Review

Outcomes related to methotrexate dose and route of administration in patients with rheumatoid arthritis: a systematic literature review

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Competing interests: B.N.C. has filed a patent on the use of adenosine $A_{2A}R$ agonists to prevent prosthesis loosening (patent number 8183225), a patent on the use of anti-netrin-1 antibodies for the treatment of bone disease (pending). B.N.C. holds patent numbers 5,932,558; 6,020,321; 6,555,545; 7,795,427; adenosine A_1R and $A_{2B}R$ antagonists to treat fatty liver (pending); $A_{2A}R$ agonists to prevent prosthesis loosening (pending). B.N.C. is a consultant for Bristol-Myers Squibb, Novartis, Eli Lilly and Co., Regeneron, Endocyte, Protalex, Allos, Inc., Savient, Gismo Therapeutics, Antares Pharma, Medivector. B.N.C. holds stock in CanFite Biopharmaceuticals.

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ABSTRACT

Objective. *Methotrexate (MTX) is considered the "anchor drug" in the therapy of rheumatoid arthritis (RA), yet many physicians do not optimise MTX regimens in spite of high RA disease activity. The recent development of an auto-injector for the subcutaneous (subQ) administration of MTX has prompted re-evaluation of MTX utilisation. The purpose of this systematic literature review is to determine the optimal dose, drug level, and route of administration for MTX in the context of relevant pharmacokinetics and pharmacogenomics.*

Methods. *A systematic literature review was performed in Medline searching specifically for randomised controlled trials, systematic reviews, case control and cohort studies evaluating outcomes related to MTX dose and route of administration. Articles fulfilling these inclusion criteria were reviewed. Data on MTX dose, route of administration, clinical response, drug levels and adverse events were evaluated.*

Results. *Our search identified 420 articles of which 6 were eligible for inclusion using the above criteria. These included 2 systematic reviews, 2 randomised open label trials, one longitudinal study and one retrospective cohort study.*

Conclusion. *Efficacy and toxicity for MTX appear related to absorbed dose of MTX, not to route of administration. While bioavailability is greater for parenteral MTX, there is no evidence yet that splitting the oral dose of MTX is less advantageous, less safe or less tolerable than administering parenteral MTX. However, there appear to be modest benefits in beginning with higher doses of MTX, and switching to parenteral MTX when the clinical response to an oral dose is inadequate.*

Introduction

In light of the safety, efficacy, and tolerability of methotrexate (MTX), it has earned its place as the "anchor drug" in the treatment of rheumatoid arthritis (RA) (1, 2). Remission and low disease activity are accepted goals of therapy for RA (3), and early therapy with disease-modifying anti-rheumatic drugs (DMARDs) – in particular, methotrexate (MTX) – is recognised as an essential step in achieving these goals. Widespread use and acceptance of the 2010 ACR-EULAR RA classification criteria has facilitated the early use of MTX (4). This is a particularly important development since early use of MTX is effective in achieving remission or low disease activity (LDA), both alone and in combination with other drugs, including the tumour necrosis factor alpha inhibitors (TNFi) (5-9). Indeed, recent international recommendations advise initiation of DMARD therapy as soon as the diagnosis of RA is made, with early MTX therapy as part of the recommended initial treatment strategy, unless specific contraindications to MTX are present (6). MTX may be effective as monotherapy, or if not, can enhance the effectiveness of biologic DMARDs when additional therapy is needed to achieve disease control (8, 10). MTX is the most commonly prescribed DMARD in RA, and is the DMARD most likely to be continued in practice over a 5-year period (9, 11). MTX use is associated with a 70% reduction in mortality for RA with the survival benefit largely related to the reduction in cardiovascular mortality (12, 13). However, in a survey of French Rheumatologists, while half of RA patients had active RA, only 20% of patients were taking doses of MTX which were higher than 15 mg/week (14). Moreover, as with all medications,

there may be problems with safety and tolerability, and 30–40% of patients fail to adequately respond to MTX alone (15, 16).

