Folate supplementation during methotrexate therapy for rheumatoid arthritis

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ABSTRACT

Methotrexate (MTX), an antifolate, is an anchor drug for the treatment of rheumatoid arthritis (RA). Both folic acid (FA) and folinic acid (FLN) supplements have been shown to reduce the toxicity of MTX when used in RA therapy. The effect of folate supplementation on MTX efficacy still needs to be studied. FA supplementation has been found to have a beneficial effect on homocysteine (hcy) metabolism and may prevent the formation of the less effective metabolite 7-hydroxy-MTX. The cost of FA *supplements is substantially less than the cost of FLN supplements. This article reviews clinical trials related to folate supplementation during MTX therapy for RA.*

Introduction

Methotrexate (4-amino-4-deoxy-10-methylpteroyl-L-glutamic acid) (MTX) is a classic structural antifolate. Figure 1 shows the structures of folic acid (FA), folinic acid (FLN), and MTX. Folic acid (FA) (pteroyl-glutamic acid), represents the oxidised, noncarbon-substituted vitamin, while FLN (FLN) ((6S) - 5 formyl, 5,6,7,8 tetrahydro pteroyl-glutamic acid) represents the fully reduced, carbon substituted coenzyme form of the vitamin. Folate is a term for the family of folate compounds.

The toxicities of MTX have been classified as Types A-D (1). Type A toxicities are dose-dependent, such as gastrointestinal (GI) and bone marrow toxicity (2-4). Such side effects are felt to be related to the effects of folate antagonism on tissues with high cell replication (5, 6). In addition, elevated mean corpuscular volume has been shown to be a predictor of haematological toxicity (7). Andersen *et al*. followed 46 subjects in a randomised, double-blind, placebo-controlled trial of MTX or D-penicillamine for rheumatoid arthritis (RA) for 28 weeks (8). Red blood cell folate levels were initially similar between the groups assigned to MTX and D-penicillamine, but declined longitudinally only in the MTX group. The patients that withdrew from MTX therapy because of toxicity had lower red cell folate concentrations than those patients remaining on MTX. Side effect scores were inversely correlated with red cell folate levels at weeks 0, 9, and 28 ($p<0.05$). The authors concluded that folate status, as measured by red blood cell folate levels, was related to the incidence of side effects and liver enzyme elevations.

Type B reactions are idiosyncratic, such as pneumonitis (9). Type C toxicity is felt to be related to long-term antifolate effects and includes hepatoxicity and hyperhomocysteinemia (10-12). Kremer *et al*. have shown that the accumulation of MTX polyglutamates in the liver is accompanied with folate deficiency (11). Type D effects are delayed effects that occur after discontinuation such as pregnancy effects and teratogenesis (1).

Toxicity is the primary reason for stopping MTX therapy during RA therapy (13), and individuals taking chronic low-dose MTX have been documented to have impaired folate status (8, 14, 15). In a prospective study to evaluate the effects of MTX on folate status, Hornung *et al*. confirmed that red blood MTX levels increased and RBC folate levels fell during therapy, except in patients taking concurrent FA supplements (16).

FA and FLN supplementation during low-dose MTX therapy

MTX toxicity symptoms and uncomplicated folate deficiency symptoms (such as stomatitis, gastrointestinal toxicity and anaemia) have some overlap which lead to a hypothesis that treatment with folate might be beneficial to lower toxicity of MTX (15). Since

