Significance of matrix metalloproteinases in norepinephrine-induced remodelling of rat hearts

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Received 27 June 2002; accepted 25 September 2002

Abstract

Objective: Norepinephrine (NE) induced hypertrophy and remodelling of the extracellular matrix (ECM) in the left ventricle (LV) of the rat heart with resulting fibrosis. However, there was no increased collagen deposition in the right ventricle (RV). To test the hypothesis that lack of RV fibrosis is the result of elevated cleavage of collagens we inhibited the activity of matrix metalloproteinases (MMP) by doxycycline (Doxy) and then measured function and collagen metabolism in the RV as compared to the LV.

Methods: Female Sprague–Dawley rats were treated with 30 mg/kg per day doxycycline alone or in combination with i.v. infusion of NE (0.1 mg/kg per h). The activity of MMP-2 was increased both in the LV and RV after 3 days of NE infusion and reduced after concomitant doxycycline treatment which also caused inhibition when given alone. Results: After 14 days of NE infusion in combination with doxycycline there was an additional increase in the NE-induced elevation of collagen accumulation in the LV (interstitial collagen fraction: NE-Doxy 1.797%, P<0.05 versus control and NE; NE 1.113%, P<0.05 versus control) and significant fibrosis in the RV (2.105%, P<0.05 versus control). This correlated with the prevention of the NE-induced elevation of RV systolic pressure (NE: 71.3 mmHg, P<0.05; NE-Doxy: 36.4 mmHg) and RV dP/dt_max (NE: 5500 mmHg/s, P<0.05; NE-Doxy: 2550 mmHg/s). Also in the NE-stimulated LV, the doxycycline-induced collagen accumulation was associated with reduced LV dP/dt_max (NE-Doxy: 13169 mmHg/s; NE: 18849 mmHg/s, P<0.05).

Conclusion: MMP inhibition leads to myocardial stiffness with negative functional consequences for the RV and LV in NE-treated rat hearts.

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Keywords: Adrenergic (ant)agonists; Extracellular matrix; Fibrosis; Hemodynamics; Remodeling

1. Introduction

The matrix metalloproteinases (MMPs) have been demonstrated to cause tissue remodelling in normal physiological processes such as tissue morphogenesis, trophoblast migration, wound healing, and mammary development. MMPs have a high specificity for components of the extracellular matrix (ECM), such as fibrillar collagen, and the degradative functions of the MMPs are thought to play a role in a number of disease processes. Increased MMP expression has been identified in pathological processes such as tumour angiogenesis and metastasis, rheumatoid arthritis and atheroma formation. The MMPs constitute a family of zinc-dependent enzymes that currently number over 20 species. There are two principal types of MMPs: the membrane-bound type and those secreted into the extracellular space. The secreted MMPs comprise the majority of known MMP species and are released into the extracellular space in a latent or proenzyme state. Activation of these latent MMPs is required for proteolytic activity. MMPs, both latent and active, bind with a second class of biological molecules, the tissue inhibitors of

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matrix metalloproteinases (TIMPs). Therefore, overall MMP activity is determined by three important mechanisms: transcription, activation, and inhibition.

Increased MMP zymographic activity has been reported in myocardial samples from patients with end-stage chronic heart failure [4–7]. Several studies have demonstrated increased MMP expression and abundance in experimental models of chronic heart failure and with end-stage cardiomyopathic disease in humans [8]. Recently it was shown that increased MMP-2 expression in patients with dilated cardiomyopathy is associated with elevated plasma NE level [9].

