

# Clinical Pharmacology Update

Donald G. Vidt, M.D.  
Alan W. Bakst, Pharm.D., R.Ph.  
Section Editors

## Methotrexate in the treatment of arthritis and connective tissue diseases<sup>1</sup>

William S. Wilke, M.D.  
Jeffrey A. Biro, D.O.  
Allen M. Segal, D.O.

Methotrexate has received much attention in recent literature as an effective agent for the treatment of rheumatoid arthritis. Potential and observed toxicity from methotrexate compares very favorably with other cytotoxic and disease-modifying agents used to treat resistant rheumatoid arthritis, especially with regard to bone marrow suppression and future oncogenesis. Long-term liver toxicity, the incidence of which appears to be rare with low-dose therapy, remains an issue. Because of its relative safety, methotrexate seems a logical agent for the treatment of other arthropathies and connective tissue diseases. This paper reviews what has been written about methotrexate as treatment for a variety of inflammatory conditions other than rheumatoid arthritis. Wider application of this agent is expected in the future for the treatment of these kinds of conditions.

**Index terms:** Arthritis • Connective tissue diseases • Methotrexate

**Cleve Clin J Med** 54:327-338, July/Aug 1987

Aminopterin and methotrexate (MTX) (amethopterin), which inhibit the enzyme dihy-

<sup>1</sup> Department of Rheumatic and Immunologic Disease, The Cleveland Clinic Foundation. Submitted for publication Dec 1986; accepted March 1987.

0891-1150/87/04/0327/12/\$4.00/0

Copyright © 1987, The Cleveland Clinic Foundation

drofolate reductase, have been used in cancer and leukemia therapy since 1947.<sup>1</sup> More recently, the antimetabolite MTX has become a valuable alternative treatment for non-neoplastic diseases.

This article will attempt to review the use of MTX in the treatment of a variety of arthritic disorders and other connective tissue diseases.

### Clinical pharmacology and mechanism(s) of action

Oral MTX (5–15 mg) is rapidly and completely absorbed.<sup>2</sup> The peak plasma concentration is reached in one to two hours. The mean elimination half-life of MTX is approximately eight hours.<sup>3</sup> Within 24 hours, 80–90% of the administered dose is excreted unchanged in the urine, renal excretion being a major route of drug elimination.<sup>4</sup> Although tubular secretion has been demonstrated, glomerular filtration plays the most important role.<sup>5</sup>

Competitive protein binding of other drugs is of little clinical importance because only 50–60% of MTX is bound to plasma albumin.<sup>6</sup> On the other hand, because glomerular filtration plays a large part in the elimination of MTX, aminoglycosides and other drugs with potential renal toxicity should be used with care in patients receiving MTX. In addition, weak organic acids including aspirin and other nonsteroidal anti-

**Table 1.** Low-dose methotrexate in refractory rheumatoid arthritis

Reference	No. patients	Study design	Effect on disease variable					Dose (mg/wk) & route	Unique findings
			Articular indexes	Grip strengths	a.m. gel	WSR	RF		
Weinblatt et al <sup>14</sup>	28	C	+	-	+	+	-	7.5-15 (po)	HLA-DR2 found in patients with marked response
Thompson et al <sup>15</sup>	48	P	+	-	+	+	NT	10 or 25 (pa)	10 mg vs 25 mg showed trend toward better efficacy, decreased toxicity
Williams et al <sup>34</sup>	189	P	+	+	+	+	+	7.5-15 (po)	Nearly one-third suffered some toxicity necessitating withdrawal from study
Andersen et al <sup>13</sup>	12	C	+	-	+	+	-	10-25 (pa)	IgG, IgA, IgM significantly lowered by MTX
Wilke et al <sup>33</sup>	19	C	+	+	+	+	± (p = 0.05)	7.5 (po)	Study employing lowest weekly dose: 71% experienced >30% improvement

*P* = parallel; *C* = crossover; *pa* = parenteral; *po* = oral; *NT* = not tested; *WSR* = Westergren sedimentation rate; *RF* = rheumatoid factor; *MTX* = methotrexate; + = statistically significant improvement; - = not significantly improved.

inflammatory drugs, penicillin, and sulfonamides can competitively inhibit the tubular secretion of MTX, resulting in raised plasma concentrations of the drug.<sup>5,7</sup> With low doses of MTX these interactions are clinically insignificant. On the other hand, probenecid can completely block the renal tubular secretion of MTX<sup>8</sup> and should be avoided.

The mechanism(s) by which MTX exerts its effects on inflammatory or autoimmune disease is poorly understood. High-dose (100-250 mg/mm<sup>2</sup>/wk) or consecutive-day therapy (15-25 mg/mm<sup>2</sup>) in human subjects depresses antibody response to a variety of common antigens<sup>9,10</sup> and blocks lymphocyte transformation as well as delayed hypersensitivity responses to the usual battery of antigens.<sup>11,12</sup> However, the information for low-dose, pulse therapy is less clear.

The prospective double-blind studies comparing MTX with placebo in rheumatoid arthritis show that low-dose, pulse MTX (7.5-15 mg/wk) therapy significantly lowers acute-phase reactants but has no consistent effect on cellular measures.<sup>13,14</sup> These studies also demonstrate that disease suppression occurs quickly (3-4 weeks) after the initiation of MTX therapy and that disease activity returns as quickly after discontinuation of the drug, timing more often associated with anti-inflammatory agents than with immunosuppressive drugs.<sup>14,15</sup> This seems in keeping with an animal study comparing physiologically equal doses of MTX, cytoxan, and 6-mercaptopurine as anti-inflammatory agents<sup>16</sup> in which MTX proved superior.

On the other hand two recent abstracts show that MTX lowered peripheral blood suppressor lymphocytes in 20 patients being treated with low-dose, pulse MTX for 28.5 months<sup>17</sup> and specifically suppressed monocyte rheumatoid factor production in treated patients and in cell culture.<sup>18</sup> MTX also appears to provide long-term effective therapy and apparently shows increasing efficacy with time,<sup>19,20</sup> and patients treated with this drug probably have a higher incidence of herpes zoster when compared with controls—both characteristics of immune modulators.

Methotrexate thus appears to act as an anti-inflammatory agent with subtle immune-modulating properties.

### Methotrexate in the treatment of arthritis

#### Rheumatoid arthritis

*Uncontrolled studies:* MTX and aminopterin were first used to treat rheumatoid arthritis in the early 1950s. Gubner<sup>21</sup> treated seven patients with rheumatoid arthritis using 1-2 mg of MTX daily for seven to 11 days, followed by drug-free periods. Clinically significant reduction of disease activity was noted in six of these patients. Subsequent German studies<sup>22,23</sup> demonstrated that other cytotoxic drugs could be combined with MTX to treat rheumatoid arthritis effectively.

