

Uric Acid and MS

Serum uric acid levels in multiple sclerosis patients correlate with activity of disease and blood-brain barrier dysfunction.

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Several findings suggest lower levels of serum uric acid in multiple sclerosis (MS) patients. The aim of this study is to investigate relationships of uric acid serum levels in relapse-remitting (RR) MS patients with clinical activity of disease and blood-brain barrier (BBB) condition. Sixty-three definite RRMS patients and 40 controls divided into two groups: 20 healthy donors and 20 patients with other inflammatory neurological diseases (OINDs) were analysed. By using a quantitative enzymatic assay according to the manufacture's protocol and a commercial uric acid standard solution, serum uric acid levels were measured and the results were standardized. To investigate BBB function, magnetic resonance imaging after administration of gadolinium was used. MS patients were found to have significantly lower serum uric acid levels (193.89 ± 49.05 micromol/l; mean value \pm SD) in comparison with healthy donors (292.7 ± 58.65 micromol/l; $P=0.000$) and OIND patients (242.7 ± 46.66 micromol/l; $P=0.001$). We found that MS patients with relapse had significantly lower serum uric acid levels (161.49 ± 23.61 micromol/l) than MS patients with remission (234.39 ± 41.96 micromol/l; $P=0.000$) and more over, MS patients with BBB disruption had significantly lower serum uric acid levels (163.95 ± 26.07 micromol/l) than those with normal BBB (252.48 ± 25.94 micromol/l; $P=0.000$). Further, we also found that serum uric acid level independently correlated with disease activity, BBB disruption, and gender. These results indicate that lower uric acid levels in MS patients are associated with relapse and suggest that uric acid might be beneficial in the treatment of MS.

Uric acid levels in sera from patients with multiple sclerosis.

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The levels of uric acid (UA), a natural peroxynitrite scavenger, were measured in sera from 240 patients with multiple sclerosis (MS) and 104 sex- and age-matched control patients with other neurological diseases (OND). The mean serum UA concentration was lower in the MS than in the OND group, but the difference did not reach the level of statistical significance ($P = 0.068$). However, the mean serum UA level from patients with active MS (202.6 ± 67.1 $\mu\text{mol/l}$) was significantly lower than that in inactive MS patients (226.5 ± 78.6 $\mu\text{mol/l}$; $P = 0.046$) and OND controls ($P = 0.007$). We found a significant inverse correlation of serum UA concentration with female gender ($P = 0.0001$), disease activity ($P = 0.012$) and duration ($P = 0.017$), and a trend towards an inverse correlation with disability as assessed by EDSS score, which did not reach statistical significance ($P = 0.067$). Finally, multivariate linear regression analyses showed that UA concentration was independently correlated with gender ($P = 0.0001$), disease activity ($P = 0.014$) and duration of the disease ($P = 0.043$) in MS patients. These findings suggest that serum UA might serve as a possible marker of disease activity in MS. They also provide support to the potential beneficial therapeutic effect of radical-scavenging substances in MS.

High-dose methylprednisolone therapy in multiple sclerosis increases serum uric acid levels.

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Uric acid, which is the final product of purine nucleoside metabolism, is a strong peroxynitrite scavenger. Several studies report on lower serum uric acid levels in multiple sclerosis. In this study, we investigated serum uric acid levels before and after high-dose methylprednisolone treatment (intravenous 1 g/day/5 days) in multiple sclerosis patients. Blood samples from 25 definite multiple sclerosis patients (11 male and 14 female) before and after methylprednisolone treatment (days 0, 6 and 30) and from 20 healthy donors (9 male and 11 female) were analyzed. Serum uric acid levels were measured using a quantitative enzymatic assay (Elitech diagnostics, Sees, France) according to the manufacturer's protocol, and the results were standardized using a commercial uric acid standard solution. We observed significantly increased serum uric acid levels 1 day after the termination of the therapy (day 6). These differences were sustained for 30 days after starting treatment (during remission period). Mean serum uric acid levels were significantly higher in the control group. These results suggest that increasing the uric acid concentration may represent one of the possible mechanisms of action of methylprednisolone in multiple sclerosis.

Inactivation of peroxynitrite in multiple sclerosis patients after oral administration of inosine may suggest possible approaches to therapy of the disease.

