

Combination Therapy With Interferon Beta-1a and Doxycycline in Multiple Sclerosis

An Open-Label Trial

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Objective: To evaluate the efficacy, safety, and tolerability of combination therapy with intramuscular interferon beta-1a and oral doxycycline, a potent inhibitor of matrix metalloproteinases, in patients with relapsing-remitting multiple sclerosis (RRMS) having breakthrough disease activity.

Design: Open-label, 7-month trial.

Setting: Louisiana State University Health Sciences Center, Shreveport.

Patients: Fifteen patients with RRMS taking interferon beta-1a with breakthrough disease activity took doxycycline for 4 months. Patients underwent monthly neurologic examination, magnetic resonance imaging of the brain using triple-dose gadolinium, and safety blood work.

Interventions: Ongoing treatment with intramuscular interferon beta-1a plus oral doxycycline, 100 mg daily, for 4 months.

Main Outcome Measures: The primary end point was gadolinium-enhancing lesion number change, and the secondary end points were relapse rates, safety and tolerability of the combination of interferon beta-1a and doxycycline in patients with MS, Expanded Disability Status

Scale score, serum matrix metalloproteinase-9 levels, and transendothelial migration of monocytes exposed to serum from patients with RRMS.

Results: Combination of doxycycline and interferon beta-1a treatment resulted in reductions in contrast-enhancing lesion numbers and posttreatment Expanded Disability Status Scale values ($P < .001$ for both). Only 1 patient relapsed. Multivariate analyses indicated correlations between decreased serum matrix metalloproteinase-9 levels and enhancing lesion activity reduction. Transendothelial migration of monocytes incubated with serum from patients with RRMS undergoing combination therapy was suppressed. Adverse effects were mild; no adverse synergistic effects of combination therapy or unexpected adverse events were reported.

Conclusions: Combination of intramuscular interferon beta-1a and oral doxycycline treatment was effective, safe, and well tolerated. Controlled clinical trials in larger cohorts of patients with MS are needed to evaluate the efficacy and tolerability of this combination.

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MULTIPLE SCLEROSIS (MS) is an immune-mediated disorder that affects genetically susceptible individuals after exposure to certain, as yet unidentified environmental antigens.^{1,2} The neuropathogenesis of MS involves destructive inflammation and progressive neurodegeneration.^{2,3} Several neuropathologic studies^{2,3} have demonstrated that these 2 mechanisms run a parallel course from disease onset. At least at the initial stages, the neuropathologic mechanism of MS consists of the interaction of activated leukocytes with cerebral endothelial cells, which ultimately leads to binding of the leuko-

cytes to the underlying endothelial bed, degradation of the endothelial basement membrane, and transendothelial migration of leukocytes into the central nervous system.⁴

Proteolysis of the endothelial basement membrane and the extracellular matrix (ECM) is essential in the movement of activated leukocytes from the periphery to the central nervous system⁵; this step later translates into the formation of perivenous demyelinating lesions. Among ECM-degrading enzymes, one important family is the matrix metalloproteinases (MMPs).⁵⁻⁷ Members of this family of endopeptidases participate in ECM remodeling in physiologic and pathologic con-

