Efficacy of Azathioprine on Multiple Sclerosis
New Brain Lesions Evaluated Using Magnetic Resonance Imaging

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Background: Azathioprine is an immunosuppressive agent that reduces relapse rates in patients with multiple sclerosis (MS), but its efficacy in suppressing new brain lesions has never been evaluated.

Objective: To evaluate the efficacy of azathioprine therapy on new brain lesion suppression in MS.

Design: Open-label treatment vs baseline study.

Setting: Outpatient MS clinical center at a university hospital.

Patients: Fourteen patients with relapsing-remitting MS of short duration and at least 3 gadolinium-enhancing (Gd+) brain lesions observed within 6 months before treatment.

Intervention: Azathioprine, up to 3 mg/kg daily, individually adjusted according to blood lymphocyte number and the occurrence of adverse events.

Main Outcome Measures: Brain Gd+ lesions evaluated by monthly magnetic resonance imaging for 6 months before and 6 months during treatment and new T2 lesions evaluated during the same periods and after an additional 6 months.

Results: The treatment reduced to 0 the median Gd+ lesion number and volume per magnetic resonance image (P<.001 for both), resulting in a Gd+ lesion number reduction of 50% or more in 12 of 14 patients (P<.01). An equivalent reduction in the new T2 lesion number was observed (P<.02); this activity also persisted during the additional treatment period evaluated using this outcome measure (P<.01). The median azathioprine dose administered (2.6-2.8 mg/kg daily) reduced the mean blood lymphocyte count to 57% of the baseline value. Adverse events were transient or reversible with dose adjustment.

Conclusions: This study indicates for the first time that azathioprine, administered at lymphocyte-suppressing doses, is effective in reducing MS new brain inflammatory lesions and is well tolerated.

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Many clinical trials have demonstrated the efficacy of new medications, such as interferon (IFN) β, in modifying the clinical course and new brain lesion accumulation of patients with multiple sclerosis (MS), but in many patients these medications show no or little efficacy or are not well tolerated. Because autoimmune pathogenetic mechanisms against central nervous system white matter underlie the development of MS lesions, immunosuppressive medications have also been successfully used in the treatment of this disease. One such medication is azathioprine, a cytostatic agent that is well tolerated, is easy to administer and monitor, and that has been used for many years in the treatment of transplant rejections and autoimmune diseases. A variety of clinical trials that meet good quality criteria showed that azathioprine is effective in modifying the course of MS, and recent metaanalyses indicated that the efficacy of azathioprine on the relapse rate in patients with MS is equivalent to that of IFNs and Copl. However, the studies involving azathioprine were performed when brain magnetic resonance imaging (MRI) was not widely available for clinical trials or was not yet considered a valid end point for treatment evaluation. Since then, MRI markers of MS brain lesions have been accepted as valid outcome measures of the disease, but the effects of azathioprine on the frequency and accumulation of new brain inflammatory lesions has never been studied in MS.

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The present open-label treatment vs baseline study evaluates, for the first time, the efficacy of azathioprine therapy in suppressing brain new lesion numbers and accumulation observed by MRI in a cohort of patients with relapsing-remitting (RR) MS. This study shows that azathioprine exerts intensive suppression on these outcome measures, at well tolerated doses.

METHODS

PATIENTS

This study was performed in the MS Outpatient Clinical Centre and the Magnetic Resonance Imaging Centre of the Careggi Florence University Hospital and was approved by the local ethics committee. All the patients gave written informed consent. Inclusion criteria consisted of a clinically and laboratory-supported definite diagnosis of MS according to the criteria of Poser et al.11 RR disease form, age 18 to 55 years, 3 or more focal brain gadolinium-enhancing (Gd+) lesions in 6 brain MRIs previously performed, an Expanded Disability Status Scale score of 1.0 to 5.5, and a disease duration of less than 10 years. Exclusion criteria consisted of clinical relapses or corticosteroid therapy within 1 month or immunosuppressive therapy within 12 months of the baseline period entry and contraindications to performing MRI or to undergoing azathioprine treatment.