Little else was written until the report of 29 patients treated for severe rheumatoid arthritis by Hoffmeister in 1972.<sup>24</sup> Moderate to marked improvement of morning stiffness, fatigue, active joint count, and erythrocyte sedimentation rate was observed in approximately half of these pa-

**Table 2.** Open studies of psoriatic arthritis treated with methotrexate

Reference	No. patients	Dose	Time of follow-up	Efficacy (%) with good or excellent result	Toxicity		
					Total %	Minimum*	Maximum†
Hunter and Millazzo <sup>40</sup>	22	2.5–5 mg/d (6-day courses)	approximately 1 month	11 (50%)	19	19	0
Kragballe et al <sup>41</sup>	59	approximately 10 mg/wk	mean 3 years	43 (82%)	10	6	4
Fledges and Barnes <sup>42</sup>	5	approximately 15 mg/wk	17 months	4 (80%)	80	80	0
Kersley <sup>43</sup>	11‡	approximately 3 mg/d (2-week courses)	—	8 (75%)	45	45	0
Chaouat et al <sup>44</sup>	26	25–50 mg 1m q 2 weeks	—	15 (58%)	88	76	12

\* Gastrointestinal, alopecia, central nervous system.

† Leukopenia, cirrhosis.

‡ One patient with rheumatoid arthritis, one patient with systemic lupus erythematosus.

tients. Since that time, at least 633 patients in uncontrolled series have been reported by numerous authors.<sup>19,20,25–32</sup> These studies suggest that when MTX is given in doses ranging from 7.5–50 mg per week it can be used to control the signs and symptoms of rheumatoid arthritis. Three of the studies were long-term prospective trials that demonstrated that the drug maintains its effect for at least two years and does not achieve its full efficacy for six to 12 months.<sup>19,20,32</sup> Two other studies<sup>25,31</sup> demonstrated that subjective and objective improvement of arthritis generally occurs within three to four weeks after institution of the drug.

**Controlled studies:** Five controlled studies of MTX have been published as full reports or abstracts<sup>13–15,33,34</sup> (Table 1). In these studies MTX was given in weekly pulse doses. Two of the studies were designed as parallel trials, the other three as crossover studies. MTX was given in a dose of 7.5 mg per week throughout the study,<sup>33</sup> or in escalating doses ranging up to 25 mg per week given either in single doses or in three equally divided doses spaced 12 hours apart. Significant improvement in global assessment, articular index, morning stiffness, and Westergren sedimentation rate was demonstrated in all studies in which these parameters of efficacy were measured. Two studies suggested lowering of rheumatoid factor.<sup>14,33</sup> Two studies showed lowering of immunoglobulin levels in treated patients.<sup>13,33</sup> Our study<sup>33</sup> demonstrated that a dose of 7.5 mg per week was effective without need for dose escalation.

Acute toxicity in these studies was usually manageable by manipulation of the dose of MTX. Nearly one-third of the patients in one study suffered minor toxicity, including elevation of

liver enzyme levels that necessitated withdrawal from that study.<sup>34</sup> This high incidence of minor toxicity was not seen in the other trials. Severe toxicity was rare and no deaths occurred. Gastrointestinal toxicity was only experienced by patients receiving 15 mg of MTX in one study,<sup>14</sup> and although other toxicity in all of the studies was not necessarily dose-related, it was usually controlled by lowering the weekly dose.

#### *Psoriatic arthritis*

The earliest, most extensive use of MTX for nonmalignant diseases was its application for the treatment of psoriasis. The first controlled study of the treatment of psoriasis with MTX was published in 1963<sup>35</sup>; the drug was found to produce statistically significant improvement in the cutaneous manifestations of the disease. Because inflammatory arthritis accompanies the skin disease in a subset of patients, these same authors were able to report their experience in uncontrolled tests with MTX given in doses of 2.5–5 mg per day for six-day courses to 22 patients with psoriatic arthritis. Nineteen of 22 patients reported improvement of joint symptoms and tolerated the drug.

Although the first controlled trial of MTX in psoriatic arthritis demonstrated its effectiveness in reducing arthritic symptoms, drug toxicity was a major problem.<sup>36</sup> Unfortunately, these investigators chose an arbitrarily high parenteral dose, beginning at 1 mg/kg/wk and raising the dose during three weeks to 3 mg/kg/wk in 21 patients. Toxicity was dramatic. Serious side effects occurred in 13 patients, including leukopenia in one and thrombocytopenia possibly contributing to the death of another patient. The authors concluded that “. . . this type of therapy requires

**Table 3.** Open studies of adult inflammatory myositis treated with methotrexate

Reference	No. patients	No. with steroid resistance	Dose (mg)	Time to clinical improvement* (weeks)	Percentage responding	Duration of treatment (months)	Toxicity
Malaviya et al <sup>48</sup>	4	3	2–100/wk	2–6	100	36	2 oral ulcers 1 leukopenia 1 fever
Sokoloff et al <sup>49</sup>	7	7	0.8/kg/wk	13	100	unknown	2 oral ulcers 1 GI/nausea 1 alopecia 1 rash
Arnett et al <sup>50</sup>	5	5	2 given 5/d 3 given 25–50/wk	5–44	80	24	1 pulmonary infiltrates (all daily dosed)
Metzger et al <sup>51</sup>	22	22	0.5–0.8/kg/wk	3–10	77	45	8 oral ulcers 1 leukopenia 1 liver enzymes 1 pulmonary infiltrates
Sarova-Pinhas et al <sup>52</sup>	2	2	initial 50–75/wk lowered to 30/wk	4	100	48	none
Giannini and Callen <sup>53</sup>	1	1	35.5/wk	10	100	12	none

\* Clinical improvement is defined as increasing muscle strength and lowering of muscle enzyme levels.

unusual caution. . . .” The serious toxicity reported in this paper, and simultaneous reports documenting fibrosis and cirrhosis in some patients who received the drug over months to years,<sup>37–39</sup> dampened most clinical investigators’ enthusiasm for methotrexate.

However, a few studies continued. From 1964 through 1982, uncontrolled trials of a total of 126 patients were reported (*Table 2*).<sup>40–44</sup> The drug appeared to be effective in 60–80% of patients but toxicity was a problem. The authors used a variety of doses and dosing schedules. A study of 59 patients, in which an initial dose of 15 mg per week was employed, reported that the drug was effective in 82%, and more importantly that toxicity was seen in only 10%.<sup>41</sup> One controlled study appears in the literature, comparing 16 patients who received 7.5–15 mg of MTX per week with 21 patients who were given a placebo.<sup>45</sup> Although this study showed statistically significant improvement with MTX in arthritic symptoms using only one parameter, physician’s global assessment, there was a trend toward improvement in all other measures of efficacy in the MTX-treated patients. In addition, this study was flawed by its short duration of only 12 weeks and relatively small patient population. Most rheumatologists view this as a positive study and think that MTX is a useful drug in the treatment of psoriatic arthritis in patients who have not

responded to maximum doses of nonsteroidal anti-inflammatory drugs.

#### *Reiter’s syndrome*

Reiter’s syndrome, another of the spondyloarthropathies, is classically defined by the clinical triad of urethritis, conjunctivitis, and arthritis. As in the treatment of psoriatic arthritis, nonsteroidal anti-inflammatory drugs are suggested as initial therapy for the asymmetric, inflammatory arthritis. MTX may be chosen for chronic, resistant disease.