MTX is usually given as an oral weekly dose, and while higher starting doses may improve the response rate and efficacy, higher doses may also produce more gastrointestinal (GI) symptoms such as nausea and diarrhoea (17). Early reports in psoriasis patients suggested that increased hepatotoxicity was associated with repeated small oral MTX doses given over 2 to 5 days. This early observation may have influenced the preference for a single weekly MTX dose (18). Folic acid and folinic acid supplementation also play a role in MTX safety and efficacy. Clear evidence supports decreased drug toxicity including liver function test (LFT) abnormalities and gastrointestinal toxicity in patients receiving folic acid supplementation, but concerns about decreased efficacy were not confirmed in a meta-analysis (19, 20).

MTX is a folic acid analogue and was developed as an anti-proliferative agent. However, its mechanism of action in RA may be through its anti-inflammatory activity (21, 22). After uptake by cells, glutamic acid moieties are bound to MTX forming MTX polyglutamates (MTX-PGs) which are more stable and may be more potent in inhibiting folate dependent enzymes. Inhibition of folate enzyme pathways leads to the intracellular accumulation of adenosine molecules, which are then released extracellularly and induce potent anti-inflammatory effects in neutrophils, macrophages, and lymphocytes (21, 23). Caffeine and theophylline are non-specific adenosine receptor antagonists and reverse the anti-inflammatory effect of adenosine in animal models. In patients with RA, regular caffeine drinkers are significantly more likely to discontinue MTX due to lack of efficacy compared to those who drink minimal caffeine, although prospective studies regarding the effect of caffeine have not been conclusive (24, 25). More recent evidence links polymorphisms in genes associated with adenosine production and metabolism to increased susceptibility to respond to MTX (21, 22). Clearly, multiple factors may contribute to the efficacy of MTX therapy, and the relationship of dose, drug levels, and route of administration with MTX efficacy is not obvious.

The recent development of an auto-injector for subQ administration of MTX has increased interest in MTX route of administration. A comprehensive systematic review of MTX dose and route of administration was published by Visser and van der Heijde who extensively reviewed the published literature to 1950, and directly compared dose and route of administration in 5 studies which will be summarised (26-31). Only one RCT directly compared subQ *vs*. po MTX administration, at 15 mg/week, which is not a maximal dose (31)**.** Given the safety and efficacy of MTX, optimising therapy with MTX is a worthwhile goal, yet there does not appear to be a clear understanding of the benefits of different MTX doses or differences in route of administration. The purpose of this study is to systematically review the literature on methotrexate dose, levels, and route of administration specifically looking for recent additions to the literature and including studies with heterogeneous outcomes, and to develop an evidence base for clinical recommendations for optimal methotrexate use in rheumatoid arthritis based on a qualitative literature review.

Methods

We formulated our clinical question into an epidemiologic question according to the PICO format and identified the *patient* as an adult with RA meeting ACR criteria on MTX (32). The *intervention* was a change in MTX administration including dose or route of MTX administration, such as oral, subcutaneous, or intramuscular. Multiple *outcomes* were utilised:

1. clinical response by standard disease activity outcome measures such as DAS-28;
2. drug retention;
3. adverse reactions.

We reviewed the literature for relevant articles included in Medline published in English between January 1, 2009 to February 1, 2014 using Medical Subject Headings (MeSH) terms and key words. We selected the start date for our search to build on the comprehensive systematic review published in 2009 by Visser and van der Heijde and have summarised their findings (33). We also reviewed bibliographies of key articles. Search terms included *rheumatoid arthritis, methotrexate, biomarkers, clinical response*. Criteria for inclusion: systematic reviews, randomised controlled trials, case control and cohort studies, and observational studies in which different MTX doses, levels, or routes of administration were assessed in RA patients with clear outcome measures related to MTX use. We searched international meeting abstracts for 2012–2013, but excluded case reports and case series. We also hand searched the literature for additional relevant studies. After titles and abstracts were screened by SG and VB, those articles which fulfilled inclusion criteria and were relevant to the research question were reviewed. Additional articles were excluded if they did not include sufficient information, if they were duplicates, or if they did not meet methodologic criteria. PRISMA guidelines for systematic reviews were followed (34).

