Review Article

Methotrexate in rheumatoid arthritis: can current knowledge and experience justify its use as a first-line disease-modifying agent?

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Introduction

The efficacy of methotrexate (MTX) in rheumatoid arthritis (RA) was first reported in 1951.¹ We now know that low-dose intermittent MTX therapy (5–15 mg/week) is well-tolerated and produces a rapid clinical response in most RA patients.^{2,3} Until recently it was reserved for patients failing other second-line agents.⁴ Now, following a number of comparative studies, MTX is recognized as a useful disease-modifying anti-rheumatic drug.^{5–8} Side effects from low-dose MTX are common^{9,10} and can be potentially serious.^{11–16} As RA usually flares on its discontinuation,^{4,17} long-term therapy with MTX has to be contemplated from the outset. This confers a special importance to its toxicity. Now, with more than 10 years experience of continuous use of low-dose MTX in adult RA,¹⁸ this therapy appears to have stood the test of time.

The mechanism of action of MTX in RA remains unclear. It probably exerts both anti-inflammatory and immunosuppressive effects^{19,20} mediated partly through folate antagonism. Both the therapeutic and toxic effects (except pneumonitis) seem to be dose dependent.^{4,21} Single weekly doses produce less hepatotoxicity than do more frequent doses suggesting that MTX toxicity is related more to the duration of exposure and less to peak concentrations.^{4,22} A theoretical risk of teratogenicity contra-indicates MTX in pregnancy.

MTX is also effective in juvenile RA,²³ though, understandably, there is greater concern regarding its long-term toxicity in children.

This review discusses the efficacy and toxicity of MTX and debates its place in current treatment of RA.

Efficacy of methotrexate in rheumatoid arthritis

Several prospective trials have confirmed the efficacy of MTX in active RA.^{9,24–26} It reduces pain, morning stiffness, the number of tender and swollen joints and the erythrocyte sedimentation rate (ESR) in most patients within 6 weeks of starting therapy, with improvement in overall function. Many patients deteriorate slightly after the initial response necessitating an increment in dose.^{4,27} In general, there is a sustained response with continued treatment.

Early trials were on patients who had failed other disease-modifying drugs.^{24,25} These trials showed a significant improvement in most outcome measures in MTX-treated groups versus placebo. Certain parameters such as grip strength, 50 ft walking time and the ESR often did not improve significantly. However, in a subsequent meta-analysis, the pooled figures for these parameters reached significance levels in favour of MTX.³ Early studies also showed marked improvement from the baseline following short-term MTX therapy.^{24–26}

In double-blind comparative studies MTX was shown to be as effective as azathioprine^{8,28} and injectable gold,^{6,29} and significantly more effective than auranofin.⁷ A recent meta-analysis suggests that MTX is equivalent to injectable gold, penicillamine and sulphasalazine in efficacy.³ Despite this, MTX remains inferior to both injectable gold and penicillamine in terms of producing a clinical remission. This shortcoming follows its rather modest effect in some reports on certain parameters of disease activity such as the rheumatoid factor and the ESR.^{3,9,24,25}

A single low dose of MTX given intravenously produces a rapid and profound drop in ESR and C-reactive protein (CRP) in RA patients who had never taken the drug previously.³⁰ Overall, about two-thirds of all patients commenced on MTX show a significant drop in ESR and CRP^{8,31} which generally parallels clinical improvement. Over a

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long term, the fall in these acute phase markers is more likely to be sustained with injectable gold, penicillamine or sulphasalazine therapy than with MTX.³ The effect of MTX on haemoglobin is usually less dramatic than its effect on the ESR. Following short-term MTX therapy one can expect a 0.5–1 g/dl rise in the haemoglobin level.^{8,32,33}

All long-term (up to 5 years) open prospective studies of MTX in RA demonstrate a sustained response.^{9,31,34} Its effect on the radiological progression of disease has also been studied. Rau and colleagues compared MTX therapy over a mean of 3.9 years with injectable gold over 2.2 years in active RA³⁵ and found that radiological progression of joints with a Larsen score of zero was significantly lower in the MTX group. However, joints with higher scores did not reveal a significant difference in progression between the two treatments. Another study found no change in the rate of radiological progression in 18 MTX-treated patients over a mean of 33 months.³⁶ The method of assessing serial radiographs in this study, though, was different from that used by Rau *et al.* Most studies investigating the effect of MTX on the radiological changes suggest that joints with no or minimal abnormalities at entry are least likely to show progression.

