REVIEW

Will pharmacogenetics allow better prediction of methotrexate toxicity and efficacy in patients with rheumatoid arthritis?

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Methotrexate (MTX) remains the most commonly used disease modifying antirheumatic drug in rheumatoid arthritis (RA) because of its cost and experience in its use, despite the availability of new treatments such as leflunomide and the biological agents. However, a significant number of patients with RA either do not benefit from the drug or are unable to tolerate it. Pharmacogenetic approaches may help optimise treatment with MTX, and also other agents, in RA.

EPIDEMIOLOGY OF RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA), a chronic disease with substantial costs to both the individual and society, affects about 0.5–1% of the general population world wide.¹ Costs include those resulting from direct medical care and those associated with work loss, pain, psychological distress, and limitations in function. Disability occurs often. About a half of the patients with RA eventually become work disabled, and mortality is increased in patients with severe, active disease.² However, deformity, disability and death can be reduced by the use of disease modifying antirheumatic drugs (DMARDs).

EFFICACY OF MTX IN RA

Among the several DMARDs available for the treatment of RA, methotrexate (MTX) is currently the most widely used. Gubner and Ginsburg first reported in 1951 that aminopterin (MTX) was successful in suppressing synovitis in six patients with RA.³ Since then, many studies have shown its beneficial effects in RA. Evidence for the efficacy of MTX in RA is primarily derived from four well designed randomised controlled trials performed in the mid-1980s.^{4–7} A multitude of subsequent studies have firmly established its efficacy in RA, with a sustained response seen in over 40% of patients.⁸ It has also been shown that patients with RA continue receiving MTX significantly longer than they continue with other DMARDs, with 60–70% of patients continuing to receive MTX for as long as 5–7.5 years.⁸

Over the past few years, several new agents for the treatment of RA have been introduced, including leflunomide and biological agents such as etanercept and infliximab. Recent evidence suggests that certain biological agents are effective in slowing the radiographic progression of

disease when used alone or in combination with MTX.^{9, 10} However, the high cost of some of these agents, especially the biological agents, and lack of long term safety and efficacy data preclude their use as the DMARDs of initial choice.¹¹ Hence, MTX continues to be used as the first line DMARD in RA.¹²

Often in clinical practice, MTX is used as the initial DMARD until the patient is deemed a non-responder or a partial responder, at which time another DMARD is added or substituted.¹¹ Thus, although several agents are available for the treatment of RA, the choice of initial DMARD (which is often MTX) remains empirical.

PREDICTORS OF RESPONSE TO MTX IN RA

Despite the well accepted efficacy of MTX, response to the drug in patients with RA is not universal. Response to MTX in patients with RA based on the American College of Rheumatology 20 response rates varies from 46% to 65%.^{9, 13} Serum levels of MTX have been considered of little value in determining drug efficacy, as the drug is eliminated from the serum within 24 hours of administration and the drug is given weekly for treatment in RA.¹⁴ By contrast, circulating intracellular levels of MTX polyglutamates in erythrocytes and polymorphonuclear cells have been shown to correlate with clinical efficacy in patients with RA, but require a difficult assay system that prohibits its availability in most clinical facilities.¹⁵

Several other factors have been studied to help predict response to treatment. Disease duration has a strong effect on the likelihood of patients responding to several DMARDs, including MTX.¹⁶ Patients with disease duration of one year or less have the best response to treatment.¹⁶ Female sex, prior DMARD use, disease functional class, and disease activity also affect patient response to treatment.¹⁶ Cytokine expression by T cells has

