Role of Uric Acid in Multiple Sclerosis

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Abstract In the past decade, a growing number of evidence has implicated free radicals in a variety of pathophysiological conditions including aging, cancer, and coronary heart disease. Analyses of different aspects of multiple sclerosis (MS) pathology with respect to oxidative damage have also revealed evidence of free radical injury to the central nervous system (CNS), although attempts to protect the CNS using various antioxidants have met with only moderate success. Several recent studies have reported lower levels of uric acid (UA), a major scavenger of reactive nitrogen species, in MS patients, while other studies found no such correlation. Here, we discuss these studies as well as current efforts to manipulate serum UA levels in MS patients.

1 Uric Acid in Evolution

The fate of uric acid (UA) in vertebrate evolution is a remarkable story in itself. The appearance of UA as an end product of purine metabolism dates back to the period when the first vertebrates emerged from an aquatic to an oxygen-rich environment [27]. In an atmosphere containing 30%–35% oxygen, early amphibians and reptiles underwent much higher oxidative stresses than did aquatic animals [9]. Loss of several enzymes responsible for sequential degradation of UA led to an

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accumulation of this powerful radical scavenger in the first terrestrial vertebrates. For about 200 million years, UA persisted as the end product of purine metabolism in reptiles. Mammals, however, returned to an earlier pathway and began converting UA into allantoin [27], a more soluble substance but without radical scavenging properties. For small, relatively short-lived early mammals, the advantages of easily excretable allantoin outweighed the benefits of accruing the poorly soluble antioxidant UA; however, with increased lifespan and the development of a more complex CNS, the need for better protection against free radicals became apparent. Between 25 and 15 million years ago, serum levels of UA increased tenfold in hominoids due to a nonsense mutation in codon 33 of the uricase (urate oxidase) gene [84, 85]. The enzyme uricase degrades UA to allantoin and is active in most mammals. The codon 33 mutation is shared among humans, chimpanzees, gorillas, and orangutans. Interestingly, independent evolutionary events led to the inactivation of the same gene in some other primates. A 13-base pair deletion in exon 2 accounts for inactivated uricase in the gibbon lineage, while other not fully characterized events, most likely mutations in a promoter region, result in reduced uricase activity in New World monkeys and, to a lesser extent, in Old World monkeys [58]. These findings suggest the presence of a common evolutionary pressure that impairs uricase activity, thereby selecting for retention of UA in most primates.

Evolutionary selection for UA occurred, even though it predisposed the organism to gout and kidney stones and probably also required some adjustments in the secretory pathways of early primates. Homozygous uricase-knockout mice, but not their heterozygous counterparts, die several weeks after birth since their kidneys cannot handle large loads of UA [83]. Perhaps the original mutation of uricase in early primates was also lethal when homozygous. Nevertheless, mutation was selected in evolution along with some adjustments to purine metabolism. Resulting serum UA levels, between 210 and 450 μM (3.5–8.0 $mgdl^{-1}$), are higher than any other natural antioxidants. However, they are dangerously close to saturation, which can be as low as 8–10 $mgdl^{-1}$ depending on minor variations in physiological conditions.

Another unsolved puzzle is the gain of UA in hominids during a similar time frame when the ability to synthesize another important radical scavenger, ascorbic acid, was lost [9]. Although hominids still depend on a dietary supply of vitamin C, normal serum levels of UA are four- to sixfold greater than those of ascorbic acid, raising the possibility that UA is partially replaced vitamin C as an antioxidant in higher primates.

Of note, there are alternative views on the role of UA in primate evolution; for example, UA may have helped to maintain blood pressure in low-salt dietary conditions encountered by early hominids during the Miocene epoch [79]. According to this hypothesis, UA merely contributes to the epidemic of cardiovascular disease and hypertension that plagues modern humans on high-salt diets. However, this does not address the question of why primates, rather than grazing animals, would be the target of pressure to adapt to low-salt conditions. Although this hypothesis is not currently in the mainstream, certain negative actions of UA cannot be disregarded. Indeed, some evidence indicates a pathogenic role for high UA levels in the development of hypertension, vascular disease, and renal disease in humans [38].

Animals other than primates demonstrate the importance of UA as a radical scavenger. Birds generally have a longer lifespan than mammals of comparable body size, body temperature, and metabolic rate [31]; they inherited UA as purine metabolic end product from reptiles and have high serum UA levels. Insects also have high levels of UA, and a potential role for UA in insect free-radical defense has been suggested based on the observation that urate-null mutants of *Drosophila melanogaster* are more susceptible to various aspects of oxidative stress than are the wild type [33]. Another study linked aging in *Drosophila* with the ability to produce UA [55].