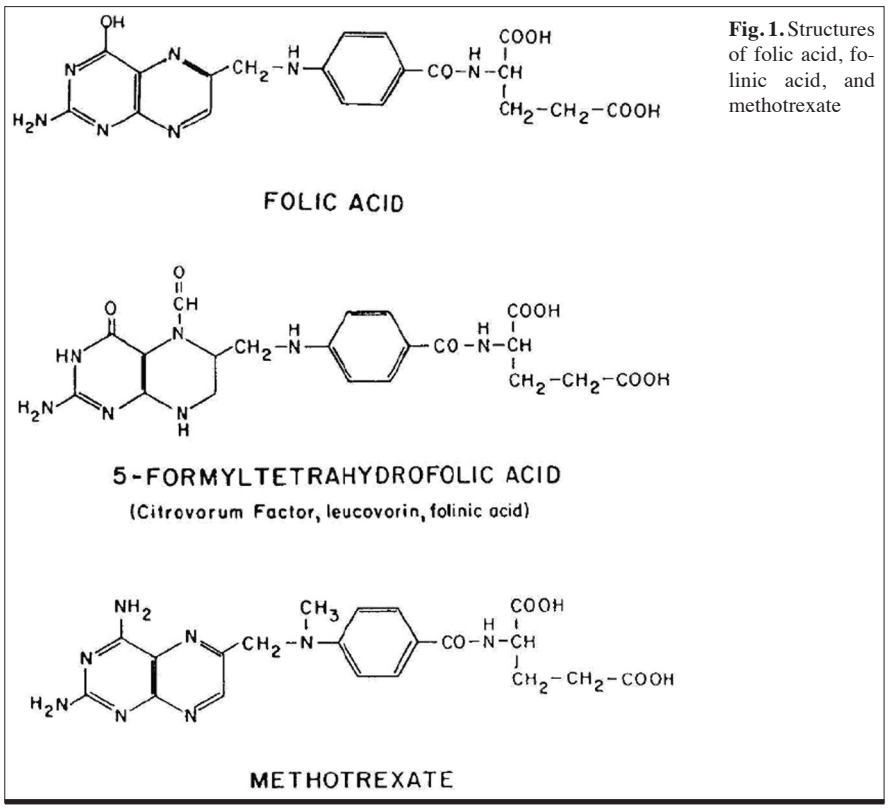

Fig. 1. Structures of folic acid, folinic acid, and methotrexate

MTX polyglutamates are only present during MTX use, the folate deficiency produced would be a complicated-type of folate deficiency. There have been a variety of case series and supplementation trials using both FA and FLN during low-dose MTX therapy (16-29). Table I displays results of clinical trial use of FA and FLN in RA and juvenile rheumatoid arthritis (JRA).

1. Effect of folate supplementation on toxic manifestations

Table I outlines the results of clinical trials which have evaluated the effect of folate supplementation on toxicity in RA and JRA (16-29). Meta-analyses have evaluated the effects of folate supplementation on MTX toxicity (30-32). Ortiz *et al*. evaluated double-blind, randomised folate supplementation trials from 1966–1996 (30). There was a 79% reduction in mucocutaneous and GI side effects with FA (OR=0.21, 95% CI 0.10–0.44) and with FLN there was a non-significant 42% reduction (OR=0.58, 95% CI 0.29–1.16). There was no difference in toxicity reduction comparing high and low doses of FA and FLN. They concluded that low doses of FA reduce mucosal and GI side effects by 80%. In a second meta-analysis, Ortiz *et al*. evaluated trials of FA or FLN supplementation during MTX therapy from 1966 to 1999 (31). There was a 79% significant reduction in GI side effects with FA supplementation (OR=0.21, 95% CI 0.10 to 0.44, $p<0.0001$) while with FLN there was a non-significant reduction of 43% (OR=0.57, 95% CI 0.28–1.15, $p=0.12$). Prey *et al*. conducted a Cochrane database and Medline search of double-blind, placebo controlled trials for individuals taking MTX for inflammatory diseases from 1997–2007 (32). The review evaluated 5 trials that met their entrance criteria and found a trend for reduction in mucocutaneous side effects in patients receiving folate supplementation (ARR= -0.072, 95% CI -0.18 to 0.037). There were no significant improvements in haematological side effects, but there was a significant reduction in elevated liver function tests with folate supplementation (ARR= -0.358, 95% CI -0.467 – -0.248).

Other studies have concluded that folate supplements are beneficial. Hoekstra *et al*. evaluated factors associated with MTX toxicity and determined that elevated body mass index (BMI) and absence of FA supplementation were associated with hepatotoxicity (33). Folate supplementation was also associated with reaching a weekly MTX dose of ≥15mg. Kent *et al*. followed 481 patients for 2,323 patient-years of MTX administration for RA (34). The lack of FA supplementation was an independent predictor of a higher aspartate aminotransferase level; of 17 patients who discontinued MTX, 65% either had no FA supplementation or hyperlipidemia. Griffith *et al*. completed a prospective, randomised, double-blind, placebo-controlled trial of 75 patients established on MTX for RA (<20mg/week) and 5mg of FA daily (35). The subjects were asked to stop their FA supplementation and were randomised to take 5mg of FA/day or an identical placebo. There were more drop-outs in the placebo group than the FA-supplemented group (46% *vs*. 21%, $p=0.02$). At 9 months of follow-up there was more nausea in the placebo group ($p=0.001$). Two patients in the placebo group discontinued the trial because of neutropenia. The authors concluded that it is useful to continue FA supplementation over the long-term.

