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Clinical research

Neurohormonal activation is associated with increased levels of plasma matrix metalloproteinase-2 in human heart failure

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Aims Development of heart failure depends on systemic and molecular abnormalities among which are the activation of neurohormonal systems and the increase of matrix metalloproteinases (MMPs). This study assessed the relationship between catecholamines and active MMPs *in vivo* in patients with severe congestive heart failure (CHF) and *in vitro* in human cardiac fibroblasts.
Methods and results Forty patients with CHF due to dilated cardiomyopathy, either idiopathic ($n = 20$) or secondary to ischaemic heart disease ($n = 20$), were compared with 20 healthy subjects. Plasma MMP-2 and MMP-9 activity, but not TIMP-2, were significantly higher in patients than in controls (median MMP-2, 270 vs. 214 ng/mL, $P = 0.006$; MMP-9 16.3 vs. 8.7 ng/mL, $P < 0.0001$). Similarly, noradrenaline, but not adrenaline, was significantly higher in patients (noradrenaline 645 vs. 157 pg/mL, $P < 0.0001$; adrenaline 86.0 vs. 72.6 pg/mL, $P = 0.68$). No difference in any parameter was observed between patient groups. The intra-group correlation between MMP-2 and noradrenaline was significant ($r = 0.33$, $P = 0.01$); indeed, noradrenaline appear to be a predictor of MMP-2. Moreover, this catecholamine increased MMP-2 in human cardiac fibroblasts.
Conclusions The positive correlation between noradrenaline and MMP-2 in severe CHF patients, together with the *in vitro* induction of MMP-2 by this catecholamine, suggests a potential biochemical link between noradrenaline and MMP-2.

Introduction

The development and progression of left ventricular (LV) dysfunction in heart failure depends on a variety of systemic, cellular, and molecular abnormalities[1] including changes in LV loading conditions, defects in myocardial perfusion and metabolism, alterations in excitation–contraction coupling, and activation of the neurohormonal system.[1,2] In addition to cardiac muscle dysfunction, the gradually progressive nature of congestive heart failure (CHF) is associated with changes in the extracellular matrix of the heart, leading to alterations in both the geometry and mechanical properties of this organ, with a significant impact on overall cardiac function.[3] Although cardiac myocytes are

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central to the contractile function of the myocardium, the cardiac interstitium and its fibrillar collagen matrix also play a critical role in maintaining LV geometry and the structural alignment of adjoining myocytes.[4]

Among the several cardiac cell types which can influence the composition of myocardial extracellular matrix, cardiac fibroblasts are undoubtedly the primary source of producing and maintaining the components of the heart extracellular matrix.[5] Matrix metalloproteinases (MMPs) are part of an endogenous family of enzymes responsible for extracellular collagen degradation and remodelling. The increased expression and activation of MMPs has been implicated in LV remodelling.[6–8] Among the known MMPs, MMP-2 is distributed throughout the cardiac myocytes and fibroblasts, whereas MMP-9 is mainly expressed in infiltrating inflammatory cells such as neutrophils and macrophages.[9,10]

Increases in myocardial MMPs and in MMP activation have been found in end-stage human CHF.[11–14] In particular, increased MMP activity, probably reflecting an increase in the gelatinases (MMP-2 and MMP-9), has been reported in myocardial tissue as well as in the pericardial fluid of CHF patients in association with LV remodelling.[15,16] More recently, plasma levels of MMPs and/or of their inhibitors have been claimed to have diagnostic or prognostic significance in CHF patients.[17]

Similarly, increased sympathetic stimulation, and specifically the activation of the myocardial β-adrenergic receptor system, has been suggested as contributing to the progression of LV dysfunction.[1] With the release of noradrenaline, sympathetic activation is the most important stimulus for vasoconstriction in CHF. In neonatal rat cardiac fibroblasts, noradrenaline stimulated cellular proliferation through transforming growth factor β (TGF-β) up-regulation, which was then followed by enhanced protein expression of fibronectin and collagen type 1.[18] Accumulation of collagen deposition, accompanied by transient increase of MMP-2 has been shown in both ventricles of rats treated for 14 days with noradrenaline.[19] In addition, β-receptor blockade attenuated MMP activity induced by angiotensin II administration and rapid pacing in dogs.[20] Thus, catecholamines may influence extracellular matrix turnover by modulating the metalloproteinases system. This study therefore examined the association between neurohormonal system activation and levels of MMPs in severe CHF patients, and the *in vitro* effect of catecholamines on MMPs in human cardiac fibroblasts.

