Published in final edited form as:
Ann N Y Acad Sci. 2012 December ; 1275(1): 23–28. doi:10.1111/j.1749-6632.2012.06804.x.

Phase II trial of methotrexate in myasthenia gravis

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Abstract

Prednisone is a frequently used treatment for myasthenia gravis (MG) but it has numerous side effects. Methotrexate is a selective inhibitor of dihydrofolate reductase and lymphocyte proliferation and is an effective immuosuppressive medication for autoimmune diseases. Given the negative results of the mycophenolate mofetil study, search for an effective immunosuppressant drug therapy is ongoing. The objective is to determine if oral methotrexate is safe and effective for MG patients who take prednisone. We have initiated a randomized, double-blind, placebo-controlled multicenter trial of methotrexate versus placebo in patients taking at least 10 mg/day of prednisone at enrollment. The methotrexate dose is increased to 20 mg and the prednisone dose is adjusted per protocol during the study. Clinical and laboratory evaluations are performed monthly for 12 months, with the primary efficacy measure being the nine-month prednisone area under the curve (AUC) from months 3 to 12. Secondary outcome measures include MG outcomes, quality of life measures, and a polyglutamation biomarker assay. A total of 18 U.S. sites and 2 Canadian sites are participating, with 48 screened cases, 42 enrolled, with 19 still active in the study.

Keywords

methotrexate; myasthenia gravis; area under the curve; prednisone

Introduction

A number of attempts have been made to suppress the immune system and the associated antibody response in myasthenia gravis (MG). Thymectomy was first performed more than a half-century ago and was the earliest form of immune-directed therapy in MG. The introduction of corticosteroids in the therapy of MG was a major clinical advance. However, corticosteroids can have dose-limiting side effects, such as generalized immunosuppression, hyperglycemia, hypertension, myopathy, weight gain, cataracts, and osteoporosis. Other approaches to immunosuppression have come into clinical use in recent years. Recently two multicenter controlled trials of mycophenolate mofetil showed no benefit in MG.[1–4] These disappointing findings have prompted interest in looking for other immunosuppressive drugs that are currently available and have led to our interest in methotrexate (MTX) for MG. The potential advantages of MTX include oral dosing once a week, a relative moderate side effect profile, inexpensive cost, easy availability, availability in a generic oral preparation, and potential use for longer periods of time.

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Conflicts of interest
The authors declare no conflicts of interest.

Methotrexate has been used and shown to be effective for autoimmune disorders such as rheumatoid arthritis[5–12] and multiple sclerosis.[13–15] Abdou *et al.* reported a small open-label series of MG patients who received 25–50 mg of MTX intramuscular weekly for up to 20 months; 87% showed some improvement.[16] In a small retrospective study of 16 patients, Hartmann showed improvement in 6 patients on MTX.[17] Recently, Heckmann *et al.* performed a single-blinded trial of MTX versus azathioprine as steroid-sparing agents in generalized myasthenia gravis and showed that MTX is an effective steroid-sparing agent 10 months after treatment initiation and that MTX has similar efficacy and tolerability to azathioprine.[18] A small retrospective study by Raja at the University of Kansas Medical Center in 2009 looked at eight MG patients on MTX, two showed improvement and prednisone dose was decreased in four patients.[19]

MTX is an analog of folic acid and is an antimetabolite and a potent inhibitor of dihydrofolate reductase,[20] which subsequently inhibits *de novo* purine and pyrimidine synthesis. It originated in the 1940s when Dr. Sidney Farber at Children's Hospital Boston was testing the effects of folic acid on acute leukemic children. MTX gained U.S. Food and Drug Administration approval as an oncology drug in 1953. Once intracellular, MTX is bioactivated to the polyglutamated form of MTX ($MTXglu_n$) by folylpolyglutamyl synthase (FPGS), which promotes cellular retention and inhibition of several enzymes.[21] No or low glutamation leads to the efflux of MTX by the ATP-binding cassette (ABC) family of transporters. *FPGS* and *ABCG2* are of particular interest as folate deprivation has been associated with increased expression of *FPGS* and decreased expression of *ABCG2,*[22] suggesting a cellular response to low folate with an increase in polyglutamation and decrease in folate export to promote retention of folate within the cell. Additionally, upregulation of *ABCG2* protein expression has been associated with MTX resistance in cancer cells.[23] Therefore, allelic variation in these genes resulting in increased or decreased activity may be associated with either increased or decreased $MTXglu_n$. This entire process is also likely dependent upon the folate status of the patient, reflected by the polyglutamation of folate itself, and the relative concentrations of the two groups of mutually antagonistic compounds.