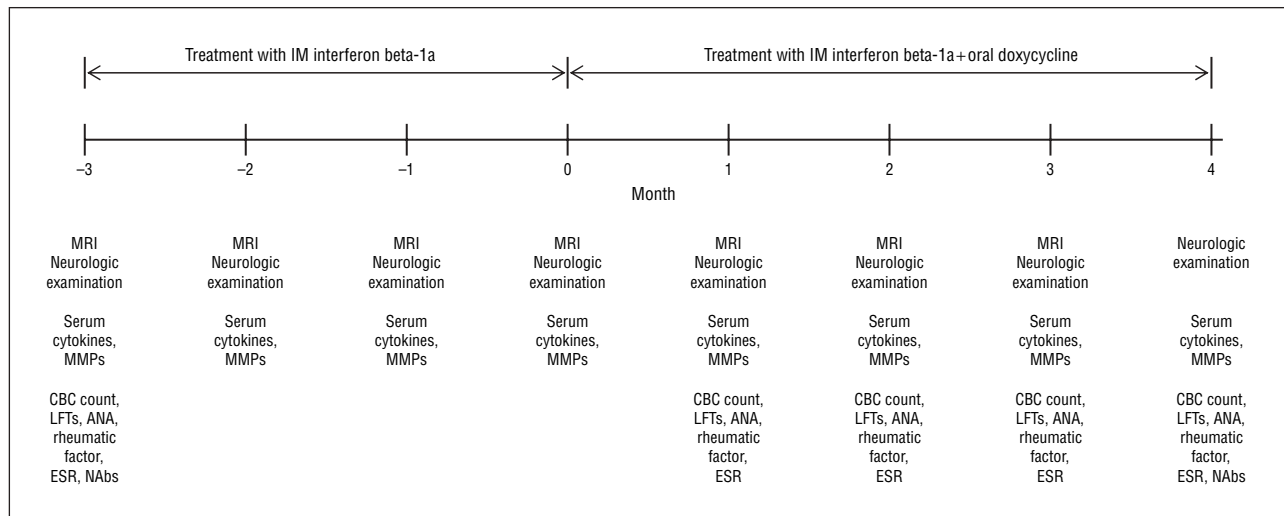


Figure 1. Study design. ANA indicates antinuclear antibody; CBC, complete blood cell; ESR, erythrocyte sedimentation rate; IM, intramuscular; LFTs, liver function tests; MMPs, matrix metalloproteinases; MRI, magnetic resonance imaging; and NABs, neutralizing antibodies.

ditions. A variety of studies⁸⁻¹¹ have demonstrated the significant roles of MMPs in the pathogenesis of MS. In the pathogenesis of MS, MMPs are involved in the transendothelial migration of leukocytes and degradation of the ECM,^{12,13} demyelination and epitope spreading, and neuronal loss.^{14,15} In addition, MMPs can render immunomodulatory therapy in MS ineffective by degrading interferon (IFN) β .¹⁶ Therefore, inhibition of MMP activity or decreasing MMP levels may be a potential mechanism to control the inflammatory cascade in MS.¹⁷

There is growing interest in using members of the tetracycline family of antibiotics for treating various diseases with an inflammatory pathogenesis; besides anti-MMP inhibitory actions, these tetracyclines also have immunomodulatory and neuroprotective effects.¹⁸ Recently, 2 members of this family have been investigated for their efficacy in the treatment of MS: minocycline¹⁹ and doxycycline.²⁰ Doxycycline has been used previously to suppress MMP-9 activity in endothelial cell cultures.²¹

Many patients with relapsing-remitting MS (RRMS), despite taking interferon beta-1a regularly, experience breakthrough disease activity, which clinically presents with relapses and neuroradiologically manifests with development of new contrast-enhancing lesions on brain magnetic resonance imaging (MRI). We hypothesized that the combination of interferon beta-1a and oral doxycycline is effective and safe for the treatment of MS. Effects of the combination of interferon beta-1a and oral doxycycline on relapse rate, brain MRIs, and serum levels of MMP-9 were measured. In addition, using an *in vitro* model, we studied the effect of serum specimens from patients with MS on transendothelial migration of monocytes before and after initiation of combination therapy.

METHODS

This open-label, single-center study was approved by the institutional review board of Louisiana State University Health Sciences Center. All the patients provided informed consent. Men and women (age range, 18-55 years) with RRMS who had received interferon beta-1a intramuscularly continuously for

at least 6 months and were experiencing breakthrough disease activity (annualized relapse rate ≥ 2 during interferon beta-1a therapy, with the most recent relapse within 60 days of study entry, and Expanded Disability Status Scale [EDSS] score of 1.5-4.5) were eligible. Eligible patients had 1 or more gadolinium-enhancing (Gd+) lesions on their baseline brain MRI (month -3). Patients who had received monoclonal antibodies, systemic corticosteroids, or immunosuppressive drugs in the previous 6 months were excluded, as were patients who had a positive result for serum neutralizing antibodies. Serum levels of neutralizing antibodies against interferon beta-1a were measured at the beginning and end of the study (Biogen Idec Inc, Cambridge, Massachusetts) using a 2-step enzyme-linked immunosorbent assay cytopathic assay.