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Abbreviations: AICAR T'ase, 5-aminoimidazole-4-carboxamide ribonucleotide transformylase; ALL, acute lymphoblastic leukaemia; CI, confidence interval; DHFR, dihydrofolate reductase; DMARD, disease modifying antirheumatic drug; FPGS, folylpolyglutamate synthase; GAR T'ase, glycylamide ribonucleotide transformylase; GI, gastrointestinal; MTHFR, methylenetetrahydrofolate reductase; MTX, methotrexate; PCR, polymerase chain reaction; RA, rheumatoid arthritis; RR, relative risk; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; THF, tetrahydrofolate; TYMS, thymidylate synthase

been correlated with response to treatment with MTX in RA. One study showed that the response to treatment with MTX correlated with a decrease in the percentage of tumour necrosis factor α producing T cells and an increase in the percentage of interleukin 10 producing T cells.¹⁷ Serum matrix metalloproteinase-3 levels parallel serum interleukin 6 and C reactive protein levels and may correlate with response to treatment with MTX in RA.¹⁸ Enhanced tissue inhibitor of metalloproteinase-1 production by peripheral blood mononuclear cells has also been shown to be associated with the clinical efficacy of MTX.¹⁹ Serum levels of chemokines such as RANTES (regulated upon activation, normal T cell expressed and secreted) and GRO- α may predict the effect of MTX on radiographic erosions.²⁰ However, the lack of availability of sophisticated cytokine and enzyme assays in most clinical settings, in addition to the expense of running them, has prevented their use in clinical practice. Also, although such assays correlate with response to treatment, they do not predict response to treatment with MTX.

MTX RESISTANCE: LESSONS FROM CANCER PUBLICATIONS

Critical determinants of MTX resistance have been identified in lymphoblasts from children with acute lymphoblastic leukaemia (ALL). These include MTX membrane transport, dihydrofolate reductase (DHFR) levels, and MTX polyglutamation.²¹ Multidrug resistance, characterised by overexpression of P-glycoprotein, a pump molecule that decreases intracellular drug concentrations by increasing drug efflux from cells, has been shown to be one of the mechanisms of MTX resistance. Transfection of multidrug resistance proteins in human ovarian carcinoma cells caused a two- to threefold lower accumulation of MTX with subsequent reduced formation of long chain polyglutamate forms of MTX and MTX resistance.²²

Variants of human DHFR with substitutions at Leu22 and Phe31 causing decreased binding to MTX have been constructed.²³ Such variants when transfected into Chinese hamster ovary cells or mouse marrow progenitor cells confer resistance to high levels of MTX.²⁴ B-lineage lymphoblasts in children with ALL have twofold lower levels of DHFR mRNA compared with T-lineage lymphoblasts.²⁵ This has been postulated as one of the potential mechanisms contributing to the worse prognosis of T-lineage ALL when treated with MTX (compared with B-lineage ALL). Also, resistance to MTX in human leukaemia cell lines and in patients with acute non-lymphocytic leukaemia is associated with a marked decrease in the intracellular level of MTX polyglutamates.²⁶ Such decreased polyglutamation is secondary to decreased activity of the enzyme folylpolyglutamate synthase (FPGS), one of the two enzymes important for the formation of intracellular MTX polyglutamates.²⁶ Thus, both the DHFR enzyme and polyglutamation have critical roles in influencing resistance to MTX.

In contrast with the oncology literature, limited evidence exists about MTX resistance in RA. One study showed expression of P-glycoprotein in the peripheral blood mononuclear cells of patients with RA. P-glycoprotein levels were higher in 16 patients with RA refractory to treatment than in eight patients who responded to treatment with MTX, either as monotherapy or in combination with other DMARDs.²⁷

Hence, several factors have been studied in an attempt to predict response to MTX in patients with RA. These have included clinical correlates such as disease duration and functional class, cytokine levels, and circulating intracellular levels of MTX. Although variations in the activities of several enzymes (such as P-glycoprotein, DHFR, FPGS) involved in the metabolic pathway of MTX have been shown to be important in determining the response/resistance to MTX, these have not yet been studied systematically.