Initial enthusiasm regarding possible arrest of radiological progression of RA with MTX, however, has waned following the results of longer term studies.^{34,37} In one report, a significant delayed worsening in the number of tender joints coupled with reduced grip strength was observed following very long-term (90 months) MTX therapy.³⁷ Nine patients of 17 in this report showed radiological progression associated with a significant rise in ESR. Of note was a concurrent reduction in the dose of MTX in the cohort at this stage of follow-up. This has importance implications. It is possible that the late deterioration of RA and its radiological progression, could in part have been prevented if the dose of MTX was kept high (at around 15 mg/week). However, increased toxicity at such a dose could lead to a rise in withdrawals.

Recently, an 18-week placebo-controlled study showed a moderate but statistically significant (up to 12%) improvement in the indices of two standard-item health status questionnaires, and a more impressive (29%) improvement using an individualized questionnaire in MTX-treated RA patients.³⁸ These data suggest that low-dose MTX not only improves the more measurable characteristics of rheumatoid disease, but it also has, at least over a short term, important beneficial effects on social and emotional aspects of the patient's quality of life.

Some authors have recently used combinations of MTX with other disease-modifying drugs in RA with variable results.^{39–41}

Toxicity

Adverse reactions from low-dose MTX in RA are common but usually mild^{27,31,34} and, over a long term, remain the main reason for withdrawal of therapy.^{10,42} By contrast, its discontinuation because of lack of efficacy is uncommon (up to 7%).^{3,7,27} Over the years, our attitude towards MTX toxicity in RA has softened as illustrated by the fact that recently some authors allowed hepatic enzymes to rise beyond three times normal before withdrawing the drug.⁴³ Further, most investigators now reserve liver biopsies for certain defined groups only. Some authors have given folic or folinic acid supplements with MTX in order to reduce toxicity. In one study, a significant reduction of gastrointestinal side effects, albeit at the expense of exacerbation of RA, was noted with folinic acid supplementation.⁴⁴ Another study reported reduced toxicity without loss of efficacy with concurrent use of 1 mg folic acid daily.⁴⁵ Folate supplementation, however, remains a matter of debate.

Studies of cohorts over several years suggest that the incidence of MTX toxicity remains unaltered with continued treatment.^{34,37} Furthermore, the patterns of toxic effects tend to remain consistent within patients.

Comparative data on toxicity profiles of various disease-modifying anti-rheumatic drugs suggest that MTX is better tolerated than injectable gold, penicillamine, auranofin and azathioprine, and slightly less well-tolerated than antimalarials and sulphasalazine.^{3,7,8} Overall, less than a third of patients on MTX require temporary interruption, and up to 10% need termination of therapy due to serious toxicity.^{26,27} The major adverse effects of low-dose MTX therapy in RA are described below.

Gastrointestinal toxicity

Adverse effects including nausea, vomiting, dyspepsia, diarrhoea and anorexia may occur in up to two-thirds of patients, and are often dose related.^{4,34} In one study there was a strong correlation between gastrointestinal toxicity and an increase in serum aspartate transaminase.³¹

High-dose MTX therapy of childhood acute lymphoblastic leukaemia can cause progressive xylose malabsorption.⁴⁶ Although a similar effect in RA patients remains unproven, there are speculations that prolonged use of low-dose MTX could lead to its own malabsorption and thereby cause a loss of efficacy. Indeed, if true, MTX-induced malabsorption could explain the unexpectedly low folate levels in some RA patients taking this drug.^{16,63}