A recent review summarizes the other published cases.<sup>46</sup> Joint symptoms improved in 75% of patients within one or two months of treatment. Cutaneous and systemic manifestations generally improved more rapidly, within the first two to four weeks. In most cases, the drug was well tolerated. Adverse reactions to MTX resulted in discontinuation of therapy in three of seven patients given daily doses, compared with only one of 13 patients receiving pulse, weekly doses, suggesting that the daily dose regimen was more toxic.

#### **Methotrexate in other connective tissue diseases**

##### *Inflammatory myositis*

Among the connective tissue diseases, corticosteroid-resistant inflammatory myositis, includ-

**Table 4.** Intra-articular methotrexate in inflammatory arthritis

Reference	No. patients	Dose and schedule	Follow-up	Compared with	Results
Bird et al <sup>69*</sup>	42	2.5 mg q wk × 2	at 3,7,14,21 days	20 mg triamcinolone	triamcinolone better by thermography
Marks et al <sup>70*</sup>	12	50 mg hydrocortisone & 5 mg MTX	at 2,3,7 days 1,3 months	50 mg hydrocortisone	"clinical & X-ray no difference"
Wigginton et al <sup>68†</sup>	4	1.5 mg 1 pt 2.5 mg 3 pts	q 48 hours	—	no effect
Hall et al <sup>71*</sup>	20	10 mg given on days 0,7,14	at 0,7,14 days 3,12 weeks	saline	no difference
Tiliakos et al <sup>67*</sup>	9	MTX 0.035 mg/kg & control	unknown	1 mL dexamethasone & 1 mL xylocaine	controls improved for 1–5 days patients improved for 2–5 months

\* Controlled study.

† Uncontrolled study.

ing polymyositis and dermatomyositis, is the only disease in which MTX is recognized as standard therapy.<sup>47</sup> There have been at least 39 patients with adult inflammatory myositis reported in the world literature<sup>48–53</sup> (Table 3).

In the earliest report, four patients who had not responded to 40–60 mg of prednisone per day were treated with parenteral MTX 25–100 mg per week, combined with corticosteroids.<sup>48</sup> All experienced improvement in disease parameters within six weeks of initiation of MTX therapy. Manageable toxicity was seen in three of four patients. The remaining 35 patients in the other studies were treated using similar dosages and schedules. Twenty-nine of 35 (83%) improved within 10–13 weeks after MTX was given. Eighteen of 35 (52%) experienced toxicity. Most of these adverse reactions were mild, including stomatitis, nausea, and alopecia, and were managed by dosage manipulation. Major toxicity in the form of pulmonary symptoms occurred in three cases.<sup>50,51</sup> In two of these patients a daily dose schedule was used. One of these two patients died of respiratory failure.<sup>51</sup> All but one of the reported 39 patients had not responded to prednisone 40–60 mg per day or its equivalent given for at least two months. In addition, six patients had not responded to other cytotoxic drugs including azathioprine and cyclophosphamide.<sup>52</sup>

One other paper deserves mention. Two cases of particularly resistant myositis, which had not responded to the combination of MTX and prednisone, did respond when daily chlorambucil was added to the treatment regimen.<sup>54</sup>

The use of MTX in the treatment of childhood

dermatomyositis has also been described in three papers that reported a total of 10 patients.<sup>55–57</sup> Disease activity was reduced in seven of the 10 patients. However, toxicity was seen in five out of 10 patients because of the high doses used. Four of these patients received 1 mg/kg of MTX per week.<sup>56</sup> Death occurred in one of these patients from sepsis following neutropenia. When a similar dose was given every two weeks, only one of five patients suffered an adverse effect.<sup>55</sup> Nine of the 10 patients reported in these papers had not responded to long-term, high-dose daily prednisone.

#### *Methotrexate in vasculitis*

Current therapy of the systemic vasculitides, which include polyarteritis nodosa, Wegener's granulomatosis, and hypersensitivity angiitis, consists of moderate to high daily doses of prednisone and cyclophosphamide. There are three reports of a total of four patients with polyarteritis nodosa in whom MTX was given as therapy, combined with high-dose corticosteroids in two cases<sup>58,59</sup> and as the sole agent in two other cases.<sup>60</sup> Parenteral doses of approximately 25 mg of MTX per week were given and response was reported in three of four patients, at 18 weeks in one<sup>58</sup> and "after the second dose" (10–14 days) in the other two.<sup>60</sup> Toxicity was neither emphasized nor reported in these papers.

There is only one report of two patients with Wegener's granulomatosis treated with MTX.<sup>61</sup> Both patients responded to initial intravenous therapy of 50 mg per week. MTX was used alone in one case and combined with 25 mg prednisone

**Table 5.** Toxicity rheumatoid arthritis patients\*

Adverse reaction	Occurrences†	Mean cumulative dose (mg)	Mean weekly dose (mg)
Elevated AST or ALT levels (drug discontinued in 3)	13	1058 (range 90–3722)	7.85
Peptic ulcer (each patient also taking NSAIDs)	3	733 (range 180–1780)	7.96
Nausea (drug discontinued in 1)	2	710, 30	8.40
Mouth ulcers	2	990, 210	7.50
Alopecia	2	180, 60	7.50
Herpes zoster	2	510, 2340	7.60
Neoplasm; adenocarcinoma of rectum (drug discontinued); metastatic adenocarcinoma (drug discontinued)	2	585, 540	7.50
Mucositis (from overdose)	1		100 in 1 week
Hepatic fibrosis	1	3875 mg‡	

AST = aspartate aminotransferase; ALT = alanine aminotransferase.

\* From Wilke et al.<sup>83</sup>

† A total of 24 of the 87 patients studied experienced toxicity. Two patients had two side effects each (herpes zoster and an elevated aspartate aminotransferase level).

‡ After 126 months of therapy.

daily in the other. Symptomatic response was observed after one week in both patients. The dose of MTX was gradually reduced in both cases. During follow-up periods of 26 and 30 months, toxicity was not seen.

#### *Lupus erythematosus*

There are no reports of the use of MTX in the treatment of systemic lupus erythematosus in the United States medical literature. In the British literature, one patient with lupus erythematosus was given MTX in a dose of 2.5 mg per day for seven weeks.<sup>43</sup> The author saw no apparent response. In the Scandinavian literature four patients received a combination of MTX and azathioprine.<sup>62</sup> The dose of MTX ranged from 12.5–20 mg per week combined with azathioprine 1–2 mg/kg/day. Only one of the four patients appeared to benefit. These authors thought that azathioprine was more effective. However, a report from Switzerland, in which MTX was given in doses of 7.5–15 mg per week alternating monthly with azathioprine 1–2 mg/kg/day in an unspecified number of patients, suggested that it was useful.<sup>63</sup>

#### *Miscellaneous*

Anecdotal reports have documented MTX use with benefit in sarcoidosis,<sup>64</sup> cyclitis,<sup>65</sup> and midline granuloma.<sup>66</sup> To our knowledge there are no reports of the use of MTX in the treatment of ankylosing spondylitis. Because of its apparent usefulness in psoriatic arthritis and Reiter's syn-

drome, its use as therapy for ankylosing spondylitis, a similar spondyloarthropathy, should be studied.

