Short communication

Serum matrix metalloproteinase-3 levels are elevated in myasthenia gravis

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Abstract

MMP-3 is capable of degrading a variety of proteins, including agrin, which plays a critical role in neuromuscular signalling by controlling acetylcholine receptor clustering. The degradation of agrin by MMP-3 may disrupt the neuromuscular junction leading to a failure of neuromuscular transmission and muscle weakness. We have therefore examined the levels of MMP-3 in 116 patients with myasthenia gravis (MG) and 90 healthy controls. A significant elevation in MMP-3 levels was observed in 10% of seronegative and 17% of seropositive MG patients, indicating that MMP-3 may play a pathogenic role in a proportion of MG patients.

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1. Introduction

Myasthenia gravis (MG) is an autoimmune disorder which causes skeletal muscle weakness. Auto-antibodies against acetylcholine receptor (AChR) are present in 85%–90% of MG patients with generalised disease (Lindstrøm et al., 1998). This is termed seropositive MG (SPMG). A thymoma is present in approximately 10–15% of patients with MG (Wilcox, 1993). Auto-antibodies to AChR impair neuromuscular transmission by complement-mediated postsynaptic membrane damage, direct blockade of ligand–receptor interaction, and/or by an increased degradation of AChR (Li et al., 1996; Lindstrom et al., 1998). Patients without detectable auto-antibodies to the AChR are termed seronegative MG (SNMG) (Mossman et al., 1986). Auto-antibodies to muscle specific kinase (MuSK) are observed in a proportion of SNMG patients (Hoch et al., 2001; McConville et al., 2004). MuSK is a key signalling protein controlling AChR clustering and the formation of the neuromuscular junction. These processes are triggered by the release of agrin from the nerve terminals. Agrin interaction with MuSK leads to the phosphorylation and clustering of AChR (Fuhrer et al., 1997; Borges and Ferns, 2001).

Agrin is a substrate for matrix metalloproteinase 3 (MMP-3) (VanSaun and Werle, 2000). MMP-3 null mice have alterations to their neuromuscular junctions, including increased AChR and agrin staining at the endplate and increased junctional folds (Vansaun et al., 2003). These observations indicate that MMP-3 is involved in controlling the neuromuscular junction structure via regulation of agrin levels.

MMP-3 is capable of breaking down various extracellular matrix components, including collagens and proteoglycans (reviewed by Sternlicht and Werb, 1999). Elevated serum MMP-3 levels have previously been reported in the autoimmune disorders systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) (Zucker et al., 1994; Kotajima et al., 1998). SLE and RA have an increased incidence in MG patients (Christensen et al., 1995; Sthoeger et al., 2006), indicating shared pathogenic mechanisms.

In MG patients, an elevated MMP-3 level may cause damage to the neuromuscular junction due to the degradation of agrin, leading to a reduction in the safety factor for neuromuscular transmission.
transmission and muscle weakness. We have therefore examined the levels of MMP-3 in the sera of patients with both SPMG and SNMG, and compared these to the levels in healthy controls.

2. Materials and methods

All reagents were obtained from Sigma Chemical Company (Steinheim, Germany), unless otherwise stated.

2.1. Patient and control sera

Serum samples from 96 SPMG patients, 20 SNMG patients, and 90 healthy blood donors (hereafter termed controls) were studied. None of the patients or controls had any recorded confounding diseases, including SLE or RA. The MG group included patients with early and late onset MG, thymoma and non-thymoma MG, and various auto-antibody profiles. No patients in this study had anti-MuSK auto-antibodies.

2.2. Serum MMP-3 levels

Total MMP-3 levels were assessed using the Quantikine MMP-3 Elisa kit (R&D Systems Europe Ltd, Abingdon, UK) as per manufacturer’s instructions. Patient sera were assayed at a dilution of 1:10 in duplicate wells.

2.3. Statistical analysis

Groups were compared using the Chi-square test. The cut-off level between a normal and a high MMP-3 level (hereafter defined as MMP-3 positive) was defined as the mean MMP-3 concentration for the 90 controls + 2 SDs; 48 ng/ml. The concentrations of MMP-3 in the two MG groups and controls were compared using the $t$-test for the difference between population means.

3. Results

16 of the 96 (17%) SPMG and 2 of the 20 (10%) SNMG patients were MMP-3 positive (Fig. 1, Table 1). The number of MMP-3 positive patients in the SPMG group was significantly higher than the number of MMP-3 positive controls ($p < 0.01$). Also the number of MMP-3 positive patients in the SPMG and SNMG groups combined was significantly higher than the number of MMP-3 positive controls ($p < 0.025$) (Table 1).

Mean MMP-3 concentrations did not differ significantly between MG patients and controls.