Hepatotoxicity

Elevation of hepatic enzymes (alanine and aspartate aminotransferase, γ -glutamyl transferase) is common. One study quoted an incidence of 88% over 53 months of MTX therapy.³⁴ Elevated enzymes correlate poorly with hepatic architectural damage.^{47,48} Minor hepatic fibrosis detectable by sensitive techniques is common in RA patients who have received a cumulative MTX dose of at least 2 g.⁴ However, similar minor changes have been detected in 5% of RA patients who never took the drug.⁴⁹ Hepatocellular anisonucleosis is the most common change in those taking MTX, and yet this has also been described in some RA patients not receiving MTX.⁴⁹ It is unclear whether patients with pre-existing minor fibrosis risk progression to overt fibrosis if treated long-term with MTX. Further, no noninvasive investigation can reliably predict the onset of MTX-induced hepatic fibrosis. However, a recent report of serial liver biopsies in 23 RA patients receiving oral MTX for more than 10 years (cumulative dose 4,690–10,230 mg) failed to show progression of hepatic changes to severe fibrosis or cirrhosis.¹⁸

MTX was associated with significant hepatotoxicity in psoriatics.⁵⁰ Fortunately, the incidence and severity of MTX hepatotoxicity in RA is much lower. This could, in part, be due to its once weekly dose in RA patients.

A meta-analysis of 15 long-term studies of MTX therapy accompanied by serial liver biopsies (a total of 636 patients) attempted to correlate the cumulative dose with progression of hepatic fibrosis.¹² It concluded that there was on average a 6.7% chance of progressing at least one histological grade (according to Roenigk classification) for each gram of MTX taken. It also calculated a 5% overall incidence of advanced changes (grades IIIB or IV) on liver biopsy. These figures, however, were not independent of the effects of concurrent intake of alcohol or others drugs. In fact, patients in this analysis who were heavy drinkers (100 g or more per week) were four times more likely to develop advanced changes and nearly three times more likely to show histological progression as compared to those who were not heavy drinkers. In one study marked elevation of liver enzymes was seen in patients taking aspirin or other non-steroidal anti-inflammatory drugs together with MTX, whereas those on a combination of MTX and hydroxychloroquine had the lowest enzyme levels.⁵¹ Non-steroidal anti-inflammatory drugs may increase MTX toxicity by interfering with its protein binding and renal excretion.⁵²

Recently, attempts have been made to delineate the indications for liver biopsy. Baseline biopsies may be required if there is a definite history of alcohol abuse or liver disease.⁵³ Some patients,

however, could be deemed unsuitable for MTX therapy on the basis of such a history alone without resorting to liver biopsy. A subsequent biopsy during therapy is indicated if liver enzymes are persistently elevated above twice the normal range,⁵³ although it is unclear as to how long one can wait for enzyme levels to drop spontaneously. Many authors classify Roenigk grades I, II and IIIA as minimal benign fibrosis^{12,47} compatible with continuation of therapy. There is debate whether patients with grades II or IIIA hepatic changes should have follow-up biopsies and, if so, after how long. Another issue is the degree of reliance on serial hepatic enzyme levels. Serial elevations in AST, checked monthly, seem to correlate well with hepatic histological deterioration detectable by light microscopy.^{53,54} Though liver biopsy remains the gold standard to assess MTX-induced damage, it is costly and has significant morbidity.^{53,55} Thus, great care is needed in selecting patients for MTX therapy and in their follow-up with regard to hepatotoxicity.

Pulmonary toxicity

High doses of MTX used in cancer chemotherapy can cause interstitial fibrosis.^{56,57} Overt pulmonary fibrosis from low-dose therapy is exceedingly rare, although acute pneumonitis can occur^{13,58} and may occasionally be life threatening. It can sometimes occur early during therapy¹³ and usually presents as dry cough, fever and dyspnoea. Recovery is the rule after discontinuation of MTX. Corticosteroids are often prescribed but their benefits remain unproven. Some authors have suggested that pre-existing pulmonary dysfunction (detected by lung function tests) or radiographic interstitial shadows could increase the risk of MTX pneumonitis.⁵⁹

Bone marrow toxicity

Leukopenia is reported in up to 4% of RA patients taking low-dose MTX.^{60,61} It resolves after temporary discontinuation or dose reduction. Thrombocytopenia can also occur and may likewise be dose related.⁶⁰ Pancytopenia is rare but may follow accidental or deliberate overdose.⁶² It is not possible to predict which patients would suffer myelotoxicity but folate deficiency could be an important risk factor.^{16,63} Close monitoring of haematological profile (at 1, 2 and 4 weeks and then monthly) has therefore been suggested.⁶³

