Serum uric acid levels in patients with myasthenia gravis are inversely correlated with disability

Dehao Yang[a,*], Yiyun Weng[a,*], Haihua Lin[b], Feiyan Xie[c], Fang Yin[e], Kangliang Lou[e], Xuan Zhou[e], Yixiang Han[d], Xiang Li[a] and Xu Zhang[a]

Uric acid (UA), the final product of purine metabolism, has been reported to be reduced in patients with various neurological disorders and is considered to be a possible indicator for monitoring the disability and progression of multiple sclerosis. However, it remains unclear whether there is a close relationship between UA and myasthenia gravis (MG), or whether UA is primarily deficient or secondarily reduced because of its peroxynitrite scavenging activity. We investigated the correlation between serum UA levels and the clinical characteristics of MG. We assessed 338 serum UA levels obtained in 135 patients with MG, 47 patients with multiple sclerosis, and 156 healthy controls. In addition, we compared serum UA levels when MG patients were stratified according to disease activity and classifications performed by the Myasthenia Gravis Foundation of America, age of onset, duration, and thymus histology (by means of MRI or computed tomography). MG patients had significantly lower serum UA levels than the controls ($P < 0.001$). Moreover, UA levels in patients with MG were inversely correlated with disease activity and disease progression ($P = 0.013$). However, UA levels did not correlate significantly with disease duration, age of onset, and thymus histology. Our findings suggest that serum level of UA was reduced in patients with MG and serum UA might be considered a surrogate biomarker of MG disability and progression. ***NeuroReport*** **27:301–305**

NeuroReport 2016, **27**:301–305

Keywords: disability, myasthenia gravis, uric acid

Departments of [a]Neurology, [b]Gastroenterology, [c]Surgical Oncology, [d]Laboratory of Internal Medicine, the First Affiliated Hospital of Wenzhou Medical University and [e]School of the First Clinical Medical Sciences, Wenzhou Medical University, Wenzhou, China

Correspondence to Xu Zhang, MD, Department of Neurology, the First Affiliated Hospital of Wenzhou Medical University, Wenzhou 325000, China
Tel: + 86 577 555 79372; fax: + 86 577 555 79318; e-mail: drzhangxu@126.com

*Dehao Yang and Yiyun Weng are co-first authors.

Received 22 December 2015 **accepted** 11 January 2016

Introduction

Myasthenia gravis (MG), caused by autoantibodies against the acetylcholine receptor (AChR) on the post-synaptic membrane at the neuromuscular junction, is an acquired autoimmune disease characterized by a defective transmission of nerve impulses to muscles [1]. Accumulating data have implicated oxidative stress in the immunopathogenesis of neuromuscular diseases [2,3].

As the final product of the common pathway of purine metabolism, uric acid (UA) is a naturally occurring anti-oxidant, with metal-chelating properties [4]. Previous studies have reported that UA can scavenge nitrogen radicals and superoxide, thus helping to block the generation of the strong oxidant peroxynitrite [5]. Peroxynitrite exerts toxic effects and irreversibly jeopardizes cellular metabolism and cell structures, including lipids, carbohydrates, protein, and DNAs [6]. Several studies have identified a therapeutic role of UA in experimental allergic encephalomyelitis and a beneficial function for increasing serum UA levels in multiple sclerosis (MS) patients [7,8]. Furthermore, UA might be a surrogate marker for monitoring MS activity [9].

Therefore, the aim of this study was to investigate whether the serum UA levels were decreased in MG patients and whether the decrease was associated with disease disability and progression.

Patients and methods

Serum samples were collected from 338 individuals: 135 patients with MG, 47 patients with MS, and 156 healthy controls (CTL). Venous blood was drawn from an antecubital vein in the morning after an overnight fast to measure the concentration of serum UA using a Clinical Analyzer Beckman Coulter AU5831 (Beckman Coulter, Brea, California, USA). In our hospital, the normal range of serum UA values is 208–428 μM for men and 155–357 μM for women. Simultaneously, concentrations of glutamate–pyruvate transaminase (normal range: 9–50 μM for men, 7–40 μM for women), glutamic–oxaloacetic transaminase (normal range: 15–40 μM, 13–35 μM for women), blood fasting sugar, and blood urea nitrogen were also measured using an enzymatic method on the same analyzer.