2. Effect of folate status and folate supplementation on MTX efficacy

If the mechanism of efficacy of MTX therapy is related to its antifolate effects, then the combination of a folate and an antifolate gives a theoretical risk of flare in disease activity. Table I also summarises the effects of folate supplementation on methotrexate efficacy in the treatment of arthritis (16-29). Also, high serum FA levels have been shown to interfere with single dose MTX therapy for ectopic pregnancy (36) and folate overproduction in *Lactobacillus plantarum WCFS1* causes MTX resistance (37). The evidence above also indicates an antifolate effect of MTX.

Two studies investigating the association of MTX polyglutamate concentrations with efficacy have suggested that high folate status may interfere with MTX efficacy (38, 39). Dervieux *et al*. conducted a cross-sectional observational study to correlate red blood cell (RBC) MTX and folate polyglutamates with disease activity measures. In a

Table I. Studies related to folic acid (FS) and folinic acid (FLN) supplementation during low dose methotrexate therapy for rheumatoid arthritis (RA).

Reference	Weekly folate: MTX ratio	Timing of folate dose	Study design	Toxicity effect	Efficacy effect
FOLIC ACID (FA)					
Morgan 1990 (23)	0.93:1	Not specified, patients took 1 mg of FA or identical placebo daily	Double-blind, placebo, controlled trial for 6 months n=32	Toxicity score of the FA group was 20% of that of the placebo group (p = 0.03).	No difference between FA and placebo
Stewart 1991 (26)	0.823:1	Not given	Prospective, non-blinded study of patients followed for a mean of 41.5 months n=200	Significantly less diarrhea, elevated liver function tests, nausea/vomiting in folate-treated group. An elevated mean corpuscular volume did not predict toxicity	Not reported
Morgan 1994 (22)	0.53:1 and 2.85:1	FA taken on 5 days of the week when MTX not taken (either 5 mg or 27.5 mg/week)	Double-blind, placebo-controlled trial for 1 year n=79	Mean toxicity scores lower in folate groups than placebo group	No difference between FA supplemented and placebo groups
Hunt 1997 (19)	0.72:1 – 0.78:1	Not specified when FA dose given	Randomised, double-blind, placebo-controlled crossover trial of juvenile rheumatoid arthritis. Each arm 6 weeks with 1 week washout n=19	Not given	No difference in clinical measures between groups
Hornung 2004 (16)	Not defined	Not specified when FA dose given administered FA n=81	Prospective study of patients with self- without FA.	No differences in clinical side effects with or There was a small but significant increase in liver function tests in patients not on FA at 15-18 weeks	No difference in drug efficacy
FOLINIC ACID (FLN)					
Hanrahan & Russell 1988 (18)	0.5:1	Received oral FLN or placebo for 2 days prior to IM MTX	Double-blind, placebo-controlled crossover study with two 40 week arms. n=13. All patients had MTX toxicity prior to trial inclusion	No difference in nausea between arms	No adverse effects on drug efficacy reported
Tishler 1988 (27)	0.95:1	15 mg oral FLN given for 3 consecutive days starting 4/6 hours after IV MTX dose	Prospective, non randomised study of 6 subjects with RA, 1 subject with psoriatic arthritis for 4 weeks	4 patients had resolution of nausea within 2 weeks of start of FLN	One patient had severe deterioration of disease. Ritchie index and grip strength deteriorated in all patients after 4 weeks
Buckley 1990 (17)	0.5:1	Oral FLN 4 hours after oral MTX dose	Randomised, double-blind, placebo-controlled crossover study duration 24 weeks n=20	Lower incidence of stomatitis and GI upset during FLN supplementation, but not statistically significant	No adverse effects on drug efficacy
Joyce 1991 (20)	2.3:1	Oral FLN 2 hours after weekly oral MTX dose	Double-blind, placebo-controlled trial lasting 12 weeks n=27	MTX toxicity, MTX withdrawal did not differ significantly between groups	Compared to placebo, significant increase in Ritchie index, global joint scores, morning stiffness, duration and ESR at 4 weeks in FLN group
Shiroky 1993 (25)	Minimum ratio 0.09:1	Oral FLN dose 24 hours after MTX dose	Double-blind, placebo controlled trial of 52 weeks n=92	Compared to placebo, 60% less elevations in transaminases, 48% lower oral ulcers, 40% lower GI symptoms in FLN group	At 52 weeks disease activity was similar in FLN *vs*. placebo. 1 FLN patient withdrew because of lack of efficacy