A clear cause–effect relationship between MMPs and the LV remodelling process has been demonstrated through the use of transgenic models or pharmacological MMP inhibitors [10–13]. A loss of MMP inhibitory control through TIMP-1 gene deletion has been shown to cause LV dilatation in mice [14]. The deletion of the MMP-9 gene in mice alters the course of LV remodelling post myocardial infarction (MI) [10]. Pharmacological MMP inhibition has been used in several animal models of LV dysfunction [12,13]. For example, MMP inhibitor treatment with chronic rapid pacing attenuated the degree of LV dilatation that invariably occurs in this model [13]. In the spontaneously hypertensive heart failure rat model, MMP inhibition resulted in attenuation of LV dilatation [11]. In the mouse MI model, MMP inhibition has also been shown to reduce the degree of post MI LV dilatation [12]. Taken together, animal models of LV dysfunction have provided compelling evidence to implicate MMPs in the myocardial remodelling process.

Norepinephrine (NE) induced LV hypertrophy in rats [15]. This was accompanied by remodelling of the ECM as a result of an increased turnover of the ECM with elevated collagen I and III mRNA expression, and elevated MMP-2 activity [16]. The balance between collagen maturation and cleavage of the collagen network was disturbed after 14 days of NE-infusion in the LV with resulting fibrosis. However, this did not occur in the right ventricle (RV). The interstitial collagen fraction was higher, and the myocytes were smaller in the RV than in the LV of control rats. The development of NE-induced RV hypertrophy was delayed and not so pronounced in comparison to the LV. There was a significantly less pronounced increase in collagen mRNA expression in the RV than in the LV after NE treatment. Despite this moderate elevation there was no increase of the collagen fraction in the RV [16], indicating that it is a result of elevated cleavage of collagens by elevated gelatinolytic activity of MMP-2. In this study we want to test the hypothesis that elevated turnover of the ECM may be the reason for the unchanged collagen fraction in the RV after NE treatment. The function and collagen metabolism in the RV was compared to the LV after inhibition of MMP activity by doxycycline.

Tetracyclines are known as antibiotics [17], but recent work has shown that doxycycline, and other derivates of tetracycline, are potent broad-spectrum MMP inhibitors [18,19]. The only MMP inhibitor so far approved as a drug, Collagenex’s Periostat, is the oral tetracycline, doxycycline, for the treatment of periodontitis [20]. Clinical data for the effect of tetracyclines are well established [18]. Their broad spectrum of activity makes them a useful tool for MMP inhibition. This is the reason why we have used doxycycline to inhibit MMP activity in rats in vivo which were stimulated with NE.

2. Methods

All experiments were performed on female Sprague–Dawley rats weighing 210–260 g at the beginning of the study. They were supplied by Charles River (Sulzfeld, Germany). The animals were maintained in accordance with the Guide for Care and Use of Laboratory Animals published by the US National Institute of Health (NIH Publication No. 85-23, revised 1996). They were allowed to move freely in their cages with access to tap water and rat chow diet (Altromin C 100, Altromin, Lage, Germany). Norepinephrine (NE) was administered at a dose of 0.1 mg/kg per h dissolved in 0.9% NaCl; NaCl-infused animals served as controls. To prevent oxidation, 100 mg/l ascorbic acid was added. NE was given as constant intravenous infusion via a catheter (Vygon, Aachen, Germany) positioned in the left jugular vein. When infusion was done for 14 days, the catheter was connected to an Alzet mini-osmotic pump (Alza, Palo Alto, CA, USA), which was implanted subcutaneously. Animals with an unconnected mini-osmotic pump replacement served as controls for 14 days. When NaCl or NE infusion was limited to 3 or 4 days, the catheter was connected to a 20-ml syringe placed in an infusion pump (Infors, Basel, Switzerland). In this set-up, the infusion rate was 4 ml/kg per h. The syringes were protected from light. Control animals were infused with 0.9% NaCl plus 100 mg/l ascorbic acid at the same infusion rate as NE infusion. Doxycycline (30 mg/kg per day) was given as tablets (cocoa butter with 15% (w/w) glucose, the total weight was 2 g) with 1 day pre-treatment. The vehicle for the administration of doxycycline had no effect on MMP-2 activity in control and after NE treatment (data not shown). The study groups are summarized in Fig. 1. NE was purchased from Sigma (Deisenhofen, Germany). L-(-+)-Ascorbic acid was obtained from Merck (Darmstadt, Germany).