2 Uric Acid and Free Radicals

UA, or urate at physiological conditions, is a powerful and selective antioxidant [1, 4, 80]. It is highly reactive with hydroxyl radicals and hypochlorous acid. Urate interferes minimally with nitric oxide ($NO^{\cdot}$), a free radical with low toxicity but with important physiological functions. Urate is an efficient scavenger of several reactive nitrogen species that form in vitro and in vivo after reaction of NO with other radicals and biological molecules [8, 81]. Free radical interactions in living organisms are extremely complex. Originally UA was described as a singlet oxygen and hydroxyl radical scavenger [1, 4]. Later, when the importance of the chemistry of NO and reactive nitrogen species became clear, UA was shown to protect live cells from the damaging actions of peroxynitrite ($ONOO^{-}$), the product of the reaction between NO and superoxide (O^{2-}) [8, 47]. Then it was shown that the chemistry of $ONOO^{-}$ may be even more complex. In a biological milieu, highly reactive peroxynitrite rapidly interacts with bicarbonate/carbon dioxide to form a nitrosoperoxycarbonate anion ($ONOOCO^{2-}$) with enhanced capacity to nitrate aromatics [18, 53] and generate intermediate radicals, such as $^{\cdot}NO2$ and $CO3^{\cdot-}$ with damaging properties [71, 72]. Despite the incomplete understanding of various proposed reactions of nitrogen species in vivo, it is clear that UA can protect living cells, including CNS cells, from the damaging actions of free radicals [13, 62, 71, 77].

A high concentration of UA in human sera is another critical factor for efficient radical scavenging. The average human body contains 1–2 g of UA, which represents a higher concentration than other nonenzymatic scavengers such as ascorbate, tocopherols, methionine, and glutathione. UA likely provides 30%–65% of the peroxyl-radical scavenging capacity in human blood plasma [4].

Biosynthesis of UA from xanthine may lead to superoxide radical production through the action of xanthine oxidase, especially under hypoxic conditions. The drug allopurinol routinely inhibits xanthine oxidase and prevents hypoxia reperfusion injury. However, under normal physiological conditions, xanthine dehydrogenase, another form of the same enzyme, oxidizes purines at the expense of NADH without significant production of free radicals [17]. Although small amounts of xanthine dehydrogenase are expressed in many organs, most of the enzyme is produced in the small intestine and the liver followed by lung and heart,

making those organs particularly sensitive to hypoxia reperfusion injury [49]. In contrast, the UA produced by xanthine dehydrogenase spreads throughout the body via the bloodstream, providing protection from oxidative damage to various tissues, including the CNS.

3 Role of Free Radicals in Multiple Sclerosis: Lessons from Experimental Allergic Encephalomyelitis and Other Models

There are multiple sources of oxidative stress in MS brain. Glutamate excitoxicity is linked to activation of metabolic pathways that lead to free radical production (reviewed in Gilgun-Sherki et al. [24]). Free radicals are generated in mitochondria of all CNS cells and in particularly high quantities by resident microglia and infiltrating macrophages (Fig. 1) (reviewed in Gilgun-Sherki et al. [25]). Activated microglia, macrophages, astrocytes, and endothelial cells can also produce $NO^{\cdot}$ and generate $ONOO^{-}$ [8]. Iron, which is present in some regions of the brain, is catalytic for the free radical reactions (reviewed in Levine and Chakrabarty 2004 [51]).

Several lines of evidence have implicated oxidative stress in the pathogenesis of MS and other neurogenerative diseases [24, 25]. In vitro studies have demonstrated that neurons, postmitotic cells, are more sensitive to oxidative stress than other CNS cells [6, 10]. Various antioxidants, including UA, protect neurons from free radical damage in tissue culture [62, 86].

The first evidence of $ONOO^{-}$ formation in brain of MS patients was reported by Bagasra et al. [3]. Since then, several independent studies have confirmed the formation of peroxynitrite in brain, cerebrospinal fluid (CSF), and blood of patients with MS and animals with experimental allergic encephalomyelitis (EAE) [15, 16, 34, 52, 63, 78]. $ONOO^{-}$ can induce a variety of effects, including oxidation of DNA and proteins, lipid peroxidation, inhibition of mitochondrial respiration, and tyrosine nitration [7, 8, 23, 29]. In addition, $ONOO^{-}$ may mediate cell death via DNA single-strand breakage and activation of the nuclear enzyme poly(ADP-ribose) polymerase (PARP) [74]. While the precise $ONOO^{-}$-dependent chemical reactivity remains unknown, there is a little doubt that $ONOO^{-}$ is a key contributor to MS and EAE.

