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Pilot study to examine the effect of antibiotic therapy on MRI outcomes in RRMS

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Abstract

This trial examined the safety and possible MRI and clinical effects of anti-chlamydial antibiotic therapy in relapsing–remitting MS (RRMS). Newly diagnosed MS patients were selected to participate if they showed *Chlamydia pneumoniae* gene in their CSF and had one or more enhancing lesions on brain magnetic resonance imaging (MRI). After a 4-month run in phase of monthly MRI, patients were randomized to receive rifampin (300 mg twice daily) and azithromycin (500 mg every other day) for 6 months or placebo (PBO). Patients then had monthly MRI on therapy and two additional scans on months 12 and 14. Lumbar punctures were repeated between months 7 and 8 and within 2 weeks of termination of the study. Data on 4 patients on treatment and 4 on PBO were available for analysis. The primary outcome measure of showing a beneficial effect on enhancing lesions was not met. However, there was a significant difference in brain parenchymal fraction loss favoring those patient receiving antibiotics compared with PBO ($p \leq 0.02$). Three of the four patients on antibiotic therapy cleared the organism from the CSF by month 12; in the PBO group one patient cleared the organism. The reduction in atrophy in patients receiving antibiotics must be viewed with caution, due to the small number of patients studied.

Keywords: *C. pneumoniae*; RRMS; Antibiotic therapy; MRI

1. Introduction

We have previously reported the prevalence of an intracellular organism, *Chlamydia pneumoniae*, in the cerebrospinal fluid (CSF) of patients with MS [1,2]. We and others have suggested that *C. pneumoniae* may act as a co-factor in the disease [1,3,4]. Other groups have confirmed our observations on the PCR based assays for the detection of *C. pneumoniae* in the CSF while some have not [5–13]. These inconsistencies might be due to methodological differences in the isolation and detection of the *C. pneumoniae* organism and DNA that are unlikely to be reconciled without some consensus regarding current methods or the development of better technology [14]. To examine a causal relationship between the presence of *C. pneumoniae* in the CSF and MS, a double-blind, placebo-controlled pilot study of oral antibiotic therapy in RRMS was performed.

2. Methods

2.1. Patient criteria for inclusion and exclusion

After obtaining approval from the IRB, RRMS patients with an EDSS of < 4.5, a positive PCR signal for *C. pneumoniae* in CSF and at least one enhancing lesion on a brain MRI at entry were enrolled [15]. Patients with potential allergy to antibiotics and those who did not fulfill the above criteria were excluded from the study. A total of

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30 patients were screened and 8 fulfilled entry criteria and were enrolled into the trial. All eight patients were women, mean age 36.8±6 and mean EDSS of 1.7.

2.2. Trial design

A double blind, placebo controlled, parallel group design was used. The study consisted of a 4-month run in phase of monthly MRI. Patients were randomized to either receive placebo (PBO) or antibiotics (rifampin 300 mg twice daily and azithromycin 500 mg every other day) for 6 months. Patients received monthly MRI on therapy until month 10. Study medication was then stopped and two additional MRI were done on months 12 and 14. Patients had repeat CSF analyses done between months 7 and 8 and within 2 weeks of termination of the study. Patients in the PBO arm of study received riboflavin 100 mg as a blind for the discoloration of urine seen following rifampin therapy. The primary outcome measure was a decrease in the number and volume of gadolinium (Gd) enhanced lesions during the period when the patients were on study medication. Secondary end point measures were a change in the clinical disability scores and the clearance of the *C. pneumoniae* gene in the CSF. Patients were followed with 3 monthly neurological exams and blood profiles to detect abnormalities in hematology and liver profiles.

2.3. MRI analysis

Patients were imaged at Vanderbilt University on a General Electric 1.5 Tesla scanner. Conventional spin echo T1-weighted sagittal images were acquired to aid in locating the region for the transaxial AFFIRMATIVE series [16]. This flow compensated, dual fast spin echo pulse sequence incorporates both fluid attenuated inversion recovery (FLAIR) and magnetization transfer (MT) and generates a set of four images for each slice location. The acquisition parameters for the AFFIRMATIVE images were 42 slices, 3 mm slice thickness, interleaved, spin-density, T2-weighted, and FLAIR with MT at two echo times; image matrix 256×256, TE=17/102 ms, echo train length=8, TI=2500 ms, TR=10 s, NEX=1. Spin echo sequence with MT pulse was used for acquiring pre- and post-contrast T1-weighted images with the following parameters: flip angle for MT pulse=700°, TR=800 ms, NEX=0.5 were obtained before and 5 min after the injection of 0.1 mmol/kg gadopentate dimeglumine. Image data was sent on magnetic storage media by courier to the MRI-AC at the University of Texas Health Science Center at Houston for analysis. The following parameters were analyzed, number and volume of Gd-enhanced lesions, number and volume of white matter plaques, volume and number of T1 hypointense lesions, gray and white matter volume, brain fraction, and construction of the Z4 MRI composite.