Teratogenicity and carcinogenicity

MTX can produce teratogenicity by breaking DNA strands and causing chromatid exchanges.^{64,65} Aminopterin, a folate analogue similar to MTX, was once used as an abortifacient; it led to infants

born with multiple congenital neurological and bone anomalies.^{66,67} A recent report on eight women conceiving while on low-dose MTX (mean of 8.33 mg/week) failed to demonstrate teratogenicity.⁶⁸ An abortifacient effect, however, could not be ruled out as there were three spontaneous abortions out of a total of 10 pregnancies conceived. This report by no means substantiates the safety of low-dose MTX in pregnancy and we should continue warning patients of child-bearing potential about the possible risks of abortion and fetal anomalies with this drug. An effective method of contraception in such patients should be mandatory to qualify for MTX therapy.

Long-term therapy with low-dose MTX does not seem to have carcinogenic potential. There was no increase in neoplasia after 3,522 patient-years in those given MTX for choriocarcinoma,⁶⁹ or in 248 psoriasis patients given MTX long-term.⁷⁰

Neurotoxicity

Headache, light-headedness, dizziness, and impaired memory occur in a minority,^{27,71} and may occasionally necessitate discontinuation of MTX. One series reported adverse central nervous system effects in 20% of patients.⁷¹ Central nervous system toxicity in this report was more common in the elderly.

Other possible or rare side effects

Skin rashes are unusual though stomatitis and mucositis may occur.^{4,72} Occasionally, MTX can

lead to accelerated nodulosis and vasculitis.^{9,73} Atypical infections in MTX-treated RA patients are rare, although *Pneumocystis carinii* pneumonia^{74,75} and cryptococcosis⁷⁶ have been described. Male impotence,⁷⁷ alopecia, gynaecomastia, and possible renal impairment if non-steroidal anti-inflammatory drugs are taken concurrently, have also been reported.

Conclusion

MTX has been variously hailed as a major advance in the treatment of active RA. It is as effective as injectable gold, penicillamine and sulphasalazine, and is well tolerated. Unfortunately, as with other disease-modifying agents, its prolonged use fails to arrest the disease. Monitoring of patients taking MTX seems as demanding and expensive as of those taking other disease-modifying drugs. Although, in future, use of MTX early in the disease coupled with better patient selection, may improve its toxicity profile and efficacy, our present knowledge and experience precludes its use as a disease-modifying drug of first choice. In conclusion, MTX has earned a place in the list of disease-modifying anti-rheumatic agents and has widened the choice available to us. Its use in combination with other agents needs further study.