 DOI: 10.1097/WNR.0000000000000535

Table 1 MGFA clinical classification

Class I	Any ocular muscle weakness May have weakness of eye closure All other muscle strength is normal
Class II	Mild weakness affecting other than ocular muscles May also have ocular muscle weakness of any severity
IIa	Predominantly affecting limb, axial muscles, or both May also have lesser involvement of oropharyngeal muscles
IIb	Predominantly affecting oropharyngeal, respiratory muscles, or both May also have lesser or equal involvement of limb, axial muscles, or both
Class III	Moderate weakness affecting other than ocular muscles May also have ocular muscle weakness of any severity
IIIa	Predominantly affecting limb, axial muscles, or both May also have lesser involvement of oropharyngeal muscles
IIIb	Predominantly affecting oropharyngeal, respiratory muscles, or both May also have lesser or equal involvement of limb, axial muscles, or both
Class IV	Severe weakness affecting other than ocular muscles May also have ocular muscle weakness of any severity
IVa	Predominantly affecting limb and/or axial muscles May also have lesser involvement of oropharyngeal muscles
IVb	Predominantly affecting oropharyngeal, respiratory muscles, or both May also have lesser or equal involvement of limb, axial muscles, or both
Class V	Defined by intubation, with or without mechanical ventilation, except when used during routine postoperative management. The use of a feeding tube without intubation places the patient in class IVb

MG, myasthenia gravis; MGFA, Myasthenia Gravis Foundation of America.

All patients in hospital care had definite MG according to the standard clinical criteria of Drachman [10]. The severity of the disease (Table 1) was estimated according to the Myasthenia Gravis Foundation of America (MGFA) clinical classification at the time of blood sampling [11]. The exclusion criteria were liver disease or abnormal ranges of glutamate–pyruvate transaminase and glutamic–oxaloacetic transaminase concentrations, as well as patients with a history of gout, diabetes, and renal failure.

The statistical software statistical program for social sciences (version 20.0; SPSS Inc., Chicago, Illinois, USA) was used for all analyses. Data in this study were expressed as the mean ± SD values. All statistical tests were two sided and *P*-values less than 0.05 were considered statistically significant. The comparisons of UA concentrations among patients with MG, patients with MS, and control participants were performed using covariance analysis with age as a covariant. Because previous evidence has shown the effect on the serum UA concentrations by sex, patients in each group were divided into two subgroups on the basis of their sex and analyzed with age as a covariant. The effect of age on serum UA levels of different subgroups of patients with MG graded according to the MGFA Clinical Classification was analyzed by covariance analysis. The levels of UA were compared when patients were stratified according to age of onset, disease duration, MGFA clinical classification, and thymus histology. Significance was calculated using covariance analysis with age as the covariant.

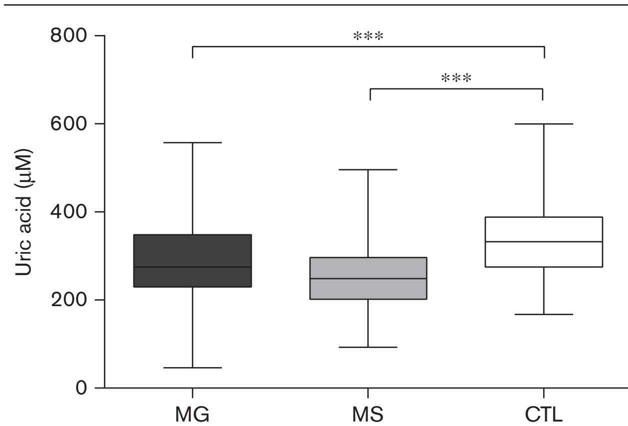
Results

The basic demographic and clinical characteristics of patients with MG, patients with MS, and healthy controls are shown in Table 1. In total, 338 individuals provided samples. These included 135 MG patients (57 men, 78 women), 47 MS patients (13 men, 34 women), and 156 controls (CTL, 69 men, 87 women), who did not differ significantly in age (MG 41.6 ± 15.8, MS 41.5 ± 10.5, CTL 42.9 ± 14.3; $P = 0.684$).