Eligible individuals were evaluated monthly for 3 months while taking intramuscular interferon beta-1a, 30 μ g weekly (before the addition of oral doxycycline), then monthly for 4 months while receiving intramuscular interferon beta-1a, 30 μ g, and oral doxycycline, 100 mg daily (**Figure 1**). Each visit included a neurologic examination, recording of adverse effects, and determination of the EDSS score. After initiation of the combination therapy, safety tests were performed during each follow-up visit and monthly safety evaluations were performed, including a complete blood cell count with a differential count, a comprehensive serum chemistry panel, and a urine pregnancy test.

MRI PROTOCOL

Brain MRIs (3-mm-thick sections) were obtained using a 1.5-T scanner. The T2-weighted, fluid-attenuated inversion recovery, and axial postcontrast T1-weighted images were obtained before and 15 minutes after intravenous administration of triple-dose gadolinium. A neuroradiologist (E.G.-T.) blinded to patients' clinical data counted the number of contrast-enhancing lesions on brain MRIs. The MRIs were obtained a minimum of 30 days after intravenous methylprednisolone sodium succinate administration to prevent corticosteroid masking of Gd+ lesions.

STUDY DRUG AND INTERVENTION

Patients with RRMS who were taking intramuscular interferon beta-1a were treated with doxycycline, 100 mg orally, once

daily. Patients were allowed to take acetaminophen or other nonsteroidal anti-inflammatory agents before weekly injections of interferon beta-1a. In cases of neurologic relapse during the study, patients were allowed to receive methylprednisolone, 1000 mg intravenously, once daily for 5 days.

MEASUREMENT OF SERUM MMP-9, TUMOR NECROSIS FACTOR α , AND IFN- γ LEVELS

Serum specimens from patients with MS were collected at months -3 and 4. The serum MMP-9, tumor necrosis factor α , and IFN- γ levels were measured using enzyme-linked immunosorbent assay (Linco Diagnostics, St Charles, Missouri).

TRANSENDOTHELIAL MIGRATION OF MONOCYTES

Brain microvascular endothelial cells (ACBRI 128; Cell Systems, Kirkland, Washington) were cultured as previously described.²² Briefly, brain microvascular endothelial cells were plated in 6.5- μ m-pore Transwell multiwell cultures precoated with collagen (Corning Inc, Corning, New York).^{23,24} Control (n=7) and pretreatment and posttreatment MS serum specimens (n=15) were added (20% vol/vol) and incubated for 18 hours. Subsequently, U937 monocytic cells (1×10^5 per well) were added and incubated for 6 hours. Filtrates were then collected and evaluated by means of flow cytometry for the presence of U937 monocytes, as previously described.²⁴ All experiments were conducted in triplicate.

STATISTICAL ANALYSIS

The mean number of Gd+ lesions during each treatment period was calculated for each patient as the total number of Gd+ lesions observed across all images divided by the number of images. Hence, the mean number of Gd+ lesions per patient represents the number of lesions per MRI. Since the distributions of within-patient changes in the mean number of Gd+ lesions and average EDSS scores were skewed, these variables were analyzed using a Wilcoxon signed rank test. A paired *t* test was used to assess changes in MMP-9 levels and the number of U937 monocytes migrating through the cerebral endothelial monolayer. All analyses were performed as 2-tailed tests. A multivariate analysis was performed using a rank-based analysis of covariance to assess the association between changes in Gd+ lesions and the following variables: age, sex, duration of MS, duration of interferon beta-1a treatment, EDSS score, the number of Gd+ lesions at study entry, and serum MMP-9 level change. A backward selection procedure was used to determine which variables were independently associated with changes on brain MRIs.

