Methotrexate Polyglutamation in a Myasthenia Gravis Clinical Trial

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ABSTRACT

Introduction. Methotrexate (MTX) is an immunosuppressive and anti-inflammatory drug used to treat rheumatoid arthritis (RA) and other autoimmune conditions. MTX is transported into cells, where glutamate moieties are added and is retained as methotrexate polyglutamates (MTXPGs). In the RA literature, it has been reported that the degree of polyglutamation correlates with the anti-inflammatory effect of MTX in RA. There are no prior studies evaluating the relationship between MTXPGs and myasthenia gravis (MG) outcome measures. The objective of this study was to assess the correlation between methotrexate (MTX) polyglutamates (MTXPGs) with Myasthenia Gravis (MG) outcome measures.

Methods. An analysis was done of blood drawn from patients enrolled in the 12-month randomized, placebo-controlled study of MTX in MG study. Red blood cell MTXPGs were measured via ultra-performance liquid chromatography and tandem mass spectrometry. MTXPG was correlated to MG outcome measures using Spearman Correlation Coefficient. A two-group t-test was used to determine the difference in MTXPG based on clinical outcome responder definitions.

Results. Twenty-one polyglutamate samples were analyzed of subjects on MTX while eight samples were analyzed from subjects on placebo. Pentaglutamate had the strongest correlation with the MG-ADL (0.99), while tetraglutamate had the strongest correlation with the QMG (0.54). Triglutamate had the strongest correlation with MGC (0.76).

Conclusion. There were variable correlations between $MTXPG_{1-5}$ and MG outcomes (rho range: 0.08 to 0.99). There are strong correlations between MTXPG and the MG-ADL, QMG, and MGC. Long chain methotrexate polyglutamates correlate better with MG outcomes. *Kans J Med 2020;13(Suppl 2):10-13.*

INTRODUCTION

Methotrexate (MTX) is a disease-modifying antirheumatic drug (DMARD) used in the management of rheumatoid arthritis (RA). Methotrexate is a folate analogue, used in treatment of cancers and autoimmune diseases. Given orally or subcutaneously, MTX is transported into cells, where additional glutamate moieties are added and is retained as methotrexate polyglutamates (MTXPGs). Prior studies suggest the degree of polyglutamation correlates with the anti-inflammatory effect of MTX in RA. There are no prior studies evaluating the relationship between MTXPGs and myasthenia gravis (MG) outcome measures.

Although the full molecular anti-inflammatory mechanism of MTX is not clearly elucidated, it is known that MTX acts as a folate antagonist. Once intracellular, MTX is bioactivated to the polyglutamated form of methotrexate (MTXglun) by folylpolyglutamyl synthase (FPGS), which promotes cellular retention and inhibition of several enzymes.[1] 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,[2] 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. Therefore, allelic variation in these genes resulting in increased or decreased activity may be associated with either increased or decreased MTXglun. 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,[3-5] the search for more stable biomarkers of diseases response to MTX have been ongoing. An association between RBC MTXglun and effectiveness of MTX in RA has been reported.[6-7] Higher levels of "long chain MTXglun" (defined as MTXglu3 or greater) were associated with improved effectiveness of MTX in RA. There has also been reported variability of MTXglun concentrations and patterns associated with MTX dosing route and dose of administration in Juvenile Idiopathic Arthritis patients prescribed this medication.[8] Since RBC folate concentrations are established during erythropoiesis and represent the average folate status over the preceding 120 days,[9] by extension, MTX concentrations in RBCs are a surrogate biomarker of average drug exposure over a similar period of time. Furthermore, methotrexate 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.[6-7, 10-11]

Methotrexate may also cause hepatotoxicity,[12] probably as a result of accumulation in the liver as polyglutamates[13] and can cause increases in serum alaninine aminotransferace (ALT) and aspartate aminotransferase (AST).[14] Renal impairment is a significant contributing factor to the occurrence of toxicities in other organ system, particularly hematopoietic and gastrointestinal systems. Whether methotrexate produces some degree of renal insufficiency has not been clearly established. Headache, dizziness, vertigo, mood changes, seizures, ataxia and cognitive impairment have been infrequently described.[15]