Results

We identified 420 articles using our screening strategy of which 58 were selected for further review. For 36 articles, there was no clear relation of outcomes to the defined change in MTX dose, level, or route of administration, 10 papers were not of sufficient quality or detail for inclusion, and 5 papers were non-systematic reviews, eliminating 52 studies. The remaining 6 papers form the framework for our study and were included in our qualitative synthesis.

In a systematic review, Visser *et al*. analysed 3 randomised controlled trials which directly compared different oral treatment regimens with different starting doses and speed of dose escalation (33). Schnabel *et al*. compared starting doses of 15 vs. 25 mg. MTX and demonstrated that higher doses were not associated with significantly greater toxicity (28). Furst *et al*. com-

Table I. Journal articles that form the framework for our study.

Study reference	Study design and quality	Study characteristics	Intervention	Outcome	Conclusion
Mouterde *et al*. 2011 (35)	Systematic review 2a	11 studies of RA patients treated with MTX	Change in MTX dose or route of administration	Disease activity (ACR response or DAS 28)	Rapid escalation of dose, switch to parenteral MTX when oral therapy fails to achieve response
Visser *et al*. 2009 (33)	Systematic review 1a	38 publications including 3 dbRCT* (481 RA) 2 open-label RCT** (484 RA)	Change in MTX dose or route of administration	Clinical, radiological, and toxicity outcomes	Rapid escalation of dose, switch to parenteral MTX when oral therapy fails to achieve response
Islam *et al*. 2013 (37)	Prospective open label randomised trial 1b	Active RA meeting ACR criteria (n=96)	subQ *vs*. oral MTX at equivalent dose	Clinical, and toxicity outcomes	subQ MTX more efficacious than PO at equal doses
Stamp *et al*. 2011 (38)	Longitudinal trial switching from PO to subQ MTX	Adult active RA (n=30)	Switch to subQ MTX from po MTX	Disease activity (DAS28, SJC) MTX PG levels	Decrease DAS28 >0.6 in active RA with increased long chain MTXPGs*
Ng *et al*. 2014 (41)	Retrospective cohort 2b	Veterans with recent diagnosis of RA (n=7,017)		MTX start and maximum dose, route of administration, and retention of MTX	Higher MTX dose and injectable MTX increased likelihood of remaining on MTX
Bakker *et al*. 2010 (40)	prospective open label randomised trial 1b	Adult RA<1yr duration, DMARD naïve	Switch from PO to subQ MTX at the same dose or MTX +CYC**	Change in disease	Switch to SubQ MTX is useful (DAS28 decrease on SubQ of 0.5)*

Oxford Levels of Evidence: 1a: SR with homogeneity of RCT; 1b: Individual RCT (with narrow CI); 2a: SR (with homogeneity) of cohort studies; 2b: Individual cohort study (including low quality RCT). [SR: systematic review; dbRCT: double blind randomised controlled trial; CDR: clinical decision rule.] *Change in DAS28>.6 is considered moderately effective; **CYC: cyclosporine.

pared patients starting on low 5 mg/m^2 *vs*. high 10 mg/m^2 oral weekly and reported a linear dose response relationship in outcome (27). Verstappen *et al*. compared an intensive MTX protocol in which oral MTX dosage was adjusted monthly based on defined criteria to standard care demonstrating improved efficacy with lower disease activity and greater likelihood of remission in the intensively treated group (29). In addition, Visser calculated effect sizes through subtraction of the mean change in the comparator group from the mean change in the index group. This reaches statistical significance if zero is outside the confidence interval (CI), with a change >0.8 indicating a large effect. MTX administered at higher doses (12.5–20 mg/week *vs*. placebo) had significantly greater effect on disease activity measures as tender joint counts (TJC) (pooled effect size 1.08, 95% CI 0.35–1.81) and global status (pooled effect size 1.58, 95% CI 0.80–2.37). Large effect sizes were seen for the clinical variables for rapid escalation of dose by 5 mg/month to 25–30 mg/week (range 1.38–1.83) compared to slow escalation (range 0.91–1.50). Patients in the fast escalation group were also more likely to have a 50% reduction in their swollen and tender joints than those in the slow escalation group (17).

