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Serum uric acid and multiple sclerosis

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Abstract Several studies indicate that patients with multiple sclerosis (MS) have low serum levels of the endogenous antioxidant uric acid (UA), although it has not been established whether UA is primarily deficient or secondarily reduced due to its peroxynitrite scavenging activity. We measured serum urate levels in 124 MS patients and 124 age- and sex-matched controls with other neurological diseases. In addition, we compared UA levels when MS patients were stratified according to disease activity (by means of clinical examination and MRI), duration, disability and course. MS patients had significantly lower serum urate levels than controls (p= 0.001). However, UA levels did not significantly correlate with disease activity, duration, disability or course. Our study favors the view that reduced UA in MS is a primary, constitutive loss of protection against oxidative agents, which deserves further pathogenetic elucidation aimed at future therapeutic strategies.

Key words Multiple sclerosis • Uric acid • Nitric oxide • Peroxynitrite • Pathogenesis

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Introduction

In rodent experimental autoimmune encephalomyelitis (EAE), the production of the free radical nitric oxide (NO) strongly correlated with the clinical severity of the disease [1]. The combination of NO with superoxide (O_2^-) during an inflammatory response leads to the formation of peroxynitrite (PN). By virtue of its potent oxidant activity, PN is believed to be responsible for the majority of damage to the central nervous system (CNS) attributed to NO [2].

Since observations on EAE may apply to multiple sclerosis (MS) one could expect that levels of uric acid (UA), a natural scavenger of PN able of penetrate and preserve the blood-brain barrier integrity [3], may be abnormal in MS patients. Regardless of clinical status, MS patients have significantly lower serum UA levels than controls [4, 5]; these studies demonstrated a protective role of UA in EAE, and showed a beneficial function of raising serum UA levels in MS patients [4, 5]. Since gout prevalence in MS is 15-times less than that estimated for the general population [1], inadequate protection against PN-mediated CNS toxicity may be a primary, constitutive MS-related defect. One study indicated that UA reduction is strictly linked to clinical and MRI activity in MS patients [6]. It is not known whether UA reduction is primarily linked to MS or simply represents an epiphenomenon related to its PN scavenging activity, or both.

In the light of such considerations, we conducted a hospitalized-based study aimed at determining possible differences in serum UA levels in relation to MS clinical status.

Patients and methods

Patients and controls

All subjects gave informed consent prior to their inclusion in the study.

A total of 124 in-patients (39 men) diagnosed as having probable or definite MS according to the criteria of Poser et al. [7] were randomly selected from the in-patient register of our Neurology

Clinic. Reasons for hospitalization were diagnostic purposes in patients with stable disease and therapeutic purposes in patients with clinically active disease (defined as the development within the previous two weeks of new neurological symptoms or signs attributable to demyelination). In a subgroup of 21 patients, 13 with active clinical disease and 8 with inactive disease, standard magnetic resonance imaging (MRI) of the brain (1.5 T) with T1-weighted sequences 15 minutes after injection of Gd-DTPA, was performed in the period from 2 days prior to serum UA determination to 2 days after.

As control population, we recruited 124 age- and sex- matched in-patients with other neurological diseases (OND) Clinical diagnosis was: syncope (19 patients), radiculopathy (16), trauma (14), neurosis (13), tumor (11), polyneuropathy (11), vertigo (13), myasthenia gravis (9), seizure (8), stroke (5), and myopathy (5).

Exclusion criteria for MS and OND patients were treatment with drugs that could increase serum levels of uric acid (e.g. steroids, acetylsalicylic acid and thiazide-type diuretics [8]) or reduce them (e.g. ibuprofen) [9]. Clinical or laboratory evidence of diabetes mellitus and renal failure were also ruled out in both groups. About 6% of controls had seizures. In general, increased urate in the postictal period may be observed and several drugs used in epilepsy may change levels of urate (e.g. carbamazepine and valproic acid may decrease whereas primidone may increase UA) [10]. Epileptic patients included in the study were not in the postictal period.

