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Communication

Matrix metalloproteinases in serum of Emery-Dreifuss muscular dystrophy patients★

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In the pathogenesis of dilated cardiomyopathy (DCM) in Emery-Dreifuss muscular dystrophy (EDMD) matrix metalloproteinases (MMPs) are supposed to be involved and may have diagnostic/prognostic value. Serum levels of MT1-MMP, MMP-2 and MMP-9 were quantified by ELISA and zymography in 22 EDMD patients and 15 age-matched controls. In the autosomal-dominant EDMD MMP-2 and MT1-MMP were increased in all cases, and MMP-9 was increased in two of the eight examined patients. In the X-linked EDMD MMP-2 expression was increased in all the cases, MMP-9 level was elevated in 3 of the 14 cases, and MT1-MMP was decreased in eight of these patients. There was no evident correlation between the MMPs level and the different cardiac parameters including left-ventricular end-diastolic diameter, left atrial diameter and left ventricular ejection fraction in either form of EDMD. The presented results indicate that a changed level of matrix metalloproteinases, especially that of MMP-2 in serum, may be of value for detection of cardiac involvement in EDMD patients, especially in those patients with no evident subjective cardiac symptoms. Further follow-up studies of MMPs are needed to check if their determination is of value for monitoring of the progression of atrial/ventricular dilatation. MMPs determinations may also be useful for monitoring DCM treatment by synthetic MMPs inhibitors.

Keywords: Emery-Dreifuss muscular dystrophy, dilated cardiomyopathy, matrix metalloproteinases

INTRODUCTION

The deficit of lamins A/C or emerin in the skeletal and heart muscle contribute to a rare, autosomal-dominant or X-linked Emery-Dreifuss muscular dystrophy (EDMD). Although the defect is generalized, selectively affected are skeletal muscles, heart and joints. Among the dominant clinical symptoms are skeletal muscle atrophy, joint contractures and dilated cardiomyopathy, the latter being at the onset either clinically silent, or preceded by arrhythmias and conduction block. The pathogenesis of dilated cardiomyopathy (DCM) in EDMD is not recognized yet. It may be also evoked by changes in myocardial extracellular matrix (ECM) which is responsible for ventricular stability and aligement of cardiomyocytes (Brilla *et al.*, 1995). Induction of matrix metalloproteinases (MMPs) in dilated cardiomyopathy is already described (Tyagi *et al.*, 1996; Thomas *et al.*, 1998; Spinale *et al.*, 1999; Schwartzkopff *et al.*, 2002; Ohtsuka *et al.*, 2003; 2007). It is suggested that determination of MMPs may serve as valuable markers

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Abbreviations: AD, autosomal dominant; AF, atrial fibrillation; AVB, atrio-ventricular block; AFL, atrial flutter; DCM, dilated cardiomyopathy; ECM, extracellular matrix; EDMD, Emery-Dreifuss muscular dystrophy; EF, ejection fraction; HTX, heart transplantation; ICD, implanted cardioverter/defibrilator; LAD, left atrium diameter; LVDD, left ventricular end-diastolic diameter; MMPs, matrix metalloproteinases; PAF, paroxysmal atrial fibrillation; RBBB, right bundle branch block; SAB, sinoatrial block; SSS, sick sinus syndrome; SVT, superventricular tachycardia; SVEB, supraventricular ectopic beats; VF, ventricular fibrillation; VPB, ventricular premature beats; VT, ventricular tachycardia; X-EDMD, X-linked EDMD.

for progression of left ventricle dilatation. The participation of the matrix metalloproteinases system in DCM in course of EDMD has not been studied yet.

The aim of this study was to analyze if determinations of the levels of circulating MMPs may be useful in detection of cardiac involvement in EDMD. The identification of cardiac status in EDMD is of special interest because of considerable mortality and risk of sudden death in patients and carriers.