Direct comparisons were also made between studies in which parenteral MTX was compared to oral MTX regimens. To evaluate differences in efficacy or toxicity for route of MTX administration, Lambert *et al*. studied patients with active RA (DAS 28>3.2) who had received at least 2 months of oral MTX (15–20 mg/week), were switched to 15 mg/week IM MTX, and were found to have a minor further improvement in disease control. No further therapeutic effect was achieved with dose escalations up to 45 mg/week (30). In a randomised double blind controlled trial, Braun *et al*. reported on patients with active RA, defined as Disease Activity Score (DAS) 28 ≥4, who received 15 mg. of oral or subQ. MTX (31). After 4 months, those who had not responded were either switched to 15 mg. subQ MTX, or if they were on subQ, had the dose raised to 20 mg. Those receiving subQ MTX had better outcomes in all measured endpoints including remission, although the subQ administered dose was higher than the oral dose. At equivalent doses over the first 24 weeks of the study, there was little difference between groups (31).

In a systematic review of studies comparing MTX dose and route of administration, Mouterde *et al*. (35) included an additional RCT by Thompson in which MTX was compared to placebo (36), in addition to the studies by Furst, Verstappen, Schnabel, Lambert, and Braun (27-31), and reached conclusions which were similar to Visser; a high oral starting dose with monthly 5 mg dose increases is more effective than slower dose escalation strategies, although gastrointestinal effects were more common at a higher starting dose. Parenteral therapy was more effective, with less GI intolerance, when oral therapy did not achieve acceptable results (35).

Islam *et al*. directly compared the safety and efficacy of oral *versus* subQ MTX

over a six month period in 92 patients with active RA on oral MTX 15 mg. Patients were randomised to dose escalate either orally or via subQ administration at the same dose, and the subQ group had significantly lower swollen and tender joint counts and better ACR 20 (93% *vs*. 80%, *p*-value=0.02) and ACR50 (89% *vs*. 72%, *p*-value=0.02) responses, with relatively fewer adverse events including nausea, vomiting, and dyspepsia (37).

Stamp *et al*. demonstrated an increase in the level of biologically active MTX polyglutamates (MTX-PGs) when 30 patients with inadequate control of RA were switched from oral MTX to subQ MTX at the same dose. A significant decrease in disease activity measured by a change in DAS28 >0.6 in 10 patients was associated with new steady state levels of long chain MTX-PG5 (*p*-value=0.035) and MTX-PG3-5 (*p*-value=0.032) were reached over 40 weeks (38).

Using drug retention as an outcome measure for efficacy and tolerability, Ng retrospectively studied a large Veteran's Administration cohort, and reported that patients achieving higher MTX doses had lower risk of changing therapy (≤15 mg/week HR 0.85, 95% CI 0.77-0.92; for ≥20mg/week HR 0.79, 95% CI 0.72–0.86). Administering parenteral MTX was also associated with better drug retention (risk of therapeutic change: HR 0.64, 95% CI 0.52–0.78). In this retrospective analysis of 7,017 veterans with RA, those receiving parenteral MTX and high dose MTX were less likely to switch to or add another DMARD (39).

Bakker *et al*. studied the effect of a switch to subQ MTX from an oral dose vs. the addition of cyclosporine as part of a protocol of tight control of disease activity. Fifty-seven of 151 RA patients were switched to subQ MTX from oral MTX, either because of adverse events or inadequate disease control, and had a statistically significant decrease in DAS 28 (0.30 points, *p*-value<0.05), and a DAS28 decrease of 0.5 over 4 months (*p*-value=0.01). No significant change was seen in the patients who added cyclosporine, while 63% of those who switched to subQ MTX improved (40).

