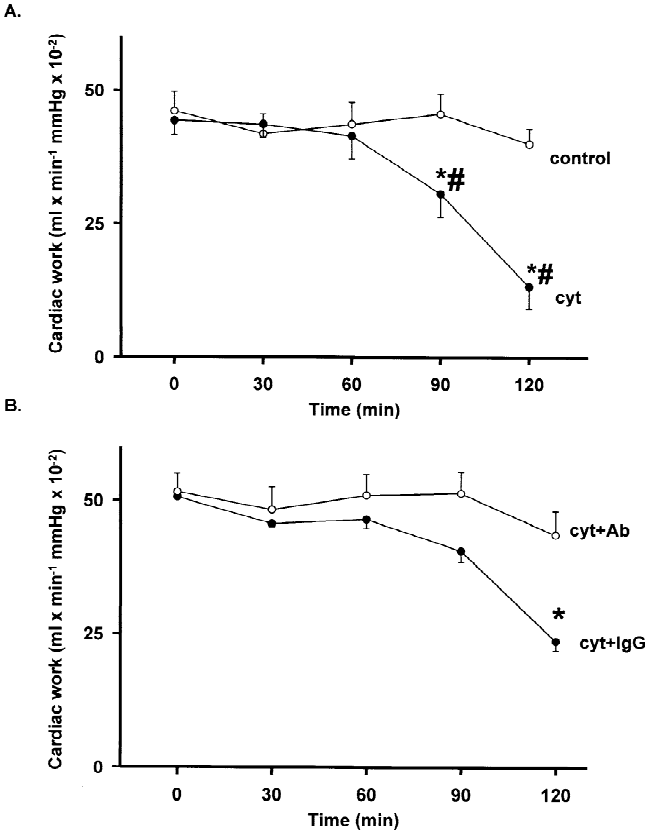

Fig. 1. Time course of changes in cardiac work in isolated working rat hearts. (A) Cardiac work of control ($n=7$) and cytokine-treated (cyt, $n=10$) isolated hearts. (B) Specific MMP-2 antibody (Ab, 30 μg/ml, $n=4$) abolished cytokine-induced myocardial depression, whereas control IgG ($n=4$) exerted no significant effect. * $P<0.05$ versus 0 min value within the same group (one-way repeated measures ANOVA); # $P<0.05$ versus control (Student's t-test).

anti-MMP-2 antibody [18] but not unrelated IgG prevented the cytokine-induced loss of cardiac work (Fig. 1B).

Fig. 2A shows that the isolated working rat heart continuously releases MMP-2 into the recirculating perfusate. Gelatinolytic activities were detected in the zymograms at 75, 72, and 62 kDa corresponding to a rodent-specific glycosylated MMP-2 (Chris Overall, University of British Columbia, personal communication), pro-MMP-2 and MMP-2, respectively, by comparison with the human HT1080 cell-derived standard. The 72-kDa activity constituted the major gelatinase activity. In perfusate from control hearts there is a time-dependent accumulation of 72 kDa activity within the first hour of perfusion. Addition of cytokines markedly increased perfusate 72 kDa activity especially in the first 60 min of perfusion (Fig. 2B and C). This preceded the onset of the delayed depression in cardiac work, which was first significantly impaired in the cytokine group at 90 min perfusion (Fig. 1A). There was a similar increase in 64 kDa activity in the perfusate from cytokine treated hearts, however, this was not within a quantifiable range (data not shown). Concomitant with the enhanced release of 72 kDa activity into the coronary effluent there was a significant decrease in its content in heart tissue (by 44%) at the end of perfusion and this decrease was attenuated in hearts treated with anti MMP-2 antibody (Fig. 3A and B).

The MMP inhibitors Ro31-9790 (0.01–3 μM) and PD166793 (0.2–20 μM) concentration-dependently inhibited the 72-kDa gelatinolytic activity of perfusate samples taken from aerobically perfused hearts (data not shown). We also tested their effects on cytokine-induced changes in cardiac work. Ro31-9790 (3 μM), PD166793 (2 μM), and anti-MMP-2 antibody (30 μg/ml) attenuated the cytokine-induced loss in cardiac work as measured at 120 min of perfusion (Fig. 4). The vehicle for both MMP inhibitors was without effect (Fig. 4).