As serum MTX concentrations have been notoriously unreliably associated with MTX clinical outcomes,[24–26] the search for more stable biomarkers of disease response to MTX has been ongoing. An association between RBC $MTXglu_n$ and effectiveness of MTX in RA has been reported.[27,28] Higher levels of "long chain $MTXglu_n$" (defined as $MTXglu_3$ or greater) were associated with improved effectiveness of MTX in RA. Since RBC folate concentrations are established during erythropoiesis and represent the average folate status over the preceding 120 days,[29] by extension, MTX concentrations in RBCs are a surrogate biomarker of average drug exposure over a similar period of time. Furthermore, MTX polyglutamates in RBCs are considered to be representative of intracellular MTX levels in target tissues, are more stable than serum levels of MTX, and may potentially predict response to the drug.[27,28,30,31]

Methods

We have designed a randomized, double-blinded, controlled trial of MTX versus placebo in MG patients who are on steroids. Patients aged 18 years or older with MGFA grade is 2, 3, or 4 are enrolled in this study. They should have an elevated acetylcholine receptor antibody (AChR-Ab) titer and be on a stable prednisone dose of at least 10 mg/day or the equivalent, with alternate day dosing for 30 days before the screening visit. They should not have thymoma, tumor, infection, or interstitial lung disease. Those excluded from this study are patients who had a thymectomy in the previous three months, those that have been on immunosuppressive therapy within the last 60 days, those using daily nonsteroidal anti-

inflammatory drugs, those having renal or hepatic insufficiency or elevated liver enzymes, and those with prior use of MTX for MG or any other condition within the prior two years. Potential patients signed informed consent and underwent baseline laboratory testing. Subjects are randomly assigned with equal allocation to the two treatment arms (MTX or placebo) stratified by baseline prednisone dose (≥30 mg day or <30 mg day, or the equivalent for every other day dosing) based on the randomization plan developed by the Department of Biostatistics at the University of Kansas Medical Center. All subjects had a baseline evaluation, including a complete history, neurological examination, quantitative MG score (QMG), MG activities of daily living (MG-ADL) score, MG composite score, and MG quality of life-15 (MGQOL-15). This is followed by a similar evaluation every four weeks for 12 months. In addition, adverse events and changes in a patient's history and medications are documented at each follow-up visit. Blood is drawn at visit 12 for MTX polyglutamate estimation in RBC. Patients receive MTX 10 mg weekly or placebo, and if there are no clinical or laboratory side effects, the dose is increased to 15 mg weekly at two weeks, and increased to 20 mg weekly at five weeks. All participants also receive folic acid to be taken daily to prevent stomatitis. Prednisone tapering is started at the month 3 visit and monthly thereafter, according to a predetermined protocol based on the MG symptoms. The dose is increased if symptoms worsen.

Outcome measures and statistical analysis

The primary measure of efficacy is the nine-month prednisone area (months 3–12) under the time dose curve (AUC),[32] which measures the total prednisone doses of each patient for nine months. A reduction of prednisone AUC demonstrates that patients improved on clinical grounds so that the prednisone dose could be decreased per protocol. Secondary outcome measures are the change of Quantitative Myasthenia Gravis Score (QMG) from baseline. We will also analyze the 12-month changes in the MG-ADL, MG QOL-15, MMT scores, composite MG score, reduction in prednisone side effects, prednisone dose change from the baseline visit to visit 15, prednisone dose AUC months 7–12, the number of patients achieving minimal manifestations or pharmacological remission, time to worsening of MG symptoms, number of worsening episodes, number of plasmapheresis patients receive from day 90 to day 360, the number of treatment failures in each group, prednisone dose at each visit, and the AUC prednisone dose only for patients who did not receive any plasmapheresis during the study. At the end of the study, a stratified log-rank test will be used to compare the treatment groups with regard to the time (from randomization) until treatment failure. Fisher's exact test will be used with regard to the number of patients achieving minimal manifestations or pharmacological remission and the numbers of worsening episodes. All the other continuous secondary outcome measures will be analyzed using the two sample *t* test. Data will be analyzed in an intent-to-treat fashion. This will be accomplished as an analysis of covariance examining the change from initial to final value as the outcome result and the initial value as a covariate.

