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Experimental heart transplantation: effect of cyclosporine on expression and activity of metzincins

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Summary[1]

Metzincins, such as matrix metalloproteases (MMP), and extracellular matrix (ECM) proteins are differentially regulated in inflammation. We hypothesised that metzincins are also dysregulated in experimental acute cardiac allograft rejection.

We investigated the Dark Agouti-to-Lewis (DA-to-Lew) rat model of acute cardiac allograft rejection. Cyclosporine (CsA) (7.5 mg/kg/d) was given from transplantation to sacrifice (day +5). At that time, mRNA levels were analysed by Affymetrix genechip and quantitative reverse transcription polymerase chain reaction (qRT-PCR). MMP protein and activities were analysed by immunohistology, fluorometry, zymography and Western blots.

In untreated rejected DA allografts, mRNA levels of MMP-2/-7/-9/-/12-/14, a disintegrin and metalloprotease (ADAM)-17, tissue inhibitor of metalloprotease (TIMP)-1/-3 were increased, whereas MMP-11/-16/-24 and TIMP-2/-4 were lowered compared to native DA hearts. With respect to these untreated allografts, CsA lowered mRNA levels of MMP-7, TIMP-1/-3 (TIMP-2/-4 remained relatively low) and ADAM17, but augmented mRNA levels of MMP-11/-16/-23 and of many ECM genes. Immunohistology showed increased staining of MMP-2 in acute rejection (AR). Overall MMP activity was augmented in both transplanted groups, but CsA reduced MMP-9 activity and MMP-14 production.

Taken together, MMP and TIMP were upregulated during acute AR. CsA ameliorated histology of rejection but showed potential pro-fibrotic effects. Thus, MMP and TIMP may play a role in acute cardiac allograft rejection, and beneficial modification of the MMP-ECM balance requires interventions beyond CsA.

Key words: cardiac allograft; acute rejection; extracellular matrix (ECM); matrix metalloproteinases (MMP); rat transplant model; transcriptomics

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Introduction

Heart transplantation remains the optimal life-saving procedure for patients with advanced cardiac failure. The success of this therapeutic modality is still marred by the occurrence of rejection episodes and allograft fibrosis or sclerosis as common final pathways of immunologic and non-immunologic origin, despite modern immunosuppressive therapy [1, 2]. Thus, elucidation of the pathophysiology of acute cardiac allograft rejection and the potential fibrogenic influence of key immunosuppressive agents, especially cyclosporine (CsA), continue to be very important for clinical transplant medicine.

Inflammatory processes in solid organs are to a significant extent controlled by the metzincin superfamily of metallo-endopeptidases, mainly matrix metalloproteinases (MMP), adamalysins (ADAM) and meprin [3–5].

MMP were in the focus of this project as the best characterized group of metzincins. These proteases are traditionally classified into four categories, consisting of interstitial collagenases (MMP-1/-8/-13), gelatinases (MMP-2/-9), stromelysins (MMP-3/-7/-10/-11/-12) and membrane-type (MT)-MMP (MMP-14, -15, -16, -17, -24, and -25) [3,6]. The majority of MMP are se-

creted in a latent proenzyme form and requires extracellular activation [3]. Naturally, MMP inhibition occurs by tissue inhibitors of metalloproteinases (TIMP1-3) [3]. MMP activity can also be inhibited by a spectrum of low molecular weight, both peptide- and nonpeptide-based synthetic MMP inhibitors [7].

The main functions of MMP consist in the degradation of extracellular matrix (ECM) proteins, and to a somewhat lesser extent in the regulation of cell proliferation [8]. MMP are increasingly recognized as important pro-inflammatory mediators [3, 8–9]. In transplantation, MMP may act as pro-inflammatory mediators in several ways: direct tissue injury, augmentation of cell proliferation and/or migration and facilitation of tissue invasion by extrinsic cells (e.g., leukocytes) [8, 10].

The purpose of the present investigations was to examine the effect of CsA on the expression of metzincins, mainly MMP and TIMP, and in a limited fashion of their substrates in an established rodent model of heart transplantation.

Materials and methods

Animals and heart transplantation

Heterotopic cardiac grafting was performed in the laboratories of Novartis Pharma AG, Basel, Switzerland, using the Dark Agouti-to-Lewis (DA-to-Lew) rat strain combination, as published previously [10]. Two groups of hearts were studied at the time of sacrifice, day +5 after transplantation: untreated DA allografts and CsA treated DA allografts (Neoral, 7.5 mg/kg/day, given from transplantation to sacrifice at day +5). Additionally, native hearts from healthy DA rats served as controls (n = 6 in each group). The study was performed according to the Swiss federal law on animal protection and approved by the Basel Veterinary Office.