Since the balance between MMPs and TIMPs might be altered in an inflammatory environment, and TIMP-4 is a potent inhibitor of MMP-2 [22] and abundantly expressed in the heart [23], we then determined myocardial TIMP-4 levels. TIMP-4 was detected in control hearts and its level was significantly decreased by 54% in cytokine-treated hearts (Fig. 5).

To evaluate whether the extracellular matrix of the heart was affected in response to cytokines we measured total collagen by determining 4-hydroxyproline content. In control, cytokine and cytokine plus anti-MMP-2 antibody groups the amount of 4-hydroxyproline was not significantly different (0.58±0.10, 0.66±0.07 and 0.65±0.08 μg/mg dry tissue weight, respectively).

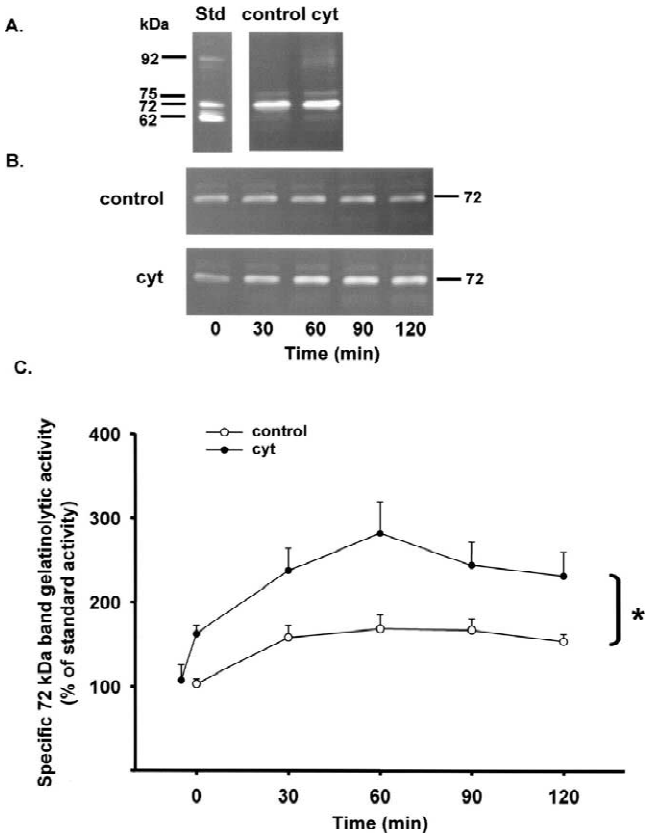

Fig. 2. (A) Representative zymogram reveals presence of 75-, 72-, and 62-kDa gelatinolytic activities in perfusate from isolated rat hearts after 60 min of aerobic perfusion. Std indicates standard (human HT 1080 cell conditioned medium). (B) Representative zymograms of perfusate time course samples from a control and a cytokine-treated (cyt) heart. (C) Densitometric analysis of specific 72 kDa gelatinolytic activity in perfusate from control ($n=7$) and cytokine-treated (cyt, $n=10$) hearts. * $P<0.05$ (two-way repeated measures ANOVA).

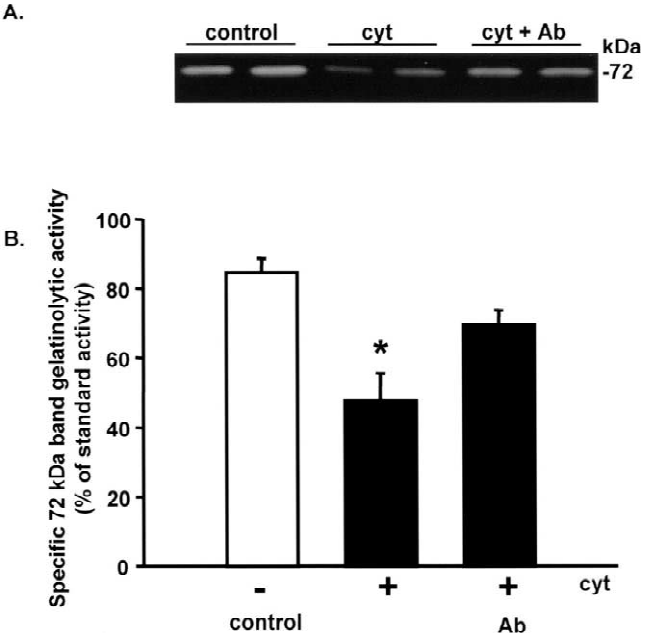

Fig. 3. (A) Representative zymogram of MMP-2 activity in heart tissue samples after 2 h perfusion. Samples from two different hearts in each group are shown. (B) Densitometric analysis of specific 72 kDa gelatinolytic activity in all heart tissue samples. * $P<0.05$ versus control (one-way ANOVA, $n=7$–10 per group).

