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Oxidative modifications of blood serum proteins in myasthenia gravis

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ABSTRACT

Myasthenia gravis (MG) is an autoimmune disease caused by production of antibodies against acetylcholine receptors of the neuromuscular junction (Ab). The aim of this study was to ascertain if oxidative stress accompanies MG by estimation of the several independent parameters of oxidative damage, mainly the levels of oxidative modifications of blood serum proteins. The group studied consisted of 50 MG patients (28 females and 22 males), 24 with ocular MG (OMG) and 26 with generalized MG (GMG), of mean age of 66.7 (30–81) years (y), mean disease duration of 9.5 (0.5–34) y, mean level of Ab of 8.9 (0.1–85) nmol/ml, and 25 age-matched healthy controls. MG patients were stratified into groups according to disease duration (<5 y or >5 y), Ab level (low, <3 or high, >3 nmol/l) as well as symptoms (GMG or OMG).
Glycophore fluorescence was increased in OMG[a]. Dityrosine was increased in both types of MG[c], in patients ill <5[b] and >5[c] y, with low[c] and high[c] levels of Ab. N-formylkynurenine was increased in OMG[a] and GMG[b], in both disease duration groups[a], in the group of low Ab[a]. Kynurenine was increased in the group with high Ab[a]. Tryptophan fluorescence was decreased in OMG[b] and GMG[c], in patients ill for <5[b] and >5[a] y, with low[a] and high[c] Ab. Serum thiol group concentration were decreased in GMG[c], in patients ill for >5 y[b]. AOPP level was elevated in OMG[a], in patients ill for >5 y[a] with high Ab[a]. Protein carbonyls were increased in both OMG[c] and GMG[c], in patients ill for >5[a] y, with low[b] and high[b] Ab. FRAP and ABTS$^{\bullet}$ scavenging by fast antioxidants were unchanged, but ABTS$^{\bullet}$ scavenging by slow antioxidants was lower in OMG[b] and GMG[c], in patients ill for >5[c] y, in patients with low[c] and high[b] Ab ([a]$p < 0.05$, [b]$p < 0.01$, [c]$p < 0.001$). These results demonstrate systemic oxidative stress in MG, suggesting therapeutic use of antioxidants.

1. Introduction

Myasthenia gravis (MG) is an acquired, chronic disease, characterized by rapid fatigue and weakness of skeletal muscles (Grob et al., 2008). MG may affect any age group and shows no geographic predilection. MG has a reported worldwide prevalence of 40–180 per million people, and an annual incidence of 4–12 per million people (Andersen et al., 2010; Carr et al., 2010; Pakzad et al., 2011). The symptoms of MG are mainly mediated by pathogenic antibody directed against the nicotinic acetylcholine receptor (AChR), which occurs in 80–85% of the patients, leading to reduced numbers of AChR molecules at the postsynaptic endplates of the neuromuscular junction (Lindstrom et al., 1976; Vincent, 2012). Apart from AChR, also muscle-specific kinase (MUSK) and lipoprotein-related protein 4 (LRP4) are well established as sensitive and specific diagnostic markers and pathogenic factors, and these autoantibodies are instrumental for subgrouping patients with MG (Gilhus, Verschuuren, 2015). MG is differentiated into two major clinical forms: ocular MG (OMG), in which the patient has predominantly ocular symptoms (*i.e.*, ptosis and/or diplopia only), and generalized MG (GMG), in which the patient develops generalized proximal weakness. The diagnosis of ocular OMG is not always clinically evident, as the pattern of deficits canmimica cranial nerve palsy, internuclear ophthalmoplegia, or thyroid eye disease. Half of patients with OMG have detectable AChR antibodies, whereas MUSK antibodies occur very rarely (Kerty et al., 2014).

Abbreviations: ABTS$^{\bullet}$, 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid free radical; Ach, acetylcholine; AchR, acetylcholine receptor; AOPP, advanced oxidation protein products; FRAP, ferric reducing antioxidant potential; GMG, generalized myasthenia gravis; MG, myasthenia gravis; MGFA, Myasthenia Gravis Foundation of America; MUSK, muscle-specific kinase; OMG, ocular myasthenia gravis; OS, oxidative stress; PBS, phosphate-buffered saline; ROS, reactive oxygen species; S-Alb, serum albumin.

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Epidemiological studies of MG have shown a bimodal pattern of incidence, with early-onset cases (defined as initial symptoms occurring before age 40 years) being predominantly women and late-onset patients being mostly men (Vincent et al., 2001; Sanders et al., 1997; Somnier, 1996). Viral infections, stressful life events, pregnancy as well as delivery may precipitate the development of MG. MG is associated with other autoimmune diseases in about 30% of cases. Even though the number of patients with MG continues to rise, it is still a rare disease (Pekmezović et al., 2006). Advanced age is associated with an increased response to autoantigens, although the implications of the age- and sex-specific frequency distribution of MG regarding pathogenesis remain unclear (Sanders et al., 1997; LeMaoult et al., 1997).

The treatment of MG involves the enhancement of neuromuscular transmission by anticholinesterase drugs (pyridostigmine) as well as by immunotherapy. Rapid improvement can be achieved when necessary by intravenous administration of immunoglobulin or plasma exchange. Intermediate rates of improvement over months involve the use of adrenal corticosteroids, the calcineurin inhibitors cyclosporine or tacrolimus, and in some patients, the B-cell inhibitor rituximab. For long-term treatment, mycophenolate and azathioprine are the most effective agents. Moreover, thymectomy is an effective treatment option for long-term remission of MG (Drachman, 2016). Thymectomy for the treatment of MG is based on several lines of evidence that support a central role of the thymus in the pathogenesis of the disease (Wolfe et al., 2016).