Results

Fifty-six subjects have been screened to date with 50 enrolled and 6 screen failures. Out of the 50 patients enrolled, 21 patients have completed the study, 1 patient died from a non-study medication–related event, and 5 patients withdrew, owing to a new diagnosis of Parkinson disease, ALT elevation, myalgia, transportation problems, and poor tolerability, respectively. A total of 23 patients are currently active in the study.

Discussion

Drugs such as azathioprine,[33,34] cyclophosphamide,[35] cyclosporine,[36,37] mycophenolate mofetil,[1–4] and intravenous immunoglobulin (IVIg)[38,39] have been studied with varying

degrees of success in MG. Like steroids, all have undesirable side effects, including hypertension, renal insufficiency, and hirsutism associated with cyclosporine, cystitis, myelosuppression, and mutagenicity with cyclophosphamide, and systemic hypersensitivity, hepatotoxicity, and myelosuppression with azathioprine.[40] Plasma exchange has been successfully used to lower the titer of AChR-Abs, with clinical improvement in some patients.[41] However, because of the technical difficulties and medical morbidity associated with chronic plasma exchange, it is a therapy that is now usually reserved for respiratory crisis. Recently two multicenter-controlled trials of mycophenolate mofetil showed no benefit in MG.[1–4] These disappointing findings have prompted interest in looking for other immunosuppressive drugs that are currently available and have led to our interest in MTX for MG. There have been few small studies that showed the efficacy of MTX in MG.[16–18] However, our study is the first randomized double-blind placebo controlled trial of methotrexate.

The three positive MG studies (i.e., cyclosporine and IVIg), where QMG was used as the primary endpoint, were short, 4- to 12-weeks.[36–38] In the positive azathioprine study, the benefit of the drug was not seen until month 12 using the prednisone dose as the primary end point.[34] One of the post hoc criticisms of the mycophenolate study was that it was only a three-month trial. Therefore, for this MTX trial we have planned a 12-month trial. In the azathioprine trial in MG, the beneficial effects of azathioprine were not seen for 12 months,[34] so we believe that new trials need to be at least this long.

The MTX polyglutamates in RBC will also be estimated in this study that has not been done previously in other MG MTX studies. MTX polyglutamates in RBCs are considered to be representative of intracellular MTX levels in target tissues, are more stable than serum levels of MTX, and may potentially predict response to the drug.[27,28,30,31]

The QMG was initially suggested as the best objective measure for use in MG clinical trials.[42] This score was used by Tindall *et al.* in double-blind, randomized, placebo-controlled studies of cyclosporine in myasthenia gravis.[36,37] Tindall found that an improvement of 4 points in QMG was associated with a sustained clinical change. This score was used in the completed MG trials of mycophenolate mofetil[1–4] and IVIg.[38,39] In the recently completed study of IVIg in MG, the patients receiving IVIg improved by 2.5 units, an additional 1.6 units compared to placebo. However, we are not using the QMG as the primary efficacy measure in this study because we are concerned that we may not observe a significant difference between these two groups in relation to QMG change even if MTX is effective. This is due to the fact that both groups are on prednisone, and the prednisone dose of each patient may be increased or decreased depending on the patient's condition. Because this is a long (12-month) study, it is very possible that increasing prednisone dose could be necessary. A dose change may subsequently affect the patient's QMG. Therefore, the prednisone dosing may confound the treatment effect with respect to the QMG. It is possible that patients in both groups will ultimately improve so that we cannot determine a difference with the QMG. For this reason, the QMG will be used as a secondary rather than the primary end point.