PATIENTS AND METHODS

Twenty-two patients (18 men, 4 women) with Emery-Dreifuss muscular dystrophy (EDMD) eight autosomal-dominant (AD-EDMD), 14 — X-linked (X-EDMD), aged 30±11 (14–54 years) were enrolled in this study. AD-EDMD patients had a gene mutation in the *LMNA*, X-EDMD in the *STA* gene. In the course of the disease a generalized cardiomyopathy was diagnosed in all cases. The moment of appearance of cardiomyopathy is, however, very difficult to define in both EDMD forms. No subjective cardiac symptoms, even with evident bradycardia, are often present. In some patients cardiac involvement was already detected at the time of neurological diagnosis of EDMD**.**

In the AD-EDMD left ventricle dysfunction was present. There were disturbances of conductivity, or contractility (Table 1a). The progress of cardiac symptoms in four of the eight patients was severe, in four it was mild or moderate. Pacemaker was implanted in three patients, cardioverter/defibrilator was implanted in one patient, in one case heart transplantation was performed, one patient (KT) suddenly died. DCM developed earlier in the AD-EDMD than in the X-EDMD group.

In ten of X-EDMD patients (Table 1b) the cardiac involvement was moderate, in three patients it was mild, in one case severe. Among the cardiac parameters at the beginning of the disease conductance and atrial involvement predominated. DCM developed later as compared to the AD-EDMD group. In 11 of the 14 patients pacemaker was implanted.

The control group consisted of 15 age-matched normal subjects with no history of cardiac symptoms.

Measurements of MMPs level in serum. MMP-2 was determined by a sandwich enzyme immunoassay kit employing two mouse monoclonal antibodies (Calbiochem, USA). In the MMP-9 determinations a sandwich enzyme immunoassay procedure employing a mouse monoclonal antibody and a sheep polyclonal antibody was used (Calbiochem, USA). MT1-MMP was determined by a two-step sandwich immunoassay (Chemicon International, USA) with enzyme-labeled antibodies against different antigenic forms (active and its precursor). The absorbance was assessed using a Sigma Diagnostics EIA Microwell Reader II. For zymography serum samples were diluted 1:50 with phosphate-buffered saline. The zymogram of MMP-2 and MMP-9 was resolved on Bio-Rad 10% precast gels with gelatin according to Azeh *et al.* (1998). The gels were stained with Coomassie Brilliant Blue R-250 and were further processed in GelDoc 1000/2000 system (BioRad), Multi-Analyst PC version 1.2 and Mitsubishi Video Printer P91.

Statistical analysis. The results were expressed as mean±standard deviation. For analysis of variance (ANOVA) on ranks the Kruskal-Wallis test was applied. Mann-Whitney test was used to compare both groups. The relations between variables were studied using Spearman's correlation coefficients. Data were analyzed by StatSoft statistical software package version 5.

RESULTS AND DISCUSSION

In serum of the AD-EDMD patients the level of MMP-2 and MT1-MMP was significantly increased in all of the cases, an increase of MMP-9 (over 2 S.D. of the normal mean) was present only in two of the eight examined patients (Table 1a). In X-EDMD the values of MMP-2 were also increased in all of the patients, the increase of the MMP-9 level was on the border of significance (a significant increase appeared in three of these cases), the MT1-MMP level was decreased (in eight of the 14 cases) (Table 1b). The difference in MT1-MMP between AD-EDMD and X-EDMD was significant. Zymograms indicated increased activity of MMP-2 in all cases of EDMD, while an increased activity of MMP-9 appeared only in some of them (Fig. 1). No difference in the circulating matrix metalloproteinases was found between male and female patients. There was no evident correlation between the levels of particular MMPs nor

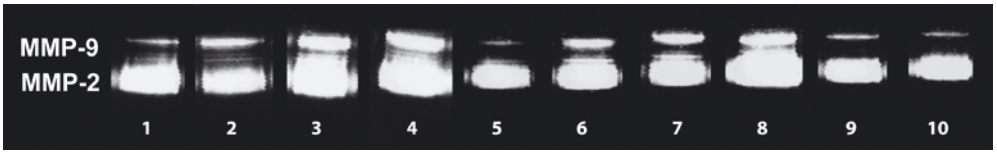

Figure 1. Representative gelatin zymogram of MMP-2 and MMP-9 in serum of EDMD patients.
AD-EDMD patients (lanes 1–4: GK, DS, NR, KT); X-EDMD patients (lanes 5–8: AD, KC, MK, TR); normal controls (lanes 9–10).