Methods

In vivo and *ex vivo* studies

Patients and sample collection

Forty patients with heart failure due to dilated cardiomyopathy (DCM) and 20 healthy volunteers were enrolled in the study. DCM was either idiopathic [normal coronary angiography, $n = 20$, New York Heart Association (NYHA) class III, $n = 11$; class IV, $n = 9$] or secondary to ischaemic heart disease ($n = 20$, NYHA class III, $n = 13$; NYHA class IV, $n = 7$). All patients were in a stable clinical condition, none had elevated liver enzymes or suffered from liver disease, osteoporosis, multiple myeloma, osteolytic metastases, rheumatoid arthritis, or cancer. Patients were receiving standard medication including ACE-inhibitors, β-blockers, digitalis, and diuretics. No patient was on systemic glucocorticoid treatment. The control group consisted of 20 healthy subjects comparable for gender and smoking habit, but were, on average, younger than the patients. Informed consent was obtained from all subjects and the study protocol was approved by the Ethics Committee of Centro Cardiologico Monzino.

Heart size was measured by echocardiography.[21] Venous blood collected from the antecubital vein was transferred into chilled sterile tubes containing heparin (68 IU, Sigma-Aldrich, St Louis, MO, USA) and immediately centrifuged at 3000 r.p.m. for 10 min at 4°C. Aliquots of plasma were frozen and stored at −80°C, and thawed just before assay.

Assay of plasma MMPs and TIMP-2

Plasma activity levels of MMP-2 and MMP-9 were measured by an activity assay system (Biotrak, Amersham Pharmacia Biotech Europe GmbH, Milan, Italy). The specific assay system used allows the protein measurement of active MMPs. The assay uses the QuickZyme™ detection enzyme, in its pro form, which can be activated by active MMPs captured by a pre-coated specific anti-MMP antibody, into an active detection enzyme, through a single proteolytic event. Repeatability was 7.49 and 0.49 for MMP-2 (at 200 ng/mL) and MMP-9 (at 10 ng/mL), respectively. Reproducibility was 17.4 and 2.23, respectively. The sensitivity (lower detection limit) was 0.19 ng/mL. TIMP-2 antigen levels were measured by an ELISA Biotrak™ System (Amersham Pharmacia Biotech Europe GmbH). Repeatability and reproducibility for TIMP-2 (at 10 ng/mL) were 0.55 and 0.58, respectively.

Heart tissue samples and MT1-MMP activity assay

Non-failing LV tissue was harvested from six donor hearts excluded from transplantation for technical reasons (mean ejection fraction: 65 ± 4%) at the Italian Homograph Bank at Centro Cardiologico Monzino, Milan, Italy and stored at −80°C until use (mean age: 44 ± 2.2 years; four males and two females). Non-necrotic tissue from six failing hearts was obtained from patients undergoing cardiac transplantation (mean age: 52 ± 5 years; four males and two females). Causes of heart failure included idiopathic ($n = 3$) and ischaemic cardiomyopathy ($n = 3$). Ejection fraction was <20%. None had been treated with LV assist devices or had received chronic intravenous inotropic support over at least 7 days immediately before transplant. The study protocol was approved by the Ethics Committee of Centro Cardiologico Monzino. Human heart specimens frozen in liquid nitrogen were ground to powder and resuspended in extraction buffer (50 mM Tris–HCl, pH 7.6, 1.5 mM NaCl, 0.5 mM $CaCl_2$, 1 μM $ZnCl_2$, 0.01% Brij 35, and 0.25% Triton X-100). Samples were homogenized at high speed (PT 1200, Polytron), sonicated 3 × 20s, and centrifuged at 2000 *g* for 10 min at 4°C. MT1-MMP activity assay was performed following the manufacturer's instructions (Biotrak, Amersham Pharmacia Biotech Europe GmbH).