Oxidative stress (prooxidative disturbance in the equilibrium between prooxidants and antioxidants; OS) is observed in the course of many diseases and plays a role in the etiopathogenesis of some of them, such as inflammatory and autoimmune diseases (Brambilla et al., 2008). Although the relationship between antioxidant status and MG has not been fully elucidated, some studies indicate that OS was implicated in the pathogenic mechanism of MG (Yang et al., 2016a, b). It seems that increased generation of reactive oxygen species (ROS) can cause inactivation of AChRs (Krishnaswamy and Cooper, 2012) and lead to serious damage to the cholinergic receptors (Venkatesham et al., 2005).Venkatesham et al. (2005) showed that G-protein-coupled muscarinic receptors are more susceptible to OS than ion-channel-linked nicotinic receptors.

A study by Zhou and Sun (2013) found reduced levels of bilirubin and uric acid in patients with MG, in comparison with the subjects in the reference group. Additionally, it was showed that women with MG have lower level of bilirubin as compared to male patients. Nevertheless, no difference existed when comparing different grades of MG patients according to the modified Osserman classification (Zhou and Sun, 2013).

A recent study by Yang et al. (2016a, b) revealed reduced concentration of bilirubin, albumin, uric acid and creatinine in MG patients as compared to healthy subjects. Moreover, it was also found that albumin, creatinine and uric acid levels in patients with MG were correlated with disease activity and classifications performed by the Myasthenia Gravis Foundation of America (Yang et al., 2016a, b). Reduced level of uric acid, a natural antioxidant and a scavenger of peroxynitrite, in MG patients has been also shown in other studies (Fuhua et al., 2012; Zhou and Sun, 2013). These results suggest that uric acid may be a useful marker for the evaluation of disease progression and disability of MG patients (Yang et al., 2016a, b).

Serum albumin (S-Alb) is a widely used biomarker of nutritional status and disease severity in patients with autoimmune diseases. S-Alb may be associated with the development of MG (Weng et al., 2016). Creatinine, a metabolite of creatine phosphate, an energy store in skeletal muscle, is one of the components contributing to the total antioxidant assays (Nyasavajjala et al., 2015).

It is intriguing whether impaired endogenous antioxidant defense results in OS in MG and if this disease belong to a large group of diseases involving OS. The aim of this study was to examine indices of OS in MG patients stratified into groups according to disease duration (<5 years or >5 years), a serum anti-acetylcholine-receptor–antibody level (<3 or >3 nmol/l) as well as symptoms (GMG or OMG) in an attempt to get insight into this question.

2. Materials and methods

2.1. Ethical permission

The study was approved by the local Ethical Committee of the Medical University of Silesia and informed consent was obtained from each patient prior to entry into the study, according to the declaration of Helsinki.

2.2. Myasthenia gravis patients and study design

A total of 50 patients with MG (26 GMG, age (years) 64.21 ± 12.85, 18 patients after thymectomy; and 24 OMG, age 57.38 ± 11.25, 11 patients after thymectomy) as well as 25 healthy donors, age 60.18 ± 15.69, nonsmokers, were recruited by the outpatient service of Neurology Clinic in Zabrze.

All control patients were screened to be free from any neurological or other major medical illnesses. MG patients were treated with Mestinon® Tablets (pyridostigmine bromide). The usual adult dose was 1/2 to 2 tablets (30 to 120 mg Mestinon® Tablets) taken three to six times daily. Pyridostigmine is a parasympathomimetic reversible cholinesterase inhibitor used for the symptomatic treatment of MG. As a quaternary amine, it is poorly absorbed in the gut and does not cross the blood–brain barrier, except possibly in stressful conditions. Pyridostigmine inhibits acetylcholinesterase in the synaptic cleft, thus slowing down the hydrolysis of acetylcholine (ACh). It is a quaternary carbamate inhibitor of cholinesterase that also does not cross the blood–brain barrier, and which carbamylates about 30% of peripheral cholinesterase enzyme. The carbamylated enzyme eventually regenerates by natural hydrolysis and excess ACh levels revert to normal.

The basic demographic and clinical characteristics of MG patients are summarized in Table 1. The diagnosis of MG was based on standard

Table 1
Demographic characteristics of the patients studied.

Patients (n)	50
Myasthenia form	
Ocular (%)	48
Generalized (%)	52
Mean age [years]	60.66
Age range [years]	30–81
Mean duration of disease [years]	9.48
Range of duration of disease [years]	0.5–34
Gender	
Female (%)	56
Male (%)	44
Antibodies against acetylcholine receptor [nmol/l]	
Mean	8.93
Range	0.1–85
Treatment	
Anticholinesterase drugs (%)	98
Steroids (%)	16
Azathioprine (%)	6
Mean BMI	24.96
BMI range	21.15–32.75
Main neurological classification	
MGFA 0 (%)	22
MGFA 1 (%)	38
MGFA 2a	16
MGFA 2b	4
MGFA 3a	12
MGFA 3b	2
MGFA 4a	4
MGFA 4b	2
MGFA 5	0