In the present study, the mean serum UA level in all participants was 304 ± 92 μM. The serum UA level was significantly lower in the MG patients than in the healthy controls (283 ± 90 vs. 335 ± 84 μM; $P < 0.001$). However, no difference was found between MG and MS (283 ± 90 vs. 257 ± 85 μM; $P = 0.072$) (Fig. 1). Previous evidence has shown that serum UA levels are significantly lower in women than in men [12]. Accordingly, we divided each cohort into men and women to eliminate the possibility that the differences that we observed were simply because of the different numbers of men and women in the disease groups. Interestingly, in all groups, the serum UA level in women was significantly lower than that in men ($P < 0.001$) (Table 2 and Fig. 2). Compared with CTL patients, MG patients had lower serum UA level, whether male or female. However, there was no statistical difference in UA levels between patients with MG and MS, whether in male or in female subgroups ($P = 0.641$ and 0.204, respectively) (Table 2 and Fig. 2).

The correlations between UA levels in MG patients, with their disease duration, age of onset, MGFA clinical classification, and thymus histology, are presented in Table 3. In MG, the UA level was not lower in patients with late-onset MG (age at onset ≥ 50 years) than early-

Fig. 1



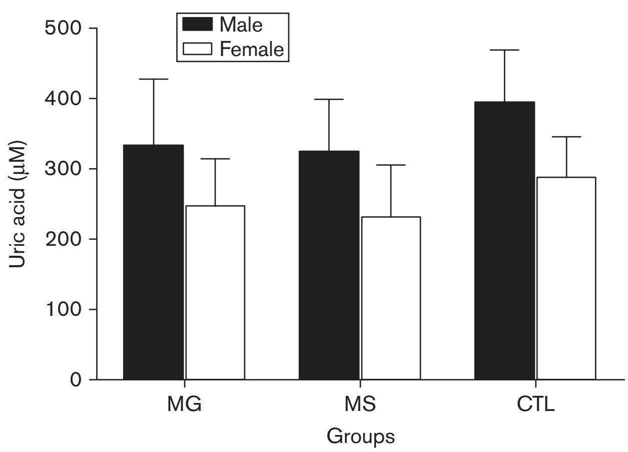
Serum UA levels in patients with MG, MS, and CTL. UA levels measured in MG patients were significantly lower than those in CTL, but were not different from those measured in MS patients. CTL, healthy control group; MG, myasthenia gravis; MS, multiple sclerosis; UA, uric acid. ***$P < 0.001$.

Table 2 Serum UA levels in male and female patients and healthy controls

Patients	Male (μM)	Female (μM)	P_1	P_2	P_3
MG	333 ± 95	247 ± 67	< 0.001		
MS	324 ± 75	231 ± 74	< 0.001	0.641	0.204
CTL	395 ± 74	288 ± 57	< 0.001	< 0.001	< 0.001

CTL, healthy control group; MG, myasthenia gravis; MS, multiple sclerosis; UA, uric acid.
P_1 = male versus female in each group.
P_2 = male patients with multiple sclerosis or the healthy control group versus male patients with myasthenia gravis.
P_3 = female patients with multiple sclerosis or the healthy control group versus female patients with myasthenia gravis.