Determination of catecholamines

Levels of catecholamines (noradrenaline and adrenaline) were determined by high performance liquid chromatography (HPLC) after isolation by adsorption on alumina, using a reverse-phase C18 column (MD-150 × 3.2 mm ESA, Chelmsford, MA, USA), eluted at 1 mL/min with a mobile phase of 75 mM NaH_2PO_4 (containing 1.7 mM octane sulphonic acid, 50 μM EDTA, and

7.5% acetonitrile) adjusted to pH 3.0 with phosphoric acid. An ESA CoulArray detector, with the electrodes set at −250 mV and +250 mV, was used for analysis. All reagents were from Sigma-Aldrich. The peak areas were measured by the software CoulArray for Windows (ESA). Repeatability for adrenaline and noradrenaline (both at 100 pg/mL) was 9.68 and 7.37, respectively. Reproducibility was 14.9 and 14.3, respectively.

In vitro studies

Isolation of cardiac fibroblasts

Samples of normal human LV myocardium were obtained from donor hearts used for valve harvest at Centro Cardiologico Monzino. After washing several times with phosphate-buffered saline, tissue was finely minced and digested at 35°C in a mixture of 0.1% trypsin and 200 U/mL collagenase (type IV, Sigma-Aldrich) for 10 min with constant shaking.[22] Cells from the third to ninth digestions were plated on 100-mm culture dishes in Dulbecco's Modified Eagle's Medium (DMEM, Invitrogen, Milan, Italy) and 10% fetal bovine serum (FBS, Sigma-Aldrich) and incubated for 2 h, at 37°C in a humidified incubator with 10% CO_2. Unattached cells (mostly cardiac myocytes) were then discarded, and attached cells were grown in DMEM + 10% FBS. For the experiments, cells from passages three to five were used. For measurement of the induction of MMP, noradrenaline was added to the serum-free culture medium in subconfluent cultures.

MMP activity by in-gel zymography

MMP-2 and MMP-9 activity was determined in conditioned media from human cardiac fibroblasts treated with noradrenaline by in-gel zymography as previously described.[23] Briefly, samples underwent electrophoresis at 4°C on 7.5% polyacrylamide gels containing 10% SDS and gelatin (1 mg/mL, Sigma-Aldrich) under non-reducing conditions and without boiling. After washing with 2.5% Triton X-100 at room temperature, gels were incubated overnight at 37°C with gentle shaking in 50 mM Tris pH 7.5, containing 150 mM NaCl, 10 mM $CaCl_2$, and 1 mM $ZnCl_2$ in order to activate the digestion of metalloproteinase substrate. MMPs were identified by estimating molecular weights against previously stained molecular weight markers and by the ability of metal chelators (10 mM EDTA) but not a serine protease inhibitor (5 mM PMSF) to inhibit activity. At the end of incubation, the gels were stained with a solution of 0.1% Coomassie Brilliant Blue R-250 (Bio-Rad Laboratories, Milan, Italy) in 25% methanol and 7% acetic acid. Unstained, digested regions representing MMP activity were quantified using an imaging densitometer.

Immunoblotting of extracellular matrix metalloproteinase inducer (EMMPRIN)

Immunoblotting for EMMPRIN was performed on cell lysate of noradrenaline-treated cardiac fibroblasts. After incubation, cells were washed with PBS and scraped in 100 μL lysis buffer.[24] Equal amounts of protein were separated on a 12%-SDS–polyacrylamide gel and transferred to nitrocellulose membrane. Western blot analysis was performed with a goat polyclonal anti-EMMPRIN (Santa Cruz Biotechnology, Inc, CA, USA) antibody (1 : 500) in TTBS containing 5% milk. After incubation with horseradish peroxidase-conjugated secondary antibody, the blot was developed using the Amersham ECL System (Amersham Pharmacia Biotech Europe GmbH).