RESULTS

Sixteen patients with RRMS who were taking interferon beta-1a and had experienced breakthrough disease activity were enrolled. One patient dropped out of the study after month -2 owing to pregnancy; hence, all the analyses were performed on 15 patients. The mean (SD) age of the patients was 44.5 (10.6) years, with a median duration of MS (from the onset of symptoms) of 4.0 years (range, 1-16 years); 80% of the patients were women. The demographic features of the patients are given in **Table 1**. The number of Gd+ lesions on brain MRIs decreased from a median of 8.8 per image (range, 2.0-24.5) during the pretreatment period to 4.0 (range, 0.7-16.7; *P* < .001) dur-

Table 1. Demographic Characteristics of the 15 Study Patients

Characteristic	Baseline Value (Month -3)
Age, y	
Mean (SD)	44.5 (10.6)
Median	45.0
Range	19-57
Female sex, No. %	12 (80)
Disease duration, y	
Mean (SD)	6.1 (4.5)
Median	4.0
Range	1-16
Duration of treatment with interferon beta-1a, y	
Mean (SD)	3.9 (2.6)
Median	3.0
Range	1-9
EDSS score, median (range)	3.5 (3.0-4.5)
Gd+ lesions, median (range), No.	4.0 (2-28)

Abbreviations: EDSS, Expanded Disability Status Scale; Gd+, gadolinium-enhancing.

Table 2. Changes in the Number of Gadolinium-Enhancing Lesions From Before to After Treatment^a

	Median	Range	<i>P</i> Value ^b
Before treatment ^c	8.8	2.0 to 24.5	< .001
During treatment	4.0	0.7 to 16.7	
During-treatment change ^d	-2.1	-9.4 to 1.0	
Reduction, %	39.2	-50.0 to 87.6	

^aN = 15.

^bBy 2-sided Wilcoxon signed rank test.

^cNumber of lesions per image from months -3, -2, -1, and 0.

^dNumber of lesions per image from months 1, 2, and 3.

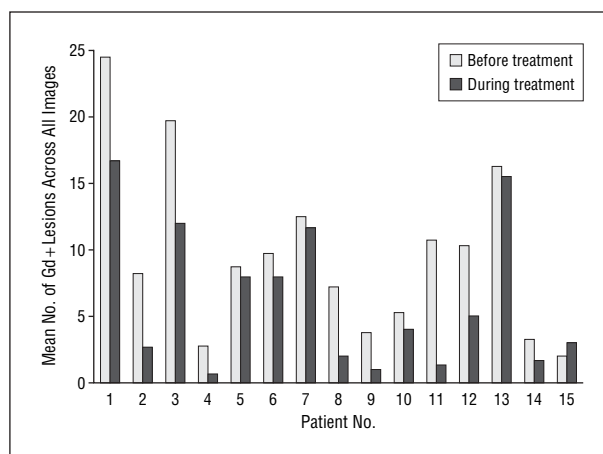


Figure 2. Mean number of gadolinium-enhancing (Gd+) lesions before and during treatment.

ing the treatment period (**Table 2** and **Figure 2**). Review of the data revealed that 3 patients had high Gd+ lesion counts during the pretreatment period; the change in Gd+ lesion counts remained significant when excluding these patients from the analysis (*P* = .002). In fact, 60%

Table 3. Changes in Serum MMP-9 Levels Measured by Means of ELISA and Change From Before Treatment in Gd+ Lesions by MMP-9 Change

	Patients, No.	Outcome	P Value ^a (95% CI ^b)
Changes in serum MMP-9 levels			
Pretreatment MMP-9, mean (SD), ng/mL ^c	15	470.1 (270.9)	.59 (-216.0 to 127.8)
During-treatment MMP-9, mean (SD), ng/mL ^d	15	426.1 (253.6)	
Change in MMP-9, mean (SD), ng/mL	15	-44.1 (310.4)	
Changes from before treatment in number of Gd+ lesions by MMP-9 change, median (range)			
MMP-9 change <0	8	-5.4 (-9.4 to -1.6)	.04 ^e
MMP-9 change ≥0	7	-0.8 (-5.3 to 1.0)	

Abbreviations: CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; Gd+, gadolinium-enhancing; MMP, matrix metalloproteinase.

^aBy 2-sided paired *t* test.

