METHOTREXATE AS AN ANTI-INFLAMMATORY AGENT: HOPE OR HYPE

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Medical Grand Rounds

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June 23, 1994

Antifolates have played an important role in the treatment of neoplastic disease. The most widely used antifolate is methotrexate. Over the past 20 years, clinicians have begun to use methotrexate as an anti-inflammatory agent in a variety of conditions. When used as an anti-inflammatory agent, the dose traditionally used is 7.5 to 20 mg/week. This has been termed "low dose" therapy to contrast it with higher doses used in treating malignancy. The purpose of this grand rounds is to review the evidence that methotrexate has anti-inflammatory properties and to discuss its mechanism of action and toxicity.

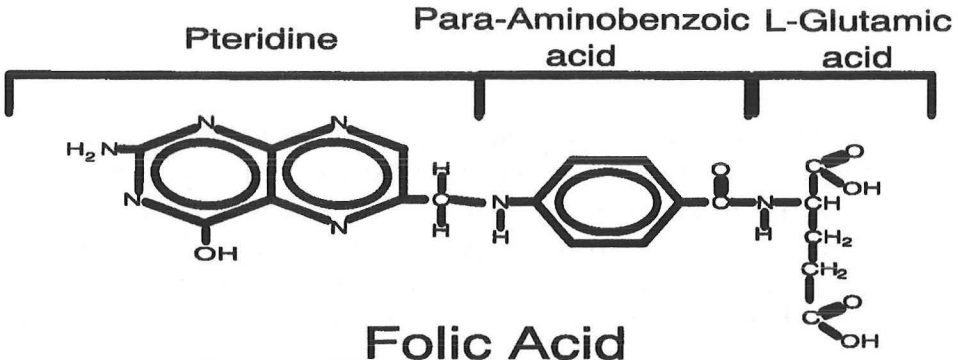
CLINICAL PHARMACOLOGY OF "LOW DOSE" METHOTREXATE

The structures of methotrexate and various forms of folic acid are provided in figure 1. Folate is pteridine compound which plays a critical role in a number of 1-carbon transfer reactions including those involved in the biosynthesis of purines, pyrimidines, serine and methionine and the degradation of histidine. Methotrexate differs from folic acid in that it contains a methyl group at N^{10} and an NH_2 group on the first ring instead of a hydroxyl group (figure 1). Folic acid is absorbed in the intestine and transported to the liver where it is reduced and methylated to form 5-methyl tetrahydrofolate (figure 1). This compound then enters the blood stream where it comes into contact with glycosylphosphatidylinositol (GPI)-anchored folate receptors expressed by nearly all cells (9). These receptors are known to cluster constitutively within caveolae (9). At various intervals these caveolae close and their pH decreases releasing folate from the receptor. Free folate then flows into the cell via a concentration gradient through an anion carrier. This process of transmembrane transport is termed potocytosis (9). Methotrexate competes with folic acid for binding to this receptor.

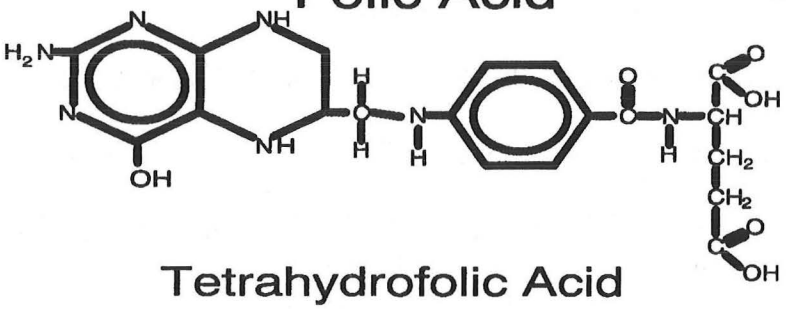
The metabolism of folic acid is provided in figure 2. The stars represent reactions that are inhibited by methotrexate or polyglutamated forms of methotrexate. Once internalized 5-methyl tetrahydrofolate is converted to tetrahydrofolate (Figure 2). This process generates methionine from homocysteine. This rids the cell of homocysteine which is toxic and provides the cells with methionine which is used for protein synthesis and to form adenosylmethionine which is a methyl donor required for protein and lipid methylation. Tetrahydrofolate is polyglutamated (4-6 glutamate residues) by the enzyme folylpolyglutamate synthetase. Methotrexate is also polyglutamated by this enzyme and is a competitive inhibitor of the polyglutamation of tetrahydrofolate. Next tetrahydrofolate is converted to N^{10}, formyl-H_4 folate which is used in purine biosynthesis or 5,10-methylene-H_4 folate which donates a carbon to uridine to form thymidine (figure 2). In both cases folic acid is oxidized. To regenerate tetrahydrofolate, the oxidized molecule must be reduced by dihydrofolate reductase (figure 3). Methotrexate inhibits dihydrofolate reductase. Methotrexate treatment of cells and folate deficiency leads to methionine, purine and thymidine deficiency, allows the accumulation of homocysteine and inhibits some protein and lipid methylation. This leads to and arrest of cell growth and eventually cell death. The latter property has been exploited in by oncologist to treated various malignancies. Recent studies, however, have demonstrated that methotrexate may have anti-inflammatory properties in addition to its anti-proliferative effects.