Table 1a. Clinical parameters and serum concentration of matrix metalloproteinases in EDMD.

				AD-EDMD									
Patient's initials	Sex	Age	Family history	Mutation	LAD (mm)	LVDD (mm)	EF (%)	Type of arrhythmia	Pacemaker (age)	Muscle atrophy	Contractures/ Spine rigidity	MMP-9 (ng/ml)	MMP-2 (ng/ml)	MT1-MMP (ng/ml)
KT	F	29	F	788T>C	36	56	55	AVB 1/2/3, AF, VT, VF	yes (28) ICD(29)	arms, calves	+/+	200	189	6.1
AŚ	M	45	F	743T>C	no data			AF, severe heart failure	HTX (31)	arms, tights	+/-	260	163	3.8
MS	F	31	S	1072G>A	37	54	44	AVB 1/2/3, PAF	yes (25)	generalized	+/+	191	173	4.3
GK	F	47	F	1357C>T	36	49	67	AVB 3	yes (41)	generalized	+/+	138	134	4.2
DŚ	M	22	F	743T>C	30	51	55	no arrhythmia	No	generalized	+/-	202	118	3.8
PK	M	31	S	1357C>T	31	42	52	SVEB, SVT, VPB, AVB1	No	arms, tights	+/+	181	130	4.4
AP	F	14	S	1337A>T	26	35	61	SVEB	No	generalized	+/+	196	129	6.8
NK	F	18	F	1357C>T	25	43	61	SVEB, SVT	No	generalized	+/+	393	174	6.3
Means ± S.D. (n)		**30±12(8)**			**32±5(7)**	**47±7(7)**	**56±7(7)**		**31±7(4)**			**220±77(8)***	**151±25(8)*****	**5.0±1.2(8)***,#
Normal					**19–40**	**35–56**	**55–65**					**177±23(15)**	**107±5(15)**	**2.3±0.7(15)**

*$P<0.05$, ***$P<0.001$ patients *vs.* normal; #$P<0.001$ AD-EDMD compared to X-EDMD.

between the MMPs and different cardiac or other clinical parameters.

Matrix metalloproteinases are a family of over 20 zinc-dependent enzymes responsible for degradation of components of the extracellular matrix (ECM), which maintains myocardial geometry during cardiac action and cardiac dysfunction (Goldsmith & Borg, 2002). Individual enzymes differ in substrate specificity and primary structure. Only some of them have been investigated to date (MMP-2, MMP-3, MMP-9, MMP-1). MMPs are detected in normal and pathologically changed myocardium (Spinale, 2002). The activity of MMPs is regulated at the transcriptional, translational, and post-translational level, by endogenous extracellular signals (cytokines, hormones, a variety of peptide growth factors) (MacNaul *et al.*, 1990; Ries & Woessner, 1991; Ries & Petrides, 1995) and specific inhibitors and regulators (TIMPs) (Weber & Brilla, 1991; Woessner, 1991; Tyagi *et al.*, 1995; Kähäri & Saarialho, 1999). The activity of MMP-2, MMP-7, and TIMP-1-4 increases, that of MMP-9 decreases with age (Bonnema *et al.*, 2007). The MMPs level is also under hormonal control. Female sex steroids inhibit MMPs (Singer *et al.*, 2000). A link between noradrenaline and MMP-2 is also recognized (Banfi *et al.*, 2005).

A number of cardiovascular diseases (dilated cardiomyopathy, hyperthropic cardiomyopathy, heart infarct, congestive heart failure, arterial hypertension) are associated with increased synthesis and degradation of extracellular matrix and disturbed maturation and incorporation of the matrix components in the myocardium.

Cardiomyopathy is accompanied by increased deposition of collagen. Little is, however, known about the temporal relationship between collagen gene transcription, the occurrence of cardiac fibrosis, and digestion of collagen by MMPs. It is not solved yet if the activation of the collagenolytic system is of primary or secondary importance in the progression of left ventricle dilation**.** Nevertheless, degradation of the fibrillar collagen matrix by collagenases is suggested to contribute to ventricular dilation (Spinale, 2002) and to coincide with the remodeling of the left ventricle and the onset of dilatation.