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Mult Scler 2001 Oct;7(5):313-9

Peroxynitrite has been implicated in the pathogenesis of multiple sclerosis (MS) and its animal model experimental allergic encephalomyelitis (EAE). Previously, we have shown that administration of uric acid (UA), a peroxynitrite scavenger, is therapeutic in EAE. We have also shown that MS patients have lower levels of serum uric acid than healthy individuals or those with other neurological diseases. The aim of this investigation was therefore to raise serum UA levels in MS patients. Oral administration of UA failed to increase low serum UA levels, evidently due to its degradation by gastrointestinal bacteria. However, serum UA could be raised and maintained at elevated levels for a year and more without reported side-effects by oral administration of its precursor inosine. Three of 11 patients given inosine showed some evidence of clinical improvement and there was no sign of disease progression in the remaining patients. Gadolinium-enhanced lesions, observed in two patients before receiving inosine, could not be detected after either 10 or 18 months inosine treatment. These data provide evidence that serum UA levels can be readily manipulated and that the benefit of higher levels to individuals with MS should be studied further in greater number of patients.

In vivo damage of CNS myelin and axons induced by peroxynitrite.

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In multiple sclerosis (MS) the mechanisms of injury caused by peroxynitrite remain uncertain. To study histological, ultra structural and molecular alterations caused by peroxynitrite in brain, the peroxynitrite donor 3-morpholinodisodoniimine was injected in rat corpus callosum. Peroxynitrite induces strong primary axonal damage with characteristics of primary acute axonopathy, together with severe myelin alteration, myelin vacuolation and demyelination, and nitrotyrosine formation as confirmed by detection of

nitrosated target proteins. Administration of the peroxynitrite scavenger uric acid inhibited these effects. In vivo, peroxynitrite leads to a disorganisation of myelin and to axonal damage presenting some similarities to the formation of MS lesions. Understanding the action of peroxynitrite in this process will open new therapeutic strategies by specific inhibition of peroxynitrite formation and action.

Uric acid levels in patients with multiple sclerosis: analysis in mono- and dizygotic twins.

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Presence of nitrotyrosine in cells surrounding plaques indicates that peroxynitrite may be the cause of brain lesions in multiple sclerosis. Low levels of uric acid, a natural scavenger of peroxynitrite, were demonstrated in blood of patients with multiple sclerosis in comparison with control individuals. These observations were now extended to 132 sets of twins with one sibling affected by multiple sclerosis. In blood of both mono- and dizygotic twins the uric acid levels were lower in the twin with the disease than in the healthy twin.

The role of uric acid in protection against peroxynitrite-mediated pathology.

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Peroxynitrite, the product of the free radicals nitric oxide and superoxide, has been implicated in the pathogenesis of inflammatory CNS disorders. Uric acid, an effective scavenger of peroxynitrite, is a purine metabolite present at high levels in the serum of hominoids relative to lower-order animals due to the functional deletion of urate oxidase. Raising the normally low levels of uric acid in mice is therapeutic for experimental allergic encephalomyelitis, an animal model of multiple sclerosis. This therapeutic activity of uric acid is associated with the inhibition of peroxynitrite-induced tissue damage, blood-CNS barrier permeability changes, and CNS inflammation. Based on these findings we have concluded that peroxynitrite has an important role in promoting enhanced vascular permeability and inflammatory cell extravasation. We hypothesize that higher uric acid levels in hominoids evolved to protect against this process.

The peroxynitrite scavenger uric acid prevents inflammatory cell invasion into the central nervous system in experimental allergic encephalomyelitis through maintenance of blood-central nervous system barrier integrity.

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Uric acid (UA), a product of purine metabolism, is a known scavenger of peroxynitrite (ONOO(-)), which has been implicated in the pathogenesis of multiple sclerosis and experimental allergic encephalomyelitis (EAE). To determine whether the known therapeutic action of UA in EAE is mediated through its capacity to inactivate ONOO(-) or some other immunoregulatory phenomenon, the effects of UA on Ag presentation, T cell reactivity, Ab production, and evidence of CNS inflammation were assessed. The inclusion of physiological levels of UA in culture effectively inhibited ONOO(-)-mediated oxidation as well as tyrosine nitration, which has been associated with damage in EAE and multiple sclerosis, but had no inhibitory effect on the T cell-proliferative response to myelin basic protein (MBP) or on APC function. In addition, UA treatment was found to have no notable effect on the development of the immune response to MBP in vivo, as measured by the production of MBP-specific Ab and the induction of MBP-specific T cells. The appearance of cells expressing mRNA for inducible NO synthase in the circulation of MBP-immunized mice was also unaffected by UA treatment. However, in UA-treated animals, the blood-CNS barrier breakdown normally associated with EAE did not occur, and inducible NO synthase-positive cells most often failed to reach CNS tissue. These findings are consistent with the notion that UA is therapeutic in EAE by inactivating ONOO(-), or a related molecule, which is produced by activated monocytes and contributes to both enhanced blood-CNS barrier permeability as well as CNS tissue pathology.