^bThe 95% CI is for the mean change in the MMP-9 level.

^cMonth -3.

^dMonth 4.

^eThis *P* value is based on a multivariate model showing a significant association between MMP-9 change and Gd+ change.

of the patients had more than a 25% reduction in Gd+ lesions from the pretreatment period. The median decrease in the EDSS score was 2.3 (range, -3.4 to -1.8; $P < .001$ by 2-sided Wilcoxon signed rank test) from a pretreatment score of 3.8 (range, 3.3-4.5). The mean (SD) change in the serum level of MMP-9 between before and after combination treatment was -44.1 (310.4) ($P = .59$) (**Table 3**). Serum levels of tumor necrosis factor α and IFN- γ did not demonstrate any significant changes between pretreatment and posttreatment with the combination therapy. Results of the transendothelial migration of monocytes assay demonstrated that incubation of U937 monocytes with posttreatment serum (compared with pretreatment serum) from patients with MS significantly decreased passage of the monocytes through the cerebral endothelial monolayers (**Figure 3**).

During the study, only 1 patient experienced a relapse (optic neuritis), which was treated with intravenous methylprednisolone, 1 g daily, for 5 days. All 15 patients with MS tolerated the combination of interferon beta-1a and doxycycline. The patients with MS reported 2 types of adverse effects: those commonly associated with doxycycline and those commonly associated with interferon beta-1a. Adverse effects related to doxycycline included nausea (n=5), vomiting (n=2), diarrhea (n=6), dyspepsia (n=9), and heartburn/reflux (n=2). None of the patients experienced photosensitivity, serum sickness, or hypertension. The most common adverse effects associated with interferon beta-1a injec-

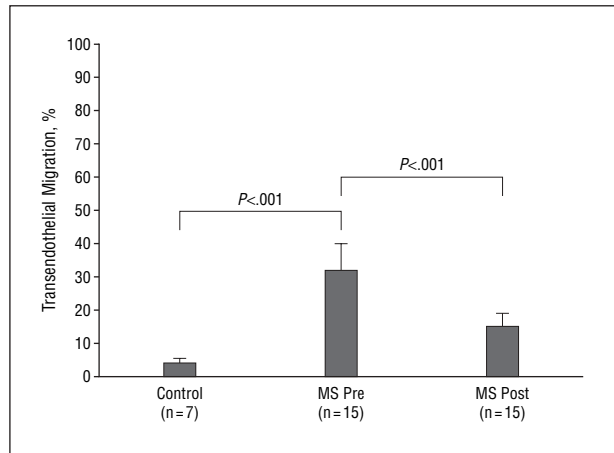


Figure 3. Induction of transendothelial migration by multiple sclerosis (MS) serum before and after treatment with the compound. Brain microvascular endothelial cells grown to confluency in 6.5- μ m-pore filters were treated with 20% control and with serum from patients with MS before treatment (MS Pre) and after treatment (MS Post). After 18 hours, 1×10^5 U937 monocytes were added. Filtrates were collected 6 hours later and were assayed for the presence of U937 monocytes. All the experiments were conducted in triplicate. Error bars represent SD.

tions were influenza-like syndrome (n=11), fatigue (n=2), headache (n=2), and muscle pain (n=4). The adverse effects were mild and resolved spontaneously. The combination of these 2 agents did not have any impact on the severity of interferon beta-1a-related adverse effects. There were no unexpected or synergistic adverse effects associated with combination therapy.

In the multivariate analysis, the duration of treatment with interferon beta-1a ($P = .02$) and changes in serum MMP-9 levels ($P = .04$) were found to be independently associated with the change in Gd+ lesions and accounted for 50.5% of the rank-based model variance. Longer treatment durations and decreased MMP-9 levels were associated with a greater reduction in the number of Gd+ lesions. The median reduction in the number of Gd+ lesions was -5.4 (range, -9.4 to -1.6) for patients who also had a decrease in MMP-9 levels, whereas the reduction was -0.8 (range, -5.3 to 1.0) for patients with an increase or no change in MMP-9 levels (Table 3).

