The Treatment of Multiple Sclerosis with Inosine

Clyde E. Markowitz, M.D.,1 Sergei Spitsin, Ph.D.,2 Vanessa Zimmerman, M.S.N.,1 Dina Jacobs, M.D.,1
Jayaram K. Udupa, M.D.,3 D. Craig Hooper, Ph.D.,2 and Hilary Koprowski, M.D.2

Abstract

Objective: The objective of this study is to evaluate the safety and tolerability of inosine in patients with relapsing–remitting multiple sclerosis (RRMS). The secondary objectives are to assess the effects of inosine administration on serum urate (UA) levels, the progression of neurologic disability, the cumulative number of new, active lesions on magnetic resonance imaging (MRI), and changes in serum levels for markers of inflammation.

Design: Oral administration of inosine was used to raise serum levels of the natural peroxynitrite scavenger UA in 16 patients with RRMS during a 1-year randomized, double-blind trial.

Outcome measures: The endpoints studied were relapse rate, disability assessed by the Kurtzke Expanded Disability Status Scale (EDSS), MRI, and analysis of serum levels of nitrotyrosine, and oxidative and pro-inflammatory makers.

Results: Increased serum UA levels correlated with a significant decrease in the number of gadolinium-enhanced lesions and improved EDSS. A number of MRI intensity-based parameters were altered by inosine treatment, in certain cases correlating with changes in serum UA levels. In a patient with low serum UA and high lesion activity, raising UA levels by inosine treatment decreased serum nitrotyrosine while increasing the ratio of Th2 to Th1 cytokines in circulating cells. The only side-effect correlated with inosine treatment was kidney stone formation in 4/16 subjects.

Conclusions: These data suggest that the use of inosine to raise serum UA levels may have benefits for at least some MS patients. The effect of this treatment is likely to be a consequence of inactivation of peroxynitrite-dependent free radicals. Close monitoring of serum UA levels as well as other measures are required to avoid the potential development of kidney stones.

Introduction

Multiple sclerosis (MS) is one of the most common neurologic diseases of young adults, accounting for more disability, treatment costs, and lost income than any other neurologic disease in this age group in Western Europe and North America.1,2 A principal anatomic feature of MS is the development of inflammatory lesions, predominantly in the white matter of central nervous system (CNS) tissues.2–4 Magnetic resonance imaging (MRI) is widely used as a diagnostic tool in MS and is beginning to be used to monitor disease progression by examining different MRI parameters.5,6 New MRI methods and analytical techniques are being developed to provide additional clinically relevant information. Active MS plaques generally contain inflammatory cells that express intracellular inducible nitric oxide synthase (iNOS) and produce peroxynitrite-dependent radicals, which can be detected by their nitration of tyrosine residues.7–9 The contribution of peroxynitrite-dependent radicals to CNS lesion formation has been extensively studied in animal models, where the therapeutic effects of the natural peroxynitrite-dependent radical scavenger uric acid (UA) has been demonstrated.10,11 These findings are significant for patients with MS who often have lower serum UA levels than normal individuals.12 In addition, an inverse correlation between the occurrence of MS and serum UA levels has been demonstrated.12 Based on these observations, a pilot study was conducted in patients with secondary-progressive MS (SPMS) to test

1Neurology Department, University of Pennsylvania, Philadelphia, PA.
2Biotechnology Foundation Laboratories at Thomas Jefferson University, Philadelphia, PA.
3Department of Radiology, University of Pennsylvania, Philadelphia, PA.
reduced. Blood work for complete blood count (CBC), blood urea nitrogen (BUN), creatinine, and UA levels was initially conducted biweekly and later at monthly intervals. Following the development of kidney stones in a few patients, detailed dietary guidelines were developed for the last 5 patients recruited. These guidelines included adequate fluid intake (6–8 glasses of water daily), a low purine and low oxalate diet, limited alcohol (especially beer), and dietary calcium intake (1000–1300 mg daily), as opposed to oral supplements.

**MRI and image analysis**

MRI scans were performed at baseline and at monthly intervals using a 1.5-T Siemens scanner. MRI scans were analyzed in a blinded fashion at the University of Pennsylvania using 3DVIEWNIX software and published approaches. Different tissue regions were delineated in the images as follows: In T2 and proton density (PD) images: brain parenchyma (BP), gray matter (GM), white matter (WM), and MS lesions (LS). The WM region was further divided into pure WM (PWM) and dirty or diseased WM (DWM) regions. Parameters were divided into two groups: (1) morphological (volumes of BP, GM, WM, PWM, DWM, T2 lesions, T2E lesions); and (2) intensity-based (for each tissue region intensity histogram in each of T2, PD, T1E, and magnetization transfer ratio [MTR] images was computed).

Changes in MR parameter values were tested from baseline to last month of treatment for both placebo and inosine groups. Baseline was considered as the month preceding the start of treatment.

**Quantitative reverse transcription-polymerase chain reaction**

Blood was collected into heparinized tubes and white blood cells were isolated by density centrifugation on Ficoll-Paque (Amersham Biosciences, Uppsala, Sweden). All details of RNA isolation, cDNA synthesis, and quantitative polymerase chain reaction using the Bio-Rad iCycler iQ Real Time Detection System (Hercules, CA) are described. Probes and primers for human gene-specific mRNAs are presented in Table 2 and primers for the corresponding standards in Table 3.