References

- Gubner, R., August, S. & Ginsberg, V. Therapeutic suppression of tissue reactivity. II. Effect of aminopterin in rheumatoid arthritis and psoriasis. *Am J Med Sci* 1951, **221**: 176–182.
- Tugwell, P., Bennett, K. & Gent, M. Methotrexate in rheumatoid arthritis: indications, contraindications, efficacy and safety. *Ann Intern Med* 1987, **107**: 358–366.
- Felson, D.T., Anderson, J.J. & Meenan, R.F. The comparative efficacy and toxicity of second-line drugs in rheumatoid arthritis: results of two metaanalyses. *Arthritis Rheum* 1990, **33**: 1449–1461.
- Furst, D.E. & Kremer, J.M. Methotrexate in rheumatoid arthritis. *Arthritis Rheum* 1988, **31**: 305–314.
- Tugwell, P., Bennett, K., Bell, M. & Gent, M. Methotrexate in rheumatoid arthritis: feedback on American College of Physicians Guidelines (editorial). *Ann Intern Med* 1989, **110**: 581–583.
- Suarez-Almazor, M.E., Fitzgerald, A., Grace, M. & Russell, A.S. A randomized controlled trial of parental methotrexate compared with sodium aurothiomalate (Myochrysine) in the treatment of rheumatoid arthritis. *J Rheumatol* 1988, **15**: 753–756.
- Weinblatt, M.E., Kaplan, H., Germain, B.F. *et al.* Low-dose methotrexate compared with auranofin in adult rheumatoid arthritis: a thirty-six-week, double-blind trial. *Arthritis Rheum* 1990, **33**: 330–338.
- Jeurissen, M.E., Boerbooms, A.M.Th., van de Putte, L.B.A. *et al.* Methotrexate versus azathioprine in the treatment of rheumatoid arthritis: a forty-eight-week randomized, double-blind trial. *Arthritis Rheum* 1991, **34**: 961–972.
- Weinblatt, M.E., Trentham, D.E., Fraser, P.A. *et al.* Long-term prospective trial of low-dose methotrexate in rheumatoid arthritis. *Arthritis Rheum* 1988, **31**: 167–175.
- Alarcon, G.S., Tracy, I.C. & Blackburn, W.D., Jr. Methotrexate in rheumatoid arthritis: toxic effects as the major factor in limiting long-term treatment. *Arthritis Rheum* 1989, **32**: 671–676.
- Szanto, E., Sandstedt, B. & Kollberg, B. Hepatotoxicity associated with low-dose long-term methotrexate treatment of rheumatoid arthritis. *Scand J Rheumatol* 1987, **16**: 229–234.
- Whiting-O'Keefe, Q.E., Fye, K.H. & Sack, K.D. Methotrexate and histological hepatic abnormalities: a meta-analysis. *Am J Med* 1991, **90**: 711–716.
- Ridley, M.G., Wolfe, C.S. & Matthews, J.A. Life-threatening acute pneumonitis during low dose methotrexate treatment for rheumatoid arthritis: a case report and review of the literature. *Ann Rheum Dis* 1988, **47**: 784–788.
- Cannon, G.W., Ward, J.R., Clegg, D.O., Samuelson, C.O., Jr & Abbott, T.M. Acute lung disease associated with low-dose pulse methotrexate therapy in patients with rheumatoid arthritis. *Arthritis Rheum* 1983, **26**: 1269–1274.

