Measuring methotrexate polyglutamates

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Received and accepted on July 22, 2010.

Clin Exp Rheumatol 2010; 28 (Suppl. 61): S24-S26.

Key words: Methotrexate, methotrexate polyglutamates

ABSTRACT

Methotrexate is the most commonly used drug in the treatment of rheumatoid arthritis, although 30–40% of patients fail to adequately respond. An accurate method for measuring methotrexate polyglutamates, the stable active metabolite of methotrexate, has recently been described. The objective of this review article is to determine if clinical use of this measurement would improve methotrexate efficacy, or decrease adverse reactions. Additionally the pharmacologic rationale for this test is discussed. Although methotrexate response improves at higher methotrexate polyglutamate levels, there is no absolute correlation of level with effect. Moreover, overlapping methotrexate polyglutamate levels between clinical responders and nonresponders limits the clinical utility of this measurement.

Methotrexate (MTX) is the most commonly used drug in the treatment of rheumatoid arthritis. It is frequently the first agent prescribed in patients who are at risk for erosive joint damage, and has become the anchor drug in combination drug regimens. It is used because of its effectiveness, as well as its favourable safety profile. Its effectiveness however is limited: 30–40% of RA patients taking MTX fail to adequately respond. Accurate measurement of the stable active metabolite, methotrexate polyglutamates (MTX-PGs) by HPLC has recently been described (1). It has been suggested that if MTXPG levels were clinically available and correlated with adverse events or therapeutic response, then the test could be used to guide the physician in providing increased response rates without an increase in toxicity.

Decades of MTX use have led to monitoring guidelines which permit dose escalations to higher and more effective levels. Measurement of liver function tests with appropriate adjustments in MTX dosage lessens the potential for liver damage. Administration of folic acid concurrently with MTX minimises potential toxicities such as mucosal ulcers, hair loss, and more significantly, hematologic effects, which can limit the escalation of MTX dose. Although higher starting doses of MTX produce higher response rates, higher starting doses are also more likely to result in higher rates of toxicity, particularly more gastrointestinal intolerance. MTX is, therefore, rarely raised above 20-25 mg orally in standard rheumatology practice. In spite of these clinical advances in MTX administration, the responder rate has not significantly improved (2). MTX levels, which vary widely between individuals, may contribute to this difference in response. Early response to therapy has both long-term implications regarding remission rates as well as lessening the likelihood of permanent joint damage. Optimal practice might benefit from a mechanism to monitor MTX drug levels, and adjust dosages to maximise response. Additionally, treatment nonresponders could be quickly recognised, permitting a shift in treatment strategies when optimal levels of MTX are reached.

MTX is absorbed from the gastrointestinal tract, and is rapidly cleared from the circulation. Clearance from the circulation occurs via uptake by nucleated cells as well as by urinary excretion, the latter accounting for 80–95% of an ingested dose within 24 hours. A small fraction of patients, approximately 10%, in fact excrete 99% of an ingested dose of MTX in the urine within 24 hours. MTX is taken into cells by the reduced folate carrier (RFC-1), competing with folinic acid for uptake. A small percentage of ingested MTX is then stabilised intracellularly by the addition of glutamic acid residues, converting the parent drug to the more stable polyglutamated form. Glutamic acid moieties are added sequentially and can be measured as MTXPG1-5, which are

Competing interests: none declared.

referred to collectively as methotrexate polyglutamates (MTX-PGs). MTX-PGs persist intracellularly and exert a sustained effect on cellular metabolism. De novo purine synthesis is inhibited by MTXPGs primarily via blockade of 5-aminoimidazole 4 carboxamide ribonucleotide transformylase (ATIC), the terminal enzyme in this pathway. ATIC inhibition leads to AICAR accumulation intracellularly and the extracellular accumulation of adenosine which has potent anti-inflammatory effects. Pyrimidine synthesis is inhibited by blocking thymidylate synthetase (3), contributing to the anti-proliferative effect of MTX. Glutamic acid residues are enzymatically cleaved from MTX, after which MTX is transported out of the cell by multi-drug resistant carriers. The level of MTXPGs is determined by multiple different enzymes, the levels and activity of which are genetically determined and which therefore produce wide variations in MTX levels among individuals. As MTX is rapidly cleared from the circulation, measuring serum levels of MTX is not useful in guiding therapy. An ability to measure MTXPGs might be more useful, as this is a stable compound which directly contributes to the therapeutic effect of MTX. This measurement could be helpful in guiding therapy, given the wide inter-person variation in MTX levels at any given dose (4).