Statistical analysis

Numerical data are given as mean ± SD or as median and interquartile range, as specified. Clinical characteristics were compared among the three groups by analysis of variance (quantitative variables) or by χ^2 test (categorical variables). Because their distributions were markedly skewed, MMPs, adrenaline, and noradrenaline levels were log-transformed before analysis. After transformations, the Kolmogorov–Smirnov test of normality was not significant in all groups for all variables, and variances were comparable (not significant *F*-test). Unadjusted comparisons were made by the Student's *t*-test. The planned sample size allowed a power of 80% for two independent comparisons (both patient groups vs. controls), each with a type-1 error probability of 0.025, assuming a mean difference equal to one standard deviation, on log-transformed data. Multivariable analysis, adjusting for potential confounding factors (age, gender, and smoking habits) was performed by covariance analysis (ANCOVA). Basic assumptions of linearity and variance homogeneity were assessed by plotting residuals vs. model predicted values, for all dependent variables. Adjustment for multiple testing was carried out by the Dunnett method. Pearson correlation coefficients between neurohormonal and MMP levels were computed on log-transformed data. Intra-group correlations were estimated from the partial correlation coefficient controlling for groups. *P*-values < 0.05 were considered significant and all tests were two-sided. All analyses were performed using SAS version 8 (SAS Institute, Cary, NC, USA). We performed quality control on our measurements by computing repeatability (standard deviation between measurements in the same experiment) and reproducibility (standard deviation between measurements in different experiments) by two-way analysis of variance. As the variability is strongly dependent on the size of the measurement, we carried out quality control at concentrations near the values observed in our population.

Results

The clinical characteristics of the patients and control subjects are summarized in *Table 1*. Gender and smoking habits were balanced in the three groups, but the control group was significantly younger. Among the variables evaluated in CHF patients, only LV ejection fraction (LVEF) reached borderline significance.

Levels of noradrenaline, MMP-2, and MMP-9 were significantly higher in both ischaemic and idiopathic CHF patients than in control subjects, whereas there was no difference in adrenaline levels. None of the variables differed significantly between ischaemic and idiopathic CHF patients (*Table 2*).

After adjusting for age, gender, and smoking habits by ANCOVA, the plasma levels of noradrenaline, MMP-2, and MMP-9 still remained significantly higher in CHF patients than in control subjects ($F = 29.0$, $P < 0.0001$; $F = 5.73$, $P = 0.006$; $F = 11.9$, $P < 0.0001$, respectively). Correlations of noradrenaline with MMP-2 and MMP-9 were positive and significant ($r = 0.439$, $P = 0.0005$ and $r = 0.321$, $P = 0.013$, respectively), when both groups of patients were considered together. The intra-group correlation, however, showed that only MMP-2 and noradrenaline were significantly and positively associated in patients ($r = 0.335$, $P = 0.012$) whereas MMP-9 showed a non-significant negative correlation with

Table 1 Clinical characteristics of patients and of control subjects

Clinical variables	Control subjects	Ischaemic	Idiopathic	*P*
Age, years[a]	60.2 ± 8.9	68.8 ± 6.6	64.1 ± 9.9	0.01
Men, *n* (%)	19 (95)	20 (100)	17 (85)	0.15
Smoking, *n* (%)	2 (10)	6 (30)	3 (15)	0.23
Hypertension, *n* (%)	–	5 (25)	10 (50)	0.10
Diabetes, *n* (%)	–	7 (35)	8 (40)	0.7
Obesity, *n* (%)	–	2 (10)	5 (26)	0.18
LVEDV, mL[a]	–	224.2 ± 58.1	188.3 ± 54.2	0.08
LVEF, %[a]	–	28.2 ± 7.8	34.6 ± 11.7	0.05
LVEDD, mm[a]	–	68.5 ± 9.3	64.5 ± 9.2	0.25

[a]Mean ± SD.
LVEDV, left ventricular end-diastolic volume; LVEDD, left ventricular end-diastolic diameter.