Biochemical analyses

Biochemical serum analyses were performed using the Beckmann Synchron CX-5 automatic chemistry analyzer.

Total protein and RNA extractions

Frozen hearts were first homogenised as powder in liquid nitrogen. Subsequently, proteins and total RNA were isolated according to the manufacturer's instructions (PARIS™ kit, AMBION INC). All aliquots of samples were stored at –70 °C until use. Protein concentration was determined using the BCA™ Protein Assay (PIERCE). RNA concentration was measured using a spectrophotometer and the quality was analysed by Bioanalyzer 2100 electropherograms (Agilent Technologies).

Genechip expression analysis

Affymetrix Rat Genome 230 2.0 Arrays were used according to the manufacturer's instructions, as previously described by us [12].

Hierarchical clustering

Hierarchical cluster was performed using dChip-software (http://biosun1.harvard.edu/ complab/dChip/). The clustering algorithm applied the "1-Pearson correlation coefficient" for the distance metric and the "centroid-linkage" method using clustering and gene function enrichment.

Quantitative reverse transcription polymerase chain reaction analysis (qRT-PCR)

Real time qRT-PCR analyses were performed as described previously [12]. Assays-on-demand gene expression products were used according to manufacturer's protocol (Applied Biosystems).

Zymography of MMP-2/-9 and fluorometry of MMP activity

MMP activity in protein extractions (see above) from frozen tissue homogenates was analyzed by gelatin substrate zymography (MMP-9) and by fluorometry (overall MMP activity), as previously described by us [12].

Western blot analysis for MMP-2/-9/-14 and TIMP-2

Western blot analyses were performed as previously described [12], using anti-MMP-2 (468-483) (Ab-3) human (mouse) (1:100), anti-MMP-9 (626-644) (Ab-3) human (mouse) (1:100), anti-MMP-14 (Ab-4) human (mouse) (1:25), anti-TIMP-2 (Ab-2) mouse mAb (67-4H11) (1:50) and as secondary antibody the peroxidase goat anti-mouse IgG. All antibodies are from Calbiochem.

Immunohistology of MMP-2

Immunohistology of MMP-2 was performed using rat heart sections, exactly as described previously, using the anti-MMP-2 (Ab-3) clone A-Gel VC2 from Neomarkers [12].

Statistics

All the investigations (qRT-PCR, immunohistology, etc.) were not done blinded to the type of rat (healthy, untreated, CsA-treated). The sample size was selected according to our resources. Results are presented as means ± standard error of mean (SEM) and were analyzed by non-parametric Mann-Whitney test using the GraphPad Prism Software version 4.0 for Windows. The analysis of MAS5 normalised genechip expression values was performed using GeneSpring 6.1.1 (Silicon Genetics). A probability of error (p-value) <0.05 (two-sided) was considered significant.

Results

Histological and biochemical characterization of cardiac allograft rejection

As expected, untreated cardiac allografts showed histological signs of severe acute rejection (AR) 5 days after transplantation (maximum survival time of untreated transplanted animals) (table 1a). Treatment with CsA reduced the severity of the AR, chiefly reflected in a decrease of mononuclear cell infiltration in the allografts.

As a result of AR-related myocardial muscle damage, possibly aggravated by surgical skeletal muscle damage, serum aspartate amino transferase concentration was elevated almost 15-fold in the Lewis rats transplanted with a DA heart, as compared to healthy Lewis animals [13]. Other serum parameters, including creatinine and albumin, remained stable (table 1b).

Affymetrix genechip and real-time PCR analyses of metzincins and ECM components

It should be noted that for all the analyses performed, the native healthy DA hearts from CsA treated animals did not show significant difference from non-treated healthy DA hearts. Thus, for the clarity of our manuscript, exclusively native DA hearts were shown as healthy controls in our experiments.

Table 1a

Histological characteristics and severity of acute rejection (Banff classification).

	DA healthy native hearts	DA graft hearts	
Cyclosporine treatment	–	–	+
Mononuclear infiltration	0	3	2MF
Polynuclear infiltration	0	0	0
Necrosis	0	0	0
Fibrosis	0	0	0
Calcification	0	0	0
Haemorrhage	0	0–1F	0–1F
Vasculitis	0	1	1
Edema	0	0–1	0
Final grade of rejection	0	3	2

DA = Dark Agouti. Mean histological scores = 1: Slight / Minimal; 2: Moderate; 3: Marked (F: Focal; MF: Multifocal); Grade of rejection = 0: no rejection, 2: moderate rejection, 3: marked rejection; (n = 6 in each group).