Evidence of free radical involvement in EAE-related damage came from attempts to treat EAE symptoms with radical scavengers. Studies in various models showed a wide spectrum of natural and synthetic antioxidants to delay or suppress EAE symptoms [11, 19, 26, 28, 54, 61]. UA administration also inhibited the development of clinical EAE and ameliorated preexisting signs of the disease [34, 36, 37, 70].

Besides EAE, other CNS conditions associated with $ONOO^{-}$-related damage have been treated successfully with UA. Increased UA levels are effective against viral and bacterial infections of the CNS. In rats infected with Borna virus, UA treatment substantially delays development of symptoms and inhibited formation of nitrotyrosine and production of proinflammatory cytokines [35]. In a rat model of pneumococcal meningitis, UA exerts dose-dependent anti-inflammatory effect at blood levels in the human physiological range [42, 43]. UA administration at the

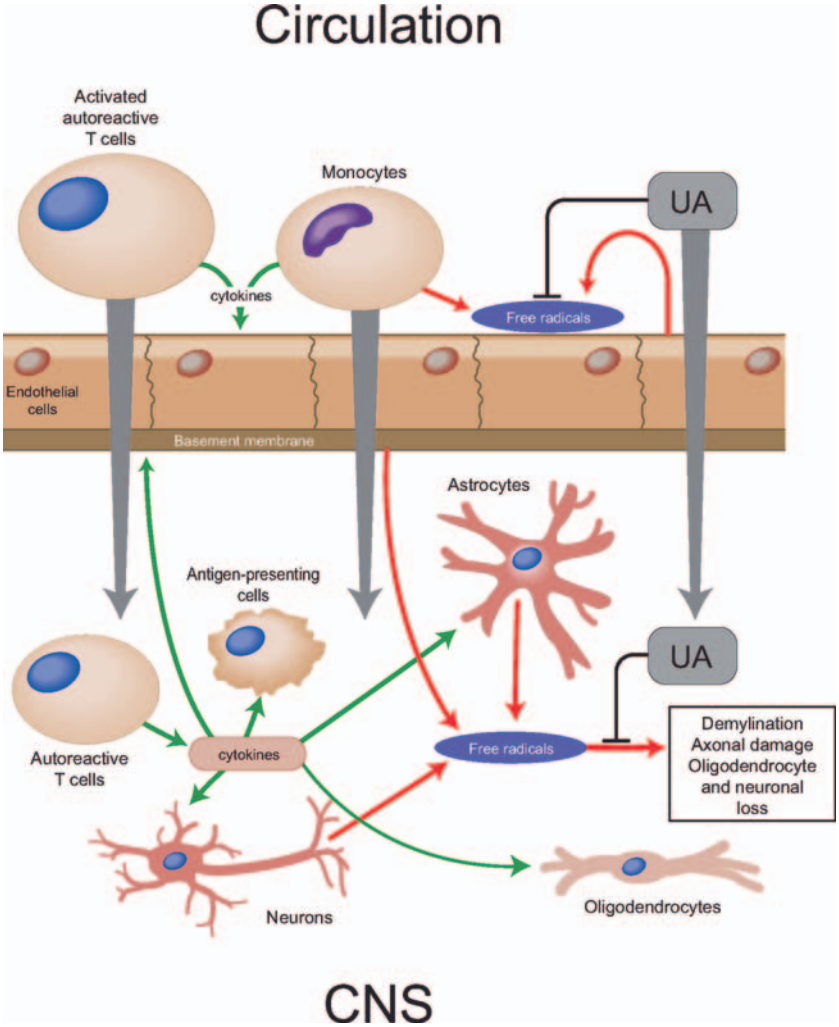

Fig. 1 Role of free radicals in demyelination. During the early stages of inflammatory response, free radicals are produced by circulating and endothelial cells and may contribute to the loss of BBB integrity through direct damage to endothelial cells or promotion of lymphocyte adhesion and infiltration. UA does not readily cross the intact BBB. During the early stages of inflammation, inactivation of certain radicals, including peroxynitrite by UA, may protect the integrity of BBB. During the late stages of CNS inflammation, free radicals are produced by a variety of resident and infiltrating cells and may cause either direct damage or participate in regulation of a variety of cytokines, chemokines, matrix metalloproteinases, and adhesion molecules. During this stage, BBB becomes compromised. UA penetrates areas of active lesions and contributes to containment of CNS pathology attributed to free radical damage such as mitochondrial dysfunction, DNA and protein damage, lipid peroxidation, myelin injury, etc