Reference	Weekly folate: MTX ratio	Timing of folate dose	Study design	Toxicity effect	Efficacy effect
Weinblatt 1993 (29)	0.05:1	Oral FLN given simultaneously with MTX	Double-blind, placebo control trial lasting 8 weeks n=16	No difference between placebo and FLN in number of adverse events	No change in efficacy
Modesto 1996 (21)	0.05:1	FLN taken 4 hours after single weekly MTX dose	Case series of 3 patients, 2 with systemic onset juvenile idiopathic arthritis 1 with poly juvenile idiopathic arthritis. All patients had previous side effects	GI side effects resolved in 2 patients, a rash resolved in a 3rd patient	Disease flare in all 3 patients
Ravelli 1999 (24)	0.25 – 0.50:1	FLN dose given 24 hours after MTX dose	Non-randomised, retrospective case series in juvenile rheumatoid arthritis. FLN started because of signs of MTX toxicity n=43	Mean number of episodes of hepatotoxicity and GI toxicity decreased after starting FLN	No disease flare while on FLN or difference in disease remission
van Ede 2001 (28)	FA 0.56 – 0.93:1 FLN 0.20 – 0.33:1	FA taken orally every morning (1 mg) FLN (2.5 mg) taken 24 hours after MTX dose. If MTX dose > 15 mg/week, the FA and FLN doses were doubled	Prospective, double-blind, placebo controlled of 48 weeks n=434	MTX discontinued for toxicity in 38% of placebo, 17% of FA and 12% of FLN supplemented groups Alanine aminotransferase levels were significantly higher in the placebo than folate-supplemented groups ($p<0.001$). There was no difference in alanine aminotransferase levels between FA and FLN groups	Disease activity improved equally in all groups. No difference in EULAR or ACR 20% or 50% response

subset of the study population (n=171), using a multivariate analysis, higher folate polyglutamates levels were associated with a higher number of tender joints (R=0.41, R^2=0.166) and a higher number of swollen joints (global =0.36, R^2=0.1290) (38). However, high RBC folate polyglutamates were not associated with worsening physician global assessment of disease or worsening in the modified Health Assessment Questionnaire (mHAQ). Stamp *et al*. conducted a cross-sectional study of patients taking MTX and correlated RBC MTX and folate polyglutamates with disease efficacy measures (39). There were no relationships between MTX polyglutamates and disease activity, however the mean RBC folate concentration was significantly greater in the group with higher disease activity compared to low disease activity (786.9±31.2 nmol/L *vs*. 664.2±27.4 nmol/L, p=0.002). These authors suggested that RBC folate levels may be more important than MTX polyglutamates as a predictive disease control measure.

Bressolle *et al*. studied 20 patients with RA who received 10mg of MTX/week IM with or without a concurrent dose of 5mg of FA for 13 days in a pharmacokinetic protocol (40). They found that plasma MTX levels were lower at 2 and 8 hours after MTX administration and the area under the curve *vs*. time was 20% lower during FA administration. There was also higher total clearance of MTX during the period of FA administration. They speculated that the lower plasma MTX levels could be related to increased cellular uptake of MTX with FA supplements causing intracellular sequestering. Hiraga *et al*. studied 29 patients with RA taking low-dose oral MTX at 12-hour intervals to evaluate MTX pharmacokinetics (41). In contradistinction to the results of Bressolle (40), higher serum folate levels were associated with an increase in the MTX area under the curve (42) and a decrease in the total body clearance divided by bioavailability (CL/F). The authors speculated that folate structural analogs inhibit cellular uptake of MTX and thereby increase the AUC. They suggested that the decrease in CL/F was related to the increase in AUC. However, no FA supplementation was used in their study and they could not rule out the possibility that higher folate doses could cause additional changes in MTX pharmacokinetics.