#### **Intra-articular methotrexate**

Five studies have reported the results of intra-articular MTX in the treatment of rheumatoid arthritis or psoriatic arthritis<sup>67–71</sup> (Table 4). In one study, 0.035 mg/kg of MTX combined with 1 mL of xylocaine and 1 mL of dexamethasone was compared with 1 mL of dexamethasone and 1 mL of xylocaine in the knees of patients with rheumatoid arthritis.<sup>67</sup> Synovial hypertrophy, joint circumference, the degree of effusion, and tenderness all responded best to the MTX combination, which had a lasting effect (mean 3 months versus 1–5 days). On the other hand, an uncontrolled study of four patients<sup>68</sup> and controlled studies comparing MTX with triamcinolone,<sup>69</sup> hydrocortisone,<sup>70</sup> and saline injection<sup>71</sup> failed to show that MTX was more beneficial than the control therapy. Most of these authors used relatively small intra-articular doses (2.5–5.0 mg). Four patients in one report<sup>69</sup> who were given up to 20 mg of intra-articular MTX showed improvement that did not reach statistical significance.

The information from three studies<sup>68,69,71</sup> suggests that when MTX is given as an intra-articular injection without the benefit of a long-acting corticosteroid, it is ineffective. In addition, the controlled study by Marks et al<sup>70</sup> of 12 patients failed to show that the combination of hydrocor-

**Table 6.** Methotrexate toxicity in uncontrolled clinical studies of rheumatoid arthritis\*

Reference	No. patients	Weekly dosage (mg) (duration)	Toxicity (%)	Elevated liver function test values	Nausea	Stomatitis	Other
Hoffmeister <sup>29</sup>	78	10–15 (4–15 years)	11	8% (6)	8% (6)	8% (6)	3% (2)
Willkens et al <sup>26</sup>	32	7.5–15 (1–10 years)	13	6% (2)	“many”	—	—
Groff et al <sup>31</sup>	28	5–12.5 (12.5 months)	50	14% (4)	18% (5)	11% (3)	25% (7)
Weinstein et al <sup>20</sup>	21	7.5–25 (38 weeks)	57	28% (10)	14% (3)	14% (3)	29% (6)
Michaels et al <sup>28</sup>	14	10–50 (7–20 weeks)	86	36% (5)	64% (9)	21% (3)	0

\* The incidence of minor toxicity shows a trend to vary directly with the magnitude of the weekly dose.

tisone and 5 mg of methotrexate was any more effective than 50 mg of hydrocortisone alone. Because the only study showing that intra-articular methotrexate was effective was the controlled study of nine patients by Tiliakos et al,<sup>67</sup> if MTX is to be used at all, it should be combined with a long-acting corticosteroid preparation.

### Methotrexate toxicity

#### Risk factors

The first clinical researchers to use folate analogs in the treatment of psoriasis administered aminopterin in daily doses of 0.5–1 mg and later MTX in daily doses of 2.5 mg per day with rest periods.<sup>72</sup> The apparent effectiveness of MTX encouraged other clinicians to adopt this regimen in the early 1960s.<sup>73</sup> By the late 1960s, it was clear that MTX was effective therapy for psoriasis but debate began concerning the safest method of administration. The oncology experience with higher-dose therapy clearly demonstrated that daily administration was more toxic than intermittent administration.<sup>74,75</sup> At the same time, it became clear that hepatic fibrosis and cirrhosis often complicated prolonged therapy with MTX, adding fuel to the debate. These histologic hepatic changes were encountered in 24–55% of patients treated with daily-dose MTX,<sup>76,77</sup> but were less often seen when the drug was given on an intermittent or pulse schedule.<sup>78,79</sup> This work and the findings of a multicenter cooperative study<sup>80</sup> served to identify risk factors associated with hepatocellular toxicity.

The most significant factors are the frequency of administration of MTX and the simultaneous use of moderate alcohol in patients receiving the drug. Daily-dose MTX or a frequency of administration exceeding 12 days per month<sup>77</sup> is clearly associated with liver toxicity, as is regular alcohol consumption of 1–3 ounces (28–84 g) or more

per day.<sup>80</sup> In addition, diabetic or obese patients are at a greater risk of steatosis at liver biopsy and might be at a greater risk of fibrosis. In some studies, a high cumulative dose (> 1.5 g) is also thought to be a factor,<sup>80</sup> although other studies have not confirmed this relationship.<sup>81,82</sup>

#### Minor toxicity

Long-term therapy with low-dose, weekly, pulse MTX is well tolerated in our experience. We reported a retrospective analysis of 87 patients who had received a mean weekly dose of 8.9 mg MTX and had been followed up for a mean of 42 months.<sup>83</sup> From this retrospective chart review we could document toxicity in only 24 patients, an overall incidence of 29% (Table 5). MTX was discontinued in only four patients as a direct result of toxicity. A similar retrospective study of 78 patients followed up for 15 years reported the same low incidence of toxicity.<sup>29</sup>

Retrospective studies provide a good estimate of the percentage of patients who prove intolerant to a drug, but they have the inherent weakness of under-reporting minor toxicity. The incidence of toxicity was higher in 21 patients from Connecticut who were followed up prospectively for a mean of 42 months and who received a mean calculated weekly dose of 12.01 mg of MTX.<sup>20</sup> Twelve (57%) of these patients experienced side effects, including transient elevation of liver transaminases in seven, gastrointestinal upset in seven, stomatitis in three, thrombocytopenia, herpes zoster, and acute “hypersensitivity” hepatitis in one patient each. MTX was discontinued in four (19%) patients.

An even higher incidence of minor toxicity (73.6%) has been reported in 72 patients.<sup>84</sup> As in other studies, this minor toxicity rarely led to discontinuation of the drug and could be managed by adjusting the dose.

**Table 7.** Methotrexate and liver histopathology\*

Reference	No. patients	Weekly dose (mg)	Cumulative dose (mg)	Fatty change	Fibrosis	Cirrhosis	Within normal limits
DiBartolomeo et al <sup>86</sup>	26	14.6	1518	1 (4%)	1 (4%)	0	10 (38%)
Mackenzie <sup>92</sup>	60	8.67	1837	30 (50%)	1 (1.7%)	0	26 (43%)
Aponte and Petrelli <sup>87</sup>	20	5–15	4690	5 (20%)	2 (10%)	0	7 (35%)
Kremer <sup>88</sup>	29	12.5	approximately 1500	2 (9%)	0	0	18 (91%)
Weinstein et al <sup>89</sup>	17	13.3	1950	12 (71%)	6 (35%)	0	3 (18%)
Koo et al <sup>90</sup>	82†	7.9	3090	26 (31%)	4 (4.9%)	0	77 (94%)
Rau and Karger <sup>91</sup>	34	11.4	1324	33 (99%)	1 (3%)	0	0

\* Hepatic histological changes in 210 patients who received mean cumulative doses of MTX  $\geq$ 1500 mg.