Any imbalance between the MMPs and TIMP systems disturbs the myocardial architecture, as both MMPs and their tissue inhibitors (TIMPs) maintain the architecture of the extracellular matrix and contribute to left ventricle remodeling and the subsequent deterioration of left ventricle performance (Tyagi *et al.*, 1995; Thomas *et al.*, 1998; Yokoseki *et al.*, 2000).

In idiopathic dilated cardiomyopathy there is an association between collagen degradation and increased expression of MMPs (Reddy *et al.*, 2004). There are, however, controversies on the presence

Table 1b. Clinical parameters and serum concentration of matrix metalloproteinases in EDMD.

			X-EDMD										
Patient's initials	Sex	Age	Family history	Mutation	LAD (mm)	LVDD (mm)	EF (%)	Type of arrhythmia	Pacemaker (age)	Muscle atrophy	Contractures/ Spine rigidity	MMP-9 (ng/ml)	MMP-2 (ng/ml)	MT1-MMP (ng/ml)
AD	M	54	S	256C>T	40	62	40	AVB 3, VPB	yes (20)	arms, tights	+/+	150	130	0.1
DK	M	22	F	450insG	34	57	56	AVB 1, VPB, SAB, RBBB	yes (18)	generalized	+/+	200	125	0.8
BB	M	20	F	187+1G>A	27	44	no data	AVB 3	yes (15)	arms, calves	+/+	153	127	1.3
MK	M	19	F	G457insC	35	53	65	SSS	yes (16)	arms, calves	+/+	156	175	0.9
KK	M	25	S	153delC	no data			PAF	yes (19)	generalized	+/+	196	103	0.1
PT	M	33	F	153delC	44	61	33	PAF, SAB, SVEB	yes (25)	arms	+/+	235	143	0.9
ZD	M	36	F	3G>A	34	45	65	AVB 1/2/3 PAF	yes (30)	arms	-/+	244	112	2.6
AP	M	33	F	153delC	34	52	68	AVB 2/3, PAF, RBBB	yes (21)	generalized	+/+	201	173	0.8
TR	M	33	F	397C>T	33	49	54	AVB 1/2/3, AFL	yes (25)	arms, calves	+/+	201	180	1.3
MP	M	40	F	153delC	37	56	45	tachy-brady	yes (28)	no atrophy	-/+	204	127	1.1
KP	M	43	F	153delC	31	51	52	AS, AVB 3	yes (31)	arms	+/+	224	126	2.1
GŁ	M	25	F	1A>G	30	47	55	SVEB, SVT	No	generalized	+/+	212	172	0.1
PL	M	17	F	153delC	no data			SVEB	No	no atrophy	-/+	176	135	0.2
KC	M	18	F	1A>G	30	45	69	AVB 1	No	arms, calves	+/+	177	121	2.9
Means ± S.D. (n)		30±12(14)			**34±5(12)**	**52±7(12)**	**55±7(11)**		**23±7(11)**			**195±29(14)[a]**	**139±26(14)[***]**	**1.1±0.9(14)[**,#]**
Normal					**19–40**	**35–56**	**55–65**					**177±23(15)**	**107±5(15)**	**2.3±0.7(15)**

[**] $P<0.01$, [***] $P<0.001$, [a] $P=0.07$ patients *vs.* normal; [#] $P<0.001$ AD-EDMD compared to X-EDMD.

of alterations of particular MMPs and TIMPs (Tyagi *et al.*, 1996; Li *et al.* 1998; Rouet-Benzineb *et al.* 1999; Spinale *et al.*, 1999; 2000; Spinale, 2002; Thomas *et al.*, 1998). In mild to moderate DCM the myocardial activity of MMP-2, MMP-3 and MMP-9 is elevated, that of MMP-1 decreased (Spinale *et al.*, 1999). In the end-stage of this disease the left ventricle MMP-1 decreases, that of MMP-3 and MMP-9 increases, the level of MMP-2 remains unchanged and TIMP-1 is up-regulated (Thomas *et al.*, 1998). An increase of MMP-2 and MMP-9, a decrease of TIMP-1 and TIMP-2 (Rouet-Benzineb *et al.*, 1999), or increase in MMP-1 and down-regulation of TIMP-1 (Tyagi *et al.*, 1996), down regulation of TIMPs and up regulation of MMP-9 have been also reported (Li *et al.*, 1998). Selectively decreased in the myocardium is TIMP-3 (Fedak *et al.*, 2004).