MTX works slowly, exerting maximal benefit over 6 months after initiation of therapy. Achieving rapid control of RA minimises joint destruction, and increases long-term disease control. There is consensus that rapid escalation of dose is more effective in achieving rapid disease control than slower dose escalation. A multinational group has recommended a starting dose of 10–15 mg/week with escalation of 5 mg every 2–4 weeks to achieve rapid disease control, concordant with demonstrated dose dependant efficacy (5). Gastrointestinal toxicity is more frequent when rapid dose escalation is employed; bioavailability thresholds limit the maximal oral dose of MTX to 20–25mg/week. Pharmacokinetic studies of MTX have determined the length of time RA patients beginning oral MTX therapy take to reach a steady state. Significant variation among individuals exists, with steady state of RBC MTXPG reached anywhere from 10 to 140 weeks after the final stable dose. This lag in achieving a steady state in measurable RBC MTXPG levels may limit the clinical usefulness of this test (6, 7). Measurement of MTX-PG levels in circulating RBCs, although convenient, further compromises the validity of the information which is generated at the site of metabolic activity in nucleated cells.

When MTXPGs levels are measured in patients and correlated with the DAS28 as a measure of RA activity, the results are inconsistent. In one cross-sectional study of 192 established RA patients treated with oral MTX, disease activity was assessed and MTXPGs levels were obtained. All patients in this study met the ACR definition of RA and had a stable dose of MTX for >3 months. Measurements of disease activity included the DAS28, swollen and tender joint counts, M-HAQ and CRP level. A questionnaire was administered to evaluate MTX toxicity. Patients with high disease activity tended to be on higher doses of MTX, on average for 12 months prior to entry into the study. There was no consistent correlation between MTX dose and disease activity in this study, or MTXPG levels and disease activity. There was significant overlap in MTXPG levels between patients with a satisfactory DAS28 (<3.2) and those with a DAS28 >3.2, signifying ongoing disease activity. Forty-three percent of patients with a DAS28<3.2 had MTXPG levels under 60 nmoles/8 x 10 12^{th} rbcs, a level reported elsewhere to correlate with efficacy. In patients whose DAS28 was above 3.2, 31% had MTXPG levels under 60 nmoles/8 x 10 12^{th} rbcs, and 69% with a DAS28 >3.2 had MTXPG levels above that threshold. MTXPG levels inconsistently predicted therapeutic response. Additionally, these authors found no correlation with adverse events (8, 9).

Other authors have reached different conclusions. As the variation in MTXPG levels may depend on genetic polymorphism in purine and folate metabolic pathways, the contribution of these polymorphisms as well as the contribution of MTXPGs to the effect of MTX was studied in a cross-sectional study. 108 RA patients over the age of 18 who met the ACR criteria for diagnosis and who had been on a stable dose of MTX for at least 3 months were entered into this study. Variant genotypes were computed as a pharmacogenetic index, and MTXPG levels were measured. Disease activity was assessed by a 22 joint count, physicians and patient's global assessments on a visual analogue scale (VAS), and M-HAQ. There was little correlation of MTX dose with these clinical outcome measures, but higher MTXPG levels were associated with less activity in these clinical measures. MTXPG levels above 60 nmoles /litre were more likely to be associated with a favourable clinical response using these measures. The combination of higher MTXPG levels with an increase in homozygous variant genotypes in the folate/purine pathways reflected in the pharmacogenetic index was associated with a more likely therapeutic response. There were associations of certain genotypes with toxicity as well. (10, 11) Although the implications of these pharmacogenetic observations are profound, these measurements have no practical role in patient management at present.

The correlation of MTXPG levels with MTX effect was also supported in a prospective longitudinal study in which 48 MTX naïve patients were enrolled and started on MTX. The dose was escalated from the starting dose of 7.5 mg/wk until a response was reached. Clinical effect was measured by the DAS28. This study also addressed the role of folate polyglutamates (PGs) on the effect of MTX. MTX competes with folinic acid for uptake into cells via RFC-1. Additional anti- folate effects are mediated through inhibition of enzymes of the folate pathway such as dihydrofolate reductase, methylene tetrahydrofolate reductase, thymidylate synthetase, and 5-aminoimidazole 4 carboxamide ribonucleotide transformylase (ATIC) which are important in the *de novo* synthesis of purines and pyrimidines and are inhibited by MTX (12). Although some authors report a decrease

in efficacy when folic acid is routinely prescribed along with MTX, most prospective trials reveal no decrease in efficacy (13) as well as less toxicity. Patients with higher MTXPG levels were more likely to have achieved a therapeutic response, an effect which was not well correlated with MTX dose, as reported in prior studies. Patients achieving a therapeutic response to MTX were more likely to have lower folate PG levels as well as higher MTXPG levels. Higher MTXPG levels correlated with lower folate PG levels as well as with beneficial effect. There was no correlation between MTXPGs, folate PGs and drug toxicity.

In summary, RBC MTXPG levels can be accurately measured by HPLC. There is no absolute correlation of MTXPG levels with beneficial effect, although efficacy is more likely at higher levels. Patients with MTXPG levels above 60 nmoles/litre are more likely to have a therapeutic benefit than those patients with lower levels. There is considerable overlap between groups, however, limiting the utility of this measurement in clinical practice. Moreover, the lag to steady state equilibrium diminishes the timeliness necessary if this were to be used to guide dose escalations. Finally, MTXPG levels did not correlate with adverse effects.

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