2.4. Image analysis

Enhanced lesion numbers and volumes were determined by expert observer using computer assisted seed growing algorithms. The post-acquisition processing steps incorporate radiofrequency inhomogeneity correction, removal of extrameningeal tissue and registration to a standard coordinate system and spatial normalization. The automated segmentation of the post-processed images uses a single Parzen map based on various combinations of the post-processed AFFIRMATIVE images for segmenting all patient images acquired across the study. The multi-spectral technique allows an estimate of the fraction of lesions showing increased amounts of loss of tissue integrity. The segmentation strategy included an expert step to remove false lesion identification due to coherent and incoherent flow artifacts. The methodology has been previously detailed [18,17]. Total segmented tissue volumes and individual lesion volumes assigned to registered brain coordinates for each subject's data were stored in a database developed for this purpose.

2.5. Statistical analysis

The data files were converted to a compatible file format integrated into a comprehensive clinical and MRI database and analyzed using SAS1 software (SAS Institute, Cary, NC, USA). Data analysis included descriptive statistics and statistical inference. The continuous variables were examined using one-way analysis of variance or student t-test when appropriate. The Z4 composite scores were constructed as previously described, with the exception that the individual component *Z* scores for the enhanced tissue volumes, total plaque volumes, hypointense plaque volumes, and normalized CSF volumes were developed based on the rank transformations of the data for each of these parameters for all subjects rather than on their means [18]. This was done to better account for the non-normal distribution of several of the components of the Z4 composite created by the large proportion of scans without enhancements. Data were analyzed as three separate epoch unless otherwise stated; run in phase, on antibiotic treatment phase, and post antibiotic treatment period.

PCR for presence of *C. pneumoniae* in the CSF. The PCR assay to detect *C. pneumoniae* MOMP DNA, was done using a nested PCR assay as described previously using similar running conditions [11].

3. Results

3.1. Patients

A cohort of 20 patients in each treatment arm was originally planned. However, changing patterns in disease

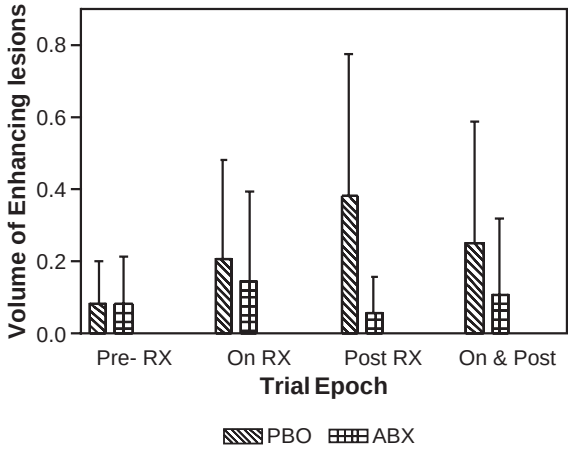

Fig. 1. Mean volume of enhancing lesions seen in during the three epochs in MS patients treated with placebo and those treated with antibiotics PBO=placebo group; ABX=antibiotic treated group; Pre RX=pre-randomization period, months 1–4; On RX=Treatment period months 5–10; Post RX=Months 10–14.

management made recruitment of patients with active disease into a PBO controlled trial difficult. Given the difficulties encountered in patient enrollment, the DSMB recommended that the trial be stopped after the eighth patient exited the study. Overall 30 patients were screened, 4 patients were randomized to PBO and 4 to antibiotic therapy. One patient on antibiotics became pregnant at month 11, and MRIs on months 12 and 14 were not performed. There were no relapses in any of the patients and no adverse event in either the antibiotic treated or placebo treated groups. Patients tolerated study medication well and there were no significant changes in the liver and hematological profiles.

3.2. Effect of antibiotic on clearance of PCR signal from CSF

In 7 of 8 patients CSF was obtained at month 0, between months 7 and 8 and within 2 weeks of termination. Three of four patients on antibiotics cleared *C. pneumoniae* from the CSF upon termination of the treatment phase of the study. In three of four PBO treated patients, CSF remained positive for *C. pneumoniae* at entry and on the lumbar puncture done following completion of the treatment phase of the study. One patient in the PBO arm cleared the organism.

3.3. Effect of antibiotic treatment on enhancing MRI lesions

There was no statistical difference in either the number or the volume of the enhancing lesions noted when comparing the three different epochs (pre-treatment, on treatment and post treatment); the primary outcome measure of the study was not met. Three of four patients who received antibiotics showed a decrease in the volume of enhancing lesions, while in the fourth patient in whom there was no decrease, scans at months 12 and 14 were not done. Two of four patients in the placebo arm showed an increase in volume, threefold over that seen during the run in phase (Fig. 1).

3.4. Effect of antibiotic treatment on brain parenchymal fraction

There was a decrease in the brain parenchymal fraction in all the 4 PBO subjects (Fig. 2). The mean decrease in volume was 1.4% in the PBO group while in the treatment group it was 0.2%. There was a statistical difference in volume loss at months 12 and 14 (post treatment epoch) when compared with that seen in the pre-treatment in those patients who were on PBO ($p<0.02$); this was not seen in patients receiving antibiotics (Fig. 2).

