

Pilot Study of Minocycline in Relapsing-Remitting Multiple Sclerosis

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ABSTRACT: Background: Current multiple sclerosis (MS) treatment is only partially effective and not all patients respond well. The goal in this study was to evaluate minocycline for its safety, tolerability, and MRI impact as a potential therapy over 36 months after a three month run-in in ten relapsing-remitting (RR) MS patients. **Methods:** Clinical assessments were at three month intervals until six months, then at six month intervals. Three Tesla MRI was performed monthly during the run-in and first six months of treatment, then at 12, 24, and 36 months. **Results:** Treatment was safe and well tolerated. Annualized relapse rate was 1.2 during the run-in and 0.25 during treatment. The proportion of active scans was lower during the first six months of treatment (5.6%, $p < 0.001$) and during the extension (8.7%, $p = 0.002$) than during the run-in (47.5%). Consistent with these outcomes, mean T2 lesion volume remained stable over three years and percent brain volume change was reduced during year three (-0.37%) of minocycline treatment. **Conclusions:** This trial is limited by small sample and no control group but suggests that minocycline is safe and potentially beneficial in RRMS. This supports further investigation of its efficacy.

RÉSUMÉ: Étude pilote sur la minocycline dans la forme cyclique rémittente de la sclérose en plaques. Contexte : Le traitement actuel de la sclérose en plaques n'est que partiellement efficace et certains patients n'y répondent pas bien. Le but de cette étude était d'évaluer la sécurité, la tolérance et l'impact sur l'IRM de l'administration de minocycline pendant 36 mois, après une phase de préinclusion de 3 mois, chez dix patients atteints de la forme cyclique rémittente de la sclérose en plaques (SEP). **Méthodes :** On a procédé à une évaluation clinique aux 3 mois pendant 6 mois, puis aux 6 mois par la suite. Les patients ont subi 3 IRM Tesla par mois au cours de la phase de préinclusion et des 6 premiers mois de traitement, puis aux 12e, 24e et 36e mois. **Résultats :** Le traitement était sûr et bien toléré. Le taux de rechute annualisé a été de 1,2 pendant la phase de préinclusion et de 0,25 pendant le traitement. La proportion de scans montrant une activité était plus faible pendant les 6 premiers mois de traitement (5,6%, $p < 0,01$) et pendant l'extension (8,7%, $p = 0,002$) que pendant la phase de préinclusion (47,5%). Le volume moyen des lésions en T2 est demeuré stable pendant 3 ans et le pourcentage de changement du volume cérébral a diminué pendant la troisième année de traitement par la minocycline (- 0,37%). **Conclusions :** Cette étude ne comportait qu'un petit nombre de patients et aucun groupe témoin. Cependant, les résultats indiquent que la minocycline est sûre et potentiellement bénéfique dans la SEP cyclique rémittente et mérite qu'on étudie davantage son efficacité.

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Multiple sclerosis (MS) is a serious and common neurological disorder of young adults. It affects over one million people worldwide. More than 80% of cases start with a relapsing-remitting (RR) course but because 40% of relapses remit incompletely¹ neurological disability accumulates. Over 25% of patients with MS are unemployed within ten years of disease onset.²

Multiple sclerosis is an immune-mediated disease of the central nervous system (CNS) that may be initiated by the activation by unknown triggers of peripheral immune cells in a susceptible subject.³ The activated leukocytes then enter the CNS with the assistance of matrix metalloproteinases (MMPs), and cause an inflammatory cascade that leads to tissue injury in the CNS including demyelination, axonal damage, and loss of neurons and oligodendrocytes.⁴⁻⁷

Current immune-modulating therapies for MS are only partially effective. Glatiramer acetate (GA), interferon beta (IFNB), and natalizumab reduce relapse rate,⁸⁻¹¹ delay the accumulation of physical disability, and decrease sub-clinical disease activity as shown by reduced number and frequency of

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contrast enhancing lesions (CELs) and new T2 lesions on magnetic resonance imaging (MRI).¹²⁻¹⁴ These therapies, however, are not effective in all patients, intolerance is common, and they are all administered by injection. In addition, they are extremely expensive. Therefore, a more efficacious, inexpensive, orally available and well tolerated and accepted treatment in MS would be of value.