onset of spinal cord injury in a mouse model inhibits several pathological changes in the spinal cord including general tissue damage, nitrotyrosine formation, lipid peroxidation, activation of poly(ADP-ribose) polymerase, and neutrophil invasion; more importantly, UA treatment improved functional recovery from the injury [62].

Based on the encouraging results in animal studies, investigators are looking into the potential benefit of dietary antioxidants for MS patients (reviewed in Carlson and Rose [12]). Thus far, no human studies have proven this hypothesis. However, it is well known that many MS patients take multiple vitamins and other purportedly antioxidant food supplements. Currently, the field of MS research lacks well-designed clinical trials to address issues of antioxidant composition, intake, and effectiveness.

4 Serum Uric Acid Levels in Patients with Multiple Sclerosis

In vitro and in vivo findings have rendered UA the most studied antioxidant in MS. In a seminal study by Hooper et al. [37], analysis of the records of 20,212,505 patients enrolled in Medicare and Medicaid for diagnosis of MS and gout revealed only four patients with both conditions instead of the 62 expected, making MS and gout almost mutually exclusive (Table 1). The same authors also observed that serum UA levels among a group of 46 MS patients were significantly lower ($p<0.001$) than in the control population comprised of patients with spinal cord injuries, cerebral palsy, Parkinson's disease, and other conditions with similar degrees of disability. Patients receiving drugs known to modulate serum UA levels were excluded from the study. To control for the significant influence of diet on serum UA levels, the institutionalized subjects all received the same diet for 5 days before collection of serum, and all subjects donated blood samples before breakfast. However, this pioneering study did not take disease activity into account. Magnetic resonance imaging (MRI) performed after the study on a selected group of 20 patients revealed gadolinium-enhancing lesions in only two patients (10%). Another study by these authors [68] analyzed sets of twins in which only one sibling had MS; the MS-affected siblings consistently revealed lower UA levels than the healthy siblings in both homo- and heterozygous twin pairs.

Since those initial reports, at least ten other independent studies on UA and MS have been conducted with somewhat conflicting results; i.e., half of the studies confirmed the low UA levels in the MS population, but the other half found no differences from controls. Drulovic et al. [21], for example, observed that average serum UA levels were about 8% lower in MS patients than in patients with other neurological diseases (OND). The difference increased to about 15% and was statistically significant for patients with active MS. Those investigators also demonstrated UA fluctuation in serum of relapsing-remitting patients with MS (RRMS), significantly higher UA levels detected during remission than during relapse ($p=0.006$), and differences reaching 20%.

In an analysis of sera of 124 MS patients vs 124 sex- and age-matched OND patients, Sotgiu et al. [67] observed a 13% reduction in serum UA levels of MS patients.

Table 1 Studies on serum UA levels in MS patients

Study	Population	Serum UA levels	Remarks
Hooper et al. 1998	46 MS patients vs 46 non-MS patients with other OND and similar degree of disabilities, sex- and age- matched. Strict dietary control. No information on disease activity	Lower in MS patients by about 10% ($p<0.01$)	Selective MRI on 20 patients several months after the study revealed that only 10% had active lesions (unpublished)
Hooper et al. 1998	Statistical analysis of 20,212,505 Medicare and Medicaid records, including 36,733 gout and 34,607 MS. No information on disease activity	15-Fold decrease of incidence of MS in patients with gout. No direct information on serum UA levels	Strongly suggestive that high serum UA levels may protect against MS
Karg et al. 1999	25 MS patients vs nine controls. 72% Males in MS population versus 50% among controls	No difference	Small study with other primary objectives. Male predominance in MS group likely influences the outcome
Drulovic et al. 2001	240 MS patients vs 104 patients with OND, sex- and age- matched	Lower in MS patients by 8% (not significant) and by 15% in patients with active MS ($p<0.05$)	Suggested that UA may be a marker of MS activity
Spitsin et al. 2001	132 Sets of twins with one sibling affected by MS, including 88 sets of the same sex and 52 monozygotic pairs. No information on disease activity	Lower in twin with MS for all 132 sets by 11%. Lower in 88 sets of the same sex by 10% and for 52 monozygotic pairs by 12%. (p Multiple Sclerosis<0.05 for all sets)	Similar decrease in UA levels in mono- and di-zygotic twins. Data from Monozygotic twins strongly support hypothesis that UA level can be driven down by MS
Sotgiu et al. 2002	124 MS patients versus 124 OND sex- and age- matched patients	Lower in MS patients by 13% ($p=0.001$)	UA levels did not significantly correlate with disease activity, duration, disability or course
Toncev et al. 2002	63 MS patients vs 20 OIND and 20 healthy controls, sex- and age-matched	Lower in MS patients by 28% ($p<0.001$)	Strong correlation between UA and MS activity. UA levels were lower in patients with disrupted blood–brain barrier ($p<0.001$)