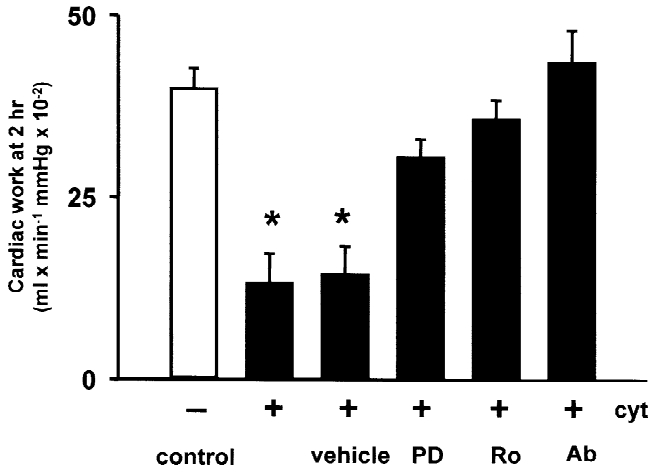

Fig. 4. Cardiac work remaining after 2 h perfusion measured in control and cytokine-treated (cyt) rat hearts and effects of MMP-2 inhibitors (PD166793, 2 μM, PD; Ro31-9790, 3 μM, Ro), their vehicle (DMSO) or anti-MMP-2 antibody (30 μg/ml, Ab). * $P<0.05$ versus control (one-way ANOVA, $n=4–10$ per group).

We recently showed that there is intracellular co-localization of MMP-2 with troponin I (TnI), the regulatory element of contractile proteins within the sarcomere of the cardiac myocyte [16]. We also found that TnI is susceptible to proteolytic degradation by MMP-2 which contributes to the mechanical dysfunction of the heart following ischemia–reperfusion injury [16]. We therefore measured the level of TnI as a target for the action of MMP-2 in the 2-h perfused heart groups (Fig. 6). In cytokine treated hearts there was a dramatic reduction in the level of 31 kDa TnI compared to control. Addition of either anti-MMP-2 antibody or the MMP inhibitor PD 166793 to the cytokine treated hearts prevented the loss in TnI content. Indeed the level of TnI in cytokine plus anti-MMP-2 antibody treated hearts was significantly higher than in control hearts. As a 2-h period of isolated working heart perfusion per se is a mild oxidative stress [17], we therefore analyzed an additional set of hearts perfused aerobically for only 15 min in vitro to wash them free of blood. Indeed the level of TnI in these hearts was comparable to the 2-h isolated working hearts perfused with cytokines plus the anti-MMP-2 antibody.

To confirm our previous results that myocardial inducible NO synthase activity and peroxynitrite production were upregulated in the heart by pro-inflammatory cytokines [4], we measured NO synthase activities in some hearts and perfusate dityrosine levels. In ventricular tissue from hearts perfused for 120 min, the Ca^{2+}-dependent NO synthase activity (between 1–2 pmol/min/mg protein) was