Blood samples were obtained before breakfast 5 days after patient's first hospitalization. Serum UA levels were determined by blinded technicians at the laboratory of Civic Hospital in Sassari, using a commercially available kit and following the manufacturer's instructions (Olympus Diagnostica, Hamburg, Germany). Normal ranges of UA, according to the Civic Hospital laboratory standardization, were 3.4–7.0 mg/dl. The levels of UA were compared when patients were stratified according to disability (scored on Kurtzke's expanded disability status scale, EDSS), disease duration, clinical activity, disease course and activity on MR images. Significance was calculated using the *t*-test and p value set at <0.05.

Results

The mean UA level in MS patients was significantly lower than that in OND patients (Table 1). Mean urate levels were higher in men than in women in both groups. Among MS patients, urate levels were lower in patients with long duration (>1 year), more disabling disease (EDSS>3.5), active disease and secondary progressive course, although these differences were not statistically significant (Table 2).

Table 1 Serum uric acid (UA) levels in hospitalized patients with multiple sclerosis (MS) and in matched control patients with other neurological diseases (OND)

		n	Age, years[a]	UA, mg/dl[b]	*t*-test
Female	MS	85	33.3 (16-61)	3.8 (1.1)	
	OND	84	33.5 (17-62)	4.2 (1.1)	p=0.0004
Male	MS	39	32.1 (18-50)	4.7 (1.3)	
	OND	40	33.3 (19-56)	6.1 (1.9)	p=0.03
Total	MS	124	33.1 (16-61)	4.1 (1.1)	
	OND	124	33.5 (17-62)	4.7 (1.7)	p=0.001

[a]Values are mean (range)
[b]Values are mean (SD)

Table 2 Serum uric acid (UA) levels in 124 patients with multiple sclerosis (MS). Values of UA are mean (SD). No differences between groups are significant

	Patients, n	UA, mg/dl
Disease duration		
<1 year	41	4.1 (1.6)
≥1 year	83	4.1 (1.0)
Disease course		
Relapsing-remitting	79	4.1 (1.3)
Secondary progressive	45	3.9 (1.1)
Disability score		
EDSS <3.5	81	4.1 (1.3)
EDSS ≥ 3.5	43	4.0 (1.2)
Clinical activity		
Active	68	3.9 (1.2)
Inactive	56	4.1 (1.3)
Activity on MR images[a]		
Active	13	4.0 (1.5)
Inactive	8	4.1 (0.7)

EDSS, expanded disability status scale; *MR*, magnetic resonance
[a]In 21 patients