15. Weinblatt, M.E. Toxicity of low dose methotrexate in rheumatoid arthritis. *J Rheumatol* 1985, **12** (Suppl 12): 35–39.
16. MacKinnon, S.K., Starkebaum, G. & Wilkens, R.F. Pancytopenia associated with low dose pulse methotrexate in the treatment of rheumatoid arthritis. *Semin Arthritis Rheum* 1985, **15**: 119–126.
17. Kremer, J.M., Rynes, R.I. & Bartholomew, L.E. Severe flare of rheumatoid arthritis after discontinuation of long-term methotrexate therapy: a double blind study. *Am J Med* 1987, **82**: 781–786.
18. Aponte, J. & Petrelli, M. Histopathologic findings in the liver of rheumatoid arthritis patients treated with long-term bolus methotrexate. *Arthritis Rheum* 1988, **31**: 1457–1464.
19. Johnson, C.A., Russell, A.S., Kovithavongs, T. & Dasgupta, M. Measures of immunologic and inflammatory responses in vitro in rheumatoid arthritis patients treated with methotrexate. *J Rheumatol* 1986, **13**: 294–296.
20. Olson, N.J., Callahan, L.F. & Pincus, T. Immunologic studies of rheumatoid arthritis patients treated with methotrexate. *Arthritis Rheum* 1987, **30**: 481–488.
21. Furst, D.E., Koehnke, R., Burmeister, L.F., Kohler, J. & Cargill, I. Increasing methotrexate effect with increasing dose in the treatment of resistant rheumatoid arthritis. *J Rheumatol* 1989, **16**: 313–320.
22. Dahl, M.G., Gregory, M.M. & Scheuer, P.J. Methotrexate hepatotoxicity in psoriasis: comparison of different dose regimens. *Br Med J* 1972, **1**: 654–656.
23. Rose, C.D., Singsen, B.H., Eichenfield, A.H., Goldsmith, D.P. & Athreya, B.H. Safety and efficacy of methotrexate therapy for juvenile rheumatoid arthritis. *J Pediatr* 1990, **117**: 653–659.
24. Weinblatt, M.E., Coblyn, J.S., Fox, D.A. *et al.* Efficacy of low-dose methotrexate in rheumatoid arthritis. *N Engl J Med* 1985, **312**: 818–822.
25. Andersen, P.A., West, S.G., O'Dell, J.R. *et al.* Weekly pulse methotrexate in rheumatoid arthritis: clinical and immunologic effects in a randomized, double-blind study. *Ann Intern Med* 1985, **103**: 489–496.
26. Williams, H.J., Wilkens, R.F., Samuelson, C.O., Jr *et al.* Comparison of low-dose oral pulse methotrexate and placebo in the treatment of rheumatoid arthritis: a controlled clinical trial. *Arthritis Rheum* 1985, **28**: 721–730.
27. Kremer, J.M. & Lee, J.K. The safety and efficacy of the use of methotrexate in long-term therapy for rheumatoid arthritis. *Arthritis Rheum* 1986, **29**: 822–831.
28. Hamdy, H., McKendry, R.J.R., Mierins, E. & Liver, J.A. Low-dose methotrexate compared with azathioprine in the treatment of rheumatoid arthritis: a twenty-four-week controlled clinical trial. *Arthritis Rheum* 1987, **30**: 361–368.
29. Morassut, P., Golstein, R., Cyr, M., Karsh, J. & McKendry, R.J.R. Gold sodium thiomalate compared to low dose methotrexate in the treatment of rheumatoid arthritis: a randomized double blind 26-week trial. *J Rheumatol* 1984, **11**: 760–763.
30. Segal, R., Caspi, D., Tishler, M., Wigler, I. & Yaron, M. Short term effects of low dose methotrexate on the acute phase reaction in patients with rheumatoid arthritis. *J Rheumatol* 1989, **16**: 914–917.
31. Tishler, M., Caspi, D., Rosenbach, T.O. *et al.* Methotrexate in rheumatoid arthritis: a prospective study in Israeli patients with immunogenetic correlations. *Ann Rheum Dis* 1988, **47**: 654–659.
32. Arnold, M.H., O'Callaghan, J., Mcredie, M. *et al.* Comparative controlled trial of low-dose weekly methotrexate versus azathioprine in rheumatoid arthritis: 3 year prospective study. *Br J Rheumatol* 1990, **29**: 120–125.
33. Shinozawa, T., Akama, H., Matsushita, Y. *et al.* Low dose methotrexate therapy in rheumatoid arthritis. *Ryumachi* 1990, **30**: 172–180.
34. Kremer, J.M. & Lee, J.K. A long-term prospective study of the use of methotrexate in rheumatoid arthritis: update after a mean of fifty-three months. *Arthritis Rheum* 1988, **31**: 577–584.