**Serum analyses**

Serum levels of cytokines, nitrotyrosine, and oxidative markers were determined using the following kits according to the manufacturers’ recommendations: 8-isoprostan, nitrotyrosine, and 8-hydroxy-2'-deoxyguanosine kits from (Oxis International, Foster City, CA), human interleukin (IL)-10 and IL-13 kits (Pelikine Research Diagnostics, Flanders, NJ), human granulocyte-macrophage colony-stimulating factor enzyme-linked immunosorbent assay (ELISA) kit (BD Pharmingen, San Jose, CA), human tumor necrosis factor-α and human interferon-γ ELISA kits (Research Diagnostics Inc., Concord, MA), soluble intercellular adhesion molecule–1 (sICAM-1) ELISA kit (R + D Systems, Minneapolis, MN). Lipid peroxidation was assessed as malondialdehyde (MDA) formation in sera by thiobarbituric acid reaction as described.

**Statistical analysis**

Evaluation of the significance of differences between parameters was performed using analysis of variance or
burden of gadolinium-enhanced lesion during the placebo phase as well as while taking inosine where serum UA levels were less than 7.0 mg/dL was significantly greater than lesion frequency when serum UA was above 7.0 mg/dL (p < 0.001, by Fisher’s exact test (two-sided) (Table 4). Detailed MRI analysis revealed a number of parameters that were significantly different between scans at baseline (Arm 2) or the final placebo assessment (Arm 1) and at the conclusion of inosine treatment. Seven intensity-based parameters showed moderate to strong correlation with the UA level, with an r value of 0.51-0.71 (Table 5). The highest correlations with UA levels were obtained for PD values in the DWM region (r = -0.71, p = 0.001). This implies that, as the UA levels are increased in WM regions that may have underlying demyelination but not yet apparent lesions, there may be reversal of this process. Eight (8) standardized PD parameters show moderate to high correlation with the EDSS scores (Table 6), with the highest value of r = 0.75, p = 0.001 observed for PD BP histogram 75th percentile. The parameters that correlated with both EDSS and UA levels are PD BP histogram peak location (r = -0.52, p = 0.06 for UA; r = 0.68, p = 0.003 for EDSS) and PD BP histogram 75th percentile (r = -0.54, p = 0.04 for UA; r = 0.75, p = 0.001 for EDSS).

Inflammatory markers

Levels of the inflammatory and oxidative markers studied varied considerably during the baseline/placebo and inosine phases of the study such that no significant difference in pooled data could be detected (data not shown). Variability was also extensive within individuals regardless of the stage of the trial. Nevertheless, in the individual in Arm 1 of the study who exhibited the greatest reduction in lesion burden between the placebo and inosine phases (averaging 10.2 ± 3.8 and 2.0 ± 1.5, respectively, p < 0.001 by the t test), significant differences were seen in several serum markers including nitrotyrosine and macrophage-1 antigen (Mac-1) mRNA levels (Table 7).

### Discussion

The main objective this study was to evaluate the safety and tolerability of the use of orally administered inosine to raise serum UA levels in patients diagnosed with MS. While it proved difficult to maintain target serum UA levels in some patients, inosine readily raised UA levels and was well tolerated in the majority of the subjects. The main concern was the development of kidney stones in 4 of the first 11 subjects. Conservative treatment, including immediate cessation of inosine treatment and improved hydration, was sufficient to promote rapid recovery without sequelae. The high incidence of kidney stones was somewhat surprising since we did not observe this complication during a previous trial involving 17 subjects and similar protocol. The principal difference between the two studies is in the nature of the subjects. Those of the first trial were ambulatory patients residing in their own domiciles without any control or monitoring of inosine compliance, diet, or liquid intake. Although their kidney stones were composed of UA, it was noted that all 4 subjects who developed this complication also took calcium supplements. Two (2) of these patients had very high blood UA levels at the time of kidney stone development (10 and 15 mg/dL), while the other 2 had UA levels within the target range (6.4 and 8.4 mg/dL). There are several factors that may contribute to the development of kidney stones during inosine treatment, including transient dehydration or acidification of urine. Another possibility is that some patients may be predisposed to the development of UA kidney stones as a consequence of alterations in the UA excretion/reuptake system responsible for maintaining UA levels. For example, this could occur through mutations in the gene encoding the UA transporter. We believe that dietary and hydration guidelines are important, and more frequent monitoring of serum UA levels than at monthly intervals would be of benefit, particularly if there is any change in dosage. The kidney cancer that was detected in the opposite kidney from one that developed a stone was an incidental finding picked up on ultrasound. The nephrologist did not feel that this was at all related to the inosine treatment, and the cancer was cured by surgery.


Address reprint requests to:
Hilary Koprowski, M.D.
Biotechnology Foundation Laboratories
at Thomas Jefferson University
1020 Locust Street
JAH, Room M-85
Philadelphia, PA, 19107
E-mail: hilary.koprowski@jefferson.edu