Minocycline is a semi synthetic tetracycline that has been in use for over 40 years. One rationale for using minocycline is to target Matrix metalloproteinases (MMPs), which are proteases up-regulated in MS and one of its animal models, experimental allergic encephalomyelitis (EAE). Matrix metalloproteinases have several detrimental effects including breakdown of the blood brain barrier (BBB), promotion of neuroinflammation, and neurotoxicity.⁵ Indeed, one of the mechanisms of IFNB therapy in MS is to reduce the production of MMPs by T cells, and therefore attenuate the infiltration of these lymphocytes into the CNS.¹⁵ Reduction of the MMP-9/TIMP-1 ratio in IFNB treated patients has been shown to be a good predictor (estimate = 0.85, $p < 0.05$) of CELs in RRMS.¹⁶ Because minocycline not only decreases the secretion of MMPs, but also inhibits their enzymatic activity^{6,17} it could be a good candidate to explore as an MS therapy. In addition to targeting MMPs, minocycline has other immunomodulatory and trophic properties in a variety of neurological disorders.¹⁷

Minocycline treatment of mice, concurrent with induction of severe acute EAE, delays the onset of clinical symptoms. In mice induced with a milder form of EAE, daily minocycline treatment decreases the severity of disease course, even when initiated as late as the appearance of first symptoms.⁶ Similarly minocycline suppresses ongoing disease activity and limits disease progression in rats with EAE.¹⁸ These favorable results were further confirmed by showing that minocycline alleviated the severity of adoptive transfer EAE.¹⁹ Additionally, there is an add-on effect when minocycline is combined with either GA²⁰ or IFNB in the treatment of EAE.²¹

Based on the encouraging EAE results, a baseline versus treatment trial of minocycline was undertaken in patients with RRMS.²² The goals of this open label pilot trial were to evaluate the safety and tolerability of minocycline in RRMS, and to estimate the impact of treatment on MRI outcomes to determine if further study of minocycline is indicated.

The primary efficacy outcome in this trial was the change in the mean number of CELs per scan during the first six months of treatment compared to the three month run-in period; as reported, there was a relative reduction of greater than 84% ($p = 0.03$).²² Here we report the final clinical and MRI results of this open label three year trial of minocycline in RRMS.

METHODS

Population and study design

This open label study included ten subjects with RRMS according to Poser criteria. They were treated with oral minocycline 100 mg twice daily for three years following a three month run-in period. RRMS patients were eligible if they were between 18 and 50 years old, had at least two relapses in the previous 24 months and had an Extended Disability Status Scale (EDSS) between 0 and 5.5. Exclusion criteria included treatment

with corticosteroid during the prior month, the presence of any unstable medical or psychiatric disorder, use of IFNB, GA, or IV gamma globulin within six months prior to enrolment, use of any investigational drug or other immunosuppressive therapy within 12 months, any prior use of cladribine or total lymphoid irradiation, allergy to tetracyclines or gadolinium, pregnancy, or breast-feeding. All patients gave written informed consent. Eight of ten patients had never been treated with approved disease modifying therapy. This study was approved by the University of Calgary Conjoint Research Ethics Board.

A treating neurologist was responsible for the overall medical management of each patient and for EDSS scoring. Clinical and laboratory assessments were completed at three-month intervals (enrolment, month 0, 3, and 6) initially, then at six-month intervals between 6 and 36 months. Symptoms suggestive of a relapse were assessed at unscheduled visits within seven days. Magnetic resonance imaging was performed at enrolment, at monthly intervals during the three month run-in and six month treatment periods, then at 12, 24, and 36 months.