(continued)

Table 1 (continued)

Study	Population	Serum UA levels	Remarks
Knapp et al. 2004	21 Patients with optic neuritis. Separate statistics for males and females. 17 Females with ON vs 22 healthy controls and four males with ON versus 16 controls	Lower in patients with optic neuritis; 22% decrease in females ($p=0.006$); 7% decrease in males (not significant)	Five of 21 patients with optic neuritis were also diagnosed with MS
Becker et al. 2004	18 MS patients vs 26 healthy controls, 11 facial palsy, 18 Guillain-Barré syndrome and 15 bacterial meningitis	No difference	Insufficient data on MS activity
Kastenbauer et al. 2005	70 MS patients vs 24 OND. At least 18 MS patients had active lesions at the time of blood collection	No difference	No evidence of increased degradation of urate to allantoin found in MS patients
Mostert et al. 2005	60 MS patients vs 29 healthy controls, sex- and age-matched. No MRI data	No difference	All MS patients were nonrelapsing
Ramsaransing et al. 2005	82 MS patients vs 29 healthy controls sex- and age-matched. No MRI data	No difference between MS patients and controls. No difference between benign and progressive MS	All MS patients were nonrelapsing
Rentzos et al. 2006	190 MS patients including 35 with MRI activity and 29 CIS vs 58 OND, including 28 with OIND, sex- and age-matched. Dietary controls	Lower in MS patients by about 20% ($p><0.03$). No difference between active and nonactive MS ($p><0.003$)	Although sex and age matching was done for the entire MS and non-MS groups, data on sex distribution in the active MRI group were not provided

OND, other neurological diseases; *OIND*, other inflammatory neurological diseases; CIS, clinically isolated syndrome, represented by patients with high risk of developing MS, with two or more silent lesions and clinical features such as optic neuritis, diplopia, hemiparesis, or cerebral ataxia

However, UA levels analyzed in MS patients selected according to disease activity showed only a 2.5%–5% reduction in active MS, and the difference was not significant. MRI data were available only for 21 patients in that study (13 with active lesions and eight with inactive), and gender distribution information was not given. Thus, the accuracy of conclusions about UA and MS activity in that study remains unclear.

Toncev et al. [76] analyzed 63 MS patients, 20 patients with other inflammatory neurological diseases (OIND), and 20 healthy controls. They reported up to a 28% reduction in UA levels in MS vs non-MS patients and a 20% reduction in MS vs patients with OIND. Both relapsing patients and those with active lesions had significantly lower UA levels than did patients in remission and without active lesions.

Recently Rentzos et al. [60] confirmed the presence of lower UA levels in MS patients in analysis of 190 MS versus 58 OND patients. However, they found no correlation between UA levels and MS activity. In fact, they reported UA levels in 35 patients with active lesions to be actually about 6% higher than in patients without lesions. They suggested that lower UA levels in MS represent a primary, constitutive loss of protection against nitric oxide and CNS inflammation.

Knapp et al. [45] reported lower serum UA levels in patients with optic neuritis (an inflammatory demyelinating condition affecting the optic nerve and closely related to MS) than in control individuals.

Two somewhat overlapping studies [57, 59] found no significant difference between MS patients and healthy controls in UA levels; they may reflect patient populations without relapse in the 3 months before the studies. Indeed, MS patients in both studies had normal serum UA levels (340 μM for males and 250 μM for females), consistent with levels reported in all previous studies for controls. About 20% of the MS patients took β-interferon, which is known to increase serum UA levels. However, β-interferon users and nonusers in these studies had similar UA levels. Patient descriptions did not allow speculation about dietary contributions to serum UA levels. No differences in UA levels were seen between patients with benign and patients with progressive MS [59]. While MS patients' NO production by peripheral blood leukocytes increased about twofold, this increase did not affect serum UA levels [57].