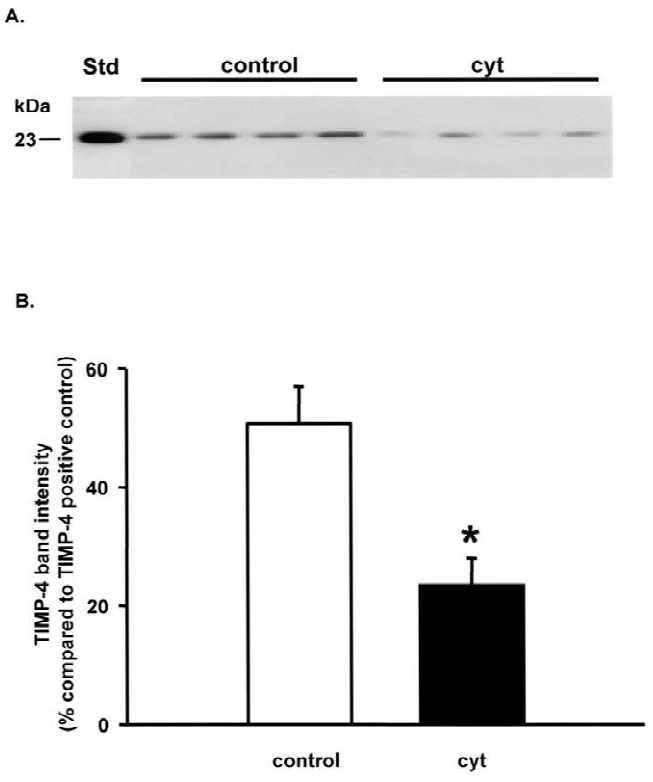

Fig. 5. (A) Representative western blots for TIMP-4 in hearts from control and cytokine-treated (cyt) rat hearts after 120 min perfusion ($n=4$ each). Std denotes TIMP-4 standard obtained from rat myocardial tissue. (B) Densitometric analysis of TIMP-4 western blots. * $P<0.05$ versus control (Student's t-test).

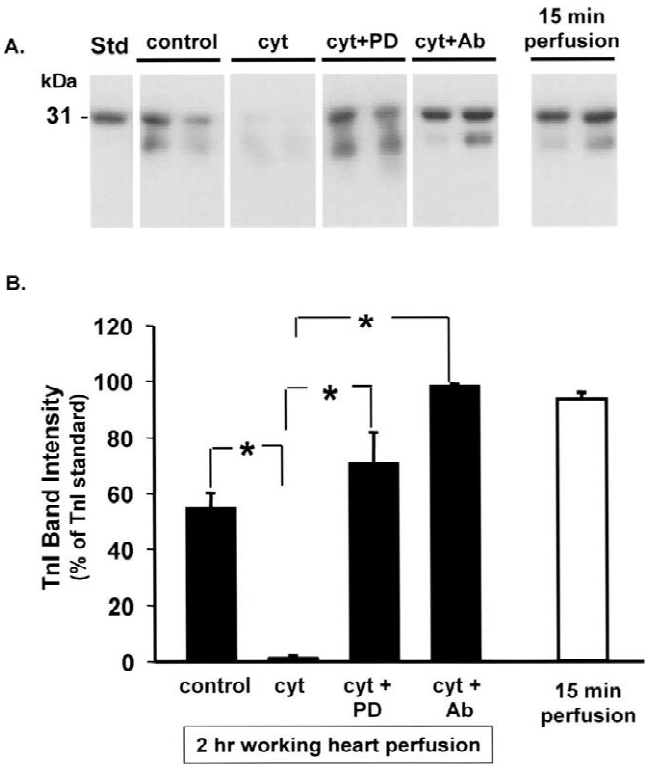

Fig. 6. (A) Representative Western blots for troponin I in hearts ($n=2$ hearts per group). Hearts were perfused for either 2 h in working mode as control hearts or in the presence of cytokines (cyt), or with addition of PD 166793 (cyt+PD) or anti-MMP-2 antibody (cyt+Ab). Another group of hearts were briefly perfused only for 15 min for comparison (open bar). (B) Densitometric analysis of 31 kDa troponin I band intensity ($n=4–7$ hearts per group). * $P<0.05$ versus control (one-way ANOVA).