Table 2 Plasma levels of noradrenaline, adrenaline, MMP-2, and MMP-9 in patients and control subjects

	Median	Interquartile interval	*P** vs. controls	*P** Ischaemic vs. idiopathic
Noradrenaline, pg/mL				
Ischaemic	678	486–831	<0.0001	0.47
Idiopathic	557	360–691	<0.0001	
Controls	157	120–210		
Adrenaline, pg/mL				
Ischaemic	85.9	34.5–131	0.4	0.83
Idiopathic	90.2	30.7–126	0.63	
Controls	72.5	34.6–97.5		
MMP-2, ng/mL				
Ischaemic	387	202–505	0.002	0.12
Idiopathic	265	212–374	0.04	
Controls	214	167–271		
MMP-9, ng/mL				
Ischaemic	19.2	11.5–26.9	0.0002	0.98
Idiopathic	16.1	11.4–19.7	0.0002	
Controls	8.7	5.6–11.4		

*Calculated by Student's *t*-test on log-transformed values and corrected for multiple testing by the Dunnett method.

noradrenaline ($r = -0.24$, $P = 0.1$) (*Figure 1*). MT1-MMP activity levels measured in human heart tissue from both non-failing and failing subjects did not differ significantly (0.26 ± 0.12 ng/mg protein and 0.31 ± 0.1 ng/mg protein, respectively).

To test the potential contribution of noradrenaline in explaining the observed group differences, we included this parameter as a co-variate in the analysis. As the differences related to aetiology were not significant, the idiopathic and ischaemic DCM patients were combined. *Table 3* shows that both MMP-2 and MMP-9 levels differed significantly across groups. Thirty-one per cent of MMP-9 and 14% of MMP-2 variances were explained by the group as shown by the partial R^2 variable (*Table 3A*). The association between groups and MMP-2 was strongly reduced after adjusting for noradrenaline: in this way only 3% of MMP-2 level variance was explained by group ($P = 0.88$, patients vs. controls) (*Table 3B*). Conversely, noradrenaline minimally affected the association between group and MMP-9. Plasma levels of TIMP-2 did not differ significantly in the three groups (controls: median, 112.6, interquartile interval 102–129; idiopathic: median, 124.1, interquartile interval 109–181; ischaemic: median, 154.0, interquartile interval 126–216). TIMP-2 levels were positively correlated with noradrenaline, adrenaline, and MMP-2 ($r = 0.45$, $P = 0.01$; $r = 0.46$, $P = 0.01$; $r = 0.59$, $P = 0.0007$, respectively). No significant correlation was found with MMP-9 ($r = 0.03$, $P = 0.87$).

To determine whether noradrenaline induces MMP-2 and TIMP-2 secretion by acting directly on cardiac cells, human fibroblasts were incubated with this catecholamine. Gelatinase activity was higher in fibroblasts incubated for 24 h with noradrenaline (50 or 100 μM) than in time-matched untreated cells (*Figure 2A*). MMP gelatinase activity was restricted to the band corresponding to 70 kDa, which is the M_r of MMP-2. Moreover, the activity of MMP-2 measured by one-step sandwich enzyme immunoassay was increased by noradrenaline treatment (*Figure 2B*). No gelatinolytic activity corresponding to MMP-9 was observed, and no MMP-9 could be detected by immunoassay. Basal levels of TIMP-2 antigen (2.56 ± 0.5 ng/mL, mean ± SD) was slightly, but not significantly, dose-dependently decreased by noradrenaline treatment (1.75 ± 0.1, 1.52 ± 0.2 ng/mL at 50 μM and 100 μM noradrenaline, respectively). Moreover, EMMPRIN protein levels were increased in human cardiac fibroblasts exposed to noradrenaline (50 or 100 μM) for 24 h (*Figure 2C*).

Discussion

This study shows that MMP-2 and MMP-9 levels are significantly elevated in severe CHF patients, compared with healthy subjects, even after adjusting for potential confounding variables. In addition, plasma levels of MMP-2 are positively correlated with noradrenaline levels, irrespective of the aetiology of the disease. Indeed, noradrenaline appears to be a significant predictor of MMP-2 levels. Moreover, in *in vitro* experiments,