Sample size was established assuming at least 50% treatment effect on the total number of Gd+ lesions, as previously reported.10,11 Considering the observed frequency of this outcome measure in patients with a mean of 0.5 Gd+ lesion per MRI, power greater than 90%, at α = .05, 2-tailed, was obtained with 10 patients. The number of patients included (n=14) took into account the possibility of 20% dropouts. Characteristics of the 14 study patients (10 women and 4 men) were as follows (median and range): age, 29 years (23-53 years); disease duration, 5.5 years (1-9 years); Expanded Disability Status Scale score, 1.5 (1.0-3.0); and number of relapses in the past 2 years, 2 (1-4). The patients were followed up according to intention-to-treat analysis. No patient interrupted the treatment.

STUDY DESIGN

This open-label, baseline-controlled, crossover (treatment vs baseline) trial was designed as previously described.9,10,12 Patients who met the clinical inclusion criteria were followed up for 6 months with monthly brain MRIs (baseline period). Patients who showed at least 3 Gd+ lesions were included in the study and began treatment; during treatment, after a gap of 6 months (induction period), the patients again underwent 6 monthly brain MRIs (evaluation period); 1 more MRI was performed after an additional 6 months (extension period) from the end of the evaluation period. The Gd+ lesions observed during the evaluation period were compared with those observed during the baseline period. To allow the evaluation of new Gd+ lesions in MRI 1, MRI was performed 1 month before the beginning of the baseline and evaluation periods, but it was not included in the analysis. The new lesions observed in T2-weighted MRIs (nT2) during either the evaluation or the extension period were compared with the nT2 lesions observed during the baseline period. Efficacy was not evaluated during the induction period to avoid initial low drug activity due to the described slow azathioprine pharmacodynamics.13

As end points for the evaluation of treatment efficacy, outcome measures of brain inflammatory activity were selected a priori: the primary end point was a 50% or more total Gd+ lesion number reduction between the evaluation and baseline periods; the secondary end points were changes between the same periods in total Gd+ lesion volumes, new Gd+ lesion numbers per MRI distribution, and number of patients who showed a 50% or greater reduction in Gd+ lesion number (“responders”). Additional secondary end points for the evaluation of treatment efficacy were reductions in the cumulative nT2 lesion number and volume per patient during either the evaluation or the extension period with respect to baseline. The outcome measure for the evaluation of azathioprine biological activity was the peripheral blood lymphocyte number.

MRI EVALUATION

The number and volume of brain lesions were assessed as follows: total and new Gd+ lesions in T1-weighted MRIs performed monthly for 6 months during the evaluation and baseline periods and cumulative nT2 lesions on fast fluid-attenuated inversion recovery imaging performed at months 1 and 6 of the baseline, evaluation, and extension periods; the number of nT2 lesions was calculated in each patient as the difference of the total brain lesions observed between images 6 and 1.

The brain MRIs were performed and evaluated as previously described.9,10,12 Briefly, magnet (Gyroscope; Philips Research, Eindhoven, the Netherlands) power was 1.5 T; section thickness, 4 mm; no interslice gap; section orientation, axial; matrix, 236 x 256; field of view, 23.6 cm; 2 signals acquired; sequences without contrast, fast fluid-attenuated inversion recovery images; sequences with contrast, T1-weighted images before and after 15 minutes from contrast administration; and contrast medium, gadolinium (0.1 mmol/kg). The bicommissural axial section has been used for precise repositioning; evaluation of the MRIs was performed by 2 independent examiners (L.M. and A.B.) blinded to the study period (baseline, evaluation, or extension). The digitized images were visualized using a software program (Analyze 1.0; Mayo Foundation, Rochester, Minn) installed on a UNIX-based workstation. The volumes were calculated using semiautomatic segmentation and single “region growing” techniques. No violations of the MRI protocol occurred.

THERAPY

Patients received oral azathioprine, up to 3 mg/kg per day, individually adjusted according to the blood lymphocyte count and the occurrence of adverse events. The target dose was reached gradually within 2 months to prevent severe adverse events in low metabolizers. Lower doses were administered to patients with low blood lymphocyte counts (<900/mm3), National Cancer Institute Common Toxicity Criteria grade 3) or Common Toxicity Criteria grade 2 for other variables. In this case, the dose was individually titrated, reducing approximately 0.5 mg/kg per day (to the nearest 25 mg).