Table 1b

Biochemical characteristics.

Laboratory values	Healthy control	Non-treated, rejected transplant
Creatinine concentration (µmol/L)	34 ± 2	36 ± 1.7
Urea (mmol/L)	3.5 ± 0.3	5.7 ± 0.4
Alkaline phosphatase (IU/L)	212 ± 16	194 ± 19
Alanine amino transferase (IU/L)	42 ± 3	62 ± 5
Aspartate amino transferase (IU/L)	49 ± 4	715 ± 228*
Albumin concentration (g/L)	13 ± 0.7	11 ± 0

Results of serum analyses are given in Mean ± SEM. Healthy control: Lewis rats (n = 5); Non-treated transplant: DA-to-Lewis rat heterotopic heart transplantation model (n = 4). * $p < 0.05$.

In untreated DA allografts (AR) 40% of the ECM genes analyzed by microarrays were up-regulated compared to native DA hearts (e.g., collagens, laminin b2 and fibronectin). The mRNA levels of TGFb1, MMP-7/-9/-/12-/14, ADAM17, TIMP-1/-3 were increased, whereas MMP-11/-16/-24 and TIMP-2 decreased (fig. 1a and supplementary table). Compared to untreated allografts, CsA lowered the mRNA levels of MMP-7, TIMP-1/-3 and ADAM17, but augmented MMP-11/-16/-23 mRNA levels (supplementary table). The increased expression of ECM genes – substrates of MMP and ADAM – in rejection was even accentuated by CsA treatment in clear-cut fashion.

By qRT-PCR, MMP-2 mRNA was significantly and almost 2-fold up-regulated in AR (fig. 2a). CsA treatment reduced MMP-2 mRNA expression. These MMP-2 data do not corroborate the array data showing no MMP-2 mRNA expression change. This was already observed and commented by us in a previous publication [14] and as follows: "*The MMP-2 Affymetrix probeset 1369825_at is based on 3' UTR sequence of GenBank entry X71466. Due to alternative splicing or different processing, this untranslated region of rat MMP-2 is not found in GenBank entry U65656. Therefore, most likely our two TaqMan probes detected all splice variants of rat MMP-2 and the Affymetrix probeset did not recognize the U65656 variant of MMP-2 over-expressed in our rat model of acute rejection*".

Furthermore, the qRT-PCR analyses showed a very high TIMP-4 mRNA level in DA healthy native hearts, which was decreased some 150-fold in untreated AR compared to these controls. In AR hearts with CsA treatment, TIMP-4 mRNA expression was increased 13.5-fold compared to non-treated AR hearts.

TNFa was used as a positive control for the inflammatory response. It was confirmed that TNFa protein levels measured by ELISA were higher expressed in AR compared to normal hearts (2.5 ± 0.2 pg/ml vs 1.1 ± 0.2 pg/ml); these levels were reduced to approximately the control levels by CsA treatment (1.0 ± 0.4 pg/ml).

MMP activity levels

As measured by fluorometry, overall MMP activity was increased almost 2-fold in acutely rejected DA hearts compared to healthy control hearts (fig. 2b). CsA did not have a significant influence.

By gelatin substrate zymography, the 92-kDa pro-form of MMP-9 was significantly increased (4.0-fold, $p < 0.05$) in AR, as compared to healthy controls (fig. 2c). MMP-9 active form was most probably under the detection limit of the technique and thus not seen here. MMP-9 pro-form was significantly reduced by exposure to CsA. These observations confirmed the mRNA results