Table 1

<table>
<thead>
<tr>
<th>MMP-3 concentrations (ng/ml) in MG patients and controls</th>
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<tbody>
<tr>
<td>Seropositive MG</td>
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<tr>
<td>----------------</td>
</tr>
<tr>
<td>Number of patients</td>
</tr>
<tr>
<td>Mean MMP-3 concentration</td>
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<tr>
<td>Number of MMP-3 positives</td>
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<tr>
<td>Mean MMP-3 concentration of MMP-3 positives</td>
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Fig. 1. Scatter plot of the MMP-3 levels in 96 seropositive MG patients (SPMG), 20 seronegative MG patients (SNMG), and 90 controls. The horizontal dotted line indicates the 2 SD cut-off of 48 ng/ml.
patients combined was significantly higher than mean MMP-3 in the four MMP-3 positive controls \( (p<0.001) \) (Table 1).

The MG patient group included a wide range of MG patient subtypes. High MMP-3 levels were found among MG patients both with thymoma and thymic hyperplasia. MMP-3 concentrations were not related to MG severity, age of debut, or to anti-muscle auto-antibody profile.

4. Discussion

We have demonstrated that 17% of SPMG patients and 10% of SNMG patients express significantly higher MMP-3 levels than healthy controls. The mean serum concentration of MMP-3 in MMP-3 positive patients was 80.4±27.2 ng/ml compared to 23.4±12.3 ng/ml for the 90 control sera in this study. In comparison, MMP-3 concentrations of 258±35 ng/ml and 187±14 ng/ml have been reported in SLE and RA respectively, with a value of 50±4 ng/ml in control sera (Zucker et al., 1994). It should be noted that the mean serum MMP-3 concentration in our control group was approximately half that observed previously. Furthermore, our studies of SLE patient sera (unpublished data) also show lower levels of serum MMP-3 than previously reported (Zucker et al., 1994), indicating that these observations arise due to methodological differences.

The difference in MMP-3 positive patients between SPMG and SNMG patients (17% vs 10%) does not represent a significant difference between these two subtypes of MG, due to the rarity of SNMG in the MG patient group, combined with the relatively small proportion of MG patients expressing high levels of MMP-3. Despite a proportion of patients expressing high levels of MMP-3, no significant difference in the average MMP-3 level was observed for MG and control sera prior to application of a 2 SD cut-off value. This would appear to be due to a higher number of MG patients expressing low levels of MMP-3 compared to control sera. The reasons, and possible biological consequences of this, remain unclear. We are currently increasing our control and MG patient cohort in order to further analyse this observation.

Since agrin is required for the clustering of AChR in the neuromuscular junction, increased degradation of agrin, and subsequent disruption of MuSK signalling, may have important consequences for the correct formation and function of the neuromuscular junction and lead to a reduction in the safety factor for successful neuromuscular transmission. Agrin is also capable of inducing the expression of the ε subunit of the AChR (Jones et al., 1996; Meier et al., 1998). Furthermore, MMP-3 null mice show alterations to their neuromuscular junctions (Vansaun et al., 2003), indicating that MMP-3 plays a critical role in neuromuscular junction formation and maintenance.

An increased MMP-3 level as a primary cause of MG is unlikely, since MMP-3 levels are increased in patients with SLE and RA who do not have MG (Zucker et al., 1994; Kotajima et al., 1998). The significance of an increased level of MMP-3 in MG, SLE and RA remains unclear and the mechanism(s) by which MMP-3 is upregulated in these autoimmune diseases is unknown. A common mechanism cannot be discounted. By expanding our MG patient database, and by including SLE and RA patient sera, it will be possible to screen for common immunological and clinical events that may explain the elevated levels of MMP-3 observed in a proportion of these patients.

Initially we hypothesised that SNMG may be explained by non-immunoglobulin mediated mechanisms, with MMP-3 being a potential mediator. Increased MMP-3 levels were observed in both SPMG and SNMG patients indicating that this is not the case. MMP-3 reducing the safety factor for neuromuscular transmission may, however, explain why auto-antibody levels and disease severity do not correlate. Unfortunately, due to the absence of patients expressing anti-MuSK auto-antibodies in the Norwegian MG population (Romi et al., 2005), this patient group could not be examined. Furthermore, due to the wide range of patient subtypes examined (patients varying in age of debut, sex, disease severity and clinical subtype), compared to the relatively low number of MMP-3 positive patients, it is not possible at this time to correlate high serum MMP-3 concentrations with a specific subset of MG patients.

In conclusion, we have shown that serum MMP-3 levels are increased in a proportion of patients with MG. A dysregulation of MMP-3 therefore appears to occur in a proportion MG patients, as has also been reported for SLE and RA (Zucker et al., 1994). A specific pathogenetic effect of MMP-3 in MG appears unlikely. Dysregulation of MMP-3 may therefore be a common feature of autoimmune diseases such as MG, SLE and RA, as opposed to a causative factor specific for MG. A greater understanding of the mechanism(s) by which MMP-3 levels are increased in these patients will improve our understanding of the pathogenesis of MG and other autoimmune diseases, and will enable correlations to be made between MMP-3 levels and disease subtypes. The mechanism(s) by which this occurs, and the biological significance of increased MMP-3 levels, remain to be established.

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References