We performed a randomized-controlled trial of oral methotrexate in myasthenia gravis involving 50 subjects at 19 sites.[16] Myasthenia gravis is an autoimmune disease with antibodies derived against the acetylcholine receptor in muscle at the neuromuscular junction.[17] Prednisone is the first line immunosuppressive therapy for MG.[18] We studied if methotrexate would be of benefit to MG patients on prednisone. In this study, we failed to show MTX subjects were on a lower prednisone dose at the end of twelve months, which was our primary endpoint. Secondary endpoints such as the Quantitative Myasthenia Gravis (QMG) and Myasthenia Gravis - Activities of Daily Living (MG-ADL) showed trends toward greater improvement in MTX subjects but did not meet statistical significance in the pre-determined intent-to-treat analysis. When alternative intent-to-treat analyses were done post-hoc, QMG and MG-ADL did meet significance. We also performed polyglutamation blood studies on participants at the conclusion of the 12-month study.

METHODS

Trial Design. Analysis was done on blood drawn from patients enrolled in the 12-month randomized, placebo-controlled study of MTX in MG. Red blood cell MTXPGs were measured via ultra-performance liquid chromatography and tandem mass spectrometry.[19] MTXPG was correlated to MG outcome measures using Spearman Correlation Coefficient.

Standard Protocol Approvals, Registrations, and Patient Consents. The trial was approved by the Institutional Review Board at the University of Kansas Medical Center. Written informed consent was obtained by all participants, in accordance with the Declaration of Helsinki and the principles of Good Clinical Practice.

Outcomes and Measures. Monoglutamate (1), diglutamate (2), triglutamate (3), tetraglutamate (4), and pentaglutamate (5) were analyzed for correlation to the MG-ADL scale, the QMG scale, and the Myasthenia Gravis Composite (QMC) scale, all standard MG research outcome measures.

Statistics. A total of 33 polyglutamate samples were collected in the study and 21 samples were used from subjects who were on active MTX for analysis. The differences of the outcome measures between the baseline and end of study visit was calculated. A two-group t-test was used to determine the difference in MTXPG based on clinical outcome responder definitions. We correlated $MTXPG_{1-5}$ to MG outcome measures using Spearman Correlation Coefficient.

RESULTS

A total of 33 polyglutamate samples were collected in the study. Twenty-five samples were from subjects on MTX and eight samples were from subjects on placebo. Twenty-one samples were used for analysis (Figure 1). The median age of subjects was 64 ± 11.3 and the male/female ratio was 16:5.
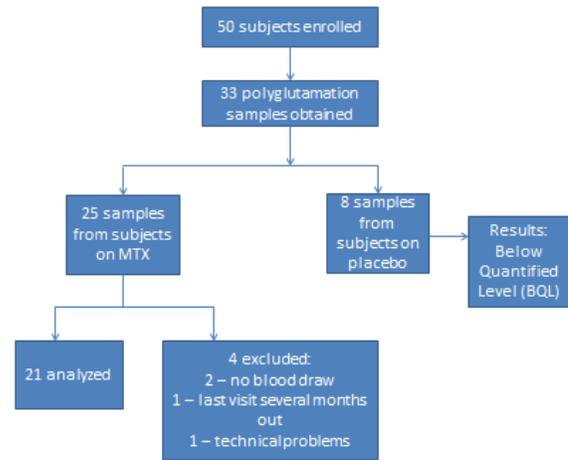
Figure 1. Study design.