The primary endpoint was change in the mean number of CELs during the first six months of treatment compared to the run-in period. Secondary outcomes included change in the proportion of active scans (scans with CELs) over six months, safety, and tolerability over three years. Tertiary outcomes included change of T2 lesion volume (LV), percent brain volume change (PBVC), proportion of CELs evolving into T1 black holes, relapse rate, and proportion of patients with disability progression defined as six-month sustained EDSS worsening of at least one point.

MRI Acquisition and Analysis

All MR images were obtained from a single 3.0 Tesla (T) MR Scanner (Signa; GE Medical Systems, Waukesha, WI). Proton density and T2-weighted MR images were acquired using an optimum dual echo spin-echo sequence (TR: 2716 ms, TE1/TE2: 20/80 ms, field of view: 24 cm², slice thickness: 3 mm, and matrix size: 512x512). This yielded 50 contiguous axial slices for each image set. The slice was set parallel to a line connecting the infero-anterior and infero-posterior parts of the corpus callosum. Two sets of T1-weighted images were also obtained using a spin-echo sequence (TR: 650 ms, TE: 8 ms) before and five minutes after the intravenous injection of gadolinium (0.1 mmol/kg). Other acquisition parameters were the same as the PD/T2-weighted MRI. Image quality was reviewed during each scan. Unsatisfactory images were discarded and corrected scans were repeated immediately.

The acquired MR images were sent online to the dedicated research picture archiving and communication system to secure data storage. The images were then transferred to the analysis computer for processing. Image analysis was completed by one neuroradiologist who was blind to patient's clinical status but who knew that all patients would receive minocycline after baseline.

Identification and quantification of CELs and T2 hyperintense lesions was done using a semi-automatic computer-assisted program (Segtool®).²³ The observer identified a lesion and informed the program by mouse-click on it, the software then "grew" the lesion to its boundary based on a k-nearest neighbor algorithm. Total lesion volume was calculated and

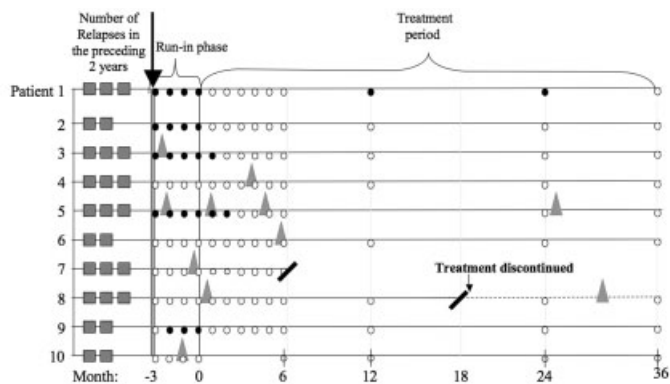


Figure 1: The impact of minocycline on relapses and MRI activity before and during 36 months of minocycline treatment. The course of each patient is displayed horizontally. ■ indicates a relapse during the 2 years before enrolment but the time of the relapse is not noted, ▲ indicates a relapse on treatment at the time of figure placement, ° indicates an MRI scan (empty if non-enhancing, black if enhancing, empty with vertical strikethrough if no gadolinium given), / indicates discontinuation of drug.

summed over the entire image set automatically by the program²³ and recorded per scan per patient. The image data sets were analyzed sequentially for each patient to minimize intraobserver variability. This software program has a relatively low inter- (0.17 cm³) and intra-observer (0.15 cm³) variability for MS lesion segmentation.²⁴

Whole brain volume was measured on T1-weighted MR images using an automated software program named SIENA (Structural Image Evaluation, using Normalization, of Atrophy).²⁵ SIENA is a longitudinal (temporal) analytic method that measures brain volume change. It first segments the brain from other tissue and estimates the outer skull surface that is used for normalization during co-registration between two time points. Local brain atrophy is then estimated as percentage brain volume change (PBVC) from the co-registered brain image sets. The PBVC was calculated between 'baseline' and months -3, 6, 12, 24, and 36 for each patient. Annualized atrophy was estimated by extrapolating the relative PBVC between time points to 365 days. SIENA is robust and accurate. The error in measuring PBVC between time points for images acquired using the same pulse sequence is 0.15%.²⁵