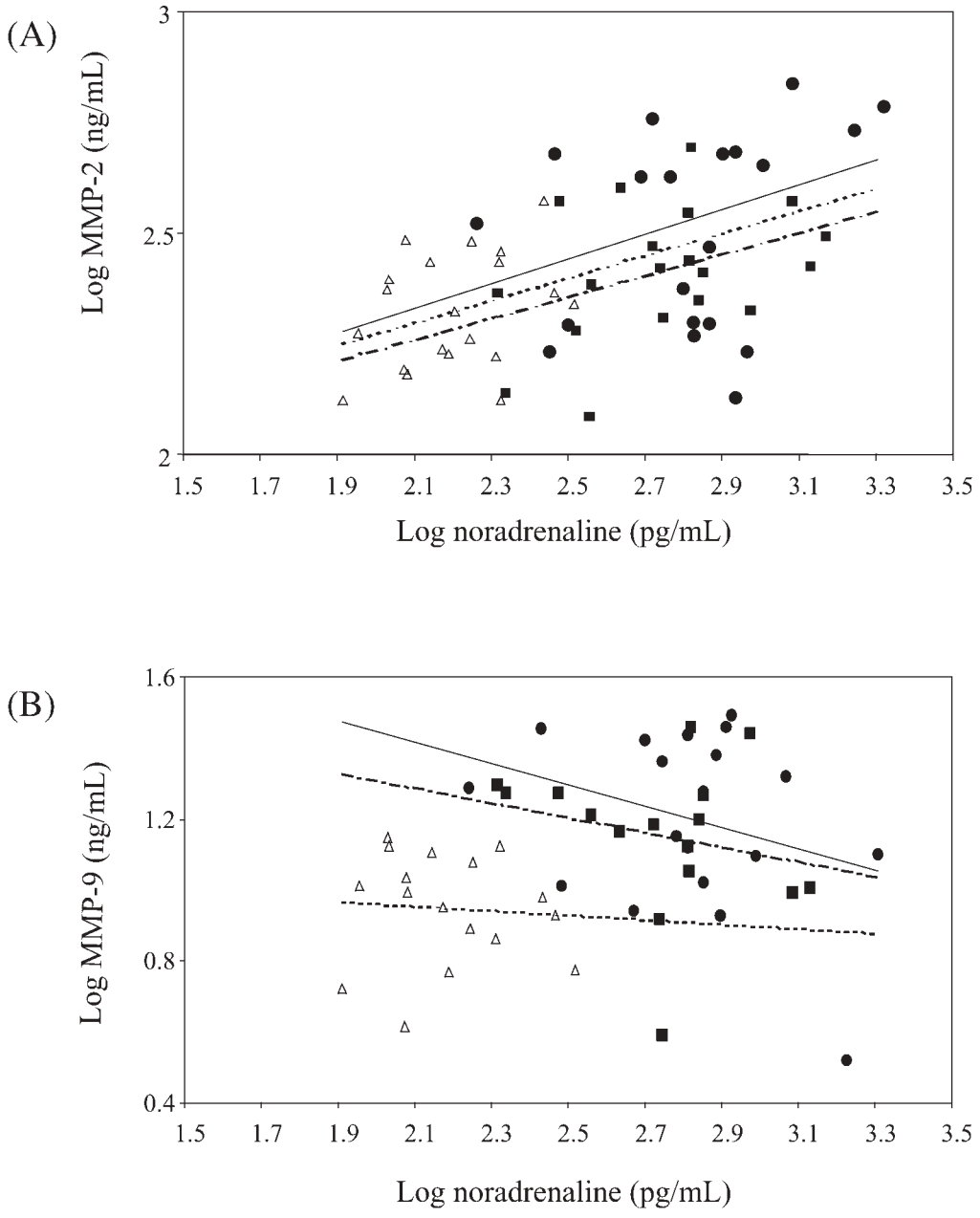

Figure 1 Scatter plot of log-transformed MMP-2 (*A*) and MMP-9 (*B*) vs. log-transformed noradrenaline. Lines are intra-group regression lines. Pearson correlation coefficients for MMP-2: 0.33, 0.32, and 0.39 for controls (triangles), ischaemic (circles), and idiopathic (squares) DCM, respectively. Overall intra-group correlation coefficient: 0.335, $P = 0.012$. Pearson correlation coefficients for MMP-9: −0.07, −0.31, and −0.23 for controls (triangles), ischaemic (circles), and idiopathic (squares) DCM, respectively. Overall within-group correlation coefficient: −0.24, $P = 0.1$.

we show that exposure of human cardiac fibroblasts to noradrenaline results in increased MMP-2 levels, pointing to a potential biochemical link between these two parameters.