35. Rau, R., Herborn, G., Karger, T. & Werdier, D. Retardation of radiologic progression in rheumatoid arthritis with methotrexate therapy: a controlled study. *Arthritis Rheum* 1991, **34**: 1236–1244.
36. Nordstrom, D.M., West, S.G., Andersen, P.A. *et al.* Pulse methotrexate therapy in rheumatoid arthritis: a controlled prospective roentgenographic study. *Ann Intern Med* 1987, **107**: 797–801.
37. Kremer, J.M. & Phelps, C.T. Long-term prospective study of the use of methotrexate in the treatment of rheumatoid arthritis: update after a mean of 90 months. *Arthritis Rheum* 1992, **35**: 138–145.
38. Tugwell, P., Bombardier, C., Buchanan, W.W. *et al.* Methotrexate in rheumatoid arthritis: impact on quality of life assessed by traditional standard-item and individualized patient preference health status questionnaires. *Arch Intern Med* 1990, **150**: 59–62.
39. Shiroky, J.B., Watts, C.S. & Neville, C. Combination methotrexate and sulfasalazine in the management of rheumatoid arthritis: case observations. *Arthritis Rheum* 1989, **32**: 1160–1164.
40. Brahn, E., Peacock, D.J. & Banquerigo, M.L. Suppression of collagen-induced arthritis by combination cyclosporin A and methotrexate therapy. *Arthritis Rheum* 1991, **34**: 1282–1288.
41. Williams, H.J., Ward, J.R., Reading, J.C. *et al.* Comparison of auranofin, methotrexate and the combination of both in the treatment of rheumatoid arthritis: a controlled clinical trial. *Arthritis Rheum* 1992, **35**: 259–269.
42. Furst, D.E., Erikson, N., Clute, L., Koehnke, R., Burmeister, L.F. & Kohler, J.A. Adverse experience with methotrexate during 176 weeks of long-term prospective trial in patients with rheumatoid arthritis. *J Rheumatol* 1990, **17**: 1628–1635.
43. Singh, Y.N., Sharma, M., Singh, R.J., Kumar, A., Malaviya, R. & Malaviya, A.N. Effects of methotrexate on clinical and immunological parameters in refractory rheumatoid arthritis: a prospective study (letter). *Br J Rheumatol* 1991, **30**: 73–74.
44. Tishler, M., Caspi, D., Fishel, B. & Yaron, M. The effects of leucovorin (folinic acid) on methotrexate therapy in rheumatoid arthritis patients. *Arthritis Rheum* 1988, **31**: 906–908.
45. Morgan, S.L., Baggott, J.E., Vaughn, W.H. *et al.* The effects of folic acid supplementation on the toxicity of low-dose methotrexate in patients with rheumatoid arthritis. *Arthritis Rheum* 1990, **33**: 9–18.
46. Craft, A.W., Kay, H.E.M., Lawson, D.N. & McElwain, T.J. Methotrexate-induced malabsorption in children with acute lymphoblastic leukaemia. *Br Med J* 1977, **2**: 1511–1512.
47. Roenigk, H.H., Auerbach, R., Maibach, H.I. & Weinstein, G.D. Methotrexate guidelines: revised. *J Am Acad Dermatol* 1982, **6**: 145–155.
48. Kremer, J.M., Lee, R.G. & Tolman, K.G. Liver histology in rheumatoid arthritis patients receiving long-term methotrexate therapy: a prospective study with baseline and sequential biopsy samples. *Arthritis Rheum* 1989, **32**: 121–127.
49. Tolman, K.G., Kremer, J.M., Lee, R.G. *et al.* Pretreatment liver histology in rheumatoid arthritis patients treated with low-dose methotrexate (abstract). *Arthritis Rheum* (Suppl) 1986, **29**, S18.
50. Dahl, M.G., Gregory, M.M. & Scheuer, P.J. Liver damage due to methotrexate in patients with psoriasis. *Br Med J* 1971, **1**: 625–629.
51. Fries, J.F., Singh, G., Lenert, L. & Furst, D.E. Aspirin, hydroxychloroquine, and hepatic enzyme abnormalities with methotrexate in rheumatoid arthritis. *Arthritis Rheum* 1990, **33**: 1611–1619.
52. Herman, R.A., Veng-Pedersen, P., Hoffman, J., Raechelle, K. & Furst, D.E. Pharmacokinetics of low-dose methotrexate in rheumatoid arthritis patients. *J Pharm Sci* 1989, **78**: 165–170.
53. Kremer, J.M. Liver biopsies in patients with rheumatoid arthritis receiving methotrexate: where are we going? *J Rheumatol* 1992, **19**: 189–191.