Supplementary Table

Gene	Affymetrix probeset	DA healthy native hearts	Untreated DA cardiac allografts	Cyclosporine treated DA cardiac allografts
Mean expression values of MMP, TIMP and ADAM				
MMP-2	1369825_at	29.5 ± 3.2	27.7 ± 0.0	28.1 ± 1.4
MMP-3	1368657_at	17.7 ± 0.4	20.2 ± 4.5	16.0 ± 1.0
MMP-7	1368766_at	20.0 ± 4.0	30.5 ± 5.4*	21.3 ± 4.3#
MMP-8	1387735_at	16.6 ± 1.6	18.8 ± 0.2	17.3 ± 2.1
MMP-9	1398275_at	20.5 ± 2.0	486.2 ± 568.2*	88.1 ± 48.8
MMP-10	1368713_at	18.1 ± 0.8	19.5 ± 1.7	24.6 ± 10.8
MMP-11	1367858_at	58.9 ± 8.0	34.3 ± 4.3*	47.8 ± 4.3#
MMP-12	1368530_at	31.4 ± 6.7	192.5 ± 112.5*	296.0 ± 50.5
MMP-13	1388204_at	13.3 ± 0.2	15.7 ± 2.6	16.1 ± 0.6
MMP-14	1378225_at	109.3 ± 7.3	261.1 ± 25.1*	256.0 ± 46.1
MMP-16	1368590_at	17.1 ± 0.3	14.5 ± 0.7*	19.3 ± 0.7#
MMP-23	1368961_at	63.7 ± 11.4	93.2 ± 26.8	153.3 ± 40.4#
MMP-24	1389833_at	243.5 ± 8.6	90.0 ± 15.1*	101.5 ± 31.8
ADAM-10	1388161_at	46.2 ± 5.6	55.3 ± 22.0	30.4 ± 6.5
ADAM-17	1367922_at	502.9 ± 45.7	1237.8 ± 86.6*	767.0 ± 225.6#
TIMP-1	1367712_at	120.0 ± 2.2	6468.3 ± 1498.2*	2616.5 ± 412.1#
TIMP-2	1367823_at	140.7 ± 4.1	98.1 ± 18.3*	96.8 ± 17.9
TIMP-3	1368989_at	31.6 ± 4.5	50.1 ± 12.5*	29.3 ± 5.9#
Mean expression values of ECM components				
Collagen I a1	1370864_at	412.6 ± 29.2	1389.5 ± 384.3*	3829.1 ± 80.0#
Collagen I a2	1387854_at	1269.6 ± 67.7	2221.2 ± 814.4*	7069.4 ± 304.2#
Collagen II a1	1387767_a_at	19.8 ± 1.1	20.7 ± 7.4	31.6 ± 25.0
Collagen III a1	1370959_at	3759.2 ± 67.9	6382.6 ± 1723.4*	12640.4 ± 1381.2#
Collagen V a1	1369955_at	126.5 ± 20.6	158.5 ± 28.1	388.9 ± 183.8#
Collagen V a2	1370895_at	561.7 ± 41.4	814.7 ± 292.4	2961.7 ± 271.8#
Collagen V a3	1368347_at	144.8 ± 14.8	189.6 ± 31.9*	306.0 ± 41.9#
Collagen X a1	1370944_at	19.3 ± 2.5	17.8 ± 1.4	18.2 ± 2.3
Collagen XI a1	1384211_at	11.4 ± 0.2	11.6 ± 0.2	13.3 ± 0.8
Collagen XI a2	1389891_at	53.5 ± 1.1	51.0 ± 7.8	55.1 ± 10.6
Collagen XII a1	1370297_at	78.1 ± 7.9	216.1 ± 67.0*	127.8 ± 23.6#
Collagen XVIII a1	1388459_at	125.0 ± 11.8	424.5 ± 110.1*	400.0 ± 75.9
Collagen XXIII a1	1393590_at	29.6 ± 3.0	33.1 ± 5.8	36.9 ± 18.5
Collagen XXVII a1	1375708_at	78.5 ± 7.3	62.9 ± 7.8*	102.2 ± 20.9#
Laminin a3	1370538_at	23.0 ± 2.6	20.7 ± 1.1	22.7 ± 1.2
Laminin a5	1388932_at	329.1 ± 17.0	367.8 ± 118.7	331.2 ± 22.8
Laminin b2	1367880_at	3003.3 ± 187.2	374.8 ± 42.1*	1676.7 ± 298.9#
Laminin c1	1370993_at	110.8 ± 18.1	197.1 ± 103.8	81.3 ± 32.6#
Fibronectin 1	1370234_at	234.1 ± 40.9	1651.7 ± 495.6*	1863.9 ± 193.7
Proteoglycan 1	1370782_a_at	14.3 ± 0.7	14.6 ± 0.8	15.1 ± 2.9
Proteoglycan 2	1387633_at	21.2 ± 0.7	20.7 ± 2.9	21.8 ± 5.2
Mean expression values of cytokines				
TGFb1	1370082_at	209.9 ± 28.5	752.9 ± 197.2*	383.2 ± 248.4
TNFa	1387691_at	42.5 ± 0.9	44.5 ± 4.2	42.3 ± 4.7

Rat Affymetrix GeneChip analyses of 41 genes (MMP, ADAM, ECM components, cytokines). Affymetrix Rat Expression Array 230 2.0 GeneChip data. Numbers represent normalized mean expression values ± SD. In bold, P <0.05. * significant compared to healthy DA native hearts. # significant compared to untreated DA heart grafts. n = 3 per group.