MMP-9 plasma levels were also increased in CHF patients, but the lack of positive correlation with plasma noradrenaline levels and the lack of induction in isolated cardiac fibroblasts suggests that the systemic MMP-9 level is independent of sympathetic stimulation in CHF. Indeed, it has been demonstrated that inflammatory stimuli, e.g. interleukin-1β, interleukin-6, tumor necrosis factor-alpha, and sources of reactive oxygen species (ROS) increase MMP-9 expression and activities in rat cardiac fibroblasts.[25–27] Since pro-inflammatory cytokines, as well as oxidative stress, are both increased in heart failure[28,29] it is plausible that these factors may contribute to changes in plasma MMP-9 levels.[30,31]

MMP activity is regulated by endogenous TIMPs through a complex mechanism. Indeed, TIMP-2 can form a complex with certain MT1-MMP species thus facilitating the activation of MMP-2.[32] On the other hand, TIMPs can bind to the latent form of certain MMPs and slow

Table 3 Multivariable analysis (ANCOVA) testing the differences between groups (controls and DCM patients)

Dependent variable	Predictor	*F*	*P*	Partial R^2
A: Association between groups and catecholamines and MMPs				
Noradrenaline	Group	82.40	<0.0001	0.59
Adrenaline	Group	0.96	0.33	0.02
MMP-2	Group	9.56	0.003	0.14
MMP-9	Group	25.67	<0.0001	0.31
B: Effect of adjustment for noradrenaline on the association between groups and MMPs				
MMP-2	Noradrenaline	7.99	0.006	0.24
	group	0.02	0.88	0.03
MMP-9	Noradrenaline	4.68	0.03	0.08
	group	24.8	<0.0001	0.28

The model also included the following co-variates: age, gender, smoking habit.