TOXICITY OF MTX IN RA

Another major factor limiting MTX use is its toxicity.²⁸ Studies with observation periods of 60 months or longer show that about 10–30% of patients with RA discontinue MTX because of toxicity.²⁸ Several toxicities are associated with the use of MTX, including nodulosis (8%),²⁹ hypersensitivity pneumonitis (2–5%),³⁰ central nervous system toxicity (1–35%),³¹ post-dosing reactions (10%),³² gastrointestinal (GI) symptoms such as nausea, vomiting, abdominal pain, and diarrhoea (60%),^{33–34} hepatitis with raised transaminases (20–58%),³⁵ haematological abnormalities (1–2%),³⁶ rash (1–2%),¹³ alopecia (5%),¹³ and osteopathy (rare).³⁷ Known risk factors for MTX toxicity include advanced age,³³ diminished renal function,³⁸ and concurrently administered drugs.³⁹ Because both the amount and duration of exposure to MTX have been shown to correlate with toxicity,⁴⁰ it has been suggested that serum levels of MTX help predict certain types of toxicity such as gastrointestinal toxicity and myelosuppression.⁴¹ However, as MTX disappears from the serum within 24 hours of administration, serum levels are not accurate enough to predict toxicity. Hence, guidelines have been established for monitoring MTX toxicity, particularly for hepatotoxicity, that do not involve measuring serum levels. Such guidelines have been defined using evidence of end organ damage already present, such as monitoring raised transaminases for liver damage.⁴² Although the drug itself is relatively inexpensive, it has been shown that MTX has the highest monitoring costs among the commonly used DMARDs.⁴³ This is particularly relevant currently, as MTX is being used increasingly in combination with other hepatotoxic agents such as leflunomide, making frequent monitoring necessary.⁴⁴

“Use of MTX is limited by its variable efficacy and toxicity”

Most of the toxicity associated with MTX is thought to be linked to drug effects on folate metabolism. MTX associated GI, myelosuppressive and possibly, hepatic toxicities are thought to be directly related to its folate antagonism in these tissues, which have a high cell turnover and a relatively high requirement for purines, thymidine, and methionine.⁴⁵ Hepatic accumulation of MTX-polyglutamates has been demonstrated in liver biopsy specimens from patients with RA, accompanied by hepatic folate deficiency, suggesting that MTX-polyglutamates may cause hepatotoxicity by folate depletion.⁴⁶ Folic acid supplementation at 1 mg/day reduces such toxicity significantly.⁴⁷ Other mechanisms thought to be important in mediating toxicity include inhibition of (a) purine metabolism; (b) adenosine deaminase with accumulation of adenosine and deoxyadenosine; (c) polyamine synthesis; and (d) homocysteine metabolism.⁴⁵

PHARMACOGENETICS AND ITS APPLICATION TO MTX METABOLISM

Thus the major factors limiting the use of MTX in patients with RA are its variability in efficacy and toxicity.^{8–28} At the present time, there are no reliable tests or assays that can predict the toxicity or efficacy of MTX. Nevertheless, there is potential to further improve the efficacy and decrease the toxicity from the drug through a better understanding of its pharmacology. This understanding can be gained by using the principles of pharmacogenetics to study genetic differences (polymorphisms) in the enzymes involved in the metabolic pathways of MTX.

The field of pharmacogenetics focuses largely on genetic polymorphisms in drug metabolising enzymes and the translation of inherited differences into drug effects.⁴⁸ Genes are considered functionally “polymorphic” when allelic variants exist stably in the population, one or more of which alters the

activity of the encoded protein in relation to the wild-type sequence. The genetic polymorphism may be associated with reduced activity of the encoded protein. Although the study of pharmacogenetics focuses largely on polymorphisms in drug metabolising enzymes, polymorphisms in drug transporters (such as P-glycoprotein) and drug targets (such as receptors) have also been studied. Such pharmacogenetic studies may soon make it feasible more precisely to select drugs and doses that are optimal for individual patients. In this regard, automated system based "gene chips" may become available soon, whereby an individual person's polymorphic genotype involved in the pathogenesis of their disease, in the metabolism and disposition of drugs, and in the targets of drug treatment can be determined. Such "gene chips" may become the blueprints for individualising drug treatment in the future.⁴⁹