Hemodynamic measurements were performed using ultraminiature catheter pressure-transducers (Millar Instruments, Inc, Houston, TX, USA) [15]. After the hemodynamic measurements were obtained, the hearts were rapidly excised, and the RV free wall was trimmed away. Both ventricles were weighed after freezing in liquid nitrogen.
Fig. 1. Design of the protocol, with number of animals receiving norepinephrine (NE, 0.1 mg/kg per h), doxycycline (CTRL, 30 mg/kg per day), combination of both (NE + Doxy) and vehicle control for doxycycline treatment: cocoa butter with 15% (w/w) glucose (CB) for various periods of time (3–14 days). Corresponding measurements are indicated: zymography (Zymo); hemodynamic measurement (Hem); RNA preparation and ribonuclease protection assay (RPA); histological analysis (Histo).

2.1. Histological changes

In another series of 14 days of NE treatment, the hearts were excised, the atria were cut off, the ventricles were weighed, and the hearts were divided by a coronal section at a midpoint between the cardiac base and apex. At the base of the heart, the RV free wall was trimmed away, and the remainder of the ventricles were frozen in liquid nitrogen. The apex of the hearts was fixed in 4% buffered formaldehyde, embedded in paraffin, and 5-μm sections were stained with hematoxylin–eosin, PAS (periodic acid Schiff) and Sirius red (0.5% in saturated aqueous picric acid). The sections were morphometrically quantified as previously described [21] to assess cardiac fibrosis.

2.2. RNase protection assay

Total RNA isolation was performed according to a modified phenol–guanidiniumthiocyanate method of Chomczynski and Sacchi [22] using Trizol™ (Gibco-BRL™, Karlsruhe, Germany). Five μg of total RNA were used in the RNase protection assay (RPA) as previously described [16].

2.3. Preparation of cardiac tissue extract and zymography

Extracellular proteins of approximately 25 mg frozen tissue were extracted with 20-fold volume of extraction buffer (10 mM Tris–Cl pH 7.5, 150 mM NaCl, 20 mM CaCl₂, 1 μM ZnSO₄, 0.01% (v/v) Triton X-100, 1.5 mM NaN₃, 0.5% PMSF) over night at 4°C. These protein extracts contained approximately 1.5 mg/ml protein (BioRad Protein assay, BioRad, München, Germany). Myocardial matrix metalloproteinase activity in the gel was measured as previously described [16].

2.4. Statistical analysis

All data were analysed and expressed as mean±S.E.M. A multiple-sample comparison (ANOVA and multiple range test using the criterion of the least significant differences) was applied to test the differences between the groups for significance. A value of $P<0.05$ was considered to be significant. For analysis of correlation the simple regression procedure with the best comparison model was used. The program Statgraphics plus 4.1 (Statistical Graphics) was used for all statistical calculations.

3. Results

3.1. Cardiac MMP-2 activity

After 3 days of continuous i.v. infusion of NE, MMP-2 activity was elevated significantly in both ventricles (Fig. 2). It was decreased in doxycycline treated controls. This decreased level of MMP-2 activity was not influenced by NE-infusion.

3.2. Morphological changes

The doxycycline-induced decrease in MMP activity was associated with an additional increase in the NE-induced elevation of collagen deposition in the LV (Table 1). In the RV, the interstitial collagen fraction was not changed after 14 days of NE treatment (Table 1 and Fig. 2C). However, it was significantly elevated after treatment with NE in combination with doxycycline. NE induced LV hypertrophy as indicated by the elevated myocyte diameter (Table 1). This elevation was reduced by doxycycline. The myocyte diameter of the RV was not changed by NE nor by NE in combination with doxycycline.
3.3. Hemodynamic changes