Protection of myelin basic protein immunized mice from free-radical mediated inflammatory cell invasion of the central nervous system by the natural peroxynitrite scavenger uric acid.

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Neurosci Lett 2000 Oct 6;292(2):137-41

Peroxynitrite (ONOO(-)), the product of nitric oxide (NO(radical)) and

superoxide ($O_2^{\cdot-}$), is believed to be a major contributor to immunotoxicity when produced by activated cells expressing inducible nitric oxide synthase (iNOS). Uric acid (UA) is a natural scavenger of ONOO $^-$ that is present at high levels in the sera of humans and other higher order primates relative to most lower mammals. We have previously shown that UA treatment is therapeutic in experimental allergic encephalomyelitis (EAE), a rodent model of multiple sclerosis (MS). In this study we have examined the effect of UA therapy on the dynamics of the appearance of iNOS-positive cells in central nervous system (CNS) tissue of mice subjected to the stimuli that cause EAE. The results indicate that UA prevents activated monocytes from entering CNS tissue where they may contribute to the pathogenesis of MS and other CNS diseases.

Uric acid, a peroxynitrite scavenger, inhibits CNS inflammation, blood-CNS barrier permeability changes, and tissue damage in a mouse model of multiple sclerosis.

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Peroxynitrite (ONOO $^-$), a toxic product of the free radicals nitric oxide and superoxide, has been implicated in the pathogenesis of CNS inflammatory diseases, including multiple sclerosis and its animal correlate experimental autoimmune encephalomyelitis (EAE). In this study we have assessed the mode of action of uric acid (UA), a purine metabolite and ONOO $^-$ scavenger, in the treatment of EAE. We show that if administered to mice before the onset of clinical EAE, UA interferes with the invasion of inflammatory cells into the CNS and prevents development of the disease. In mice with active EAE, exogenously administered UA penetrates the already compromised blood-CNS barrier, blocks ONOO $^-$ -mediated tyrosine nitration and apoptotic cell death in areas of inflammation in spinal cord tissues and promotes recovery of the animals. Moreover, UA treatment suppresses the enhanced blood-CNS barrier permeability characteristic of EAE. We postulate that UA acts at two levels in EAE: 1) by protecting the integrity of the blood-CNS barrier from ONOO $^-$ -induced permeability changes such that cell invasion and the resulting pathology is minimized; and 2) through a compromised blood-CNS barrier, by scavenging the ONOO $^-$ directly responsible for CNS tissue damage and death.

Uric acid, a natural scavenger of peroxynitrite, in experimental allergic encephalomyelitis and multiple sclerosis.

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Uric acid, the naturally occurring product of purine metabolism, is a strong peroxynitrite scavenger, as demonstrated by the capacity to bind peroxynitrite but not nitric oxide (NO) produced by lipopolysaccharide-stimulated cells of a mouse monocyte line. In this study, we used uric acid to treat experimental allergic encephalomyelitis (EAE) in the PLSJL strain of mice, which develop a chronic form of the disease with remissions and exacerbations. Uric acid administration was found to have strong therapeutic effects in a dose-dependent fashion. A regimen of four daily doses of 500 mg/kg uric acid was required to promote long-term survival regardless of whether treatment was initiated before or after the clinical symptoms of EAE had appeared. The requirement for multiple doses is likely to be caused by the rapid clearance of uric acid in mice which, unlike humans, metabolize uric acid a step further to allantoin. Uric acid treatment also was found to diminish clinical signs of a disease resembling EAE in interferon-gamma receptor knockout mice. A possible association between multiple sclerosis (MS), the disease on which EAE is modeled, and uric acid is supported by the finding that patients with MS have significantly lower levels of serum uric acid than controls. In addition, statistical evaluation of more than 20 million patient records for the incidence of MS and gout (hyperuricemic) revealed that the two diseases are almost mutually exclusive, raising the possibility that hyperuricemia may protect against MS.

Prevention of experimental allergic encephalomyelitis by targeting nitric oxide and peroxynitrite: implications for the treatment of multiple sclerosis.

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Proc Natl Acad Sci U S A 1997 Mar 18;94(6):2528-33

In this study we provide further evidence associating activated cells of the monocyte lineage with the lesions of multiple sclerosis (MS). Using a combination of immunohistochemistry and reverse transcriptase-dependent in situ

polymerase chain reaction analysis, we have identified monocytes expressing inducible nitric oxide synthase (iNOS) to be prevalent in the plaque areas of post mortem brain tissue from patients with MS. In addition, we have obtained evidence of the nitration of tyrosine residues in brain areas local to accumulations of iNOS-positive cells. In parallel studies we have assessed the effects of inhibitors of iNOS induction, as well as scavengers of nitric oxide and peroxynitrite in the experimental allergic encephalomyelitis model. Significant therapeutic effects were seen with the inhibitor of iNOS induction, tricyclodecan-9-yl-xanthogenate, a nitric oxide scavenger, 2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide, and a peroxynitrite scavenger, uric acid. In particular, treatment with high doses of uric acid virtually prevented clinical symptoms of the disease. Together with our demonstration of the presence of activated macrophages expressing high levels of iNOS and evidence of peroxynitrite formation in brain tissue from patients with MS, these findings are of importance in the development of approaches to treat this disease.