† 58 additional patients (includes 24 patients of Mackenzie's 1974 study).

A review of uncontrolled clinical trials suggests that there is a trend toward a higher incidence of minor toxic events directly related to the magnitude of the weekly dose (Table 6).

#### Major toxicity

The occurrence of major toxicity in reported series is low. Mild thrombocytopenia ( $< 100,000/\text{mm}^3$ ) may occur<sup>17</sup> in approximately 10% of patients (personal communication). However, clinically significant cytopenia or serious infection is very rare. Acute "hypersensitivity" hepatitis that occurred in the first few weeks of treatment has been reported<sup>20</sup> but has not been encountered by others. Herpes zoster can be expected in 1–2% of treated patients.<sup>83</sup>

**Pulmonary toxicity:** Reports of acute, life-threatening pulmonary toxicity are worrisome. Patients with this problem present with acute or subacute nonproductive cough and dyspnea. Diffuse interstitial and alveolar infiltrates are seen on the chest radiograph. Hypoxemia with arterial oxygen pressure falling below 50 mmHg is common. Treatment consists in the discontinuation of MTX and institution of supportive measures. Some authors<sup>84</sup> administer corticosteroids (1 mg/kg/day of prednisone) after infection has been excluded. We believe that the dose schedule employed may be an important factor in the etiology of pulmonary toxicity.<sup>85</sup> This kind of toxicity appears to occur more often in patients who receive the drug each week in three equal doses separated by 12-hour intervals than in patients receiving the entire dose within 12 hours each week.

**Liver toxicity:** Chronic liver toxicity taking the form of grade III fibrosis or cirrhosis is another major potential problem with methotrexate. As

already discussed, the incidence of fibrosis or cirrhosis in patients treated with long-term MTX for psoriasis has been shown to be 6–55%.<sup>76,77,80</sup> At the present time there are seven prospective studies<sup>86–92</sup> in which liver biopsies have been obtained from patients with rheumatoid arthritis who have been treated with the drug (Table 7). Although the incidence of fatty change is reported to be as high as 99%<sup>91</sup> and that of low-grade fibrosis as high as 35%,<sup>89</sup> cirrhosis has not been encountered. Histopathologic abnormalities are common in untreated patients with rheumatoid arthritis. In fact, the incidence of fatty change and fibrosis was found to be no higher in treated patients than in control patients with rheumatoid arthritis.<sup>87,92</sup> These studies demonstrate that the incidence of serious long-term liver toxicity in patients with rheumatoid arthritis treated with pulse, low-dose MTX is considerably lower than the incidence seen in the earlier psoriasis literature.

At present, our group does not require interval liver biopsy nor do we require pre-MTX biopsy. Although in some patients, hepatic enzymes may be normal despite histologic pathologic findings,<sup>80</sup> we continue to monitor these enzymes on a regular basis. Obesity, suspected alcohol abuse, insulin-dependent diabetes, or chronic elevation of hepatic enzymes (including alkaline phosphatase) influence our decision regarding liver biopsy.

**Potential toxicity:** When cytotoxic drugs are used to treat patients for conditions other than malignant disease, clinicians are faced with the potential of teratogenesis and oncogenesis. MTX is clearly teratogenic when given at conception or during the first three months of gestation.<sup>93</sup> In the male it may cause reversible oligosper-



mia.<sup>94</sup> However, unlike the alkylating agents cyclophosphamide and chlorambucil<sup>95-97</sup> and the antimetabolite azathioprine,<sup>98</sup> MTX has not been shown to be carcinogenic in either high-dose or low-dose therapy.<sup>99-103</sup>

### Therapeutic implications

The treatment of all of these diseases is palliative. In rheumatoid arthritis and most of the other conditions, it is often necessary to maintain therapy for years. Therefore, the agents used should not only be effective but also must be safe and carry a low rate of dropout from adverse effects. These agents must also be compatible with many other drugs or modalities. Low-dose MTX can be used to maintain control of rheumatoid arthritis for many years<sup>19,20,29,83</sup> and in most patients appears to be safe for long-term use. It is also compatible with a variety of drugs used in the treatment of connective tissue diseases, including hydroxychloroquine, nonsteroidal anti-inflammatory drugs, and prednisone.<sup>83</sup> We believe that the incidence of minor toxicity can be limited by maintaining the dose of MTX at or below 12.5 mg per week, by giving the total dose at one time, and by careful monitoring of renal function. We also use folic acid as another theoretical measure to reduce toxicity. This is given in equal-milligram oral doses five days after MTX is administered. Animal studies suggest that folate might provide a direct protective effect by displacing MTX from the enzyme dihydrofolate reductase,<sup>104,105</sup> however, this is controversial. Weekly folate may simply prevent subclinical folate deficiency, which is associated with a higher incidence and severity of toxicity in cancer patients treated with MTX.<sup>106</sup>

Patient selection to limit toxicity is also important. Regular alcohol use should be discouraged. Patients with a previous history of liver disease are not considered candidates for this drug without a pretreatment biopsy. Because the elimination of MTX largely depends on renal excretion, patients with kidney disease and creatinine clearance below 60 mL/min should be excluded from treatment. Proteinuria has been shown to interfere with the renal excretion of MTX,<sup>30</sup> and because this can result from previous treatment with gold compounds or d-penicillamine in patients with rheumatoid arthritis, patients so treated should be monitored carefully. Neither obesity nor mild diabetes appear in our experience to be associated with a higher incidence of

side effects,<sup>83</sup> but insulin has been shown to enhance MTX's cytotoxicity to human breast cancer cells.<sup>107</sup> This combination might theoretically lead to increased toxicity.

When MTX is compared with other cytotoxic agents and with the disease-modifying drugs used to treat rheumatoid arthritis, it appears to be at least as safe. Our 29% incidence of toxicity with MTX<sup>83</sup> compares very favorably with short-term toxicity from cyclophosphamide (90% of patients in a 32-week study).<sup>108</sup> Toxicity with MTX appears to be similar to that encountered with azathioprine, the use of which resulted in an overall incidence of toxicity of 33% of 12 patients receiving the drug for a period of 40 months.<sup>109</sup> Certainly, toxicity from MTX is less frequent than the 48% incidence seen in 200 patients treated with d-penicillamine and followed up for at least two years,<sup>110</sup> and is comparable to the 29% long-term toxicity reported for parenteral gold.<sup>111</sup> In a recent prospective report, the dropout rate with MTX was lower than that seen with either parenteral gold or d-penicillamine<sup>18</sup> during a mean follow-up period of 2.5 years.

With careful selection and regular monitoring for toxicity, MTX can be used safely for the treatment of rheumatoid arthritis. It may also have a role in the treatment of a variety of other inflammatory or autoimmune conditions.