Statistical Methods

Clinical outcomes were described but not evaluated statistically. The mean number of CELs per scan, and the proportion of active scans were calculated pre (months -3 to 0) and post treatment (month 0 to 36). The mean number of CELs per scan was compared using the Wilcoxon signed-rank test and the proportion of active scans was compared using the paired Students *t*-test. The difference in T2 LV and PBVC between multiple time points was analyzed using a non-parametric analysis of variance method (Friedman). Patient number was adjusted for each analysis. Two-tailed $p \leq 0.05$ was set as

significant for all comparisons. Last observation carried forward imputation was implemented to account for missing data.

RESULTS

Study Completion

All ten patients completed six months of treatment;²² 36 month outcomes were available for nine patients (Figure 1). One patient declined participation in the extension as she lived in another city. One patient discontinued minocycline treatment at month 18 due to transient worsening of pre-existing MS symptoms (truncal dysesthesia) following an influenza vaccination, but follow-up continued without minocycline or other disease modifying therapy (Figure 1). Another patient declined gadolinium after the three month run-in phase due to venous irritation. Therefore, three-year MRI contrast enhancing activity outcomes are available for eight patients although one patient declined the month 12 scan. Three scans had only CEL data available because other MR images were lost due to an error in electronic data transfer. Overall, 90% of MRI data were available for analysis.

Safety and Clinical Outcomes

Eight of ten participants were women, mean age was 42.8 years (SD 4.0), and mean MS duration was 11.8 years (SD 6.3). Median EDSS at baseline was 2.5 (range 1.5-5.5); mean relapse rate in the two years prior to enrolment was 2.6 (annualized relapse rate = 1.3).²² During this trial one serious adverse event occurred. A 48-year-old woman using oral contraceptives (pre-trial as well) suffered a large left hemisphere ischemic infarction due to a carotid dissection shortly after her month 30 study visit. During the first 48 hours of care her minocycline was discontinued by her caregivers. This event was thought unlikely to be related to minocycline by her stroke neurologist and her MS trial treating neurologist because the mechanism was dissection. Also, review of the literature did not find any suggestion of an association between stroke or other ischemic events and use of tetracyclines. This patient chose to continue minocycline thereafter. Her month 36 brain MRI was only used to assess contrast enhancing activity.

There were no clinically significant or sustained laboratory abnormalities over the three-year period. Treatment was generally well tolerated although at onset of dosing five patients experienced transient, mild lightheadedness and nausea that was managed in two patients by a two-week dose reduction. Persistent nausea in one patient necessitated reduction of the minocycline dose to 50 mg twice daily between month 6 and month 27. Because this patient had MRI CEL activity at months 12 and 24 full dose minocycline was resumed between months 27 to 30. Additional MRI scans were undertaken at months 30 and 33 which showed no CELs but persistent nausea led to use of an intermediate dose (50 mg each morning and 100 mg at bedtime) from months 30 to 36. The month 36 MRI remained inactive (no CELs).

During the three month run-in phase, four relapses occurred (annualized rate = 1.2) but during the three years on treatment only seven more relapses occurred (annualized rate = 0.25 based on actual patient follow-up of 27.5 patient-years). Six of ten patients remained relapse free. Five of the on-treatment relapses

occurred within the first six months of treatment, one occurred at month 27, 11 months after this patient discontinued minocycline, and another occurred at month 25 of treatment in a patient who also had two of the five relapses within the first six months of therapy and one relapse during the three month run-in phase. This patient had previously failed disease modifying therapy.

