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Serum Levels of Matrix Metalloproteinases-2 and -9 and Their Tissue Inhibitors in Inflammatory Neuromuscular Disorders

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Key Words

Intravenous immunoglobulins · Matrix metalloproteinases · Neuromuscular disorders · Tissue inhibitor of matrix metalloproteinases · Intravenously applied immunoglobulin therapy

Abstract

We monitored serum levels of matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) before and during intravenously applied immunoglobulin (IVIG) therapy in 33 patients with chronic immune-mediated neuropathies and myopathies and 15 controls. Baseline MMP-2 and TIMP-2 serum levels were lower and MMP-9 and TIMP-1 serum levels higher in all patients compared to age-matched controls. Eight days after IVIG treatment, MMP-2, TIMP-2, and TIMP-1 serum levels increased, while MMP-9 serum levels decreased, indicating tissue repair. After 60 days, MMP-9 levels increased, MMP-2 approached normal levels, while TIMP-1 and TIMP-2 serum levels were below day 8 levels, indicating relapsing tissue damage. Comparing the MMP/TIMP results with the clinical courses, IVIG treatment tended to change MMP/TIMP levels in a way that paralleled clinical improvement and relapse. In sum, during a distinct time period, IVIG therapy seems to be able to modulate MMP-mediated tissue repair.

Introduction

Matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) participate in extracellular matrix (ECM) tissue remodeling in both normal and pathological conditions [1–3]. TIMPs form non-covalent bimolecular 1:1 complexes with MMP pro-forms; therefore ratios between MMPs and TIMPs reflect to a certain extent the overall proteolytic activity. An increase in active MMPs may indicate a disparity between TIMPs and pro-MMPs [1–3]. The constitutively expressed matrix metalloproteinases type 2 (MMP-2) and type 9 (MMP-9) seem to be involved in mechanisms of T-cell migration into the peripheral nerve and skeletal muscle [2, 4–7]. Inflammatory myopathies such as polymyositis (PM) and sporadic inclusion body myositis (sIBM) show a strong upregulation of MMP-9 and to a less significant extent of MMP-2 at atrophic and inflamed myofibers [2, 4, 6, 7]. In wallerian degeneration and regeneration of peripheral nerves, macrophages and other epi- and endoneural cells, involved in nerve repair in vivo, secrete e.g. MMP-2 and MMP-9 [5, 8].

Chronic severe immune-mediated neuromuscular disorders like chronic inflammatory demyelinating neuropathy (CIDP), multifocal motor neuropathy (MMN) and inflammatory myopathies are treated with intrave-

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nously administered immunoglobulins (IVIG) [for reviews, see 9, 10]. Apart from clinical and electrophysiological follow-up parameters, the correlation of disease activity to specific antibodies or immune-mediated serum protein levels is often uncertain.

The aim of this pilot study was to investigate effects of high-dose IVIG on serum levels of MMP-2, MMP-9, tissue inhibitor of metalloproteinases type 1 (TIMP-1), type 2 (TIMP-2) and their ratios. A change in ratio was supposed to be helpful detecting treatment-related effects in patients with PM, sIBM, CIDP, and MMN.

Patients and Methods

Fifteen patients with electron microscopically-proven sIBM (5 women and 10 men, mean age 68.4 years, range 59–82 years, mean disease duration 5.1 years), 4 patients with biopsy-proven PM (3 women and 1 man, mean age 58 years, range 46–73 years, mean disease duration 3.6 years), 11 patients with electrophysiologically-proven MMN (4 women and 7 men, mean age 57.7 years, range 48–71 years, mean disease duration 2.8 years), and 3 patients with clinical, cerebrospinal fluid and electrophysiological signs of CIDP (1 woman and 2 men, mean age 58 years, range 40–72 years, mean disease duration 4.2 years) were prospectively investigated. A sequential electrophysiological analysis (days 0 and 60) was performed in all patients, but did not show any significant differences in the chosen follow-up period (data not shown). Clinical follow-up was monitored by neuromuscular scores (Medical Research Council (MRC) scale, Neuromuscular Symptoms Score (NSS, 18) and the Angelini score at admission and on days 8, 30 and 60. Patients with sIBM, MMN and CIDP did not receive any other immunomodulator during this study. Only patients with PM received 20 mg methylprednisolone and 150 mg azathioprine as a concurrent treatment. Finally, age- and sex-matched control blood specimens were obtained from 15 healthy volunteers (8 women and 7 men) with a mean age of 59.4 years (range 34–82 years).