William S. Wilke, M.D.  
Department of Rheumatic and Immunologic Disease  
Cleveland Clinic Foundation  
9500 Euclid Avenue  
Cleveland, OH 44106

### References

1. Jolivet J, Cowan KH, Curt GA, et al. The pharmacology and clinical use of methotrexate. *N Engl J Med* 1983; **309**:1094-1104.
2. Henderson ES, Adamson RH, Oliverio VT. The metabolic fate of tritiated methotrexate. II. Absorption and excretion in man. *Cancer Res* 1965; **25**:1018-1024.
3. Edelman J, Biggs DF, Jamali F, et al. Low-dose methotrexate kinetics in arthritis. *Clin Pharmacol Ther* 1984; **35**:382-386.
4. Shen OD, Azarnof DL. Clinical pharmacokinetics of methotrexate. *Clin Pharmacokinetics* 1978; **3**:1-13.
5. Leigler EG, Henderson ES, Hahn MA, et al. The effect of organic acids on renal clearance of methotrexate in man. *Clin Pharmacol Ther* 1969; **10**:849-857.
6. Taylor JR, Halprin KM. Effect of sodium salicylate and

- indomethacin on methotrexate-serum albumin binding. *Arch Derm* 1977; **113**:588-591.
7. Thys A, Milano G, Kubar J, et al. Clinical and pharmacokinetic evidence of a life-threatening interaction between methotrexate and ketoprofen. *Lancet* 1986; **1**: 256-258.
  8. Bourke RS, Chheda G, Bremer A, et al. Inhibition of renal tubular transport of methotrexate by probenecid. *Cancer Res* 1975; **35**:110-116.
  9. Gerebetzoff A, Lambert PH, Miescher PA. Immunosuppressive agents. *Annu Rev Pharmacol* 1972; **12**:287-316.
  10. Hersch EM, Wong VG, Freireich EJ. Inhibition of the local inflammatory response in man by antimetabolites. *Blood* 1966; **27**:38-48.
  11. Mitchell MS, Wade ME, DeConti RC, et al. Immunosuppressive effects of cytosine arabinoside and methotrexate in man. *Ann Intern Med* 1969; **70**:535-546.
  12. Thomas ED, Storb R. The effect of amethopterin on the immune response. *Ann NY Acad Sci* 1971; **186**:467-474.
  13. Andersen EA, West SG, O'Dell JR, et al. Weekly pulse methotrexate in rheumatoid arthritis: Clinical and immunologic effects in a randomized, double-blind study. *Ann Intern Med* 1985; **103**:479-486.
  14. Weinblatt ME, Coblyn JS, Fox DA, et al. Efficacy of low-dose methotrexate in rheumatoid arthritis. *N Engl J Med* 1985; **312**:818-823.
  15. Thompson RN, Wats C, Edelman J, et al. A controlled 2-center trial of parenteral methotrexate for refractory rheumatoid arthritis. *J Rheumatol* 1984; **11**:760-763.
  16. Stevens JE, Willoughby DA. The anti-inflammatory effect of some immunosuppressive agents. *J Pathol* 1969; **97**:367-373.
  17. Kremer JM. Lymphocytes subset analysis after long-term methotrexate (MTX) therapy for rheumatoid arthritis (RA). *Arth Rheum* 1986; **29**(abstr):S75.
  18. Olsen N, Baer A, Pincus T. Methotrexate induces early, specific decreases in IgM-rheumatoid factor synthesis in rheumatoid arthritis patients. *Arth Rheum* **29**(abstr):S75.
  19. Boh L, Schuna A, Pitterle M, et al. Long-term use of methotrexate (MTX) in inflammatory arthritis: Clinical and x-ray evaluation. *Arthritis Rheum* 1985; **28**(Suppl):S46.
  20. Weinstein A, Marlow ES, Korn J, et al. Low-dose methotrexate treatment of rheumatoid arthritis: Long-term observations. *Am J Med* 1985; **79**:331-337.
  21. Gubner R. Therapeutic suppression of tissue reactivity. II. Effect of aminopterin in rheumatoid arthritis and psoriasis. *Am J Med Sci* 1951; **122**:176-182.
  22. Beickert VA, Geidel H. Erfahrungen mit 6-merkaptopurin und Methotrexate bei primar chronischer Polyarthritits und anderen Kollagenosen. *Deutsche Gesundheitswesen* 1968; **23**:685-693.
  23. Enderlin DM, Gross D. Beitrag zur Behandlung der Progredient chronischen Polyarthritits mit Antimetaboliten und Cytostatica. *Zeitschrift fur Rheumaforschung* 1966; **26**:26-35.
  24. Hoffmeister RT. Methotrexate in rheumatoid arthritis. *Arthritis Rheum* 1972; **15**(Suppl):S114.
  25. Wilke WS, Calabrese LH, Scherbel AL. Methotrexate in the treatment of rheumatoid arthritis: Pilot study. *Clev Clin Q* 1980; **47**:305-309.
  26. Willkens RD, Watson MA, Paxson CS. Low-dose pulse methotrexate therapy in rheumatoid arthritis. *J Rheumatol* 1980; **7**:501-505.
  27. Bachman DM. Pulsed intravenous methotrexate treatment in rheumatoid arthritis. *Arthritis Rheum* 1982; **25**(Suppl):S65.
  28. Michaels RM, Nashel DJ, Leonard A, et al. Weekly intravenous methotrexate in the treatment of rheumatoid arthritis. *Arthritis Rheum* 1982; **25**:339-341.
  29. Hoffmeister RT. Methotrexate therapy in rheumatoid arthritis: 15 years experience. *Am J Med* 1983; **75**:69-73.
  30. Edelman J, Russell AS, Biggs DF, et al. Methotrexate levels, a guide to therapy? *Clin Exper Rheum* 1983; **1**:153-156.
  31. Groff GD, Shenberger KN, Wilke WS, et al. Low-dose oral methotrexate in rheumatoid arthritis: An uncontrolled study and review of the literature. *Sem Arthritis Rheum* 1983; **12**:333-347.
  32. Kremer JM, Joong KL. The safety and efficacy of the use of methotrexate in long-term therapy for rheumatoid arthritis. *Arthritis Rheum* 1986; **26**:822-831.
  33. Wilke WS, Segal AM, Calabrese LH, et al. 24 week double-blind crossover study of methotrexate in rheumatoid arthritis. *Clin Res* 1985; **33**:884A.
  34. Williams HJ, Willkens RF, Samuelson CO, et al. Comparison of low-dose oral pulse methotrexate and placebo in the treatment of rheumatoid arthritis: A controlled clinical trial. *Arth Rheum* 1985; **28**:721-730.
  35. Hunter GA, Turner AN. Methotrexate in the treatment of psoriasis: A controlled clinical trial. *Aust J Med* 1963; **7**:91-92.
  36. Black RL, O'Brien WM, Van Scot EJ, et al. Methotrexate therapy in psoriatic arthritis: Double-blind study in 21 patients. *JAMA* 1964; **189**:743-747.
  37. Coe RO, Bull FE. Cirrhosis associated with methotrexate treatment of psoriasis. *JAMA* 1968; **206**:1515-1520.
  38. Dubin HB, Harrell ER. Liver diseases associated with methotrexate treatment of psoriasis patients. *Arch Derm* 1970; **102**:498-503.
  39. Epstein Jr EH, Croft Jr JD. Cirrhosis following methotrexate administration for psoriasis. *Arch Dermatol* 1969; **100**:531-534.
  40. Hunter GA, Millazzo SC. Response of psoriatic arthropathy to methotrexate. *Aust J Med* 1963; **7**:137-141.
  41. Kragballe K, Zachariae E, Zachariae H. Methotrexate in psoriatic arthritis: A retrospective study. *Acta Dermatovener (Stocholme)* 1982; **63**:165-167.
  42. Fledges DH, Barnes CG. Treatment of psoriatic arthropathy with either azathioprine or methotrexate. *Rheumatol Rehab* 1972; **13**:120-124.
  43. Kersley GD. Amethopterin (methotrexate) in connective tissue disease—psoriasis and polyarthritits. *Ann Rheum Dis* 1968; **27**:64-66.
  44. Chaouat Y, Kanovitch B, Faures B, et al. Le rhumatisme psoriasique traitement par le methotrexate. *Revue du Rhumatisme* 1971; **38**:453-460.
  45. Willkens RF, Williams HJ, Ward JR, et al. Randomized, double-blind, placebo controlled trial of low-dose pulse methotrexate in psoriatic arthritis. *Arthritis Rheum* 1984; **27**:376-381.
  46. Lally EV, Ho Jr G. A review of methotrexate therapy in Reiter syndrome. *Sem Arthritis Rheum* 1985; **15**:139-145.
  47. Bradley WG. Polymyositis. [In] Kelley WN, Harris Jr ED, Rudd S, Sledge CB, eds. *Textbook of Rheumatology*. Philadelphia, London, Toronto, Saunders, 1981, pp 1255-1276.
  48. Malaviya AN, Many A, Schwartz RS. Treatment of dermatomyositis with methotrexate. *Lancet* 1968; **1**:485-488.