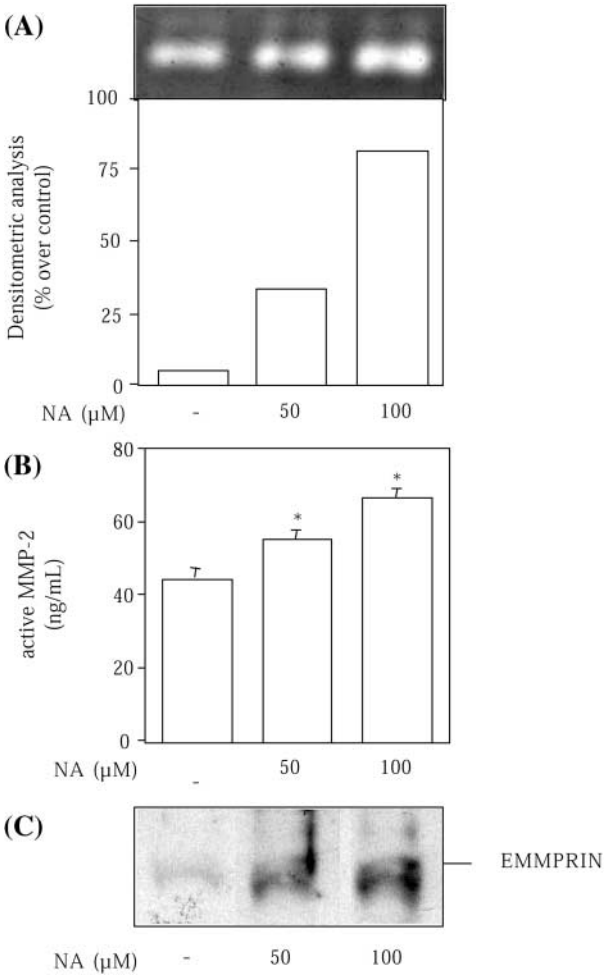

Figure 2 (*A*) zymography in the *in vitro* assay. Cardiac fibroblasts were incubated for 24 h with 50 or 100 μM noradrenaline. Gelatin released was measured by densitometric scanning and shown as % over control in the bar graph. (*B*) MMP-2 activity was measured by one-step sandwich enzyme immunoassay in the conditioned medium of cells incubated for 24 h with different concentrations of noradrenaline. $^*P < 0.001$ vs. untreated cells. (*C*) Cells were incubated for 24 h with 50 or 100 μM noradrenaline. After incubation, cells were washed with PBS and lysed in 100 μL Laemmli buffer. Equal amounts of protein were separated on a 12%-SDS–polyacrylamide gel and transferred to a nitrocellulose membrane. Western blot analysis was performed with a goat polyclonal anti-EMMPRIN antibody (1 : 500) in TTBS containing 5% milk. $^*P < 0.05$ vs. untreated cells. NA: noradrenaline.

the MMP activational process.[33] In the present study we did not find a significant modulation of circulating levels of TIMP-2 between control and CHF subjects.

MT1-MMP has been clearly shown to participate in the MMP activational process, specifically of MMP-2 and MMP-13, in a number of cell systems.[34,35] Our data show that MT1-MMP activity levels were not significantly modified in human myocardium from severe CHF patients with respect to non-failing hearts, suggesting that an alternative MMP-2 activation pathway, not including active MT1-MMP, may exist.[36,37]

The development of heart failure has been shown to be accompanied by ventricular remodelling and LV dilation, a process in which MMPs play an essential role in the high turnover of collagen in the extracellular matrix.[6–8,15]

Indeed, increased activity or expression of MMPs, which can degrade a wide spectrum of extracellular matrix components, has been identified in myocardial tissue samples from patients with end-stage CHF.[8,11–13,15] Studies with transgenic mice or with MMP inhibitors show a causal relationship between MMP activity and the LV remodelling process.[38] Indeed, targeted deletion of the MMP-2 gene reduces early LV rupture and late remodelling in mice after myocardial infarction,[39] MMP-9 gene deletion attenuates LV enlargement,[40] and the loss of MMP inhibitory control through gene deletion of tissue inhibitor of MMP-1 has been shown to cause LV dilation.[41] Finally, pharmacological inhibition of MMP in an experimental model of spontaneous or induced heart failure reduces the degree of LV dilation.[42,43] Taken together, these results provide evidence for the involvement of MMPs in myocardial remodelling.

LV dysfunction is known to be accompanied by the activation of a number of neurohormonal systems,[1,44] but the molecular mechanisms underlying this process are not fully understood. Chronic activation of specific neurohormonal pathways results in increased MMP activation in isolated porcine LV myocytes.[45] Moreover, β-blockade treatment, which inhibits catecholamine receptors, limits chamber remodelling and diastolic dysfunction, and prevents MMP activation by offsetting angiotensin II-mediated toxicity in dogs.[20] Our results are in accordance with these experimental data.

A large number of extracellular stimuli can alter the expression of MMPs in different cell systems.[35,46] It has been hypothesized that EMMPRIN induces MMP expression by a cell–cell interaction or a paracrine-mediated effect. Treatment of human fibroblasts with recombinant EMMPRIN induced MMP-1, MMP-2, and MMP-3.[47] The present study clearly shows that EMMPRIN is up-regulated in human cardiac fibroblasts exposed to noradrenaline, which suggests that EMMPRIN may facilitate MMP expression in failing human myocardium.

However, the intracellular signalling pathway by which noradrenaline modulates EMMPRIN protein levels remains to be elucidated; our preliminary experiments (data not shown) suggest that ERK1/2 is involved.

Thus we have shown that increased plasma levels of noradrenaline in severe CHF patients appear to increase plasma levels of active MMP-2. In addition, our *in vitro* data indicate a biochemical link between noradrenaline and MMP-2 in human cardiac fibroblasts.

However, future pharmacological studies are needed to support the hypothesis that a neurohormonal pathway determines the activation of MMPs in cardiac remodelling. The use of β-blockers, which attenuate or reverse the adverse effects of chronic adrenergic stimulation,[48] may throw some light on this issue.

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