Three other studies also found no reduction in serum UA levels in MS patients [5, 40, 41]. In the first two studies, the MS patient population was small (25 and 18 patients, respectively), and comparison of UA levels was not a primary objective so that critical factors such as disease activity and sex distribution were not fully considered. Kastenbauer et al. [41] compared serum UA levels of 70 MS patients (at least 18 with active lesions and 36 with acute exacerbation) with those in 24 OND patients. Although the OND control group was small and heterogeneous, average serum UA levels for the MS group (238 μM) were closer to those of the OND control than to MS populations reported in other studies.

Recently, Koch and Keyser [46] suggested that MS patients are not primarily deficient in UA but that serum UA decreases with inflammatory disease activity. Thus, UA levels might be a marker of MS activity rather than a protective factor. In fact, a substantial amount of data supports this hypothesis. In most reported cases, serum UA levels of MS patients are only 10%–15% lower than those in normal

controls or patients with OND. It seems unlikely that such a small difference in antioxidant defense could be so critical in the development of MS. Data from monozygotic twins also support an environmental rather than a genetic basis for the differences in UA levels [69]. Benign and progressive MS populations reported no differences between serum UA levels, although higher levels might be expected in the benign MS group. Oxidative stress may act to decrease UA levels in MS patients. Allantoin is one of the possible products of UA oxidation by free radicals. Increased allantoin levels were detected in human sera and CSF of patients with other inflammatory conditions; however, limited studies did not reveal an increase in samples from MS patients [5, 41]. On the other hand, Kanabrocki et al. [39] demonstrated an altered relationship between serum NO and UA in MS patients. Temporal reduction in serum UA level apparently correlates with increased $ONOO^-$ production in MS patients.

Methodological issues may account for the lack of correlation between MS activity and serum UA levels reported in some studies. For example, human serum UA levels are dependent on gender, age, diet, medication, and other factors, and not all studies could address these factors equally. Moreover, disease activity can be monitored by the presence of gadolinium-enhancing lesions and/or clinical relapses, but both criteria have their own limitations. MS patients also often have evidence of activation in different lymphocyte populations; however, most studies could not take account for conditions of the peripheral immune system. Small patient sample size may be another critical factor. A study demonstrating lower serum UA levels in MS patients enrolled a total of 795 MS patients and 21 patients with optic neuritis but looked at a total of 195 patients when no difference was found and included an apparently overlapping patient population [5, 41, 57, 59]. Definite conclusions about the primary or secondary role of UA levels in the development of MS await further research. In our experience, about 10% of MS patients have very low UA levels (≤ 3 mgdL^{-1}). Possibly, slightly lower UA levels reflect disease activity in some patients, and very low UA levels predispose them to MS. We need appropriately designed studies to answer this question.

Generally, 50 patients should be sufficient to apply logistic regression analysis and/or *t*-tests to demonstrate a 10%–15% reduction in serum UA levels. However, due to differences in UA levels between male and female populations, a total of 200 people (four groups: MS/non-MS, male/female) are needed for such a study. Further attempts to break the MS cohort into groups (e.g., relapsing-remitting, primary progressive, secondary progressive, benign) require bringing the number of patients in each group to 50 for proper statistical analyses.

Although logical attempts have been made to correlate serum UA levels and disease severity, these studies may not fully address whether UA deficiency is a primary or secondary function in MS. This is because UA level is only one of several factors contributing to the development of MS symptoms including genetic background and environmental factors. Conceivably, a person with a weak predisposition for MS may develop symptoms because of a lower UA level. On the other hand, higher UA levels may partially diminish a stronger predisposition for MS. As a result, disease severity will be similar in both cases. Consequently,

studying existing cases of MS may not address the question about a primary or secondary role of UA. Another approach involves monitoring serum UA levels in individuals before the development of MS. This type of study requires a very large population. Even by performing the study in individuals with a genetic predisposition (risk for the development of MS, 1%–2%), 5,000 to 10,000 individuals highly predisposed to MS would need to submit to monitoring of serum UA levels from childhood through adulthood.