Determination of MMP-2, MMP-9, TIMP-1 and TIMP2

Whole blood samples were obtained after written informed consent by venous puncture before IVIG therapy, on day 8, and 60 days after starting a 5-day administration of a total of 120–160 g high-dose IVIG (2 mg/kg b.w.). Samples were immediately stored on ice, directly centrifuged and stored frozen at –80°C.

Commercially available ELISA kits were used to determine the concentration of MMP-2, MMP-9, TIMP-1 and TIMP-2 in EDTA serum (Chemicon, Temecula, Calif., USA). According to the manufacturer's directions, the MMP-9 and MMP-2 ELISA measure the pro-form of MMP-9 and the pro-MMP-2/TIMP-1 complex or the pro-form of MMP-2 and pro-MMP-2/TIMP-2 complex, respectively. The TIMP-1 ELISA detects TIMP-1 and the complex of TIMP-1 with the pro- and active forms of MMPs. Assays were performed following the manufacturer's instructions. Samples of each individual patient were analyzed on one plate. All standards and samples were assayed in duplicate. In addition, sample vials of IVIG were tested for MMP and TIMP contamination.

Statistical Analysis

The statistical analysis was performed using the paired t-test. Wilks' multivariate test of significance was performed to compare MMP-2, MMP-9, TIMP-1 and TIMP-2 levels at different times. The Mann-Whitney test was used to compare the results obtained in patients and control subjects. $p < 0.05$ was considered as statistically significant.

Results

Clinical Course. The course of disease in all patients was relapsing and refractory to other immunomodulators. We could not detect any containment of MMPs or TIMPS in the IVIG vials administered. A slight statistically non-significant clinical improvement was evident between days 8 and 30 after IVIG therapy in almost all chronically ill patients (table 1). Thereafter, a decline in clinical condition was obvious in all patients and a repeated course of IVIG treatment was administered.

Baseline Results. Before therapy, baseline MMP-2 serum levels were significantly lower and MMP-9 serum levels significantly elevated in all patients compared to healthy controls with the exception of PM (fig. 1).

Sporadic Inclusion Body Myositis. In sIBM patients, a significant increase of MMP-2 and TIMP-1 was found on day 8, but after 60 days only the MMP-2 serum levels were still significantly elevated compared to pretreatment levels. The elevated levels of MMP-2 on days 8 and 60 reached values comparable to untreated age-matched controls.

Polymyositis. In PM patients on days 8 and 60, only elevated TIMP-1 levels were found. An up to 50% increase compared to controls was found.

Multifocal Motor Neuropathy. In MMN patients, a significant MMP-2 serum level elevation was found on days 8 and 60. The day 60 MMP-2 levels almost approached control levels. In addition, on day 8 a significant TIMP-2 elevation was found followed by a decrease on day 60.

Chronic Inflammatory Neuropathy. The preliminary results in 3 CIDP patients showed differences of MMP-2 serum levels only on day 60 (fig. 1).

MMP to TIMP Ratio. The change in the MMP-9 to TIMP-1 ratio paralleled the course of MMP-9 showing a decrease on day 8 and an increase on day 60 (table 2). During the whole study period, the MMP-9 to TIMP-1 ratio for all diseases was higher than values of the control group with the exception of PM and MMN on day 8. The differences were not statistically significant either on day

Table 1. Prospective clinical follow-up data in IVIG-treated patients with neuromuscular diseases

	sIBM		PM		MMN		CIDP	
	mean	SE	mean	SE	mean	SE	mean	SE
Patients, n	15		4		11		3	
MCR sum score (max. 130)*								
Day 0	109.5	7.5	101.5	5.5	118.5	7.5	113.0	4.5
Day 8	110.0	5.0	102.5	6.0	120	4.5	115	5.0
Day 30	111.5	5.5	105.5	4.5	123.0	6.0	119.5	7.0
Day 60	108.4	6.5	100.3	3.5	114.1	5.5	110.3	2.5
Angelini score (max. 12)*								
Day 0	8.6	0.4	8.0	0.2	9.5	0.3	9.7	0.5
Day 8	8.6	0.2	8.2	0.2	9.5	0.1	10	0.3
Day 30	9.0	0.5	8.4	0.1	9.5	0.5	10.2	0.4
Day 60	8.1	0.4	7.8	0.3	9.0	0.5	9.7	0.4
Neuromuscular symptoms score (max. 35)*								
Day 0	28.7	0.2	27.5	0.4	33.7	0.7	33.3	0.1
Day 8	29.0	0.7	27.5	0.9	33.0	0.4	33.3	0.8
Day 30	31.5	0.9	28.7	0.6	34.5	0.6	33.9	0.6
Day 60	30.1	0.7	28.0	0.4	33.6	0.7	33.7	0.5

* All differences are statistically non-significant ($p < 0.5$).