Fig. 2



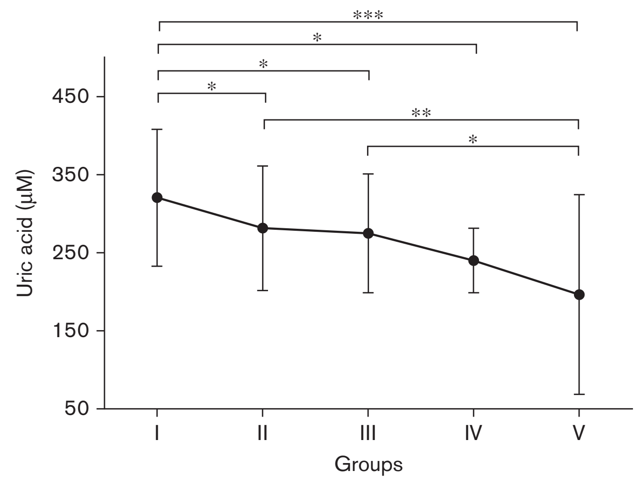
Serum UA levels in male and female patients with MG, MS, and CTL. UA levels measured in male or female MG patients were significantly lower than those in CTL, but were not different from those measured in MS patients. CTL, healthy control group; MG, myasthenia gravis; MS, multiple sclerosis; UA, uric acid.

Table 3 Serum UA levels in patients with MG

Variables	Mean	SD	Range	P
Age of onset (years)				0.664
< 50 (*n* = 92)	280	94	46–500	
≥ 50 (*n* = 43)	291	82	118–558	
Duration of disease (years)				0.261
≤ 1 (*n* = 101)	290	87	46–558	
> 1 (*n* = 34)	264	97	87–469	
MGFA				0.013
I (*n* = 43)	321	88	151–558	
II (*n* = 48)	282	80	118–436	
III (*n* = 25)	275	76	153–500	
IV (*n* = 8)	240	41	193–319	
V (*n* = 11)	197	128	46–469	
MGFA				0.707
IIa, IIIa, IVa (*n* = 34)	285	74	162–500	
IIb, IIIb, IVb (*n* = 47)	269	77	118–426	
Thymus histology				0.867
Nonthymoma (*n* = 83)	284	90	118–558	
Thymoma (*n* = 52)	282	92	46–500	

Correlation of serum UA levels with age of onset, disease duration, MGFA clinical classification, and thymus histology.
MG, myasthenia gravis; MGFA, Myasthenia Gravis Foundation of America; UA, uric acid.

Fig. 3



Serum UA levels in five subgroups according to the MGFA clinical classification. MGFA, Myasthenia Gravis Foundation of America; UA, uric acid. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

onset MG (age at onset < 50 years) ($P = 0.664$) (Table 3). There was no significant difference ($P = 0.261$) in UA levels between patients with longer disease duration (>1 year) and those with short disease duration (≤ 1 year), although the former was somewhat lower than the latter (Table 3). The serum UA level was not lower in patients with thymoma shown on MRI or computed tomography than in patients without thymoma ($P = 0.867$). However, we found that the relative decrease in the mean serum UA level of the patients with MG correlated inversely with the degree of disease progression, as expressed by the MGFA clinical classification (Table 3 and Fig. 3). On grouping MGFA IIa, IIIa, and IVa categories (absence of bulbar involvement) and comparing them with IIb, IIIb, and IVb categories (presence of bulbar involvement), no significant difference was observed between bulbar independent and involved bulbar MG patient groups (Table 3).

Discussion

MG is a severe autoimmune disease characterized by its tendency to selectively affect AChR of the postsynaptic membrane, which is associated with B and T cell activation [13]. The role of oxidative stress in MG has not been fully studied. Reactive oxygen species play a role in injuring the body's cells and tissues through multiple pathways, including direct damage to the biological structures, such as cell membrane, genetic material, and enzymes, and indirectly stimulating the expression of genes associated with apoptosis. Furthermore, accumulating evidence has shown that oxidative stress contributes toward the pathogenesis in inflammatory and autoimmune-mediated tissue destruction [14]. Skeletal muscle is strongly dependent on oxidative metabolism, and corresponding antioxidant protection mechanisms are present. Venkatesham *et al.* [3] have shown that reactive oxygen species might contribute toward damage to the AChR. Moreover, Krishnaswamy and Cooper [15] has suggested that the highly conserved cysteine residues in nicotinic AChR are the major targets of reactive oxygen species producing receptor dysfunction. Highly conserved cysteine residue, a component of several nicotinic AChRs on neurons, locates near the intracellular mouth of the receptor pore and enables ganglionic transmission and sympathetic reflexes to function normally.