One finding clearly stands out among all others: the significant inverse correlation between incidence of MS and gout suggests that gout protects against development of MS [37]. However, patients with gout have a substantially larger increase in serum UA levels when compared with the difference between MS and non-MS populations in these levels. Most patients with gout have UA levels greater than 8.0 $mgdl^{-1}$; i.e., about twofold higher than the average level in men. Another feature of gout is formation of urate crystals, mainly in the joints but also throughout the body. These crystals may trigger a local inflammatory response that activates macrophages and produces various cytokines. Whether those events might suppress the development of MS remains to be addressed.

5 Possible Mechanisms of Uric Acid Action in Multiple Sclerosis, Protection of Blood–Brain Barrier Integrity

There are at least two components underlying MS pathogenesis: inflammation and neurodegeneration (reviewed in Hauser and Oksenberg [30]). Both components may be associated with free radical-related damage.

In vitro experiments have shown UA to block multiple actions of $ONOO^-$ and some other free radicals [1, 4, 80]. Findings in both MS and EAE demonstrate that UA treatment suppresses the permeability changes in the blood–brain barrier (BBB). In turn, immune cell invasion into the CNS is inhibited, and TNF-α production and ICAM-1 upregulation in CNS tissue are blocked [36, 44, 65]. On the other hand, the presence of UA does not alter immune function parameters such as antigen presentation, T cell proliferation, antibody production, and monocyte activation [44, 70]. Similar effects of UA have been described in other models such as neurotropic virus-induced encephalitis [35], bacterial meningitis [42, 43], and spinal cord injury in mice [62]. Thus, certain $ONOO^-$-dependent reactions may play a key role in the functional changes occurring at the BBB in EAE and other CNS inflammatory diseases [64]. They may directly modify structural elements of the BBB or modulate neurovascular endothelial cell function through an effect on signal transduction by $ONOO^-$-mediated, UA-sensitive reactions. UA treatment restores the integrity of the BBB and blocks 3-nitrotyrosine formation, but not inducible nitric oxide synthase (iNOS) expression, in focal areas of inflammation [36].

Only limited data exists on possible mechanisms of CNS protection by UA. However, in a pilot human clinical trial aimed at increasing serum UA levels in MS

patients, disappearance or reduction of gadolinium-enhanced lesions and decreased nitrotyrosine blood levels were observed ([48, 68] and unpublished observations). This suggests that the mechanisms of UA action in humans are similar to those observed in animal models and are directed at maintaining BBB integrity. Of note, UA does not penetrate the intact BBB in either animals or humans; thus, normal CNS concentrations of urate are approximately tenfold lower than in the blood stream. However, UA has constant access to CNS microvasculature and can penetrate the compromised BBB in both human MS and mouse EAE conditions [66, 69]. As a result, UA levels are higher in areas of active lesions [50].

Recently, it has become evident that axonal degeneration is an important factor in MS pathogenesis (reviewed in Hendriks [32] and Andrews et al. [2]). While the mechanisms underlying this phenomenon are not fully understood, free radicals generated by activated resident and infiltrating cells as well as by mitochondria of demyelinated axons are likely to contribute to axonal loss. Increased UA levels in these areas may be protective against such damage. For example, recently it was reported that UA protects spinal cord neurons in vitro against glutamate toxicity by a mechanism other than purely binding peroxynitrite [22].

6 Treatment of MS Patients by Raising UA Levels

Although more research is needed to pinpoint the exact mechanisms of CNS protection by UA, initial attempts to manipulate UA levels in MS patients have been made. From the data discussed in Sect. 4 of this review, it is unlikely that raising UA levels in MS patients by 10%–20% (the difference between MS and non-MS populations) will produce any therapeutic effect. More likely, serum UA levels of 8 $mgdl^{-1}$ or higher, such as those observed in gout patients, are needed for protection [37]. On the other hand, levels much higher than 8 $mgdl^{-1}$ might precipitate a gout attack and/or kidney stone formation. In the original attempt to raise serum UA levels in MS patients and maintain these levels at around 8 $mgdl^{-1}$, UA was administered orally [69]. However, oral UA proved ineffective in raising serum UA levels due to poor absorption and sensitivity to bacterial uricase in the gut. Thus, investigators chose the UA precursor to raise the typically low serum UA levels of MS patients. Inosine is approved for human consumption as a food supplement and a muscle performance enhancer. Although the capacity of inosine to act as a muscle stimulant remains controversial [20, 56, 73, 82], professional athletes use it routinely and extensively at dosages of 1–6 g per day for periods ranging from several days [73, 82] to weeks [20] and years [14] with no reported side effects. The EAE animal model of MS confirmed inosine's efficacy as a therapeutic agent [66]. A phase I clinical trial proved that inosine administration effectively raised UA levels of MS patients into the high/normal range [69]. Only two of the 11 enrolled patients had gadolinium-enhancing lesions, and all 11 were free of active lesions after 1 year of inosine therapy. At least two more trials followed. Toncev [75] reported that 32 MS patients receiving 1–2 g of inosine per day for approximately