Heart rate (HR), RV systolic pressure (RVSP) and RV contractility (RV $dP/dr_{max}$) were elevated by NE after 14 days of i.v. infusion of NE (Table 2 and Fig. 3A,B). The increase of these parameters was prevented by doxycycline. Doxycycline alone had no effect on the hemodynamic parameters nor on the interstitial collagen fraction (Fig. 3C). It induced a significant increase in the RV collagen accumulation of NE-treated rats (Table 1 and Fig. 3C). When correlating RVSP with RV collagen fraction, the best fit with an exponential regression analysis was a correlation coefficient of $-0.96$ ($P<0.01$) for all results from animals which were treated with NE and doxycycline (Fig. 4A, only the filled squares). When these results were analysed together with the results from animals which were treated only with NE this relationship was $-0.81$ (Fig. 4A). Similar results were obtained when RV $dP/dr_{max}$ was correlated with RV interstitial collagen fraction. There was a strong relationship between both parameters, when all results of NE treatment alone and NE with doxycycline treatment were combined (Fig. 4B). A similar correlation was observed when RV relaxation was chosen as hemodynamic parameter (data not shown).

In the LV, the hemodynamic changes after doxycycline treatment were not so pronounced as in the RV (Table 2). After 14 days, NE had a positive chronotropic and inotropic effect in the LV. End-diastolic pressure and total peripheral resistance (TPR) were not changed. After

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Table 1
Changes in collagen accumulation and myocytes diameter of the left (LV) and the right ventricle (RV) after 14 days of norepinephrine (NE, 0.1 mg/kg per h), of doxycycline treatment (Doxyc, 30 mg/kg per day), and the combination of both (NE-Doxy)

<table>
<thead>
<tr>
<th></th>
<th>Control ($n=7$)</th>
<th>NE ($n=7$)</th>
<th>Doxy ($n=4$)</th>
<th>NE-Doxy ($n=5$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV interstitial collagen fraction (%)</td>
<td>0.741±0.084</td>
<td>1.133±0.087*</td>
<td>1.062±0.14</td>
<td>1.797±0.21††</td>
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<tr>
<td>LV mean myocyte diameter (μm)</td>
<td>13.75±0.41</td>
<td>18.02±0.901*</td>
<td>12.24±0.05</td>
<td>14.01±0.196‡</td>
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<tr>
<td>RV interstitial collagen fraction (%)</td>
<td>1.225±0.1</td>
<td>1.248±0.109</td>
<td>1.253±0.324</td>
<td>2.105±0.398*‡</td>
</tr>
<tr>
<td>RV mean myocyte diameter (μm)</td>
<td>11.24±0.257</td>
<td>13.07±0.159</td>
<td>11.15±0.327</td>
<td>12.19±0.468</td>
</tr>
</tbody>
</table>

Values are mean±S.E.M.; *$P<0.05$ versus CTRL, †$P<0.05$ versus Doxy, ‡$P<0.05$ versus NE.

Table 2
Changes in functional parameters of the left (LV) and right ventricle (RV) and of circulation after 14 days of norepinephrine (NE, 0.1 mg/kg per h), of doxycycline treatment (Doxyc, 30 mg/kg per day), and the combination of both (NE-Doxy)