During the run-in phase one of ten patients suffered a relapse that led to six-month sustained worsening of EDSS but none developed sustained disability progression during the treatment period. The patient who suffered her third on treatment relapse at 25 months had not completely recovered five months later then went on to have a stroke two weeks later. Her EDSS was 2.0 at baseline and before this third relapse but increased to 4.0 during the relapse and was still 3.5 five months later at her 30 month visit. While this patient likely would have suffered sustained worsening on treatment because it is unlikely she would have completely recovered within the following month she did not meet the protocol defined definition of sustained EDSS worsening.

MRI OUTCOMES

Contrast Enhancing Activity

Five patients had inactive scans during the run-in phase. Four of these patients remained inactive during the initial six-month treatment period and the other declined gadolinium injection. One of these inactive patients then discontinued follow-up but the three who continued to undergo MRI scans with contrast injection remained inactive to 36 months including one patient who discontinued treatment after month 18 (Figure 1). The other five patients accounted for all MRI CEL activity, which quickly resolved as reported previously.²² There were no new or recurrent CELs after month three in any patient on full dose minocycline up to 36 months. The only patient with active MRI scans during the extension was the patient taking half dose minocycline. This patient had one CEL at month 12 (0.14 mm³) and one at month 24 (0.31 mm³). Her MRI scans became inactive (Figure 1) after the dose was increased to 100 mg bid then stayed inactive with an intermediate dose. The mean total number of CELs was 0.09 per scan during the 30 month treatment extension representing a relative reduction of 59% compared to the mean total CEL number (0.22) during the first six months of treatment ($p=NS$), and a relative reduction of 93% compared to the mean total CEL number (1.38) during the run-in period ($p=0.03$). The proportion of active scans was 5.6% (3/54) during the first six months of treatment ($p<0.01$) and 8.7% (2/23) during the extension ($p<0.01$) compared to 47.5% (19/40) during the run-in phase.

Evolution of CELs into T1 Black Holes

Only one CEL arose during treatment extension from a region of previously normal appearing white matter (NAWM) in a patient on half dose treatment. It did not evolve into a black hole. Estimation of the rate of conversion of CELs to black holes is therefore limited by sample size.

T2 Lesion Volume

The mean T2 LV, including data from the patient who discontinued minocycline after month 18, was lower at all time

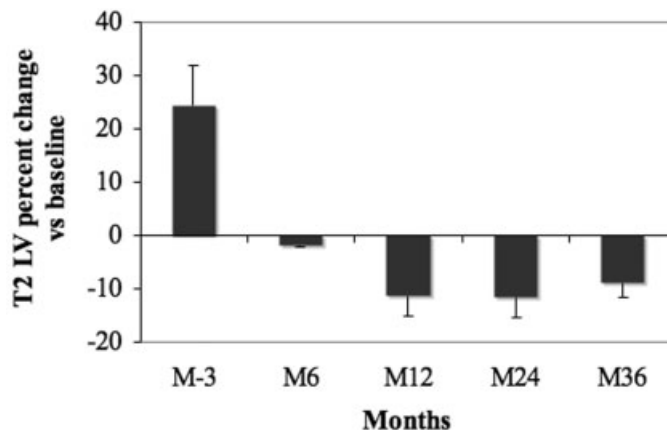


Figure 2: Percent change of mean T2 LV compared to baseline before and during minocycline treatment. T2 LV at each time point during treatment tend to be lower than at baseline in contrast to an increase of 24.3% during the three month run-in period. Error bars represent standard error.

points compared to baseline (Figure 2) ($P=NS$). This contrasts with an increase of 24.3% in mean T2 LV during the run-in period. Median T2 LV displayed a similar trend (Table).