COMMENT

There is growing interest in combination therapy in patients with MS to stabilize the clinical course, reduce the rate of clinical relapses, and decelerate the progressive course of the underlying pathologic mechanism.²⁵ The scientific rationale behind the concept of combination therapy in MS is to block different pathogenic pathways by applying different agents with dissimilar mechanisms of action, which translates into additive or synergistic therapeutic effects. Combination therapy in MS may be particularly useful in patients who have failed treatment with a single immunomodulatory agent. To achieve this goal, various therapeutic combinations, such as methotrexate and interferon beta-1a²⁶ or interferon beta-1a and glatiramer acetate,²⁷ have been used in clinical trials.

Results of the present clinical trial suggest that the combination of oral doxycycline and interferon beta-1a may

be effective in some patients with MS. Except for 1 patient who developed a relapse, all the other patients became clinically stable, with improvement of their EDSS scores. Furthermore, the significant decrease in the number of contrast-enhancing lesions on brain MRI provides objective evidence that the combination of oral doxycycline and interferon beta-1a reduces the inflammatory cascade of MS and stabilizes the blood-brain barrier. The combination of oral doxycycline and interferon beta-1a was safe and well tolerated in this cohort of patients with MS. The adverse effects were consistent with the adverse event profiles that have been reported for the individual agents,²⁸⁻³⁰ and there was no evidence of synergistic or unexpected adverse events.

The MMPs are involved in disintegration of the underlying endothelium and subendothelial ECM, which in turn aids transendothelial migration of activated leukocytes,^{15,31} a step that is crucial in the formation of MS lesions. In addition, overexpression of MMPs in the pathogenesis of MS is associated with promotion of the inflammatory cascade in the central nervous system³² and axonal loss.³³ It has previously been demonstrated that increased levels of MMP-9 are associated with increases in Gd+ lesions in patients with RRMS.^{34,35} Thus, the addition of doxycycline, an MMP inhibitor, to interferon beta-1a may reduce the number of contrast-enhancing lesions on brain MRIs. This theory is supported by the results in this cohort, in which the effect was more pronounced in patients with lowered serum levels of MMP-9.

The results of the in vitro model of monocyte transendothelial migration in the presence of MS serum before and after treatment with oral doxycycline and interferon beta-1a clearly indicated that doxycycline can decrease transendothelial migration of monocytes, and this is probably one of the therapeutic mechanisms of oral doxycycline in patients with MS. Because IFN- β also decreases MMP production^{31,35} and activity³⁶⁻³⁸ in patients with MS, the combination of oral doxycycline and interferon beta-1a may exert a synergistic impact on MMP levels and activity, and this effect can potentially stabilize the cerebral endothelial barrier integrating the blood-brain barrier. It is already known that MMP-9 can degrade the interferon beta molecule and render it ineffective¹⁶; such disintegrative effects of MMP-9 on the interferon beta molecule may be inhibited or decreased by doxycycline. Alternatively, doxycycline may exert its stabilizing effects on the integrity and function of the cerebral endothelial component of the blood-brain barrier by inducing the expression of claudin-1,³⁹ an essential protein for optimal functioning of the cerebral endothelial tight junctions.

Another significant point is that when brain MRIs demonstrate the presence of multiple contrast-enhancing lesions while the patient is taking an immunomodulatory agent, this should raise clinical concerns about the suboptimal response to therapy and the potential need for combination therapy.

One potential limitation of this small study is that the results may be partially due to regression to the mean. However, the percentage reduction in the number of Gd+ lesions was 55% ([8.8–4.0]/8.8), a substantially greater reduction than would be expected for changes arising

solely from regression to the mean. Another limitation is that the open-label study design may introduce bias into the results, particularly with subjective measures such as the EDSS. In blinded pivotal studies^{29,30} of interferon beta-1a, EDSS scores did not decline; however, EDSS score progression was delayed.

Overall, data from this cohort suggest that the treatment combination of oral doxycycline and interferon beta-1a may be safe and effective in some patients with MS; however, further controlled clinical trials are warranted to demonstrate safety and efficacy in a larger patient population.

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