UA, a naturally occurring product of purine metabolism, is an important natural antioxidant that could scavenge superoxides and reduce oxidative stress [16]. Numerous studies have shown that there is a reduced serum UA level in inflammatory and autoimmune diseases [17–19]. Previous studies in MS showed that UA may be a surrogate marker of MS activity and it is one of the antioxidants evaluated for their effect on MS disease progression [20]. Moreover, UA treatment has been proven to prevent inflammation and destruction of central nervous tissue and ameliorate established disease in the animal models of MS [21].

In this study, MG patients presented significantly lower serum UA than healthy controls. However, the difference in serum UA between MG patients and MS patients was not significant. Furthermore, similar results were also observed when the female and male cohorts were investigated separately. A relatively high proportion of patients with MG, in our hospital, had serum UA levels below the lower limit of the normal range, which was similar to the previous report [22]. However, the correlation between the serum UA and the clinical characteristics of MG was not investigated in the previous study. To better clarify the underlying mechanisms of antioxidant status in MG, patients were divided into five subgroups according to the MGFA clinical classification. Patients with clinically active disease had significantly lower UA levels than those with clinically inactive disease. However, comparison of UA levels in patients stratified according to MG disease duration, age of onset, and thymus histology showed no significant difference. Nevertheless, it was also uncertain whether a low concentration of UA was a cause or a consequence of MG progression and activity. Further studies are necessary to clarify the role of UA and its underlying mechanisms in patients with MG. Nonetheless, patients with MG were unable to protect neuromuscular junction against oxidative stress with low antioxidant status.

Conclusion

Our findings of a significant inverse correlation between serum UA level and disease activity and disability, as assessed by the MGFA clinical classification, indicate that serum UA might serve as a possible biomarker of disease disability in MG. To our knowledge, the present study is the first description of an inverse correlation of serum UA level with MG disability as assessed by the MGFA clinical classification. Confirmation of these conclusions with a much larger sample will be an important next step.

Acknowledgements

The present study was supported by the Natural Science Foundation of Zhejiang Province (No. LY13H090010) and the Wenzhou Municipal Sci-Tech Bureau Program (No. Y20140278).

Conflicts of interest

There are no conflicts of interest.