There is evidence that provision of the fully reduced, carbon substituted coenzyme FLN, in excessive doses can cause a flare in disease activity (17, 27). There has also been concern voiced about the effect of FA supplements on mean MTX doses required for efficacy. In the study of van Ede *et al*. (28) which evaluated the effect of folate supplementation on disease activity, there was no difference in disease activity between the FA, FLN, and placebo supplemented groups, however, the mean dose of MTX was higher in the FA and FLN groups than placebo (18mg/week

and 16.4mg/week *vs*. 14.5mg/week). Arabelovic *et al*. evaluated trends in mean annual MTX before and after the implementation of FA food fortification of grain and cereal products (43). The mean annual MTX dose between 1988 and 1996 (prior to FA fortification of flour) was 12.4±4.0 mg. After folate fortification of flour it rose to 16.6±5.1 mg. The authors suggested that FA fortification contributed to the increased mean MTX doses but could not rule out the possibility that rheumatologist's increasing comfort with higher MTX doses was responsible.

Data from the Phase III trial of leflunomide in RA demonstrated that 52% of MTX-treated patients in the United States achieved the criteria of a 20% improvement in the United States where 98% received FA supplementation. However, 65% of international patients achieved a ACR 20 response and only 11% of patients were reported to receive folate supplementation (44-46). Khanna *et al*. in a post-hoc analysis evaluated the efficacy of MTX therapy during FA administration at 12 months in two phase III randomised controlled trials of leflunomide *vs*. MTX. Multinational patients and patients from the United States were recruited (47). A propensity score mechanism was used to match for differences in baseline characteristics of FA *vs*. non FA users. Nine to 21% fewer patients taking FA and MTX achieved ACR 20%, 50% or 70% improvement at one year compared to individuals who did not receive FA supplements. It has been suggested that the difference in disease response was related to the folate supplementation (44). However, this conclusion has been criticised since the analysis was *post hoc*, the demographics of the American and international treatment groups were different and only the American trial was placebo-controlled. There was also a difference in the mean disease duration between the patients in the European *vs*. American trials (3.8 *vs*. 6.5 years), a larger proportion of patients received nonsteroidal anti-inflammatory drugs in the multinational cohort, and the similarity of ACR 20% or better response rates at 2 years (67% American *vs*. 72% multinational) despite the fact that the proportion of patient receiving folate supplements was unchanged (48, 49). We argued that the use of propensity score analysis could not compensate for the lack of a placebo group in the multinational study (33, 34).

Continuation of MTX therapy in patient cohorts can also be considered as an efficacy measure. In a retrospective evaluation of 1,072 MTX treatment episodes of 1022 patients, FA supplementation was associated with continuation of MTX therapy (50) with significant increased drug survival with FA supplementation ($p<0.001$). The 5-year survival probability for continuing MTX was 67% with FA and 31% without FA. In a multivariate analysis, FA, attending rheumatologist and concurrent prednisolone use were associated with MTX therapy survival.

3. Effect of folate supplementation on homocysteine (Hcy) metabolism

A folate-dependent enzyme, methionine synthase, is involved in the methylation of Hcy to form methionine. It has been postulated that elevated serum Hcy levels are causally related to increased risk for atherosclerosis (51). However, recent trials have generally not shown a benefit to cardiovascular risk in lowering Hcy levels by supplementation with FA, pyridoxine, and vitamin B_{12} (52, 53). A recent double-blind, randomised controlled trial in the United Kingdom documented that reductions in blood Hcy levels did not have beneficial effects on vascular outcomes (54). In participants from the Third National Health and Nutrition Examination Survey (ages 40–79) individuals with a high 10-year risk of coronary artery disease had elevated levels of C-reactive protein (CRP), fibrinogen, and Hcy, raising the possibility that elevated Hcy is a marker of increased inflammation (55) rather than an independent risk factor. Yxfeldt *et al*. in a study of patients with RA treated with MTX and B-vitamins found, using a multiple regression model, that changes in CRP were significantly associated with changes in Hcy levels (56), a finding which is consistent with the above.

Several studies have documented that Hcy levels are elevated in MTX-treated patients, presumably related to enzyme inhibition and/or folate deficiency mechanism (12, 57, 58). Hoekstra *et al*. followed 15 patients with RA taking a median dose of MTX of 30mg/week and FA supplementation (5–30mg/week) (59). The time to maximum Hcy concentration was 8–48 hours after MTX administration. The median plasma Hcy concentration prior to MTX therapy was 10.1μmol/L (range 6.6–12.7μmol/L) and the median rise in Hcy concentration was 2.5μmol/L range 0.7–5.1μmol/L). There was no correlation between serum FA and Hcy concentration, conceivably because all patients were on folate supplementation. The addition of the antifolate sulfasalazine to MTX therapy appears to have an additive effect on Hcy levels (60). Hornung *et al*. followed 81 individuals on low dose MTX therapy and documented that plasma Hcy levels were inversely associated with RBC folate levels ($R^2=0.26$, $p<0.001$) and that plasma Hcy levels increased over time, especially among individuals not on FA supplements (16). Tiftikci *et al*. also showed a negative relationship between Hcy and plasma folate levels during low-dose MTX therapy (61).