The primary measure of efficacy in our study will be the nine-month prednisone AUC (months 3–12), which measures the total prednisone doses of each patient in nine months. A reduction of prednisone AUC demonstrates that patients improved on clinical grounds so that the prednisone dose could be decreased. If the patients receiving MTX have a smaller prednisone AUC compared to the placebo patients, this will have demonstrated the efficacy of MTX. Our biostatisticians will determine if both placebo and MTX groups are equivalent for subject weight as part of the analysis. A daily prednisone drug diary is maintained by patients, and this information will be used to calculate the AUC measurements. The two-

sample *t* test will be used to test the difference between the mean AUCs of the two treatment groups under the assumption that the distribution of AUC is approximately normal. The normality assumption was satisfactorily tested in a comparable study,[34] which used the median maintenance prednisolone dose as the primary measure to test the efficacy of azathioprine plus prednisone versus prednisone plus placebo. Without prior information about prednisone AUC with MTX, we used information from the Palace study for the sample size consideration because these two studies have a similar design. Data derived from the Palace study showed a mean AUC that is nearly three times its standard deviation in the prednisone plus placebo group, and the mean/SD value based on the pooled data from both groups is about 2. To be more conservative, we assume a mean/SD ratio of 2.5 for the placebo group. Twenty patients in each study arm provides 0.8 power of detecting a 0.784 effect size (mean change/SD), which is equivalent to a 31.4% reduction in total prednisone doses in nine months for the MTX group over the placebo group. Assuming a drop-out rate of 20%, anticipated enrollment is a total of 50 participants (25 patients in the treatment group and 25 patients in the placebo group).

Similar to other previous MG studies, the enrollment has been a challenge and a slow process. However, we anticipate complete enrollment by December 2012 and clinical follow-up for another 12 months. We started with six sites initially, and due to challenges with enrollment, other sites were included. Presently there are 18 U.S. sites and two Canadian sites participating in this study.

Acknowledgments

This study was funded by Food and Drug Administration Orphan Products Division RO1 FD 003538. Additional funding was provided in part by Grants UL1 RR 033179 (which is now UL1 TR 000001) from the University of Kansas Medical Center Clinical and Translational Science Awards (CTSA). Muscle Study Group: University of Kansas Medical Center: R.J.B., MD (PI); M.P., MD (coPI); M.D., MD; L.H. (project manager); University of Texas Southwestern Medical Center: Sharon Nations, MD (PI); University of Texas Health Science Center San Antonio: Carlayne Jackson, MD (PI); University of Virginia: Ted Burns (PI); University of Miami: Michael Benatar, MD (PI); Ohio State University: Bakri Elsheikh (PI); University of California, Irvine: A.W., MD (PI); University of San Francisco–Fresno (J.R., MD (PI); Indiana University: R.P., MD (PI); University of North Carolina: J. Howard, MD (PI); Massachusetts General Hospital: David Walk, MD (PI); California Pacific Medical Center: Jonathan Katz, MD (PI); University of Iowa Hospitals and Clinics: Andrea Swenson, MD (PI); Nerve and Muscle Center in Texas: Aziz Shaibani, MD (PI); The Methodist Hospital System: Ericka Simpson, MD (PI); Penn State Hershey: Matthew Wicklund, MD (PI); Phoenix Neurological Center: David Saperstein (PI); University of Florida-Jacksonville: Michael Pulley, MD (PI); University of Toronto: Vera Bril, MD (PI); McGill University: Angela Genge, MD (PI); and Children's Mercy Hospital and Clinics (for polyglutamation assays), Mara Becker, MD. Muscle Study Group (MSG) Steering Committee: Annabel Wang, MD, T.B., MD; R.J.B., MD; M.P., MD; L.H. and J. (Wendy) H., PhD. Safety Monitoring Committee: Kevin Latinis, MD (chair); Anthony Amato, MD; Erik Ensrud, MD; and Jonathan Goldstein, MD.

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