3 years had significantly lower relapse rates and smaller increases in EDSS score than 32 patients in a nontreated control group matched for age, gender, disability, and disease duration. Daily doses of 1–2 g of inosine have boosted serum UA levels in treated patients from an average of 200 μM (3.4 $mgdl^{-1}$) to 250–300 μM (4.2–5.0 $mgdl^{-1}$). Thus, even modest increases in serum UA levels might provide a therapeutic value for MS patients.

A current, more comprehensive phase I/II clinical trial is assessing the therapeutic efficacy of raising UA levels by inosine administration in a larger group of MS patients with active disease. Of particular importance is the effect of treatment on quantifiable BBB permeability and on lesion activity evaluated by gadolinium-enhancing MRI. This study is still in progress, but preliminary results for the first group of 11 patients who have completed 1 year of study are available for discussion. The patient with the highest number of active lesions prior to treatment showed the most dramatic improvement (Fig. 2). During 6 months of inosine treatment, the average serum UA level in this individual increased from 4.2 to 8.7 $mgdl^{-1}$ ($p<0.001$), the number of active lesions decreased from an average of ten to one by the end of the trial ($p<0.001$), and the Kurtzke expanded disability status scale (EDSS) score dropped from 2.0 to 1.0.

The remaining patients had fewer active lesions than the patient presented above. Nevertheless, analysis of the combined data for these patients revealed a significant correlation ($p<0.007$ by two-sided Fisher's exact test) between raising serum UA level and reduction in disease activity (Table 2). This suggests that UA may naturally protect against the loss of BBB integrity and inhibit lesion formation. Experiments to assess possible measures of disease activity in sera have revealed nitrotyrosine levels to be the most active predictor of treatment outcome.

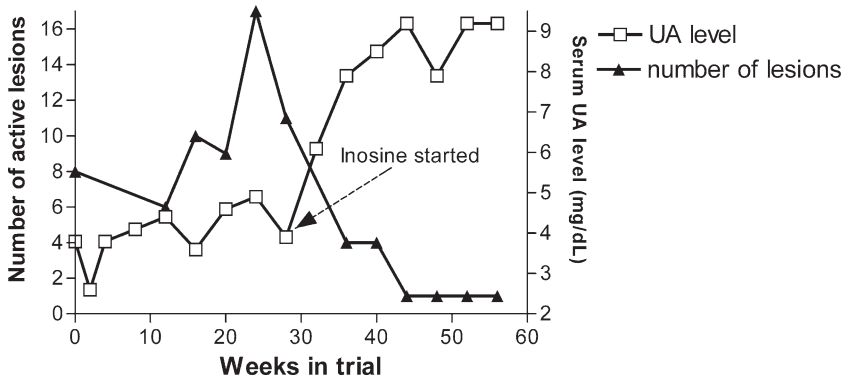

Fig. 2 Reduction of disease activity during inosine treatment in an MS patient with high lesion burden. Patient was treated with placebo for the first 6 months and with inosine for the second 6 months. Blood drawings and gadolinium-enhanced MRI were performed monthly

Table 2 Comparison of serum UA levels and disease activity in MS patients during placebo/inosine treatment

Serum UA level	Number of visits	Visits with active lesions	Visits with exacerbations
>6.5 mg/dl	49	3	0
<6.5 mg/dl	29	11	4
Total	78	14	4

The association between UA level and lesion activity is significant by Fisher's exact test (two-sided) $p<0.007$. The association between UA level and exacerbation is significant by Fisher's exact test (two-sided) $p<0.03$

Conclusions

UA acts as a part of a sophisticated, but not infallible, antioxidant defense system consisting of multiple components [9]. To be fully effective, UA must interact with numerous enzymes, scavengers, and quenchers. A better understanding of these mechanisms may aid the development of optimal therapeutic approaches to target free radicals in MS. Low UA levels apparently reflect disease activity in the majority of MS patients and can be used as a diagnostic tool. A high UA level, approaching those in patients with gout, may be a therapeutic tool in itself or in combination with other treatments. The question of whether the very low serum UA levels found in some MS patients might predispose to the development of MS remains to be answered.

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