The results of the degree of polyglutamation for each sample are shown in Figure 2. There were variable correlations between $MTXPG_{1-5}$ and MG outcomes with a rho range: 0.08 to 0.99 (Table 1). Strong correlations were seen between $MTXPG_{2,4,5}$ and MGADL, $MTXPG_{4,5}$ with QMG and $MTXPG_3$ with MGC. Both coefficients lie between -1 and +1. The more the correlation coefficient comes closer to -1 or 1, the more there is a correlation between two variables. Our interpretation of strength of correlation in this study is as follows: < 0.15 = very weak, 0.15 – 0.25 = weak, 0.25 – 0.40 = moderate, 0.40 – 0.75 = strong, >0.75 = very strong (Table 1).
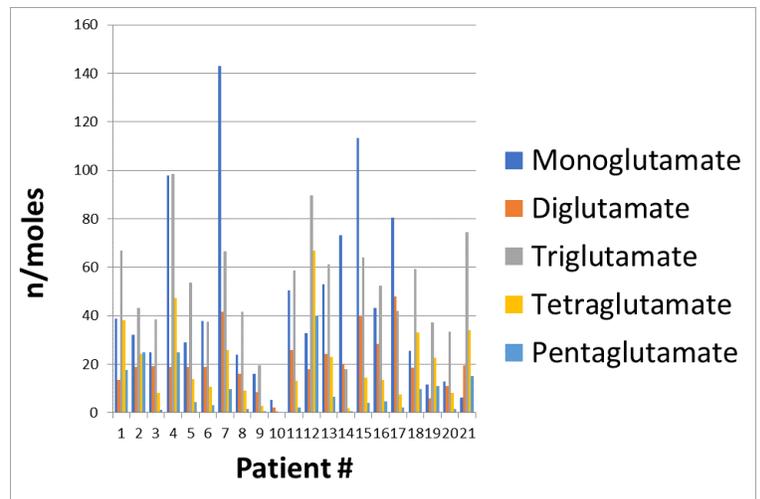
Figure 2. Degree of methotrexate polyglutamation in MG subjects.

Table 1. Correlation of methotrexate polyglutamation and MG.

Polyglutamates*	Correlation with MG outcomes (rho)			
	MG-ADL	QMG	MGC	Prednisone dose
Monoglutamates	0.20	0.08	0.08	0.17
Diglutamates	**0.60**	0.02	0.39	0.14
Triglutamates	0.38	0.19	**0.76**	0.36
Tetraglutamates	**0.71**	**0.54**	0.30	0.30
Pentaglutamates	**0.99**	**0.53**	0.15	-0.18

MG-ADL = Myasthenia Gravis Activities of Daily Living; QMG = Quantitative Myasthenia Gravis; MGC = Myasthenia Gravis Composite
*Bolded are strong and very strong correlations.

DISCUSSION

We found that the therapeutic effect of MTXPGs correlates with MG clinical outcome measures used in our prospective randomized trial. The therapeutic effects of MTX in RA depend on its conversion to MTXPGs, so measuring intracellular MTXPGs has been proposed as an objective method to guide MTX therapy in various disease conditions. Most of the information on MTXPG is from rheumatoid arthritis literature. Methotrexate helps stabilize rheumatoid arthritis conditions. Long chain MTXPGs stay in the cell longer than short chain MTXPG; therefore, long chain MTXPG keeps MTX in active form longer inside the cell, hence possibly allowing MTX to work longer. Rheumatoid arthritis literature showed higher concentrations of RBC long chain MTXPGs were associated with increased therapeutic response to MTX. However, literature is limited in other conditions.

This is the first study studying MTXPGs in Myasthenia Gravis and correlating it with outcomes in a randomized-controlled trial. We showed a wide inter-patient variability of RBC MTXPG concentrations in patients receiving similar dose of MTX therapy, which is similar to the rheumatoid arthritis literature. Also similar to the rheumatoid literature, the long chain polyglutamates (tetra and penta) correlated well with outcomes. In this case outcomes used were QMG, MG-ADL, and MGC. Prior studies showed no statistical significance of the MTXPGs levels with side effects; however, this analysis was not performed in our study. Of note, none of the subjects who were part of this sub-study developed liver toxicity.

Although the phase II Methotrexate in MG trial was negative (did not show significant reduction in the prednisone dose under the curve), the polyglutamate analysis showed strong correlations with MG outcome measures. This supported our other findings that secondary outcomes showed trends in improvement when patients were on methotrexate versus placebo.

As a result of this analysis, we believe that polyglutamation may be a useful personalized medicine biomarker to predict clinical improvement in MG when patients are on methotrexate. However, further studies should be done using this tool in myasthenia gravis.

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