49. Sokoloff MC, Goldberg LS, Pearson CM. Treatment of corticosteroid-resistant polymyositis with methotrexate. *Lancet* 1971; **1**:14-16.
50. Arnett FC, Whelton JC, Zizic TM, et al. Methotrexate therapy in polymyositis. *Ann Rheum Dis* 1973; **32**:536-546.
51. Metzger AL, Bohan A, Goldberg LS, et al. Polymyositis and dermatomyositis: Combined methotrexate and corticosteroid therapy. *Ann Intern Med* 1974; **81**:182-189.
52. Sarova-Pinhas I, Siegal T, Turgman J, et al. Methotrexate treatment in dermatomyositis. *Eur Neurol* 1977; **16**:149-154.
53. Giannini M, Callen JP. Treatment of dermatomyositis with methotrexate and prednisone. *Arch Dermatol* 1979; **115**:1251-1252.
54. Wallace DJ, Metzger AL, White KK. Combination immunosuppressive treatment of steroid-resistant dermatomyositis/polymyositis. *Arthritis Rheum* 1985; **28**:590-592.
55. Jacobs JC. Methotrexate and azathioprine. Treatment of childhood dermatomyositis. *Pediatrics* 1977; **59**:212-218.
56. Fischer TJ, Rachelefsky GS, Klein RB, et al. Childhood dermatomyositis and polymyositis. Treatment with methotrexate and prednisone. *Am J Dis Child* 1979; **133**:386-389.
57. Goel KM, Shanks RA. Dermatomyositis in childhood. Review of 8 cases. *Arch Dis Child* 1976; **51**:501-506.
58. Tannenbaum H. Combined therapy with methotrexate and prednisone in polyarteritis nodosa. *Can Med Assoc J* 1980; **123**:893-894.
59. Leib ES, Restivo C, Paulus HE. Immunosuppressive and corticosteroid therapy of polyarteritis nodosa. *Am J Med* 1979; **67**:941-947.
60. Fraga A, Mintz G, Orozco JH. Immunosuppressive therapy in connective tissue diseases other than rheumatoid arthritis. *J Rheumatol* 1974; **4**:374-391.
61. Capizzi RL, Bertino JR. Methotrexate therapy of Wegener's granulomatosis. *Ann Intern Med* 1971; **74**:74-79.
62. Cruchaud A, Pometta D, Rouso C. Treatment of systemic lupus erythematosus (SLE) with immunosuppressive drugs. An analysis of immunologic data. *Helvetica Med Acta* 1967; **34**:5-22.
63. Miescher PA, Berris PH. Immunosuppressive therapy in the treatment of autoimmune diseases. *Springer Semin Immunopathol* 1984; **7**:69-90.
64. Lacher MJ. Spontaneous remission or response to methotrexate in sarcoidosis. *Ann Intern Med* 1968; **69**:1247-1248.
65. Wong GV, Herst EM. Methotrexate in the therapy of cyclitis. *Trans Am Acad Ophthalmol Otolaryngol* 1965; **1**:279-293.
66. von Leden H. Midline lethal granuloma. *Trans Am Acad Ophthalmol Otolaryngol* 1964; **68**:620-624.
67. Tiliakos NA, Lawrence TAB, Wilson Jr TH. Intra-articular methotrexate in intractable rheumatoid knees. *Arthritis Rheum* 1982; **25**(Suppl):S65.
68. Wigginton SM, Chu BCF, Weisman MH, et al. Methotrexate pharmacokinetics after intra-articular injection in patients with rheumatoid arthritis. *Arth Rheum* 1980; **23**:119-122.
69. Bird HA, Ring EFJ, Daniel R, et al. Comparison of intra-articular methotrexate with intra-articular triamcinolone hexacetone by thermography. *Current Med Res Opinion* 1977; **5**:141-146.
70. Marks JS, Stewart IM, Hunter JAA. Intra-articular methotrexate in rheumatoid arthritis. *Lancet* 1976; **2**:857-858.
71. Hall GH, Jones BJM, Head AC, et al. Intra-articular methotrexate. Clinical and laboratory study in rheumatoid and psoriatic arthritis. *Ann Rheum Dis* 1978; **37**:351-356.
72. Rees RB, Bennett JH, Hamlin EM, et al. Aminopterin for psoriasis. A decade's observation. *Arch Dermatol* 1964; **90**:544-552.
73. Rees RB, Bennett JH, Maibach HI, et al. Methotrexate for psoriasis. *Arch Derm* 1967; **95**:2-11.
74. Louis J. Methotrexate toxicity: Relationship to rate of administration surface area, plasma volume and renal function. *J Lab Clin Med* 1970; **70**:888-889.
75. McDonald CJ, Bertino JR. Parenteral methotrexate for psoriasis. *Lancet* 1968; **1**:864.
76. Dahl MGC, Gregory MM, Scheuer PJ. Methotrexate hepato-toxicity in psoriasis—comparison of different dose regimens. *Brit Med J* 1972; **1**:654-656.
77. Podurciel VJ, McGill DB, Ludwig J, et al. Liver injury associated with methotrexate therapy for psoriasis. *Mayo Clin Proc* 1973; **48**:787-791.
78. Weinstein GD, Frost P. Methotrexate for psoriasis—a new therapeutic schedule. *Arch Derm* 1971; **103**:33-38.
79. Warin AP, Landells JW, Levene GM, et al. A prospective study of the effects of weekly oral methotrexate on liver biopsy. Findings in severe psoriasis. *Brit J Derm* 1975; **93**:321-327.
80. Cooperative Study Group. Psoriasis-liver-methotrexate interactions. *Arch Dermatol* 1973; **108**:36-42.
81. Lanse SB, Arnold GL, Gowans JDC, et al. Low incidence of hepato-toxicity associated with long-term, low-dose oral methotrexate in treatment of refractory psoriasis, psoriatic arthritis, and rheumatoid arthritis. An acceptable risk/benefit ratio. *Dig Dis Sci* 1985; **30**:104-109.
82. Roenigk Jr HH, Bergfeld WF, St. Jacques R, et al. Hepato-toxicity of methotrexate. *Arch Derm* 1971; **103**:250-261.
83. Wilke WS, Calabrese LH, Krall PL, Segal AM. Incidence of toxicity in patients with rheumatoid arthritis treated with methotrexate. [In] Rau R, ed. *Rheumatology*. 9th ed. Basel, Munchen, Paris, London, New York, New Delhi, Singapore, Tokyo, Sidney, Karger, 1986, pp 134-144.
84. St. Claire EW, Rice JR, Snyderman R. Pneumonitis complicating low-dose methotrexate therapy in rheumatoid arthritis. *Arch Intern Med* 1985; **145**:2034-2038.
85. Wilke WS, Mackenzie AH. Methotrexate therapy in rheumatoid arthritis. Current status. *Drugs* 1986; **32**:103-113.
86. DiBartolomeo AG, Mayes MD, Bathon JM. Methotrexate in rheumatoid arthritis: a longitudinal study of liver biopsies. *Arthritis Rheum* 1984; **27**(Suppl):S61.
87. Aponte J, Petrelli M. Hepatic histology following prolonged treatment of rheumatoid arthritis with bolus methotrexate. *Arthritis Rheum* 1985; **28**(Suppl):S37.
88. Kremer JM. A long-term prospective study of methotrexate (MTX) in rheumatoid arthritis (RA). *Arthritis Rheum* 1985; **28**(Suppl):S68.
89. Weinstein A, Marlow S, Korn J, et al. Low-dose methotrexate treatment of rheumatoid arthritis: long-term observations. *Am J Med* 1985; **79**:331-337.
90. Koo AP, Mackenzie AH, Tuthill RJ, et al. Liver histology in rheumatoid arthritis before and after prolonged methotrexate therapy. *Arthritis Rheum* 1985; **28**(Suppl):S14.
91. Rau R, Karger T. Liver biopsy findings in patients with rheumatoid arthritis (RA) and psoriatic (PsA) on long-term treatment with methotrexate. *Arthritis Rheum* 1986; **29**:S76.
92. Mackenzie AH. Hepatotoxicity of prolonged methotrexate