CLINICAL EVALUATION

At each scheduled examination and at exacerbations (as defined by Pozzilli et al14), medical and neurologic evaluations were performed, and the presence of possible adverse events by Common Toxicity Criteria was established. Clinical state, adverse events, blood cell counts, serum chemistry values, and concomitant treatments were also evaluated monthly (or when needed). Neurologic exacerbations were treated with 1 g of intravenous methylprednisolone sodium succinate daily for 3 days.

DATA ANALYSIS

Analysis of the brain MRI results were performed using non-parametric statistics, as described elsewhere.13 Accordingly, the

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data are reported as medians, but mean values are also included. Lymphocyte counts are reported as mean±SD and were analyzed using the *t* test.

**RESULTS**

**BRAIN LESIONS**

The median (99% CI) Gd+ lesion number per MRI was 2 (1-3) during the baseline period and 0 (0-1) during the evaluation period, equivalent to a mean±SD of 2.26±2.21 and 0.82±0.31 (−64%) (Table 1 and Figure 1). The Gd+ lesion volumes per MRI showed a similar reduction (Table 1 and Figure 2). Twelve of 14 patients had total Gd+ lesion number suppression to 50% or less (Table 1). During the baseline period, the median lesion number was stable across time (Figure 1). The distribution of new Gd+ lesions per MRI significantly changed during the evaluation period (Figure 3).

The cumulative nT2 lesion number and volume per patient during the evaluation and extension periods decreased consistently with the Gd+ lesions (Table 1, Figure 4, and Figure 5). Most patients showed a reduction of 50% or more in nT2 lesion number during the evaluation and extension periods (Table 1).

**LYMPHOCYTE COUNT, ADVERSE EVENTS, AND CLINICAL EVALUATION**

During the evaluation and extension periods, the mean lymphocyte count was 57% of that of the baseline period (Table 2). Six adverse events occurred during the treatment period, and 1 occurred during the extension period, showing an 83% reduction across time due to spontaneous and dose-associated reversibility (Table 2). Four patients experienced a relapse during the baseline period, 1 during the evaluation period, and 1 during the extension period (Table 2). Median neurologic disability did not change in any of the study periods (data not shown).

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**Table 1. Results of the Brain Lesion Evaluation Using Magnetic Resonance Imaging**

<table>
<thead>
<tr>
<th>End Point</th>
<th>Baseline</th>
<th>Evaluation</th>
<th>Extension</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gd+ lesions per MRI, No.</td>
<td>2 (1 to 3)</td>
<td>0 (0 to 1)*</td>
<td>ND</td>
</tr>
<tr>
<td>Median (99% CI)</td>
<td>NA</td>
<td>−2 (−1 to −2)</td>
<td>ND</td>
</tr>
<tr>
<td>Mean ± SD (%)</td>
<td>2.26 ± 2.21</td>
<td>0.82 ± 0.31 (−64%)</td>
<td>ND</td>
</tr>
<tr>
<td>Gd+ lesions per patient, No.</td>
<td>12 (4 to 24)</td>
<td>3 (1 to 9)</td>
<td>ND</td>
</tr>
<tr>
<td>Median (99% CI)</td>
<td>NA</td>
<td>−10 (−3 to −14)</td>
<td>ND</td>
</tr>
<tr>
<td>Gd+ lesion volume per MRI, mm³</td>
<td>300 (144 to 404)</td>
<td>0 (0 to 52)*</td>
<td>ND</td>
</tr>
<tr>
<td>Median (99% CI)</td>
<td>NA</td>
<td>−272 (−144 to −338)</td>
<td>ND</td>
</tr>
<tr>
<td>Mean ± SD (%)</td>
<td>382 ± 118</td>
<td>131 ± 64 (−66%)</td>
<td>ND</td>
</tr>
<tr>
<td>nT2 lesion number per patient</td>
<td>7 (2 to 9)</td>
<td>2 (0 to 4)†</td>
<td>1 (0 to 4)‡</td>
</tr>
<tr>
<td>Median (99% CI)</td>
<td>NA</td>
<td>−4 (−1 to −8)</td>
<td>5 (0 to −9)</td>
</tr>
<tr>
<td>nT2 lesion volume per patient, mm³</td>
<td>868 (117 to 2200)</td>
<td>223 (0 to 1050)§</td>
<td>58 (0 to 460)</td>
</tr>
<tr>
<td>Median (99% CI)</td>
<td>NA</td>
<td>−341 (+18 to −1990)</td>
<td>−750 (−0 to −1990)</td>
</tr>
<tr>
<td>Patients with ≥50% reduction, No./total No.</td>
<td>Gd+ lesions</td>
<td>NA</td>
<td>12/14¶</td>
</tr>
<tr>
<td>nT2 lesions</td>
<td>NA</td>
<td>9/14¶</td>
<td>10/14¶</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; Gd+, gadolinium enhancing; MRI, magnetic resonance image; NA, not applicable; ND, not detected; nT2, new T2.