Folate supplementation has been shown to lower Hcy levels during low-dose MTX therapy (12, 57, 62) . Jensen *et al*. found that plasma Hcy levels in patients with RA receiving MTX and FA did not differ significantly from Hcy levels in patients with RA receiving other therapies (62). In a double-blind, placebo-controlled trial of 79 patients taking either 5mg or 27.5mg of FA per week during low-dose MTX therapy there was a significantly lower Hcy concentration at 3, 6, 9, and 12 months compared to placebo therapy (12). However, the usefulness of lowering plasma Hcy levels, to reduce cardiovascular disease risk during low-MTX therapy has not yet been established.

4. Effects of folate supplementation on other aspects of MTX metabolism

Folate supplementation may also confer other positive benefits related to MTX metabolism during RA therapy. We have shown that in 24-hour urine samples that relatively high amino

imidazolecarboxamide (AICA) levels were correlated with reduced disease activity (*i.e.* increased efficacy) and that FLN supplementation, but not FA supplementation normalised urinary AICA levels (63). In addition, we have postulated that 7-OH-MTX is less efficacious than MTX and that FA supplementation, (but not FLN supplementation), lowers the formation of 7-OH-MTX. We have found that FA but not FLN inhibited *in vitro* aldehyde oxidase which is the enzyme that catalyses the formation of 7-OH-MTX from MTX. Patients with a marked improvement in swelling and pain/tenderness indices had a significantly lower 7-OH-MTX excretion ($p<0.05$) and patients receiving FA supplements also had a decreased 7-OH-MTX excretion ($p=0.03$). Relatively high 7-OH-MTX excretion was correlated with relatively high MTX excretion and therefore with relatively low MTX retention in vivo ($p<0.05$). Consequently, there may be additional benefits to FA supplementation in MTX therapy, which could produce efficacious MTX biochemistry. This hypothesis needs to be tested in a systematic way.

Guidelines related to MTX and folate supplementation

A variety of guidelines have been proposed regarding the prescription of folates during MTX therapy for RA (64-68). Using a Delphi procedure Pavy *et al*. evaluated guidelines for MTX use (67). They concluded that "folate supplementation can be given routinely to patients treated with MTX for RA" and recommended 5mg of FA per week at a time distant from the oral MTX administration. Visser *et al*. convened an international panel of rheumatologists in 2007–2008 (68). Their recommendation was that "at least 5mg of FA per week with methotrexate therapy is strongly recommended". Guidelines for use of MTX in juvenile idiopathic arthritis recommend a dose of FA 1mg/day (66). Several other guidelines have now been written which recommend the use of routine folate supplementation without specifying a specific dose during low-dose MTX therapy (69, 70). The British Society for Rheumatology states that "regular FA supplements are thought to reduce toxicity (71).

Summary

MTX serves as an anchor drug, in low doses, for a variety of inflammatory diseases. Since some MTX toxicities can be seen as complicated-folate deficiency, supplementation with various folates has been studied. Excessive doses of FLN can negate MTX efficacy in RA, however more moderate doses of both FA and FLN have been shown to reduce toxic manifestations. However, the question of whether folate supplementation affects efficacy is still incompletely studied. One interpretation of the data may be that FA supplementation allows a higher dose of MTX to be tolerated, thereby increasing the possibility of better efficacy. In addition, FA supplementation has been found to have a favourable effect on Hcy metabolism and may have other effects on MTX metabolism such as lowering the formation of its less active metabolite, 7-OH-MTX. There is considerable room for a future clinical trial to continue to investigate these important areas. The optimal dosing regimen for FA and FLN is not known and undoubtedly a balance between MTX dose and folate dose is important. There seems to be a broader margin of safety using FA than FLN. In most countries, a lower cost of FA than FLN would argue for FA supplementation. The potential of FA interfering with MTX absorption can be overcome by giving FA 24 hours prior to the weekly MTX dose or 24 hours following the dose.

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