Reaction of uric acid with peroxynitrite and implications for the mechanism of neuroprotection by uric acid.

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Arch Biochem Biophys 2000 Apr 15;376(2):333-7

Peroxynitrite, a biological oxidant formed from the reaction of nitric oxide with the superoxide radical, is associated with many pathologies, including neurodegenerative diseases, such as multiple sclerosis (MS). Gout (hyperuricemic) and MS are almost mutually exclusive, and uric acid has therapeutic effects in mice with experimental allergic encephalomyelitis, an animal disease that models MS. This evidence suggests that uric acid may scavenge peroxynitrite and/or peroxynitrite-derived reactive species. Therefore, we studied the kinetics of the reactions of peroxynitrite with uric acid from pH 6.9 to 8.0. The data indicate that peroxynitrous acid (HOONO) reacts with the uric acid monoanion with $k = 155 \text{ M}^{-1} \text{ s}^{-1}$ ($T = 37 \text{ degrees C}$, pH 7.4) giving a pseudo-first-order rate constant in blood plasma $k(\text{U}(\text{rate}))(\text{plasma}) = 0.05 \text{ s}^{-1}$ ($T = 37 \text{ degrees C}$, pH 7.4; assuming $[\text{uric acid}](\text{plasma}) = 0.3 \text{ mM}$). Among the biological molecules in human plasma whose rates of reaction with peroxynitrite have been reported, CO_2 is one of the fastest with a pseudo-first-order rate constant $k(\text{CO}_2)(\text{plasma}) = 46 \text{ s}^{-1}$ ($T = 37 \text{ degrees C}$, pH 7.4; assuming $[\text{CO}_2](\text{plasma}) = 1 \text{ mM}$). Thus peroxynitrite reacts with CO_2 in human blood plasma nearly 920 times faster than with uric acid. Therefore, uric acid does not directly scavenge peroxynitrite because uric acid can not compete for peroxynitrite with CO_2 . The therapeutic effects of uric acid may be related to

the scavenging of the radicals $\text{CO}(\cdot)(3)$ and $\text{NO}(\cdot)(2)$ that are formed from the reaction of peroxynitrite with $\text{CO}(2)$. We suggest that trapping secondary radicals that result from the fast reaction of peroxynitrite with $\text{CO}(2)$ may represent a new and viable approach for ameliorating the adverse effects associated with peroxynitrite in many diseases.

Increase in serum levels of uric acid, an endogenous antioxidant, under treatment with glatiramer acetate for multiple sclerosis.

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Free radicals including peroxynitrite are induced in Multiple Sclerosis (MS). Antioxidant and peroxynitrite inhibitor uric acid (UA), suppresses the MS animal model experimental autoimmune encephalomyelitis (EAE). MS patients have lower average serum UA than controls. An inverse relationship exists between MS and gout. Glatiramer acetate (GA) suppresses EAE and is beneficial in relapsing MS. We investigated serum UA changes during open-label treatment of relapsing MS with GAA. Ten patients (six females, four males, aged 19 to 39 years, mean age 32 years) completed 6 months of GAA (Copaxone 20 mg s.c daily). Of these, nine completed 12 months. After 6 months on GAA, serum UA (normal, 173-359 micromol/ml for women, 258-491 micromol/ml for men) increased in nine and marginally decreased (302 to 300 micromol/ml) in a single patient. Mean UA significantly increased from 240 to 303 micromol/ml ($P=0.0014$). At 12 months, UA remained significantly higher than at start ($P=0.006$) decreasing in only one patient. In contrast, we found no significant UA changes after 6 and 12 months of treatment in 21 MS patients treated with interferon beta1-a (Avonex), or in 11 treated with interferon beta1-a (Rebif), or in five placebo-treated controls. Increasing UA, a natural inhibitor of free radicals, may represent a mechanism of action of glatiramer acetate in MS.

If uric acid may benefit multiple sclerosis patients ("Uric acid linked to multiple sclerosis," SN: 1/31/98, p. 68), could a diet high in organ meats and other purines prove beneficial?

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Immunologist D. Craig Hooper says any food rich in purines will raise uric acid concentrations and might be beneficial. -- M.N. Jensen