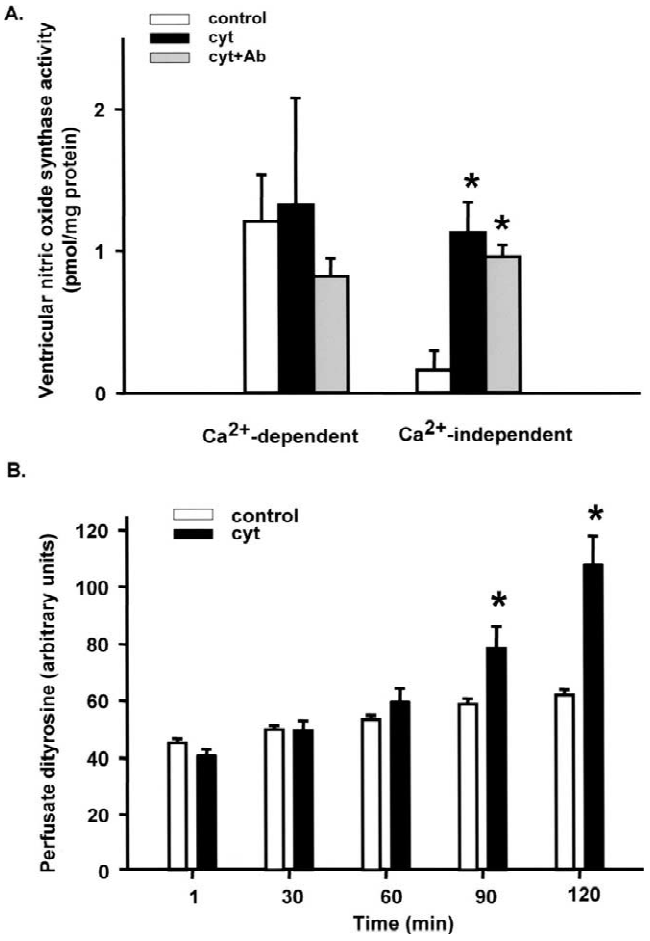

Fig. 7. (A) Ca^{2+}-dependent and Ca^{2+}-independent nitric oxide synthase activities in ventricular homogenates from control and cytokine-treated (cyt) working rat hearts at 120 min perfusion and effect of MMP-2 antibody (Ab, $n=5$ hearts per group; * $P<0.05$ versus control). (B) Time course of changes in perfusate dityrosine level in control ($n=7$) and cytokine-treated hearts ($n=10$), * $P<0.05$ versus 1 min value (one-way ANOVA).

not significantly different between control and cytokine-treated hearts (Fig. 7A). In contrast, cytokine treatment resulted in a near seven-fold increase in Ca^{2+}-independent activity, which was not abolished by anti-MMP-2 antibody. In accordance with the increase in Ca^{2+}-independent NOS activity, the level of perfusate dityrosine (a marker for peroxynitrite) in cytokine treated hearts showed a time-dependent increase which was statistically significant at 90 and 120 min (Fig. 7B).

4. Discussion

We show here for the first time that pro-inflammatory cytokines cause a rapid and enhanced release of MMP-2 activity from the heart into the perfusate. This preceded the onset of a marked depression in cardiac mechanical function. At the end of perfusion both the remaining MMP-2 activity and the level of its endogenous inhibitor TIMP-4 in heart tissue were reduced in cytokine-treated hearts. The collagen content of the heart was unaltered by cytokine treatment, however, the level of TnI was diminished. Pharmacological inhibitors of MMP or a neutralizing MMP-2 antibody protected the heart from the loss in TnI as well as the myocardial dysfunction caused by cytokines.

We have recently shown using this model of acute myocardial contractile dysfunction that pro-inflammatory cytokines enhance the myocardial generation of the pro-oxidant species peroxynitrite through an enhancement in both superoxide and NO generating enzyme activities, which are together responsible for the depression in contractile function [4]. This was confirmed in the present study with the enhanced inducible NO synthase activity in heart tissue and perfusate dityrosine level, an indicator of peroxynitrite generation [2,4]. We also recently found that the activation and release of MMP-2 from the heart contributes to another oxidant stress induced injury to the heart caused by ischemia and reperfusion [18]. The oxidant stress of acute myocardial ischemia–reperfusion injury is also mediated by the generation of peroxynitrite [2,3]. As MMPs can be activated by oxidant stress, we determined here whether cytokine-induced cardiac dysfunction is also mediated through MMPs.

In accordance with our previous report [18], the main gelatinolytic activity in coronary effluent from the aerobically perfused heart is 72 kDa, corresponding to pro-MMP-2. A rapid and significant increase in 72 kDa activity was observed within the first hour in the perfusate from hearts exposed to cytokines along with some enhanced release of 64 kDa activity corresponding to MMP-2, albeit at a reduced level. Cytokine treatment caused a clear-cut depletion of MMP-2 activity in the myocardium. Pro-MMPs may be activated by breaking a zinc–cysteine bond in the catalytic centre [24], which then exposes the catalytic site followed by proteolytic activation, or through oxidant-induced conformational changes [6]. Previous studies suggested oxygen free radicals may be capable of activating matrix metalloproteinases in perfused rat hearts [25]. Indeed, the powerful oxidant peroxynitrite can activate pro-MMP-2 in human smooth muscle cells [7] and 1–20 μM peroxynitrite activated purified pro-MMPs without a change in molecular weight [6]. Recently we have shown that the acute mechanical dysfunction caused by the infusion of peroxynitrite into the heart is mediated through MMP-2 [26]. It is therefore more accurate to specify the MMP-2 activities reported here by molecular weight than by the use of the designations ‘pro-MMP-2’ and ‘MMP-2’ which imply inactive and active forms of the enzyme, respectively, when this indeed may not be the case. Endogenous tissue inhibitors of metalloproteinases (TIMPs) also control MMP activity. Interestingly, peroxynitrite was shown to inactivate TIMP-1 in vitro [8]. We chose to investigate TIMP-4 as it is the most abundantly

expressed TIMP in heart tissue compared with other organs [23] and is found in both human [23] and murine hearts [27]. Moreover, it has been demonstrated that TIMP-4, unlike TIMP-2, does not promote but actually inhibits the activation of MMP-2 [22]. There was also a decrease in TIMP-4 levels resulting from the exposure of the heart to cytokines. It is interesting to speculate that this loss in TIMP-4 protein also contributed to enhanced MMP activity and the depression in myocardial function.