Figure 1

Hierarchical cluster analysis of gene expression profiles from rat hearts. 1 = DA healthy native hearts, 2 = Untreated DA cardiac allografts (AR), 3 = CsA treated DA cardiac allografts. Each column represents a sample; each row represents a gene from the gene list of Supplementary Table. Transcripts abundance is displayed on a red-green colour scale, with red expression above and green expression below the median. The cluster dendrogram sorts the samples based on the degree of similarity of gene expression profiles. Based on our genes of interest, rat samples with untreated AR can be clearly separated from the control and CsA-treated groups.

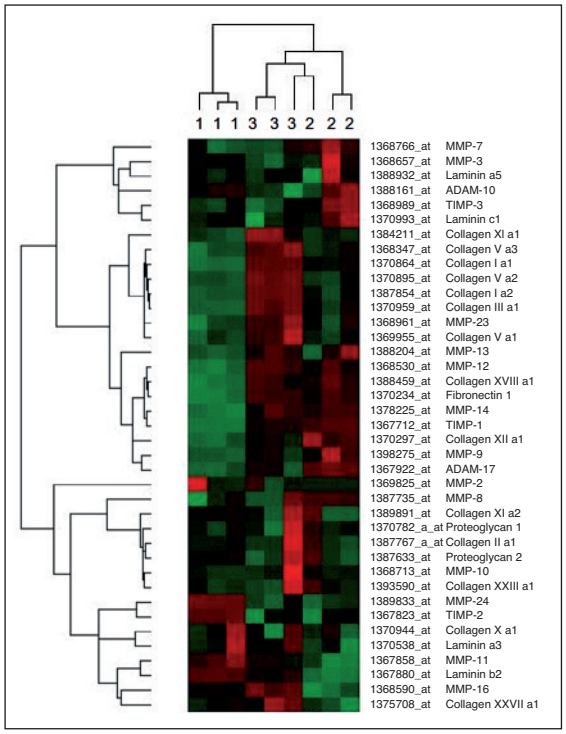
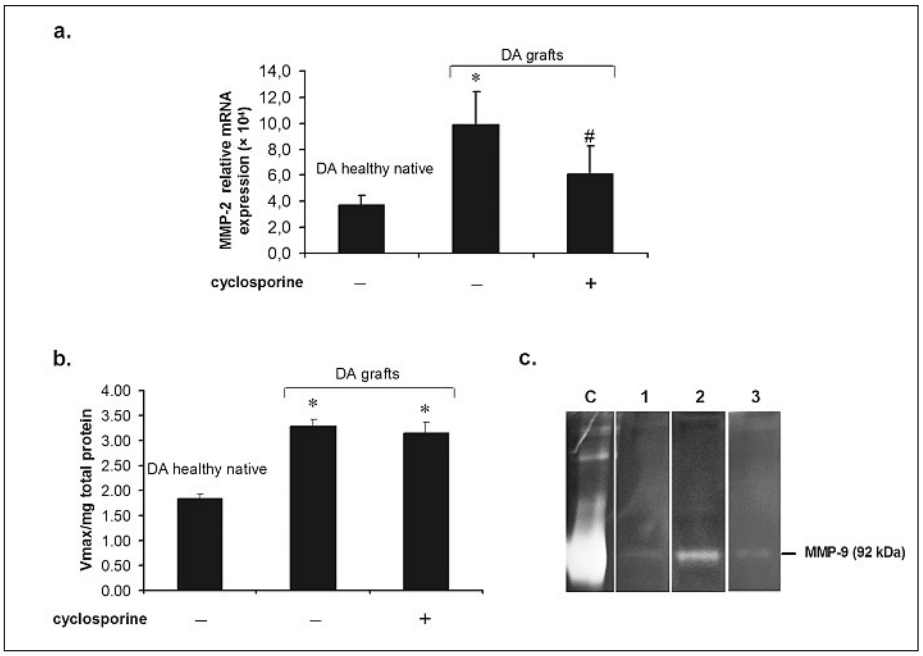