54. Kremer, J.M., Tolman, K.T. & Samowitz, W. Long-term prospective sequential liver biopsies in patients with rheumatoid arthritis on weekly oral methotrexate (abstract). *Arthritis Rheum* (Suppl) 1990, **33**: S40.
55. Piccinino, F., Sagnelli, E., Pasquale, G. & Guisti, G. Complications following percutaneous liver biopsy: a multicentre retrospective study on 68,276 biopsies. *J Hepatol* 1986, **2**: 165–173.
56. Schwartz, I.R. & Kajam, M.K. Methotrexate therapy and pulmonary disease. *JAMA* 1969, **210**: 1924.
57. Everts, C.S., Westcott, J.L. & Bragg, D.G. Methotrexate therapy and pulmonary disease. *Radiology* 1973, **107**: 539–543.
58. St Clair, E.W., Rice, J.R. & Synderman, R. Pneumonitis complicating low-dose methotrexate therapy in rheumatoid arthritis. *Arch Intern Med* 1985, **145**: 2035–2038.
59. Bell, M.J., Geddie, W.R., Gordon, D.A. & Reynolds, W.J. Pre-existing lung disease in patients with rheumatoid arthritis may predispose to methotrexate lung (abstract). *Arthritis Rheum* (Suppl) 1986, **29**: C28.
60. Thompson, R.N., Watts, C., Edelman, J., Esdaile, J. & Russell, A.S. A controlled two-centre trial of parenteral methotrexate therapy for refractory rheumatoid arthritis. *J Rheumatol* 1984, **11**: 760–763.
61. Howland, W.L. Methotrexate-associated bone marrow suppression following surgery (letter). *Arthritis Rheum* 1988, **31**: 1586–1587.
62. Brown, M.A. & Corrigan, A.B. Pancytopenia after accidental overdose of methotrexate: a complication of low-dose therapy for rheumatoid arthritis. *Med J Austral* 1991, **155**: 493–494.
63. Doolittle, G.C., Simpson, K.M. & Lindsley, H.B. Methotrexate-associated early-onset pancytopenia in rheumatoid arthritis. *Arch Intern Med* 1989, **149**: 1430–1431.
64. Li, J.C. & Kaminskas, E. Accumulation of DNA strand breaks and methotrexate cytotoxicity. *Proc Natl Acad Sci USA* 1984, **81**: 5964–5968.
65. Lindskov, R., Wulf, H.C., Wantzin, G.L. & Niebuhr, E. Sister chromatid exchange in patients treated with methotrexate for psoriasis. *J Invest Dermatol* 1984, **82**: 458–459.
66. Thiersch, J.B. Therapeutic abortions with a folic acid antagonist, 4-aminopteroglutamic acid (4-amino PGA) administered by oral route. *Am J Obstet Gynecol* 1952, **63**: 1298–1304.
67. Meltzer, H. Congenital anomalies due to attempted abortion with aminopterin (4-aminopteroglutamic acid). *Am J Dis Child* 1959, **97**: 279–281.
68. Kozlowski, R.D., Steinbrunner, J.V., MacKenzie, A.H., Clough, J.D., Wilke, W.S. & Segal, A.M. Outcome of first-trimester exposure to low-dose methotrexate in eight patients with rheumatic disease. *Am J Med* 1990, **88**: 589–592.
69. Rustin, G.J.S., Rustin, F., Dent, J., Booth, M., Salt, S. & Bagshawe, K.D. No increase in second tumours after cytotoxic chemotherapy for gestational trophoblastic tumours. *N Engl J Med* 1983, **308**: 473–476.
70. Nyfors, A. & Jensen, H. Frequency of malignant neoplasms in 248 long-term methotrexate-treatment psoriatics. *Dermatologica* 1983, **167**: 260–261.
71. Wernick, R. & Smith, D.L. Central nervous system toxicity associated with weekly low-dose methotrexate treatment. *Arthritis Rheum* 1989, **32**: 770–775.
72. McKendry, R.J.R. & Cyr, M. Toxicity of methotrexate compared with azathioprine in the treatment of rheumatoid arthritis: a case control study of 131 patients. *Arch Intern Med* 1989, **149**: 685–689.
73. Segal, R., Caspi, D., Tishler, M., Fishel, B. & Yaron, M. Accelerated nodulosis and vasculitis during methotrexate therapy for rheumatoid arthritis. *Arthritis Rheum* 1988, **31**: 1182–1185.
74. Leff, R.L. & Case, J.P. Rheumatoid arthritis, methotrexate therapy, and pneumocystis pneumonia (letter). *Ann Intern Med* 1990, **112**: 716.
75. Wallis, P.J., Ryatt, K.S. & Constable, T.J. Pneumocystis carinii pneumonia complicating low dose methotrexate treatment for psoriatic arthropathy. *Ann Rheum Dis* 1989, **48**: 247–249.
76. Altz-Smith, M., Kendall, L.G., Jr & Stamm, A.M. Cryptococcosis associated with low dose methotrexate for arthritis (letter). *Am J Med* 1984, **83**: 179–181.
77. Blackburn, W.D., Jr & Alarcon, G.S. Impotence in three rheumatoid arthritis patients treated with methotrexate (letter). *Arthritis Rheum* 1989, **32**: 1341–1342.