3.5. Analysis of Z4 composite between the placebo and antibiotic treatment group

The Z4 composite scores were constructed to represent values for enhanced tissue volumes, total plaque volumes,

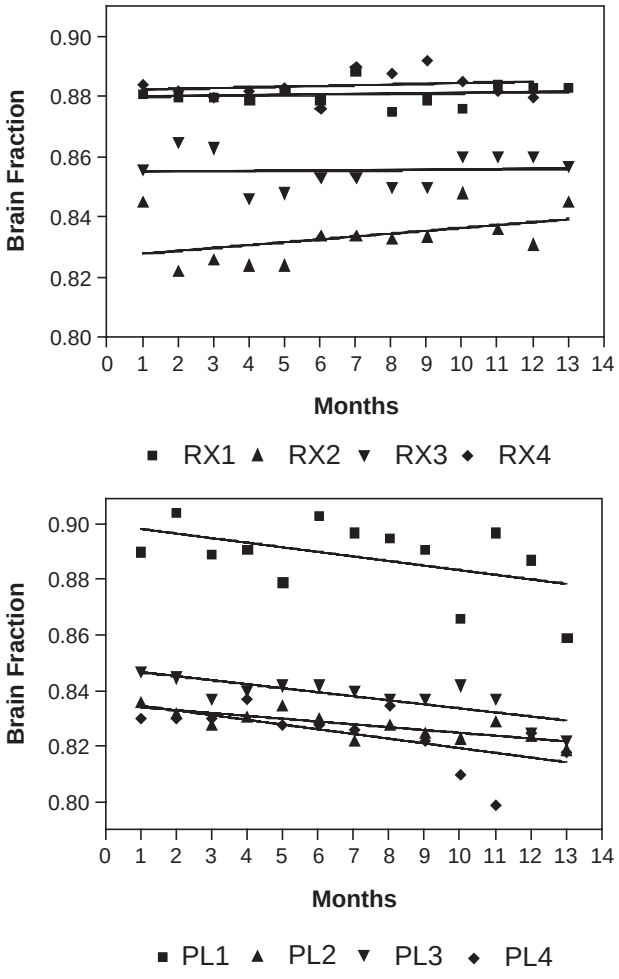

Fig. 2. Brain parenchymal fraction in MS patients treated with antibiotics upper panel and those treated with placebo (lower panel). Lines represent linear regression curves for the progression of atrophy.

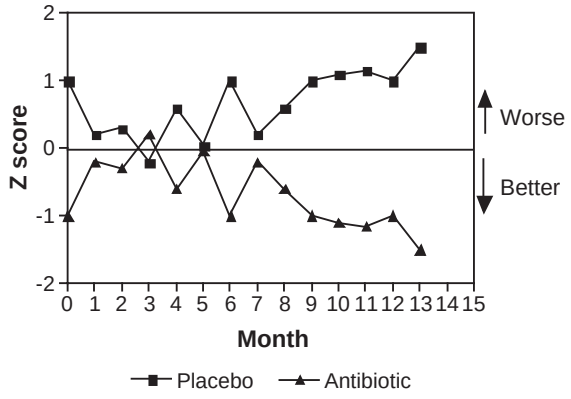

Fig. 3. Z4 values of the placebo and antibiotic treated groups throughout the study. Positive values represent a worsening of MRI measures of disease activity and negative values represent improvement.

hypointense lesion volumes and normalized CSF volumes. An increase in *Z* score reflects a worsening of MRI measures of disease burden while a lowering of Z4 score suggests an improvement. There was an increase in the Z4 values for the PBO group from 0.459 ± 0.641 to 0.911 ± 1.77 while the group that received antibiotics showed a decrease in the Z4 values from -0.459 ± 2.6 to -1.21 ± 1.61. These values did not reach statistical significance but suggested a trend toward benefit of therapy with antibiotics (Fig. 3). The improvement in the Z score was not driven by a single patient but essentially by the stabilization in atrophy scores.

4. Discussion

The primary outcome measure of the number of enhancing lesions was not significantly altered with antibiotic therapy. However, a delayed reduction in volume of enhancing lesions was seen in a post hoc analysis in three of four patients on antibiotics. Moreover, anti-chlamydial therapy may have stabilized brain atrophy over a 14-month period in this small number of patients with RRMS. Treatment was safe and well tolerated over 6 months. There was no difference in the clinical outcomes of EDSS and relapse rate.

In spite of the small number, we believe that these preliminary study suggests unexpected stabilization in brain atrophy. These results mirrors the results of a study done with RRMS and scheduled pulsed intravenous methylprednisolone (IVMP) [19]. Although macrolide antibiotics are known to possess anti-inflammatory properties, the anti-inflammatory properties of azithromycin are weak [20]. Azithromycin, unlike roxithromycin does not impair the cytokine response to lipopolysaccharide and did not decrease joint swelling in carrageen induced paw edema in rats [21,22]. Anti-infective therapy likely impacts brain atrophy through a different process than IVMP and its mechanism remains unexplained. Larger studies will be needed to confirm this treatment effect. Given the difficulties in performing double blind placebo controlled studies, we would suggest that a larger trial is feasible as a combination therapy with existing therapies.

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