<table>
<thead>
<tr>
<th></th>
<th>Control ($n=11$)</th>
<th>NE ($n=11$)</th>
<th>Doxy ($n=4$)</th>
<th>NE-Doxy ($n=5$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (bpm)</td>
<td>372±9.4</td>
<td>484±20.4*</td>
<td>397±2.5</td>
<td>402±14.7‡</td>
</tr>
<tr>
<td>LV systolic pressure (mmHg)</td>
<td>147±7.8</td>
<td>163±11</td>
<td>166±15.5</td>
<td>172±5.2</td>
</tr>
<tr>
<td>Diastolic aortic pressure (mmHg)</td>
<td>114±6.2</td>
<td>117±9.6</td>
<td>128±13.1</td>
<td>133±3.9</td>
</tr>
<tr>
<td>Cardiac output index (ml/min per kg)</td>
<td>338±15.2</td>
<td>367±19.2</td>
<td>306±22.1</td>
<td>298±27.9</td>
</tr>
<tr>
<td>Total peripheral resistance (mmHg/kg-min/ml)</td>
<td>0.388±0.038</td>
<td>0.384±0.022</td>
<td>0.478±0.026</td>
<td>0.536±0.055*‡</td>
</tr>
<tr>
<td>LV $dP/dr_{max}$ (mmHg/s)</td>
<td>10 585±808</td>
<td>20053±2682*</td>
<td>10 846±1759</td>
<td>13 169±2120</td>
</tr>
<tr>
<td>RV $dP/dr_{max}$ (mmHg/s)</td>
<td>−12 078±893</td>
<td>−11559±1284</td>
<td>−10 923±1615</td>
<td>−9661±625</td>
</tr>
<tr>
<td>RV $dP/dr_{max}$ (mmHg/s)</td>
<td>2882±167</td>
<td>5813±585*</td>
<td>1938±136</td>
<td>2550±726‡</td>
</tr>
</tbody>
</table>

Values are mean±S.E.M.; *$P<0.05$ versus CTRL, †$P<0.05$ versus Doxy, ‡$P<0.05$ versus NE.
Fig. 3. Effect of doxycycline (Doxy, 30 mg/kg per day) on the increase in right ventricular (RV) systolic pressure (RVSP, A) and RV contractility (RV \(\frac{dP}{dt_{\text{max}}}\), B) induced by norepinephrine (NE, 0.1 mg/kg per h) treatment after 14 days of stimulation, as well as the effect of doxycycline on the interstitial collagen fraction of the RV (C). Animals with an unconnected mini-osmotic pump replacement served as controls (CTRL). *\(P<0.05\) versus CTRL, †\(P<0.05\) versus Doxy, ‡\(P<0.05\) versus NE; number of measurements in parentheses.

Fig. 4. Correlation of the level of interstitial collagen fraction (ICF) of the right ventricle (RV) to RV systolic pressure (RVSP, A) and contractility of the RV (RV \(\frac{dP}{dt_{\text{max}}}\), B) from rats which were treated with norepinephrine (NE, 0.1 mg/kg per h) alone (open squares) and in combination with doxycycline (30 mg/kg per day, filled squares) for 14 days. The data were analysed with the exponential model with a \(r^2\) value of 66.3% for (A): RVSP = \(\exp(4.98–0.575 \times \text{ICF})\), and with a \(r^2\) value of 72.1% for (B): RV \(\frac{dP}{dt_{\text{max}}} = \exp(9.67–0.88 \times \text{ICF})\).

4. Discussion

4.1. Inhibition of collagen degradation

The main findings of this study are that inhibition of MMPs by doxycycline in NE-treated rats led to an additional increase in the NE-induced collagen accumulation in the LV and elicited fibrosis in the RV which was not present after NE stimulation alone (Table 1). This fibrosis after 14 days of NE infusion in combination with doxycycline seems to be the result of MMP inhibition which occurred in both ventricles to the same extent (Fig. 2). With NE treatment alone, fibrosis had developed predominantly in the LV. This seems to be due to the fact that the mRNA of collagen I and III was increased to a higher extent in the LV after 3–4 days of NE infusion (Fig. 5), while MMP-2 activity was elevated to the same extent in both ventricles (Fig. 2). This increased MMP-2 activity may have been sufficient to degrade any originating collagen in the RV so that no fibrosis developed there within 14 days of NE treatment. In addition, NE induced myocyte hypertrophy only in the LV which required remodelling of connective tissue as well.

MMP inhibitors have been advocated as a potential...
Fig. 5. Collagen I, collagen III, MMP-2 and TIMP-2 mRNA expression in the left (LV) and right (RV) rat hearts after 3 and 4 days of norepinephrine (NE, 0.1 mg/kg per h) stimulation. 0.9% NaCl-infused animals served as controls (CTRL). RNA was subjected to RNase protection assay. Data are given as mRNA abundance relative to GAPDH mRNA expression. Mean values±S.E.M.; *P<0.05 versus CTRL, §P<0.05 versus RV; number of measurements in parentheses.