References

1 Meriggioli MN, Sanders DB. Autoimmune myasthenia gravis: emerging clinical and biological heterogeneity. *Lancet Neurol* 2009; **8**:475–490.
2 Stuerenburg HJ. The roles of carnosine in aging of skeletal muscle and in neuromuscular diseases. *Biochemistry (Mosc)* 2000; **65**: 862–865.
3 Venkatesham A, Sharath Babu P, Vidya Sagar J, Krishna D. Effect of reactive oxygen species on cholinergic receptor function. *Indian J Pharmacol* 2005; **6**:366–370.
4 Davies KJ, Sevanian A, Muakkassah-Kelly SF, Hochstein P. Uric acid–iron ion complexes. A new aspect of the antioxidant functions of uric acid. *Biochem J* 1986; **235**:747–754.
5 Whiteman M, Ketsawatsakul U, Halliwell B. A reassessment of the peroxynitrite scavenging activity of uric acid. *Ann N Y Acad Sci* 2002; **962**:242–259.
6 Chen W, Feng L, Huang Z, Su H. Hispidin produced from *Phellinus linteus* protects against peroxynitrite-mediated DNA damage and hydroxyl radical generation. *Chem Biol Interact* 2012; **199**:137–142.
7 Hooper DC, Spitsin S, Kean RB, Champion JM, Dickson GM, Chaudhry I, Koprowski H. Uric acid, a natural scavenger of peroxynitrite, in experimental allergic encephalomyelitis and multiple sclerosis. *Proc Natl Acad Sci USA* 1998; **95**:675–680.
8 Koprowski H, Spitsin SV, Hooper DC. Prospects for the treatment of multiple sclerosis by raising serum levels of uric acid, a scavenger of peroxynitrite. *Ann Neurol* 2001; **49**:139.
9 Miller A, Glass-Marmor L, Abraham M, Grossman I, Shapiro S, Galboiz Y. Bio-markers of disease activity and response to therapy in multiple sclerosis. *Clin Neurol Neurosurg* 2004; **106**:249–254.
10 Drachman DB. Myasthenia gravis. *N Engl J Med* 1994; **330**: 1797–1810.
11 Jaretzki A 3rd, Barohn RJ, Ernstoff RM, Kaminski HJ, Keesey JC, Penn AS, *et al.* Myasthenia gravis: recommendations for clinical research standards. Task Force of the Medical Scientific Advisory Board of the Myasthenia Gravis Foundation of America. *Ann Thorac Surg* 2000; **70**:327–334.
12 Zoccolella S, Tortorella C, Iaffaldano P, Direnzo V, D'Onghia M, Luciannatelli E, *et al.* Low serum urate levels are associated to female gender in multiple sclerosis patients. *PLoS One* 2012; **7**:e40608.
13 Luo J, Lindstrom J. AChR-specific immunosuppressive therapy of myasthenia gravis. *Biochem Pharmacol* 2015; **97**:609–619.
14 Park H, Bourla AB, Kastner DL, Colbert RA, Siegel RM. Lighting the fires within: the cell biology of autoinflammatory diseases. *Nat Rev Immunol* 2012; **12**:570–580.
15 Krishnaswamy A, Cooper E. Reactive oxygen species inactivate neuronal nicotinic acetylcholine receptors through a highly conserved cysteine near the intracellular mouth of the channel: implications for diseases that involve oxidative stress. *J Physiol* 2012; **590** (Pt 1):39–47.
16 Glantzounis GK, Tsimoyiannis EC, Kappas AM, Galaris DA. Uric acid and oxidative stress. *Curr Pharm Des* 2005; **11**:4145–4151.
17 Moccia M, Lanzillo R, Costabile T, Russo C, Carotenuto A, Sasso G, *et al.* Uric acid in relapsing-remitting multiple sclerosis: a 2-year longitudinal study. *J Neurol* 2015; **262**:961–967.

18 Oh SI, Baek S, Park JS, Piao L, Oh KW, Kim SH. Prognostic role of serum levels of uric acid in amyotrophic lateral sclerosis. *J Clin Neurol* 2015; **11**:376–382.
19 Peng F, Zhong X, Deng X, Qiu W, Wu A, Long Y, *et al*. Serum uric acid levels and neuromyelitis optica. *J Neurol* 2010; **257**:1021–1026.
20 von Geldern G, Mowry EM. The influence of nutritional factors on the prognosis of multiple sclerosis. *Nat Rev Neurol* 2012; **8**:678–689.
21 Hooper DC, Scott GS, Zborek A, Mikheeva T, Kean RB, Koprowski H, Spitsin SV. Uric acid, a peroxynitrite scavenger, inhibits CNS inflammation, blood–CNS barrier permeability changes, and tissue damage in a mouse model of multiple sclerosis. *FASEB J* 2000; **14**:691–698.
22 Fuhua P, Xuhui D, Zhiyang Z, Ying J, Yu Y, Feng T, *et al*. Antioxidant status of bilirubin and uric acid in patients with myasthenia gravis. *Neuroimmunomodulation* 2012; **19**:43–49.