Brain Volume Measurement

The annualized PBVC was 71% less during the third year of treatment than during the run in ($P=NS$). The mean (\pm SD) annualized PBVC was -1.25% (\pm 3.72) during the run-in phase, -1.04% (\pm 1.74) during the first six months of minocycline treatment, -1.14% (\pm 0.68) during 6-12 months of treatment,

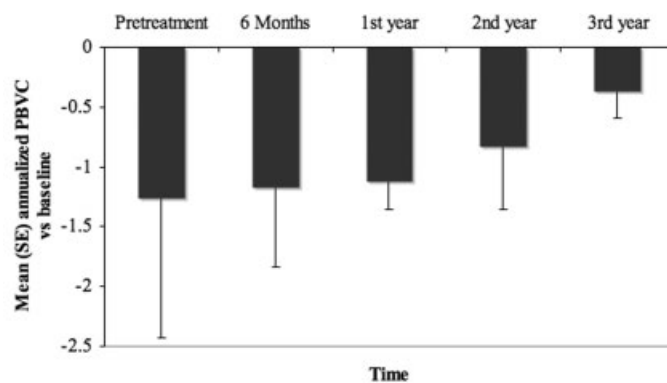


Figure 3: Percent brain volume change (PBVC) over time. The PBVC displayed a trend of decrease (less atrophy) yearly. The lowest rate of atrophy was observed during the third year (-0.375%) of minocycline treatment in contrast to the annualized atrophy of -1.25% during run-in period. Error bars represent standard error (SE).

-0.82% (± 1.50) during the second year, and -0.37% (± 0.63) during the third year (Figure 3).

DISCUSSION

This small study suggests that minocycline, known to be relatively safe for general use over several years, is also safe and tolerable for long term use in people with MS. The only serious adverse event that occurred, a carotid dissection causing stroke, was unlikely to be caused by minocycline treatment. Nausea limited dosing in one patient. This is a recognized side effect of minocycline that starts early and usually settles but limits use in some people.

Table: Mean (SD) and median T2 LV over time

	Mean (SD) (mm ³)	Median (mm ³)
Run-in phase		
M -3	*5093.2 (7221.1)	2106.4
M 0	*6332.2 (10259.6)	2092.9
Treatment phase		
M 6	*6234.2 (9735.8)	1956.5
M 12	6138.8 (9420.3)	1752.8
M 24	6125.3 (9987.7)	1547.1
M 36	6313.9 (10095.5)	2104.4

* Refers to values obtained from 10 patients, otherwise from 8 patients. Multiple comparisons of means (corrected for patient numbers) yielded no significant difference on T2 LV between time points ($p=NS$).

The treatment outcomes also appear promising. There were only seven on-study, and six on-treatment, relapses during the entire three year treatment period. One patient would likely have suffered sustained EDSS worsening if an adverse event, which was unlikely due to minocycline, had not made evaluation of relapse recovery impossible. Therefore, at most, one patient would likely have suffered disability progression over three years of treatment. However, without a placebo group interpretation of these data is limited. While a placebo effect is unlikely to cause the relapse rate to drop after six months of treatment regression to the mean could be an explanation. Furthermore, clinical assessment at six month intervals, rather than three month intervals, may have reduced relapse detection. Further study is therefore required before use of minocycline can be recommended.

Favorable clinical outcomes were supported by MRI results. The contrast enhancing activity was continuously suppressed for 36 months in patients taking full dose minocycline and a statistically significant decrease of CEL number was observed during the initial six month treatment phase compared to the run-in period.²² T2 LV was also stable over the entire treatment period. This suggests that inflammatory disease activity remained controlled between yearly scans. In fact, while not statistically significant there was a decrease of T2 LV during treatment, in contrast to a 24.3% increase during the three month run-in phase. T2 LV reduction was observed during treatment with both GA (-12.3% in nine months)¹² and IFNB (-3.8% in two years).¹⁴ The sustained decrease in T2 LV during three years of minocycline treatment is consistent with the low rate of relapses and CELs, and the absence of sustained disability worsening over three years.