Figure 2

Rat heart MMP-2 mRNA expression and overall MMP activity. (a) MMP-2 TaqMan qRT-PCR showed a significant increase of MMP-2 mRNA expression in DA allografts compared with DA healthy native control hearts. (b) Fluorometry from total protein extractions showed a significant, almost two-fold increase in overall MMP activity in DA allografts compared with the DA healthy native control hearts. (c) Zymography demonstrated increased pro-MMP-9 in untreated DA grafts (lane 2) compared with DA healthy native control hearts (lane 1). Pro-MMP-9 was significantly reduced by CsA treatment (lane 3). Purified MMP-9 was used as positive control (lane C). *p<0.05 compared to the DA healthy native hearts; #p <0.05 compared to the DA grafts CsA-treated; n = 6 per group.



described above. However, it cannot be ruled out that CsA causes a suppression of MMP-9 activity independent of any change in gene expression. Concurrent results for MMP-2 were less conclusive, mainly due to a much lower degree of enzymatic activity in zymograms not allowing reliable quantification.

MMP and TIMP protein levels

In Western blot analyses, the pro-form of MMP-2 (72 kDa) was significantly increased in untreated DA grafts with AR, compared to the healthy control hearts (1.8-fold, p <0.05) and decreased by CsA treatment compared to the untreated DA grafts with AR (1.4-fold, p <0.05) (fig. 3a).

As observed by zymography, the level of the MMP-9 pro-form was strongly increased during AR (2.9-fold; p <0.05), compared to the DA healthy control hearts, and again had a tendency to decrease upon CsA treatment (1.3-fold) (fig. 3b). The active forms of MMP-2/-9 remained unchanged in all three experimental groups.

MMP-14 appeared not to be regulated in AR, but its protein expression was strongly decreased in DA grafts treated with CsA (2.2-fold; p <0.05) (fig. 3c).

In accordance with the mRNA results, TIMP-2 protein levels were decreased during the AR process, an effect maintained by CsA (fig. 3d).

We extended the MMP-2 analyses with immunohistology of heart tissue sections. MMP-2 staining was visibly increased in the myocardium of acutely rejected DA hearts. Since only the pro-form was significantly regulated in Western blot