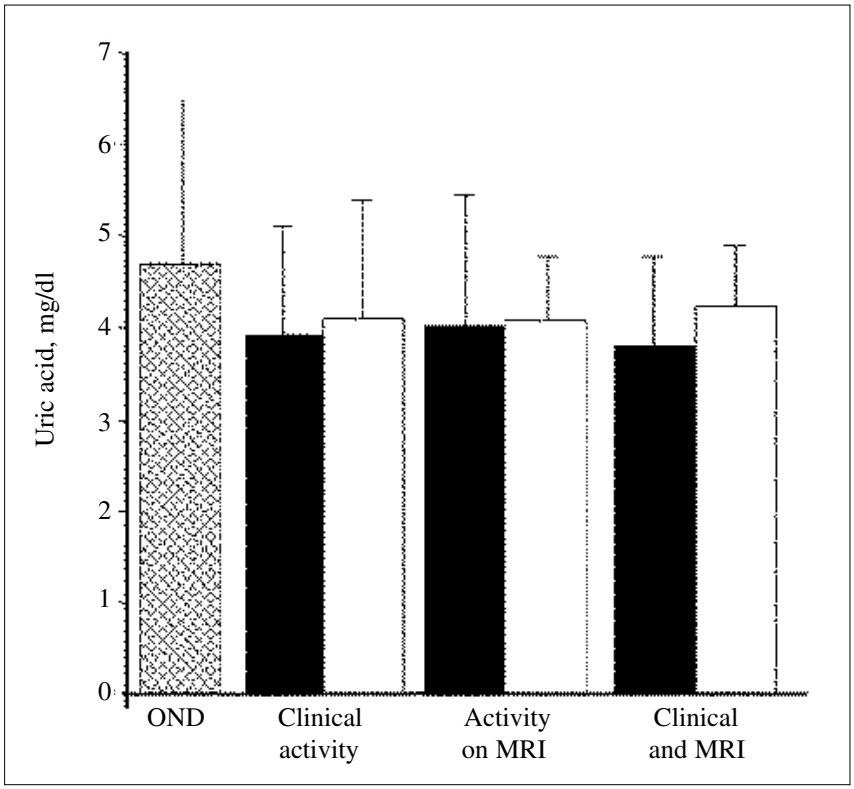

Fig. 1 Serum levels of uric acid (UA) in 124 patients with multiple sclerosis (MS) and in 124 matched subjects with other neurological diseases (OND). *Black bars*, active disease; *white bars*, inactive disease

In the subgroup of 21 patients who underwent brain MRI, 13 (62%) patients, including 9 with clinically active disease and 4 with inactive disease, had one or more Gd-DTPA-enhancing lesions. The highest mean UA level (4.2 mg/dl) was found in the four clinically and MRI inactive patients whereas the lowest (3.8 mg/dl) was observed in the 9 clinically and MRI active ones (Fig. 1). However, these differences were not significant.

Discussion

Among the toxic effects of PN on CNS tissues [11], lipid peroxidation [12], release of oxygen species and tyrosine nitration [13–15], and DNA strand breakage or mutation virtually leading to apoptosis of resident cells [13,16] are possibly the most relevant ones. A role of PN in the pathogenesis of MS is sustained by the detection of nitrotyrosinase residues on cell debris in MS plaques [16], anti-nitrocysteine antibodies in MS sera [17], lipid peroxidation [18] and oxidized lipids and proteins found by means of MS lesion microspectroscopy [19]. However, myelin vacuolation and primary acute axonopathy are found in mice as a UA-treatable intrathecal PN effect, even in the absence of inflammation [20].

High levels of NO and metabolites (nitrite and nitrate) are found in the cerebrospinal fluid (CSF) of MS patients during an exacerbation [21, 22] despite some controversies [23]. Urinary nitrite and nitrate levels are increased in early and relapsing-remitting MS but not in patients with progressive disease, thus confirming a participation of NO in modulating tissue damage [24] and in amplifying the effector stage of the immune attack against myelin [25, 26].

Induction of NO synthase (NOS) in CNS is commonly associated with cells of the monocyte lineage [27, 28] and with dendritic cells [29]. Interferon (IFN)-γ is a major contributor to NOS induction [30], while IFN-γ showed inhibitory effects on the IFN-β-induced NOS expression in astrocytes [31]. When treated with the NOS inhibitors aminoguanidine [32] and lovastatin [33] and with an antisense oligodeoxynucleotide complementary to NOS [34], mice failed to develop EAE. Nonetheless, there is also evidence favoring the view that NO has a protective role in the CNS: (i) NOS knock-out mice had a greater incidence of EAE and higher clinical score than wild-type mice [35], (ii) locally activated dendritic cell-derived NO promotes apoptosis of auto-reactive T cells in EAE, therefore acting as a protective factor during the course of a T cell-mediated immune attack [29] and (iii) macrophages excrete large amounts of UA during active phagocytosis [36]. Additionally, a recent study suggested that specific inhibition of NO production in vivo rendered glucocorticoid receptor knock-out mice susceptible to EAE [37], thus indicating a complex, still debated contribution of NO in CNS tissue damage or repair. Finally, a genetic study in Swedish multiplex MS families clearly excluded NOS as a MS susceptibility gene region in this population [38].

Increased levels of UA in the spinal cord of rats during active EAE have been reported [39], and UA was increased within the MS plaque, reduced in adjacent white matter and even lowered in distant white matter [40]. Consistently, healthy monozygotic twins discordant for MS have higher serum levels of UA than the affected twin [41]. However, though the number of relapses showed a decremental rate, UA serum levels monitored during the course of IFN-β treatment for MS did not change over time [42].