Brain atrophy and T1 black holes are generally considered markers of tissue damage. Because of the rarity of CELs that occurred on treatment it was not possible to estimate the proportion that convert to black holes. Also, the sample size of this study was too small to expect detection of a statistically significant impact on PBVC even when using the efficient baseline versus treatment trial design that reduces the impact of between patient variability. We did however find that the annualized PBVC observed pretreatment (-1.25%) was within the range of annual atrophy (-0.8% to -1.5%)²⁶ expected to occur during the natural course of MS and that the low PBVC during year three of minocycline treatment (-0.37%) was comparable to that reported during year three of IFNB treatment (-0.378%).²⁶ While an early decrease in PBVC may reflect reduced tissue water content from suppressed inflammation²⁷ the ongoing reduction in PBVC suggests a possible neuroprotective benefit of minocycline in MS. It has been shown that the ability to detect reduced tissue injury is delayed until the third year of IFNB treatment,^{27,28} and that only patients without CELs during their first year of IFNB treatment have stable T1 black hole volume during the following two years.²⁹ The changes we observed in brain volume may result from resolution of inflammation, decrease in toxic molecules such as MMPs, and inhibition of apoptotic pathways.^{17,30} Indeed, we have observed favorable immunologic outcomes over 18 months of minocycline therapy, such as a persistent reduction in serum activity of MMP-9 compared to baseline values.³¹ While neuroprotection may be largely related to resolution of inflammation Maier et al³⁰ recently showed neuronal and axonal protection with minocycline treatment in optic neuritis in a murine model of EAE, independent of the anti-inflammatory properties of minocycline. Therefore, our observations may indicate a neuroprotective potential of minocycline but this requires confirmation in larger studies as well as elucidation of the mechanisms involved.

Long-term use of antibacterial therapy may be associated with opportunistic infections and with pigment deposition in the skin. While they were not an issue in this trial these potential effects must be evaluated in larger populations and with even longer use. Modified tetracyclines may avoid these issues. Minocycline should also be evaluated in combination with other immunomodulating therapies. In vitro studies suggest that minocycline may reduce the degradation of IFNB, thus

prolonging its activity or potentially allowing lower doses³² and evidence that minocycline may have a synergistic effect on EAE with either GA20 or IFNB.²¹ Studies to evaluate these combinations for safety and preliminary efficacy in MS are ongoing.^{33,34}

This is the first MS clinical trial that we are aware of that was performed entirely using a 3.0 T MR System. Higher field MRI provides higher resolution, better image contrast, and greater signal-to-noise ratio than lower field MRI, which allows more accurate quantification of disease activity and therefore more precise monitoring of therapeutic efficacy. Studies have shown that 3T MRI increases detection of the number and volume of CELs by 21% and 30%, and increases detection of T2 LV by 10% compared to 1.5T MRI.³⁵ This elevated sensitivity of 3.0 T suggests we were unlikely to have missed evident tissue injury. One possible confounder to the MRI outcome could be that the analyzing radiologist was not blinded to treatment status of the patients in this study.

This trial therefore provides support for the safety of minocycline in MS. The sample size was too small to support conclusions regarding efficacy but the favorable clinical and MRI outcomes observed here were consistent and sustained. These outcomes appear similar in magnitude and time course to those observed during treatment with established therapies. This data, while limited to a small group of patients without a placebo group for comparison, therefore supports the continued investigation of minocycline as a potential MS therapy. In countries where public funding covers the cost of current MS therapies of up to \$22,000.00CAN per year per patient, it seems that the opportunity to reduce this cost by 95% should be an incentive for government-sponsored trials. Indeed, the Multiple Sclerosis Society of Canada has recently approved funding of a phase III randomized, placebo-controlled clinical trial of minocycline in patients experiencing a clinically isolated syndrome that is likely to evolve into MS based on clinical and MRI features. If this inexpensive, oral treatment is shown to be effective in preventing conversion to MS, or in managing MS, either alone or by improving the efficacy of current immune modulating therapy, this could reduce treatment cost, improve efficacy and thus disease management costs, or both. Without proof of efficacy, however, minocycline should not be considered as an alternative to proven therapy.

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