"Gene chips" may be used to individualise drug treatment"

Until recently, clinically important genetic polymorphisms in drug metabolism were typically discovered on the basis of familial occurrence or extreme phenotypic differences among individual subjects in the population. Recent advances in the field of genetics, specifically pharmacogenetics, have changed this. With molecular sequencing technology, genetic polymorphisms such as single nucleotide polymorphisms can now be detected. When translated to clinical studies, such technology may permit the elucidation of polymorphisms in drug metabolising enzymes that may have clinically important consequences, such as interindividual variability in drug response or drug toxicity.⁴⁹ Such polymorphisms may be readily detectable to help assess drug response or toxicity even when traditional approaches are not fruitful.

MTX was developed to be a highly selective competitive inhibitor of the enzyme DHFR.⁴⁴ MTX enters cells through an active transport mechanism. Once inside the cell, it is converted into a polyglutamate form by the enzyme FPGS (fig 1). This process can be reversed by the enzyme folylpolyglutamate hydrolase. The polyglutamate form of MTX which can have up to four glutamic acid moieties (a) retains MTX within the cell for long periods⁵⁰; (b) inhibits DHFR which mediates the conversion of dihydrofolate to tetrahydrofolate (THF), the precursor of biologically active folate cofactors such as 5-methyl-THF, important for the conversion of homocysteine to methionine and in the synthesis of polyamines⁴⁵; (c) increases the potency for inhibiting thymidylate synthase (TYMS), which converts deoxyuridylate to deoxythymidylate in the de novo pyrimidine biosynthetic pathway⁵¹; and (d) increases the potency for directly inhibiting enzymes, such as glycylamide ribonucleotide transformylase (GAR Tase) and 5-aminoimidazole-4-carboxamide ribonucleotide transformylase (AICAR Tase),⁵² which play a part in de novo purine biosynthesis. The ability of MTX to inhibit these enzymes increases as the number of glutamate moieties increases. The pentaglutamate form of MTX is about 2500 times more potent in inhibiting AICAR Tase, 250 times more potent in inhibiting TYMS, and 32 times more potent in inhibiting GAR Tase than the native monoglutamate form.⁴⁴

MTX, by affecting the intracellular folate pool, influences the activity of the enzyme methylenetetrahydrofolate reductase (MTHFR), one of the enzymes in the folate pathway (fig 1). MTHFR is important in the generation of 5-methyl-THF, which is the methyl donor for the conversion of homocysteine to methionine. Methionine is used for the synthesis of S-adenosylmethionine (SAM), which is converted to S-adenosylhomocysteine (SAH) and homocysteine.⁵³ SAM is the methyl donor for over a 100 different transmethylation reactions, including methylation of DNA and proteins, phospholipid synthesis, and neurotransmitter synthesis.⁵⁴

Recently, heterozygous and homozygous Mthfr knockout mice have been developed, which have raised plasma homocysteine and disrupted SAM and SAH levels that are associated with DNA hypomethylation in several tissues. Such mice have reduced survival, delayed development, cerebellar abnormalities and abnormal lipid deposition in the proximal aorta.⁵⁵ Thus, the MTHFR enzyme impacts several crucial cellular processes and its deficiency can have widespread consequences.

More than a dozen polymorphisms have been described in the MTHFR gene.⁵⁶ Of these, the C677T and A1298C polymorphisms have been associated with altered phenotypes and adverse drug events. The C677T polymorphism, first described in the mid-1990s, results in an alanine to valine substitution in the codon at nucleotide 677 of the MTHFR gene.⁵⁷ It leads to a thermolabile variant of MTHFR with decreased enzyme activity, and subsequent increased plasma homocysteine levels.⁵⁸ The homozygous C677T variant, with about 30% of wild-type activity, is present in about 8–10% of the general population. Heterozygotes have about 60% activity and form approximately 40% of the population. The C677T polymorphism has been shown to be associated with decreased risk for ALL and colorectal neoplasia, and increased risk for neural tube defects and cardiovascular disease.^{59–63} It has also been shown to influence the clinical effects of drugs such as anticonvulsants, levodopa, oestrogen, and cholestyramine.^{64–67}