Table 2. Ratios of MMP and TIMP serum levels in inflammatory neuromuscular diseases and healthy controls (mean values are shown)

	Patients				Controls
	sIBM	PM	MMN	CIDP	
Samples, n	15	4	11	3	15
MMP-9/TIMP-1					
Day 0	8.51	8.06	7.38	15.61	4.44
Day 8	5.73	3.84	4.04	9.18	
Day 60	10.0	5.7	9.55	16.4	
MMP-2/TIMP-2					
Day 0	9.74	11.70	9.45	10.03	12.67
Day 8	11.63	9.55	8.49	9.93	
Day 60	12.4	13.86	13.86	15.02	

8 ($p < 0.065$) or day 60 ($p < 0.086$) (table 2). In addition, before and 8 days after treatment the MMP-2 to TIMP-2 ratio was statistically lower compared to controls ($p < 0.017$ and $p < 0.018$–0.024). On day 60, a non-significant increase of the MMP-2 to TIMP-2 ratio approaching control levels was found (table 2).

Discussion

MMPs are proteolytic enzymes involved in degrading and remodeling the ECM in response to e.g. inflammatory autoimmune processes like PM or CIDP. This study is one of the first attempts to monitor peripheral blood MMP and TIMP levels in inflammatory myopathies and immune-mediated neuropathies before, during and after 60 days of high-dose IVIG therapy.

In contrast to a previous study on expression of MMPs and TIMPs in inflammatory myopathies, we were able to measure MMP-2, MMP-9 and TIMP-1 and TIMP-2 in sera of controls and all patients [6]. Although in the prior study MMPs and TIMPs were present in human muscle tissue, in sera of the same patients neither MMP-9 nor TIMP-1 activity was found using gelatin zymography and ELISA analysis [6]. This result is in obvious contrast to other reported studies of MMPs and TIMPs in multiple sclerosis and Guillian-Barré syndrome patients [11–13]. Consequently, we suppose an ELISA assay-dependent effect for this inconsistency.

Regarding IVIG effects on MMP expression, so far only one study has reported dose-dependent inhibition of IVIG on MMP-9 protein activity and its mRNA expression in human monocytic cells [14]. $F(ab)_2$, but not Fc fragments, led to suppressed MMP-9 activity. Neverthe-