Although results of studies of particular MMPs conducted on dilated, ischaemic and valvular cardiomyopathies are divergent, they show that down/up-regulation of collagenolytic activity conducted by MMPs is present (Tyagi *et al.*, 1996; Spinale *et al.*, 1999) and leads to ECM interruption and its disorganization (Beltrami *et al.*, 1995; Pauschinger *et al.*, 1999; Li *et al.*, 2000). Overexpression of MT1-MMP and TIMP-1 mRNA in DCM also indicates increased collagenolytic activity (Picard *et al.*, 2006). Besides their action on collagen, MMPs are also involved in disorganization of the contractile apparatus in DCM hearts by cleaving myosin heavy chain (Rouet-Benzineb *et al.*, 1999).

Changes in the level of collagen degradation markers are reflected in serum of patients with different cardiovascular diseases. It is higher in idiopathic, as compared to ischemic DCM (Tziakas *et al.*, 2005). An association between serum MMP-9 level and clinical cardiac symptoms with a risk of cardiovascular death has been also indicated (Blankenberg *et al.*, 2003). Serious cardiac events also correlate with an increased MMP-3 level (Ohtsuka *et al.*, 2007). In patients with congestive heart failure an increase of circulating MMP-2 (Yamazaki *et al.*, 2004), or MMP-2, MMP-9 and TIMP-1 is observed (George *et al.*, 2005), which

correlates with the severity of the disease (Yamazaki *et al.*, 2004). It is also indicated that MMP-1 and TIMP-1 levels in serum of DCM patients may serve as markers of progression of the left ventricle dilatation (Schwartzkopff *et al.*, 2002). The progressive activation of MMPs in the course of DCM may be evoked by increased levels of circulating specific cytokines (Marriott *et al.*, 1996; Munger *et al.*, 1996).

In EDMD cardiac risk and the expression of cardiac involvement is highly variable. At the beginning of this disease no clinical cardiac symptoms are usually present. In the course of the disease EDMD patients and carriers often need a permanent cardiac pacemaker, in some cases sudden death occurs (Rowland *et al.*, 1979; Miller *et al.*, 1985; Galassi *et al.*, 1986; Pinelli *et al.*, 1987; Fishbein *et al.*, 1993; Buckley *et al.*, 1999). Progressive atrial fibrillation/flutter, atrial paralysis, heart block with slow junctional rhythm, and later on ventricular dilatation and ventricular failure appear. The myocardium is progressively replaced by fibrous and adipose tissues (Yoshioka *et al.*, 1989). Early onset of severe myocardial fibrosis before clinical and echocardiographic signs and cardiac dysfunction is already manifested in a family with a deletion of the 5′ end of the *LMNA* gene (van Tintelen *et al.*, 2007). An analysis of the MMPs/TIMPs system in EDMD patients, in the myocardium or serum has not been conducted until now.

Here we evaluated the potential significance of circulating MMPs levels in patients with EDMD. Serum level of MMP-2 was higher in all patients of both groups of EDMD. The difference in MT1-MMP expression between AD-EDMD and X-EDMD may be connected with the fact that a more severe cardiac involvement is present in the AD-EDMD form. MMP-9 level appears to be less informative, as its increase was only present in a small number of patients.

Further examination of MMPs, particularly of MMP-2, in EDMD patients, their relatives and carriers, especially in follow up studies, is needed. MMPs determination may be of value in prediction of the susceptibility to the dilated cardiomyopathy, especially important in cases with no preceding clinical cardiac symptoms. The MMPs determination may be also conducted in monitoring DCM treatment by means of synthetic MMPs inhibitors, which could prevent left ventricular dilatation and delay the impairment of left ventricular systolic function. The introduction, however, of synthetic inhibitors to the strategy of DCM treatment has to be preceded by detailed studies of the whole MMPs family, especially as the sensitivity of the individual enzymes to the inhibitors greatly differs.

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