In 1998, another polymorphism in the MTHFR gene, A1298C, causing a glutamine to alanine substitution in the codon at nucleotide 1298, was described.^{68–69} Both the homozygous and the heterozygous polymorphisms lead to reduced activity of the MTHFR enzyme, although not to a thermolabile variant. The homozygous genotype with ~60% of enzyme activity in lymphocytes has been found in ~10% of the Canadian population (prevalence world wide unknown).⁶⁹ The A1298C polymorphism, by itself, does not result in increased plasma homocysteine levels. However, subjects heterozygous for both the C677T and A1298C polymorphisms have significantly decreased activity of the MTHFR enzyme and raised plasma homocysteine levels comparable with subjects homozygous for the C677T polymorphism.⁶⁸ An increased frequency of the A1298C polymorphism has been seen in children with neural tube defects.⁶⁸

The effect of the C677T polymorphism on the toxicity of MTX in patients undergoing bone marrow transplantation was studied recently. Two hundred and twenty patients with chronic myelogenous leukaemia who underwent marrow allografts and received MTX for prevention of graft versus host disease were assessed for MTX toxicity prospectively. Toxicities assessed included oral mucositis, speed of engraftment (platelet and granulocyte counts), and hepatotoxicity (measurement of bilirubin). All patients were genotyped for the C677T polymorphism. Forty two per cent of the patients were wild type, 42% heterozygous, and 16% homozygous. There was a higher incidence of oral mucositis in patients with the homozygous and heterozygous genotypes, reaching statistical significance with the homozygous genotype. Recovery of platelet counts was slower among patients with the polymorphism than among patients with the wild type. There was no effect of the genotype on recovery of granulocyte counts or bilirubin levels. This study demonstrated a relation between the C677T polymorphism and MTX toxicity, with patients with the homozygous genotype experiencing higher toxicity.⁷⁰

Research that examines the influence of pharmacogenetics on both the efficacy and toxicity of MTX in RA is just beginning to appear. Plasma homocysteine levels were measured prospectively in 105 patients with RA, 35 of whom were treated with MTX, 34 with sulfasalazine, and 36 with a combination of MTX and sulfasalazine. All patients were genotyped for the C677T polymorphism in the MTHFR gene by polymerase chain reaction (PCR) and restriction enzyme analysis. The two treatment groups receiving MTX showed a

Figure 1 Putative targets for MTX pharmacogenetic analysis (shown in *italics*).

persistent, greater increase in plasma homocysteine than the group receiving sulfasalazine alone, with the patients receiving a combination of MTX and sulfasalazine showing the greatest increase. The MTHFR genotype influenced the rise in homocysteine. Patients with the heterozygous mutation had higher plasma homocysteine after one year than patients without the mutation. Patients homozygous for the mutation had a high plasma homocysteine level at baseline which did not change significantly, possibly because of a ceiling effect. A statistically significant higher rise in plasma homocysteine (an increase of 17%, $p < 0.05$) was found in patients experiencing a GI adverse event (nausea, abdominal discomfort/pain) than in patients without an adverse event. Also, patients treated with the combination of MTX and sulfasalazine had the highest homocysteine levels and the highest incidence of GI side effects. There was no direct relation between the occurrence of adverse events and the MTHFR genotype. The authors concluded that patients with RA receiving MTX have increased plasma homocysteine levels, which may be further enhanced by the C677T polymorphism in the MTHFR gene. Plasma homocysteine may be important in mediating the gastrointestinal toxicity from MTX.⁷¹