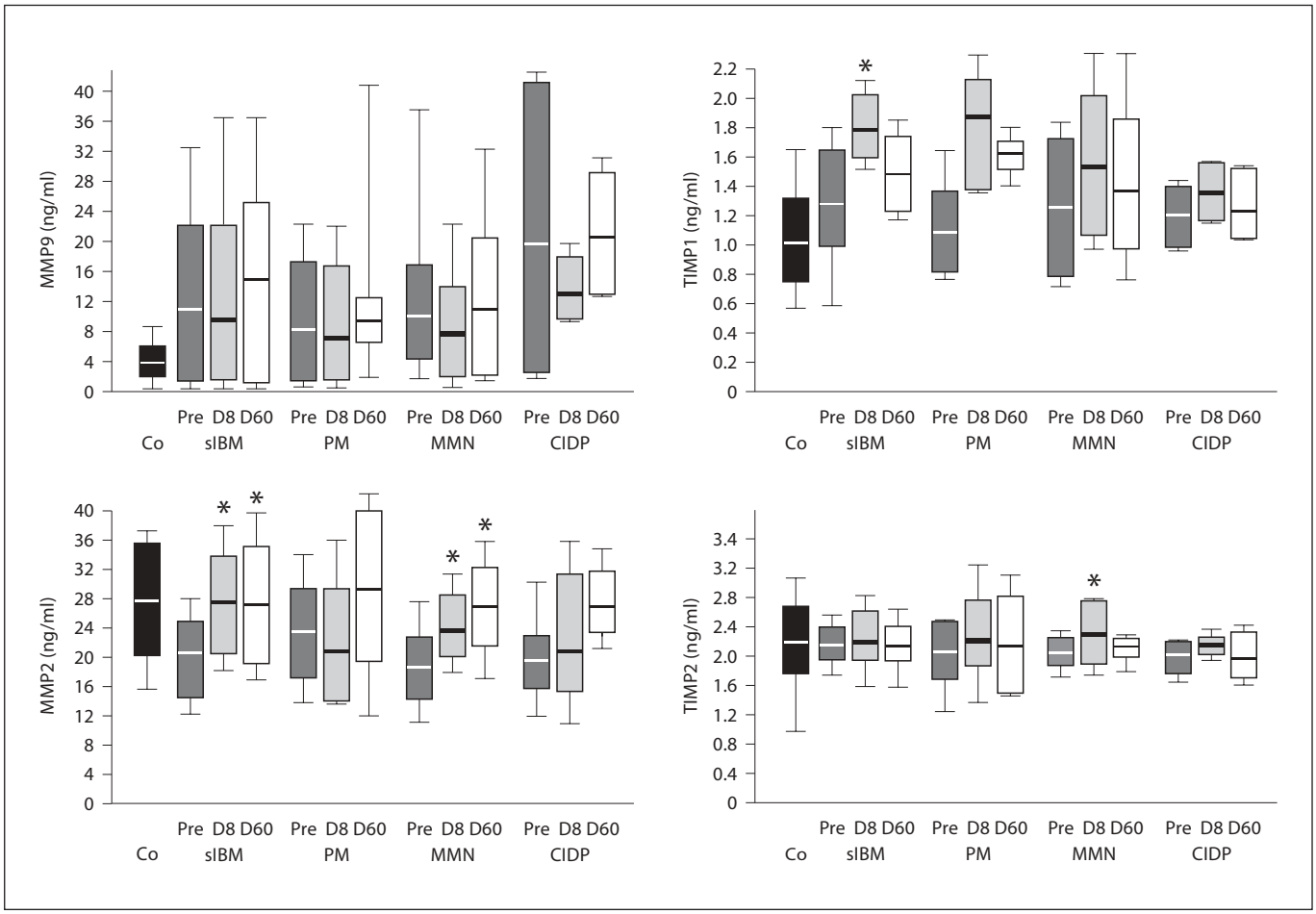

Fig. 1. Mean, standard deviation and minimal and maximal values of MMP-2, MMP-9, TIMP-1 and TIMP-2 serum levels, presented for all diseases and controls. Mean values are indicated by cross-bars. The box-blot represents standard deviations, and vertical lines mark minimal and maximal values of serum levels. * $p > 0.5$ is expressed on top of the min-max lines. All values are given in ng/ml. Co = Controls; Pre = before IVIG therapy, D8 = day 8, D60 = day 60.

less, competitive experiments demonstrated that Fc but not $F(ab)_2$ fragments reversed IVIG-induced inhibitory effects. Additionally, no changes in MMP-2 activity levels were found using gelatin zymography. The authors concluded that the whole IgG molecule might be needed for relevant IVIG-induced MMP-9 downregulation [14]. Thus, IVIG may introduce stabilization of altered ratios of MMPs and their TIMPs in peripheral nerves and skeletal muscles.

Our results are in accordance with these findings. Baseline elevated MMP-9 serum levels significantly dropped after IVIG treatment and may indicate tissue repair, while posttreatment increased MMP-2 levels indicate relapsing tissue damage. Moreover, 60 days after treatment, all the immune-mediated disorders investigated here showed relapsing MMP and TIMP levels and no long-lasting significant change in MMPs/TIMPs ratios was found. This parallels the well-known clinical improvement and relapse of these diseases under IVIG therapy. Thus, even high-dose IVIG seems to modulate the inflammatory ECM attack for a short period only.

In summary, ELISA quantification of MMPs and their inhibitors in serum appears to be sensitive to provide a method for monitoring enzyme levels longitudinally under IVIG therapy. At this stage of investigation we cannot completely exclude that our observations may be solely due to a non-specific effect of immunoglobulins. Further studies may provide evidence on whether MMP/TIMP

serum level changes are a biomarker for clinical response to IVIG and related immunosuppressive treatment that may lead to a prolonged anti-inflammatory ECM remodeling in distinct diseases.

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