Fig. 6. Effect of doxycycline (Doxy, 30 mg/kg per day) on the increase in collagen I, collagen III, MMP-2 and TIMP-2 mRNA induced by norepinephrine (NE, 0.1 mg/kg per h) treatment in the left (LV) and right (RV) rat hearts after 14 days of stimulation. Animals with an unconnected mini-osmotic pump replacement served as controls (CTRL). RNA was subjected to RNase protection assay. Data are given as mRNA abundance relative to GAPDH mRNA expression. Mean values±S.E.M.; *P<0.05 versus CTRL, ‡P<0.05 versus NE; number of measurements in parentheses.
therapeutic approach for the prevention of cardiovascular diseases [10,11,13,23]. Until now, they have been studied in animal models of chronic volume overload [23], rapid pacing-induced heart failure [13], hypertension [11] and myocardial infarction [24]. The direct MMP inhibition can attenuate LV dilatation and progression to LV dysfunction and RV dysfunction [24]. The NE-induced LV hypertrophy described in this study, however, is not a model of LV dilatation.

MMP-inhibition could reduce or elevate myocardial fibrosis depending on the species studied. Treatment of spontaneously hypertensive heart failure (SHHF) rats with the MMP-inhibitor PD 166793 prevented cardiac dilatation, preserved contractility, and reduced myocardial fibrosis compared with untreated SHHF controls [11]. This suggested that the beneficial effects of MMP inhibition are mediated by limiting cardiac remodelling, thereby slowing the progression to heart failure. Similar MMP inhibition studies by Spinale et al. [13] found that concomitant treatment with PD 166793 in pigs undergoing rapid pacing attenuated the degree of LV dilatation, but was associated with a qualitative increase in interstitial collagen and an abnormal increase in myocardial stiffness. They concluded that the increase in ventricular stiffness was due to a greater amount of fibrillar collagen in the hearts of treated animals, suggesting that MMP inhibition might also have negative effects by inhibiting normal collagen turnover. This increase of collagen fraction was seen after combined treatment with NE and doxycycline too (Fig. 3C and Table 1), with an attenuation of the elevation of systolic pressure and contractility by NE especially in the RV.

NE elevated collagen I and III as well as MMP-2 and TIMP-2 mRNA expression after 14 days of treatment (Fig. 6). The reduction of collagen I and III mRNA expression to control level by doxycycline seems to be a result of a negative feedback mechanism in response to the elevated collagen accumulation. In a mouse model with a constitutively expressed human MMP-1 there was an opposite situation in that cardiac collagen mRNA expression was increased [25]. The elevation of TIMP-2 mRNA expression by doxycycline alone and the further increase after combination with NE was a surprising result (Fig. 6). It cannot be explained by the inhibitory characteristics of TIMP-2. Paradoxically, the specific physiological MMP-2 inhibitor, TIMP-2, at low levels, promotes this activation by forming a membrane complex with membrane type 1 (MT1)-MMP, anchoring the pro-MMP-2 to the cell surface [26,27]. TIMP-2 regulates the activity of MMP-2 and not the mRNA expression of MMP-2. The activation of the zymogen proform to the active MMP is the main point of regulation. Only 2–5% of MMP-2 is active in normal rat myocardium [28]. This may also explain the paradoxical reduction of MMP-2 mRNA to control levels after doxycycline treatment (Fig. 6). The mRNA level of MMP seems to be not so important for the regulation of MMP activity.