In another study, 236 patients with RA receiving MTX were assessed prospectively for toxicity and disease activity every three and six weeks, respectively. All patients were genotyped for the C677T MTHFR polymorphism using genomic DNA by PCR and restriction enzyme analysis. Nineteen patients (8%) had the homozygous polymorphism, 95 patients (40%) had the heterozygous polymorphism, and 122 patients (52%) did not have the polymorphism. The presence of the C677T poly-

morphism (homozygous or heterozygous) was associated with an increased risk (relative risk (RR) 2.01; 95% confidence interval (95% CI) 1.09 to 3.70) of MTX discontinuation because of adverse events such as GI symptoms, hair loss, and hepatotoxicity. The polymorphism led to MTX discontinuation mainly due to an increased risk of raised liver enzymes, specifically alanine aminotransferase (RR 2.38; 95% CI 1.06 to 5.34). The predictive power of the genotype was independent of folate supplementation. No relation was seen between the polymorphism and the efficacy of MTX in this study. The authors postulated that an increase of transaminases during MTX treatment in RA is mediated by its effects on homocysteine metabolism. Such effects may be more pronounced in patients with the C677T polymorphism receiving MTX.⁷²

In a retrospective analysis, 106 patients with RA were genotyped for the C677T and A1298C polymorphisms in the MTHFR gene using the PCR-restriction fragment length polymorphism method. All patients were receiving MTX at the time of enrolment or had discontinued MTX in the past because of adverse events. The efficacy and toxicity of MTX were assessed in patients and correlated with the presence or absence of the C677T and A1298C polymorphisms. Patients homozygous or heterozygous for the A1298C polymorphism were receiving lower doses of MTX than patients without the polymorphism ($p < 0.05$, RR 2.18; 95% CI 1.17 to 4.06). The presence of the polymorphism was associated with improvements in C reactive protein levels and erythrocyte sedimentation rates ($p < 0.05$), but not in tender or swollen joint counts.

No such associations were found with the C677T polymorphism. However, overall MTX toxicity, such as increase in transaminases, stomatitis, nausea, vomiting, hair loss, fatigue, and rash, was more common in patients homozygous or heterozygous for the C677T polymorphism than in patients without the polymorphism ($p < 0.05$, RR 1.25; 95% CI 1.05 to 1.49). There was no effect of the A1298C polymorphism on toxicity. Thus, the C677T polymorphism made patients with RA more sensitive to MTX toxicity, whereas the A1298C polymorphism made them more responsive to treatment.⁷³

Thus, so far, only genetic polymorphisms in the MTHFR enzyme, one of the enzymes in the metabolic pathway of MTX, have been studied. These studies have demonstrated the predictive power of the MTHFR genotype in determining MTX toxicity, with one study showing that two different polymorphisms in the MTHFR gene are related to two different phenotypes—that is, MTX efficacy and toxicity in RA. Single nucleotide polymorphisms in other enzymes involved in the metabolic pathway of MTX may be better predictors of MTX efficacy and toxicity. In vitro studies have already established the important roles of such enzymes, including DHFR (which is the substrate for MTX and may be the rate limiting enzyme in the folate pathway) and FPGS (critical for MTX polyglutamation), in mediating MTX resistance. Also, certain polymorphisms may translate into potent functional differences in enzyme activity while others may not. Hence, further studies are needed to study polymorphisms in other enzymes in the MTX pathway and their correlations with drug efficacy and toxicity in RA.

SUMMARY

Despite the availability of several new agents for the treatment of RA, MTX remains the mainstay because of both cost and experience with its use. However, there is considerable variation in the response to MTX, with toxicity limiting treatment in some patients. At present, there are no standardised tests to predict the drug's efficacy or toxicity in RA. It may be possible to develop such tests in the future based on the principles of pharmacogenetics. There is some initial evidence supporting the influence of pharmacogenetics on the efficacy and toxicity of MTX in RA. Potentially, the principles of pharmacogenetics can be applied to optimise drug treatment, not only with MTX, but also with other drugs, including the newer agents such as leflunomide and the biological therapies. Such applications will have tremendous impact, given our current inability to predict which patients with RA will respond to these drugs or develop toxicity and the high costs of some of these agents.

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