Discussion

This review demonstrates that MTX efficacy is related to dose, but not necessarily route of administration, although a switch to parenteral route may increase efficacy for some patients. Both Visser and Mouterde concluded that higher starting doses and rapid escalation of MTX, with a switch to parenteral MTX in cases where the response was inadequate, was the optimal dosing strategy for MTX, based on evidence reported in their systematic reviews. When clinical response to MTX is inadequate, increases in the dose of MTX can successfully re-establish a response, due to the approximately linear relationship between MTX dose and effect previously demonstrated (42, 43). Further support for this strategy comes from an early RA cohort demonstrating that oral MTX monotherapy beginning at a dose of 15 mg with rapid escalation based on disease activity resulted in remission in 60% of patients (44). Other early studies have also demonstrated an increase in clinical benefit when patients who failed oral MTX were switched to parenteral MTX at a higher dose (45, 46). Bioavailability, or the amount of drug delivered to the systemic circulation, can be limited for oral MTX by incomplete absorption from the GI tract as well as "first pass" hepatic metabolism. In a recent crossover study, SubQ MTX demonstrated a bioavailability increase in a linear fashion without reaching the plateau seen with oral doses above 15 mg. per week given as a single dose (47). Absorption of oral MTX is limited by saturation of the reduced folate carrier 1 (RFC1), a ubiquitous transmembrane transporter, so oral administration at doses of 20–25 mg may saturate this mechanism, impeding absorption. However, if doses above 15 mg are split into 2 doses ≥8 hours apart, GI absorption is improved (48-50).

Using drug retention as a measure of drug efficacy and tolerability, Ng *et al*. demonstrated that patients receiving parenteral or high dose MTX were more likely to remain on MTX as monotherapy (39). However, dose of MTX, not route of administration, was found to be significant in a prospective cohort study of early RA patients, which was achieved through a higher use of both oral split dose as well as subQ use of the drug. Although better DAS-28 scores were seen in the patients receiving subQ MTX, once the analysis was corrected for dose the difference was no longer significant, although the subQ route may have been more sustainable (51). Using a tight disease control strategy, Bakker *et al*. described an improvement in DAS-28 when patients were switched from oral to subQ MTX, however, the there was little clinical significance of the change in DAS 28 of 0.3, as a decrease of 0.6 is considered a moderate improvement (52). Additional observations were made in 81 patients treated with parenteral MTX in complete remission of inflammatory arthritis after a shortage of parenteral MTX forced a switch to oral MTX. Forty-nine of the patients were switched to the same oral dose and 8 flared, while 10 were switched to a higher MTX dose and remained stable (53). This inadvertent experiment demonstrates the inter-patient variability in MTX bioavailability. Administering MTX by the subcutaneous or intramuscular (IM) route provides reliable absorption into the systemic circulation (54, 55) most likely explaining the improved efficacy described for subQ MTX. Recent studies performed to assess new proprietary MTX autoinjector formulations have replicated the demonstration of increased bioavailability of subQ MTX *versus* oral, measuring area under the curve (AUC) and maximum concentration. While the AUC of oral dose MTX plateaued at 15 mg, the AUC for the subQ dose continued to increase in a dose dependent fashion (56, 57). GI effects were greater with the oral drug (57).

After uptake by nucleated cells, MTX is stabilised by the enzymatic addition of 1–5 glutamic acid residues, forming bioactive long-chain MTX polyglutamates (MTX-PG1-5) which accumulate within the cell, remain for long periods of time, and confer the anti-inflammatory activity of MTX (38, 58, 59). Stamp demonstrated an increase in MTX-PG levels in association with a decrease in disease activity in patients switched from PO to subQ MTX (38).

Long-chain MTX-PGs reach a steady state after months of stable dosing (60). Measurement of stable intracellular long-chain MTX-PG 1–5 has been used to assess drug effect, as the long chain MTX-PGs are more effective inhibitors of enzymes such as 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR), which is important in the anti-inflammatory effects of MTX (61, 62). As higher MTX doses appear to confer more benefit, measurement of MTX-PG levels might improve management of RA by informing when poor response is related to low drug levels and poor absorption of MTX, providing a rationale for safe dose adjustments or a switch to subQ administration. However, a steady state in drug levels was reached over 40 weeks, and while concentrations of long-chain MTX-PGs increased more over a 24 week period in those who had improvements in disease activity than those without improved disease activity (measured as DAS-28), overlap between groups was significant (38). A recent longitudinal study also found that while an increase in the concentration of long-chain MTG-PGs over a 9 month period was associated with lower RA disease activity, no relationship to adverse events was seen (63). As there is no absolute relationship between the level of MTX-PGs and either toxicity or efficacy, and MTX-PG steady state is reached after months of a stable dose, these measurements are challenging to use clinically (64, 65). While long-chain MTX-PGs are an interesting target for understanding MTX effects, measurements of drug level do not seem to be practical to determine or predict treatment response or toxicity, and cannot be effectively used to assess a MTX dose or route of administration.