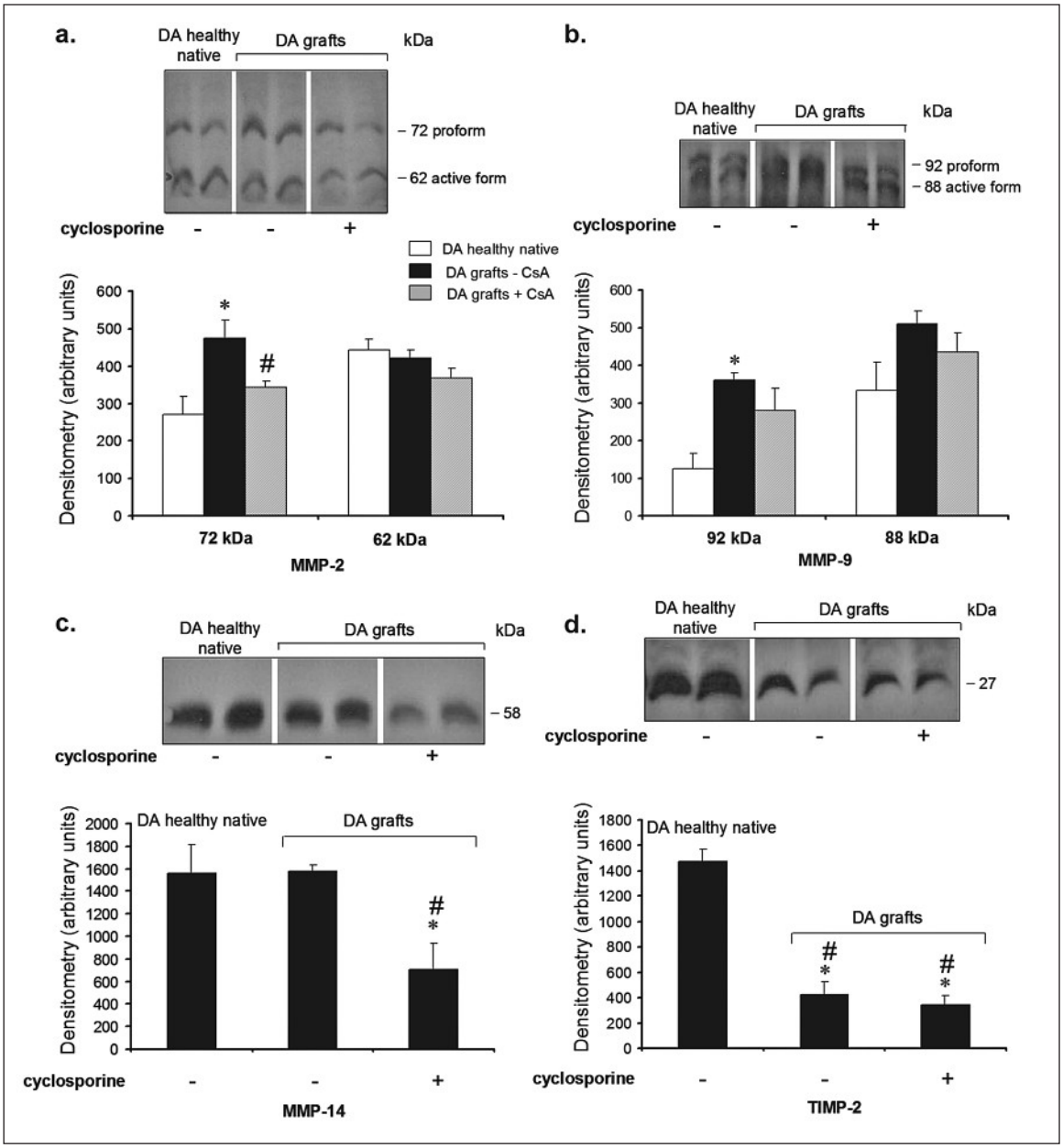


Figure 3

Rat heart MMP protein expression. Western blot and densitometry analyses of MMP-2 (a), MMP-9 (b), MMP-14 (c), TIMP-2 (d) in rat hearts. Two representative samples for each group are depicted. *p <0.05 compared to the DA healthy native hearts, #p <0.05 compared to the DA grafts CsA-treated; n = 4 per group.

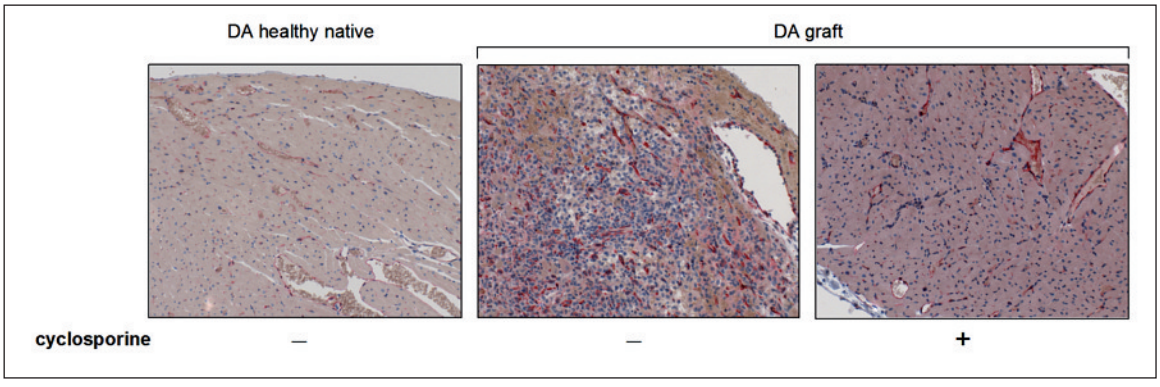


Figure 4

MMP-2 immunohistology in Dark Agouti (DA) heart sections. The increased presence of MMP-2 was observed in the myocardium of acutely rejected DA hearts (middle panel), as compared to the healthy control (left panel). CsA reduced acute rejection and consequently also the MMP-2 signal (right panel).

analysis (fig. 3a), it is most probable that the immunohistology represents pro-MMP-2 form and that day +5 after transplantation does not render the active form visible. However, we cannot determine to what extent the increased MMP-2 production resulted either from the cell infiltrates or from the myocardium itself. Nevertheless, this result was in accordance with the PCR and Western blot results. The immunosuppressant CsA reduced the MMP-2 signal.