Our results provide compelling evidence that MMP-2 acts as a mediator to cytokine-induced myocardial dysfunction. Several observations support this: (1) cytokine-treated hearts showed a significant loss in cardiac work between 60 and 120 min of perfusion, (2) this was preceded by a rapid and marked increase of MMP-2 activity in perfusate, (3) this occurred in conjunction with a decrease of MMP-2 and TIMP-4 in heart tissue following cytokine treatment, and (4) Ro31-9790 and PD166793, inhibitors of MMP activity, prevented and a neutralizing MMP-2 antibody abolished the depression in mechanical function. The MMP-2 antibody, however, did not affect the cytokine-induced increase in inducible NO synthase activity, excluding the possibility that it nonspecifically inactivated cytokines or interfered with the expression of inducible NO synthase.

In accordance with our findings, activation of MMPs is implicated in cardiac injury resulting from diverse pathologic insults such as ischemia, infarction, and inflammation. Cheung et al. [18] found in isolated rat hearts that MMP-2 was rapidly released into the perfusate during the first minute of reperfusion after ischemia. Inhibitors of MMP or a neutralizing anti-MMP-2 antibody improved the recovery of post-ischemic mechanical function [18]. Li et al. [12] demonstrated a selective downregulation of both TIMP-1 and -3 transcripts and proteins along with upregulation of myocardial MMP-9 gelatinolytic activity in cardiomyopathic ventricles. Much evidence supports that MMPs directly mediate crucial steps during the pathogenesis of myocardial infarction and also determine the clinical outcome. MMP activity rapidly increases in the myocardium during infarction and remains elevated through the healing phase [10]. Also infarcted hearts from MMP-9 deficient mice show a reduction in both early myocardial rupture [28] as well as progressive ventricular dilation [29]. Experiments from several laboratories indicate that in both humans and experimental animal models of heart failure that there is increased activation of myocardial MMPs and that inhibition of MMPs can block ventricular dilation [30–32]. Furthermore, Kim et al. [33] demonstrated that transgenic overexpression of the human MMP-1 gene in the mouse ventricle leads to myocyte hypertrophy and ventricular dysfunction.

We showed here that the total collagen content of the heart was not changed following cytokine treatment, suggesting that other actions of MMP-2 may have occurred apart from degradation or remodeling of the extracellular matrix. An intracellular association of MMP-2 with the sarcolemma was found in hearts from patients with dilated cardiomyopathy [34]. This indicates that elements of the contractile apparatus may represent a molecular target for the detrimental actions of MMP-2. We recently showed that the contractile protein regulatory element TnI is susceptible to rapid proteolytic cleavage by MMP-2 and that inhibition of MMP activity prevents TnI degradation while improving the recovery of mechanical function in ischemic-reperfused hearts [16]. Moreover several lines of evidence showed the co-localization of MMP-2 with TnI in cardiac myocytes [16]. Indeed, results from the present experiments show that cytokine treatment caused a marked loss in myocardial TnI content which was prevented by the neutralizing anti-MMP-2 antibody. This provides further evidence for the intracellular action of MMP-2 and that TnI is a target for MMP-2 in the setting of cytokine-induced myocardial depression. Recently, several other novel substrates and resulting biological activities of MMP-2, independent of its well-described activities on the extracellular matrix, have been described. This includes roles for MMP-2 in the control of vascular tone [14], platelet aggregation [13], and inflammation [15] suggesting that this protease can have effects on a more rapid time scale and with both intracellular and extracellular loci of action.

In summary, we have demonstrated that pro-inflammatory cytokines induced depression of mechanical function of the heart is mediated by MMP-2. Inhibition of MMP activity protects the heart from cytokine-induced myocardial injury, thus MMP-2 may be a viable target for the therapeutic intervention of inflammatory heart disease.

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