Islam *et al*. demonstrated an improvement in disease activity in patients with active RA switched to subQ MTX compared to po MTX at equivalent doses with less gastrointestinal adverse toxicity (37). Others have also found that parenteral MTX has better GI tolerance (66), and that gastrointestinal symptoms of nausea and diarrhoea may be more frequent with oral MTX, but this has not been consistently described. In another comparison of oral *versus* parenteral MTX, those patients receiving parenteral MTX were more likely to discontinue the drug due to all toxicities including GI reactions, with fewer patients reporting diarrhoea and more reporting nausea with the subQ route (31).

Although GI toxicity for MTX is well described, the relationship between other toxic effects and drug dose or drug level is more difficult to assess. The wide variation in MTX efficacy and toxicity between individuals may be related to the multiple genetically coded enzymes involved in MTX metabolism and transport as well as drug level (67). Genetic polymorphisms have been associated with MTX toxicity and efficacy. MTX, a folate analogue, is absorbed, transported and metabolised by multiple enzymes in the folate pathway. GI toxicity, alopecia, and hepatotoxicity have been associated with specific transporter gene polymorphisms (68). Racial and ethnic differences in allele frequencies of folate pathway enzymes associated with adverse events have also been reported (69). For a meta-analysis of pooled pharmacogenetic data, single polymorphisms in methylenetetrahydrofolate reductase (MTHFR), an enzyme relevant to MTX, was associated with increased toxicity (70). Recent work has also found an association of MTX efficacy with certain RFC-1 polymorphisms, with no association with toxicity. Interestingly, the effect was not seen with higher MTX doses (71) suggesting pharmacogenetic analysis may ultimately be most helpful for clinical assessments of MTX efficacy and toxicity.

Hepatotoxicity may be mediated by adenosine, as adenosine can stimulate hepatic steatosis and also contribute to hepatic fibrosis (72, 73). Transient liver function test elevations are described in up to 30% of RA patients, and typically resolve with drug discontinuation or dose decrease, suggesting a dose dependent effect. While increases in liver enzymes in patients on MTX are frequent, they are rarely clinically significant (11, 74, 75). Folic acid supplementation reduces the incidence of MTX associated liver enzyme elevations, but concern about an effect on efficacy has been raised (76, 77). Liver histologic change is associated with MTX dose (78), and long-term use of MTX for ≥2 years may contribute to liver damage (79). Hepatic fibrosis is also dose dependent, and although mild fibrosis is relatively common, affecting 15% of patients on long-term MTX therapy, only 0.5% develop cirrhosis and 1.3% severe fibrosis on biopsy (80). If "first pass" hepatic effects are minimised by subQ or IM administration of MTX, significantly less of a given dose would be metabolised by the liver. However, no association has been described for hepatotoxicity with the route of administration (80).

Toxic effects such as stomatitis, alopecia, and cytopenias, are linked to the anti-proliferative effects of MTX and may be dose dependent. Given the greater bioavailability of parenteral MTX, these reactions might be more frequent with parenteral MTX, although this has not been reported. Clinically, cytopenias have been associated with impaired renal function, which delays renal excretion and increases MTX levels (17, 82).

Conclusion

Efficacy and toxicity for MTX appear related to absorbed dose of MTX, not to route of administration. While bioavailability is greater for parenteral MTX, there is no evidence that splitting the oral dose of MTX is less advantageous, less safe or less tolerable than administering parenteral MTX. However, there appears to be modest benefits in beginning with higher doses of parenteral MTX, or switching to parenteral MTX when the clinical response to an oral dose is inadequate. Measuring MTX drug levels has little clinical applicability. Further study is needed to determine the ideal initial dosing strategy in specific patients in the context of potential for rapid control of inflammation and tolerability. Studies are needed to assess the risk-benefit and cost effectiveness of split dose MTX *versus* subQ MTX as well as optimal MTX levels to ensure that poor clinical response is not related to inadequate dose. Patient-specific biomarkers relating to safety and effectiveness of MTX will enhance the use of MTX in clinical practice.

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