4.2. Alteration in heart function and peripheral circulation

The increased interstitial collagen fraction correlated with the prevention of the NE-induced elevation of RVSP and RV dP/dt max (Fig. 3). Also in the NE-stimulated LV, doxycycline induced an additional collagen accumulation which was associated with reduced LV contractility (Table 2). The deterioration of heart function by fibrosis may be due to myocardial stiffening. There are different hypotheses for the development of myocardial stiffening. Studies using the Dahl-S rat diastolic heart failure model demonstrated that the transition from the compensatory stage to the overt heart failure was associated with the progression of LV hypertrophy, fibrosis and myocardial stiffening [29]. Those results suggest a crucial role of myocardial stiffening in the development of heart failure. In view of the contribution of LV hypertrophy and fibrosis to myocardial stiffening, previous studies yielded different results. Narayan et al. [30] and Matsubara et al. [31] concluded that collagen accumulation, not LV hypertrophy, was responsible for myocardial stiffening. Schraeger et al. showed that LV hypertrophy, not fibrosis, is closely related to myocardial stiffening [32]. The attenuation of the NE-induced functional effect after doxycycline treatment seems to be the result of collagen accumulation and not of myocytes hypertrophy, since the diameter of LV myocytes was smaller after MMP inhibition (Table 1).

Myocardial stiffness together with relaxation abnormality is normally connected with ventricular diastolic dysfunction [33]. However, the relaxation was not significantly changed by NE and after doxycycline treatment in the LV. RV dP/dt max was more pronounced after NE and normalized after doxycycline like dP/dt max (Table 2). A similar relation between contractility and fibrosis was detected in SHHF rats [11]. Lower collagen content was accompanied by higher contractility. This effect can hardly be separated from the compensation of dilatation. We may therefore speculate, that fibrosis led to myocardial stiffening and this prevented NE induced elevation of systolic pressure and contractility especially in the RV. In this ventricle the increase of collagen accumulation was also more pronounced after doxycycline treatment (Table 1).

There was not a significant effect of doxycycline on diastolic aortic pressure (DAP), LVSP and cardiac output (CO), although DAP and LVSP were slightly higher and CO lower after combined treatment with NE and doxycycline (Table 2). In addition there was a significant elevation of TPR. The elevated DAP could be a result of stiffer resistance vessels. The inhibition of vessel enlargement by MMP inhibition was shown in an animal model of chronic volume overload [34,35]. It was demonstrated that flow-mediated arterial enlargement is limited by competitive MMP inhibition in a dose-dependent fashion. Furthermore, it was shown that doxycycline elevated pulmonary artery pressure in rats with 15 days of chronic hypoxic...
pulmonary hypertension [36]. This increased pressure was accompanied by collagen accumulation in pulmonary arteries.

The elevated TPR (Table 2) in doxycycline-treated NE-infused rats may induce reactive myocardial fibrosis. Fibrosis is well known in hypertensive heart disease [37], and thus may contribute to the additional increase observed with the combination (Table 1). Myocardial fibrosis is one of the histological constituents of myocardial remodelling present in hypertensive patients with hypertensive heart disease. In fact, an exaggerated interstitial and perivascular accumulation of fibrillar collagen type I and type III has been found in the myocardium of patients with arterial hypertension and left ventricular hypertrophy [37].

4.3. Myocyte hypertrophy

There was a significant inverse relationship between LV myocyte diameter and interstitial collagen fraction (Fig. 7). The more pronounced collagen network was associated with the inhibition of the enlargement of myocytes. This may indicate that there is a cross-talk between myocytes and the ECM which was disturbed by MMP inhibition. Myocytes may need the information from the environment so that there is room for enlargement. This may be a new important function of MMP inhibition. An inhibition of ventricular hypertrophy by blocking MMP activity was seen in the TNF-α transgenic mouse model of dilated cardiomyopathy [38]. This seems to be a result of prevented enlargement of myocytes too. Additionally, an extensive remodelling of ECM by over-expression of MMP resulted in compensatory myocyte hypertrophy [25].

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft (ZI 199/10-3, ZI 199/10-4) and by a grant of the Medical Faculty of the University of Leipzig (formel.1-10). The excellent technical assistance of Grit Marx and Brigitte Mix is gratefully appreciated.

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