- therapy for rheumatoid arthritis. *Cleve Clin Q* 1985; **52**:129-135.
93. Anderson TF. Psoriasis. *Med Clin N Am* 1982; **66**:769-794.
94. Grunnet E, Nyfors A, Brogaard-Hansen K. Studies on human semen in topical corticosteroid treated and methotrexate treated psoriatics. *Dermatologica* 1977; **154**:78-84.
95. Baltus JAM, Boersma JW, Hartman AP, et al. The occurrence of malignancies in patients with rheumatoid arthritis treated with cyclophosphamide; a controlled retrospective follow-up. *Ann Rheum Dis* 1983; **42**:368-373.
96. Pedersen-Bjergaard J, Larsen SO. Incidence of acute non-lymphocytic leukemia, pre-leukemia and acute myeloproliferative syndrome up to 10 years after treatment of Hodgkin's disease. *N Engl J Med* 1982; **307**:965-971.
97. Cameron S. Chlorambucil and leukemia. *N Engl J Med* 1977; **296**:1065.
98. Kinlen LJ, Sheil AGR, Peto A, et al. Collaborative United Kingdom-Australasian study of cancer in patients treated with immunosuppressive drugs. *Aust Med J* 1979; **2**:1461-1466.
99. Rustin GJS, Rustin F, Dent J, et al. No increase in second tumors after methotrexate chemotherapy for gestational trophoblastic tumors. *N Engl J Med* 1983; **308**:473-476.
100. Stern RS, Zierler S, Parrish JA. Methotrexate used for psoriasis and the risk of non-cutaneous malignancy. *Cancer* 1972; **50**:869-872.
101. Bailin PL, Tindall JP, Roenigk HH, et al. Is therapy for psoriasis carcinogenic? A modified retrospective analysis. *JAMA* 1975; **232**:359-362.
102. Nyfors A, Jensen H. Frequency of malignant neoplasms in 248 long-term methotrexate-treated psoriatics. A preliminary study. *Dermatologica* 1983; **167**:260-261.
103. Grunewald W, Rosner F. Acute leukemia in immunosuppressive drug use. A review of patients undergoing immunosuppressive therapy for non-neoplastic diseases. *Arch Intern Med* 1979; **139**:461-466.
104. Werkheiser WC. Specific binding of 4-amino folic acid analogs by folic acid reductase. *J Bio Chem* 1961; **236**:888-893.
105. White JC. Reversal of methotrexate binding to dihydrofolate reductase by dihydrofolate. *J Bio Chem* 1979; **254**:10889-10894.
106. Hellman S, Ianotti AT, Bertino JR. Serum folate activity in patients with tumors of the head and neck treated with methotrexate. *Proc Am Assoc Cancer Res* 1963; **4**:27.
107. Alabaster BOH, Vonderhaar BK, Shafie SM. Metabolic modification by insulin enhances methotrexate cytotoxicity in MCF-7 human breast cancer cell. *Eur J Cancer Clin Oncol* 1981; **17**:1223-1228.
108. Cooperating Clinics Committee of the American Rheumatism Association. A controlled trial of cyclophosphamide in rheumatoid arthritis. *N Engl J Med* 1970; **283**:883-889.
109. Hunter T, Urowitz MB, Gordon DA, et al. Azathioprine and rheumatoid arthritis. A long-term follow-up study. *Arthritis Rheum* 1975; **18**:15-20.
110. Rothermich NO, Thomas MH, Phillips VK, et al. Clinical trial penicillamine in rheumatoid arthritis. *Arthritis Rheum* 1981; **24**:1473-1478.
111. Rothermich NO, Phillips VK, Bergen W, et al. Chrysotherapy: A prospective study. *Arthritis Rheum* 1976; **19**:1321-1327.