Serial observations [1, 4, 5] indicated that low UA levels are possibly a primary feature of MS patients. Instead, another study suggested that UA levels serve as a marker of MS activity, being inversely correlated with disease activity and duration [6]. In the present study, we obtained confirmatory evidence for an association of low serum UA levels with MS, even though some control neurological diseases (such as epilepsy and stroke) were potentially related to hyperuricemia. Drulovic and colleagues [6] found confirmatory evidence that patients with active relapsing-remitting and secondary-progressive forms of MS have significantly lower UA levels than controls. On the other hand, in the overall group (including MS patients with primary progressive and clinically inactive forms of MS) serum UA levels did not differ significantly from those of controls, although a strong tendency was evident (p=0.068). It may be observed that the two study populations (MS and OND) of Drulovic et al. [6] differed in terms of numerosity (240 and 104, respectively) and that about 30% of their control patients had seizures. Since carbamazepine and valproic acid can decrease serum UA levels [10], they may possibly have lowered the mean UA level in the OND population.

In our study, comparison of UA levels in patients stratified according to MS course and disability showed no significant difference. This observation may favor the view that, although a concomitant disease activity-related effect is present, low UA levels might represent a primary "MS-specific" deficiency. In fact, despite the fact that the UA level was lower in MRI- and clinically-documented active patients, therefore partially confirming Drulovic et al's [6] results, a general mean UA decrease is evident also in clinically and MRI inactive MS patients as compared to OND.

Therefore, the question whether reduced UA level in MS is a primary deficit or an epiphenomenon related to its oxidation by PN and free radicals remains open, although the two alternative hypotheses are not mutually exclusive and concomitance of either facts is likely. In the latest metabolic stage, UA is transformed into allantoin which does not have PN scavenging activity. In other pathological conditions, such as myocardial infarction [43] and chronic lung disease of preterm infants [44] or during muscle metabolic stress induced by intense exercise [45], serum allantoin is a useful early predictor of the subsequent free radicals generation. To our knowledge, no study has assessed allantoin levels in MS patients so as to determine whether UA is primarily deficient or secondarily reduced by virtue of its protective role against oxidant compounds. Investigations aimed at determining such an in vivo surrogate marker of free radical production in MS are clinically relevant. In fact, although the therapeutic value of raising UA levels in already established MS has been indicated [5, 46, 47], further pathogenetic investigations are needed so as to propose antioxidant prevention treatments in individuals at high-risk of developing MS (e.g. unaffected monozygotic twins, first-degree relatives of MS patients and individuals residing in areas at high risk for MS) and before MS clinical appearance.

Sommario *Numerosi lavori indicano come nei pazienti con sclerosi multipla (SM) vi siano ridotti livelli sierici dell'antiossidante endogeno acido urico (AU), sebbene non sia chiaramente stabilito se l'AU sia primariamente deficitario o secondariamente ridotto in seguito all'attività di scavenger del perossinitrito. Il nostro studio è volto a fornire evidenze in favore dell'una o l'altra ipotesi. Sono stati comparati i livelli medi ematici di AU da 124 pazienti con SM e 124 controlli neurologici appaiati. Inoltre, i livelli di AU dei pazienti con SM sono stati stratificati in base alle principali caratteristiche cliniche quali l'attività di malattia (clinica e di RM), la durata, la disabilità e il tipo di decorso. I dati indicano che i pazienti con SM presentano livelli ematici di AU significativamente ridotti rispetto a controlli con altre patologie neurologiche (p=0.001). Tuttavia, i livelli di AU non correlano significativamente con il grado di attività, la durata, la disabilità, né col tipo di decorso della malattia. I risultati del nostro studio sembrano favorire l'ipotesi che la riduzione dell'AU nella SM costituisca un deficit primario di protezione contro gli agenti ossidanti. Tale ipotesi necessita di ulteriori conferme e delucidazioni al fine di intraprendere possibili nuove strategie terapeutiche.*

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