Azathioprine and Steroids Are Not More Effective in Decreasing Multiple Sclerosis Intra–Blood-Brain-Barrier IgG Synthesis than Steroids Alone

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Intra–blood-brain-barrier IgG synthesis rates and oligoclonal IgG banding patterns were examined in 9 patients with multiple sclerosis who were treated with azathioprine and steroids for 2 to 4.5 years. The IgG synthesis rates of 5 patients were significantly decreased from the pretreatment mean values 1 month after treatment, and their synthesis rates remained at the decreased levels throughout treatment. However, among the remaining 4 patients, the rates exceeded the pretreatment means. This continuous suppressive effect of the combined azathioprine and steroids upon the IgG synthesis rate was similar to that of steroids, suggesting that azathioprine and steroids in combination were not more effective in reducing intra–blood-brain-barrier IgG synthesis than steroids alone. Oligoclonal IgG patterns in all cerebrospinal fluid samples were not significantly altered during the study.

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Numerous immunosuppressive drugs have been administered to patients with multiple sclerosis in an attempt to control the disease [1,2]. Among the treatments, adrenocorticotropic hormone (ACTH) and corticosteroids are considered somewhat effective in temporarily reducing clinical symptoms and in producing a marked and lasting reduction of intra–blood-brain-barrier (BBB) IgG synthesis [1, 7]. Since elevated intra-BBB IgG synthesis is a by-product of inflammatory demyelination that characterizes multiple sclerosis, treatments that can normalize the synthesis may be of value. Azathioprine without steroids has been reported not to alter the intra-BBB IgG synthesis rate or oligoclonal IgG pattern [10]. In our preliminary trial, we sought to determine whether azathioprine enhances the effects of steroids on the synthesis rate and oligoclonal pattern.

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Materials and Methods

Patients

Nine patients with clinically definite multiple sclerosis [4] and either frequent relapses (three or more within the preceding 24 months or two within the preceding 12 months) or progressive worsening of neurological function over the preceding 6 months were entered into the study.

Treatment

Azathioprine was administered to these 9 patients in three to four divided oral doses, starting at 2 to 2.5 mg per kilogram of body weight per day and increased by 25-mg increments at monthly intervals to a dose that would lower total leukocyte counts to 3,000 to 4,000 per mm^3 without producing serious adverse effects. In addition, these patients received methylprednisolone intravenously, 1 gm in 250 ml D5W, administered over 20 to 30 minutes on 3 consecutive days, once a month for the first 3 months. They also received prednisone orally as a single morning dose every other day for 4 weeks starting with a 100 mg dose that was tapered over the subsequent 6 months. For acute exacerbations, 2 patients also received ACTH for 2 weeks [5]. Three of the 9 patients resumed continuous alternate-day oral prednisone because of progression of their disease.

Cerebrospinal Fluid and Serum Analysis

Cerebrospinal fluid (CSF) and serum were obtained just before treatment, then monthly for the first 3 months, and every 3 months thereafter during treatment (2 to 4.5 years). IgG and albumin concentrations were quantified in fresh unfrozen CSF and serum by electroimmunodiffusion [9], and the daily rate of intra-BBB IgG synthesis was calculated [6]. Specimens were stored at −70°C until oligoclonal band analysis. IgG from unconcentrated CSF, 0.5 μg, and diluted serum were applied to polyacrylamide gels (LKB PAG plate, pH 3.5 to 10), and the gel was focused for a total of 2,000 volt-hours in 2 hours. The samples were then immunofixed for 2 hours using rabbit antihuman IgG (Dako) and silver-stained using modified Oakley procedures [8]. All specimens from a given patient were analyzed simultaneously on the same gel by isoelectric focusing. Oligoclonal bands were defined as IgG bands unique to CSF or more intense in CSF than in serum.

Results

For the 9 patients in the study, the intra-BBB IgG synthesis rate during pretreatment ranged from 8 to 115 mg/day (Table). In 5 of 9 patients (Nos. 7004,

Intra–Blood-Brain-Barrier IgG Synthesis Rate[a] in Multiple Sclerosis Patients Treated with Azathioprine and Steroids

Patient No.	Pretreatment Mean (No. of Samples)	Treatment		
		1 Mo	6 Mo	2 Yr
7004	8 (3)	2	−1	−2
6596[b,c]	15 (1)	12	20	17
7007[b,c]	18 (1)	13	15	36
7006[d]	16 (3)	22	15	19
5354[b,d]	23 (2)	21	22	43
6226	25 (3)	8	7	9
6947[c]	31 (3)	16	11	19
4469	51 (2)	13	22	36
6828	115 (3)	38	37	59

[a]In mg/day.
[b]Patient taking prednisone before azathioprine administration.
[c]Patient resumed taking prednisone.
[d]Patient received adrenocorticotropic hormone before or during treatment.

6226, 6947, 4469, and 6828), the synthesis rate during treatment dropped below pretreatment values. For the remaining 4 patients, the rates during treatment rose above the pretreatment values. The greatest and most steady decrease in synthesis rate was observed in the patient who had an initially high synthesis rate (Patient 6828). Three of 9 patients received prednisone before the combined azathioprine and steroid treatment. Their synthesis rates increased during treatment. Of the 3 patients who resumed taking prednisone, 1 (Patient 6596) returned to about the pretreatment value after an initial reduction, and the other (Patient 7007) had a synthesis rate markedly exceeding the pretreatment baseline at the end of the experiment (2 years). No patient had a change in oligoclonal banding patterns during the treatment.

Discussion

Combined azathioprine and steroid treatment had a continuous suppressive effect on intra-BBB IgG synthesis in 5 of 9 patients. These results are similar to those of a previous study on the effect of steroids alone [7]. Azathioprine administered to patients with multiple sclerosis has been reported to decrease IgG synthesis by peripheral blood lymphocytes in vitro [3]. However, azathioprine alone has not been found to decrease the intra-BBB IgG synthesis rate [10]. Our results indicate that combined azathioprine and steroids do not enhance the suppression of intra-BBB IgG synthesis, which can be achieved by steroids alone.

Wurster and Patzold [10] also reported changes in the oligoclonal banding pattern in some of their patients, but attributed these changes to storage of the samples at −20°C and accidental thawing. In contrast, there were no qualitative banding pattern changes in our samples, which were stored from 2 months to 5 years at −70°C. In addition, we have shown that repeated freezing and thawing (up to 9 times) had no apparent effect on IgG banding patterns, although the staining intensity decreased overall [Tourtellotte and co-workers, in preparation]. These data suggest that CSF and serum obtained during long-term clinical trials should be stored at −70°C, and that repeated freezing and thawing should have little effect on the results of isoelectric focusing.

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