*P<.001, by Wilcoxon signed rank test.
†P<.02, by Wilcoxon matched pairs test.
‡P<.01, by Wilcoxon matched pairs test.
§P<.05, by Wilcoxon matched pairs test.
||P<.006, by Wilcoxon matched pairs test.
¶P<.01, by Newcombe test.

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*Figure 1.* Gadolinium-enhancing (Gd+) lesion numbers at each month of the baseline and evaluation periods in 14 patients with relapsing-remitting multiple sclerosis. Azathioprine treatment, administered starting from the induction period, significantly decreased the number of lesions (*P*<.001 by Wilcoxon signed rank test). During the baseline period, the lesion number per month did not decrease across time. Error bars represent the upper quartiles.

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The results of this study show, for the first time, that the immunosuppressive agent azathioprine suppresses new brain lesions evaluated using MRI in patients with RRMS. This result corroborates the previous clinical data obtained through randomized controlled trials, which showed a reduction in the relapse rate during azathioprine therapy. In addition, the present result is consistent with an MRI trial performed with identical design and end points that included 6 patients with MS receiving IFN therapy, nonresponding to the treatment. A previous retrospective evaluation of azathioprine activity on total T2 lesion load changes in a large RRMS patient cohort is also consistent with the present study, as lesion load is an outcome measure that closely depends on Gd lesions. The dimension of the new brain lesion suppression observed in the present study is similar to that observed with different IFNs using the same study design, indicating that the treatment effect of azathioprine on brain lesion accumulation is probably equivalent to that of these medications and suggesting that phase 3 clinical trials...
Directly comparing the efficacy of azathioprine with that of IFNs are worth planning.

Suppression of the cumulative nT2 lesion number and volume was equivalent to that of the Gd+ lesions, but allowing treatment activity analysis for an additional 6 months confirmed the efficacy for a whole year. The demographic and clinical characteristics of the patients were typical, suggesting that the results obtained can probably be extended to a larger RRMS population with new brain lesion activity.

Adverse events were observed mainly at the beginning of the evaluation period in 6 patients, but they were transient or reversed after dose reduction, and no patient interrupted the therapy. The treatment was well tolerated for the rest of the study (approximately 1 year), indicating that, overall, treatment compliance was good. Further adverse events, and particularly the most severe ones, probably were prevented by the careful individual dose adjustment.

Neurologic disability was stable, and the relapse rate decreased consistently with the new brain lesion rate. The study was not powered to evaluate clinical outcomes; however, these results confirm the previously reported azathioprine efficacy on the relapse rate5-7 and suggest that the clinical activity of this medication is mediated by the suppression of new brain lesions. In this study, the median number of Gd+ lesions per patient was stable across the baseline period, confirming that this trial design can minimize the regression of extreme values to the mean, as previously reported.9,10

In conclusion, the present study supports the use of azathioprine in clinically active and MRI-active RRMS with low disability and short disease duration. Indeed, the treatment induces remarkable new brain lesion reduction, stable for 12 months. This activity was obtained at doses that can be well tolerated and that are associated with low circulating lymphocyte numbers. If considered in the context of previous clinical trials, the present study indicates that azathioprine may represent an alternative to immunomodulatory medications specifically approved for RRMS.

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Statistical analysis: Massacesi. Obtained funding: Massacesi and Amaducci. Administrative, technical, and material support: Parigi, Barilaro, Repice, Pellicano, Konze, and Taiuti.

Study supervision: Massacesi and Amaducci.

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