Discussion

Taken together, the results of our investigations confirmed that most of the essential MMP and also ADAM17 were up-regulated during experimental AR of heart allografts. The important TIMP were divergently regulated. Amongst these key MMP inhibitors, TIMP-1 was increased and TIMP-2 was decreased as a result of the rejection process. It should be noted that TIMP-2 regulates MMP-2 activity in the extracellular environment after pro-MMP-2 activation by MMP-14. Thus, the remaining expression of TIMP-2 in rejection is not enough to inhibit MMP-2 activity in significant fashion and leads to an imbalance with increased MMP-2 activity. Consequently, overall MMP activity, including MMP-9, was enhanced in our model of allograft rejection. Furthermore, our rat data were in principle in accordance with human microarray data from endomyocardial transplant biopsies recently made publicly available [15]. These expression data were obtained from three biopsies per patient: one before AR, one during AR and after rejection (patients under immunosuppressant).

Alterations in the regulation of MMP and TIMP have already been investigated in various human and experimental cardiovascular diseases (e.g., vascular remodelling, cardiomyopathy, etc.) [16,17]. MMP were also shown to contribute to atherosclerosis and to be associated with the acute coronary syndrome, and they may thus be important therapeutic targets for future drug development [18]. However, in another study investigating rats with congestive heart failure, MMP-2 and -9 were not noticeably activated [19]. The antibiotic tetracycline significantly inhibited collagenase activity in cardiomyopathic tissue [19]. Interestingly, in another recent study it was suggested that the distribution of ECM proteins and MMP differ between the two ventricles in human hearts in end-stage cardiomyopathies [20].

With respect to transplantation medicine, MMP-2,-9,-14 and TIMP-1,-2 proteins were found to be differentially expressed in different regions of coronary vessels at different time points of acutely rejected transplanted primate hearts [21]. Furthermore, it was concluded that MMP-2, TIMP-1/-2 expressions were sensitive indicators of acute and chronic cardiac rejection, as analyzed in myocardium and coronary arteriosclerosis in cardiac allografts of non-human primates [22].

MMP-2 and MMP-9 were up-regulated in another rat heart strain combination three days after transplantation and the treatment with a specific MMP inhibitor decreased the levels of both MMP efficiently [23]. Our results are together in accordance with these two studies.

Since the minimum efficacious dose of CsA was 5.0 mg/kg/day in the DA-to-Lew heart transplantation model [24], the cyclosporine dose in our study was certainly sufficient to have a potential effect on metzincins and ECM proteins. CsA did not show a clear effect on the expression of MMP by decreasing as well as increasing various MMP. However, TIMP were decreased or kept low in acute cardiac allograft rejection. CsA did not influence overall MMP activity, though this agent reduced the production of MMP-14 in accordance with the literature reporting that MMP-14 mRNA expression is indeed modified by CsA [25].

The main effect of CsA was to raise RNA levels of ECM components, such as many types of collagens and laminin. It is also likely that molecular changes indicative of fibrosis precede visibly recognizable fibrosis. In this respect, CsA showed signs of fibrosis on the gene expression level. However, these changes were not (yet) evident on our histology sections. Thus, although this calcineurin inhibitor showed a clear benefit in the prevention of acute rejection, in the long run it may contribute to organ fibrosis and sclerosis.

In our study we cannot distinguish to what extent CsA has a direct effect on MMP production and an indirect effect via attenuation of AR. However, since the results of untreated DA native hearts and DA CsA treated native hearts were identical, these effects of CsA appeared to be predominantly indirect under our study conditions.

In another rat model of cardiac transplantation (Wistar Furst-to-Lewis), TGFb, collagen, fibronectin, MMP-2/-9 and TIMP-2 were found to be increased by PCR in rats treated with cyclosporine for 180 days, compared to untreated animals [26]. These findings are in principle concordant with our results, although we used a slightly different animal model and acute instead of chronic treatment.

In another experimental study, the treatment with the immunosuppressant tacrolimus had to be supplemented by the administration of an MMP inhibitor during 14 days to decrease the high expression of MMP-2 in a heterotopic rat heart transplantation model [27]. Thus, for effective MMP-inhibition, a specific MMP-inhibitor needs to be added to an immunosuppressant regimen of this kind.

To definitely elucidate the pathogenetic role of metzincins in cardiac allograft rejection, interventional studies with the aim of selectively inhibiting individual members of this enzyme family are needed. Besides the model of AR used in our investigation, a more chronic experimental transplant model (LEW to F344) may also be used for such investigations [28].

Taken together, most MMP, TIMP and ECM genes were up-regulated during AR in heart allografts. The short-term therapy with CsA ameliorated the histology of acute rejection but also showed a potential concomitant pro-fibrotic effect, reflected by the regulation of TIMP and ECM

proteins. Thus, MMP and TIMP may play a role in acute cardiac allograft rejection and beneficial modification of the MMP-ECM balance requires interventions beyond the application of CsA.

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