Bioavailability and Pharmacokinetics

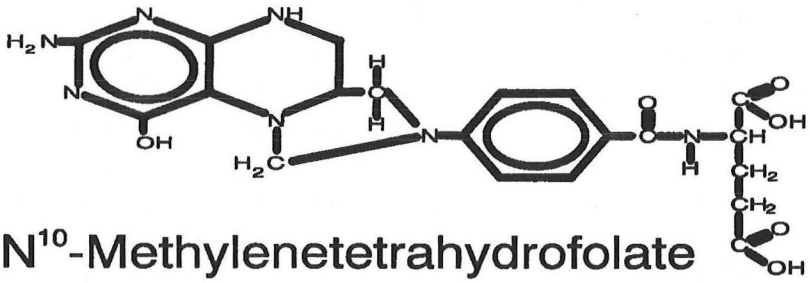
Methotrexate is well absorbed from the intestine (40-70%) at doses below 30 mg/m^2



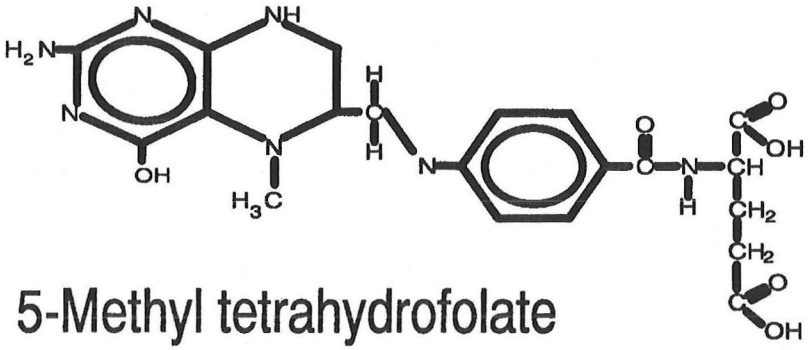
Folic Acid



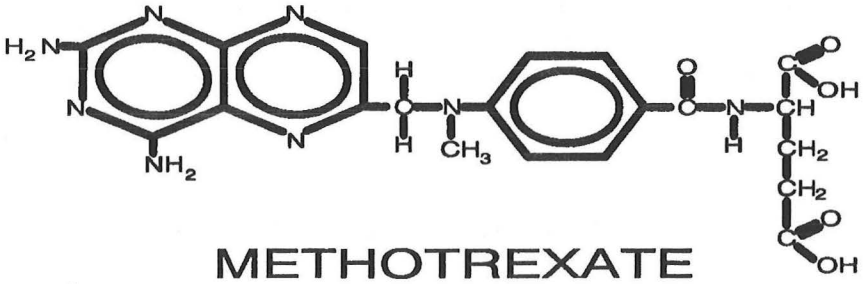
Tetrahydrofolic Acid



N^5, N^{10}-Methylenetetrahydrofolate



5-Methyl tetrahydrofolate



METHOTREXATE

Figure 1

INHIBITORY EFFECTS OF METHOTREXATE

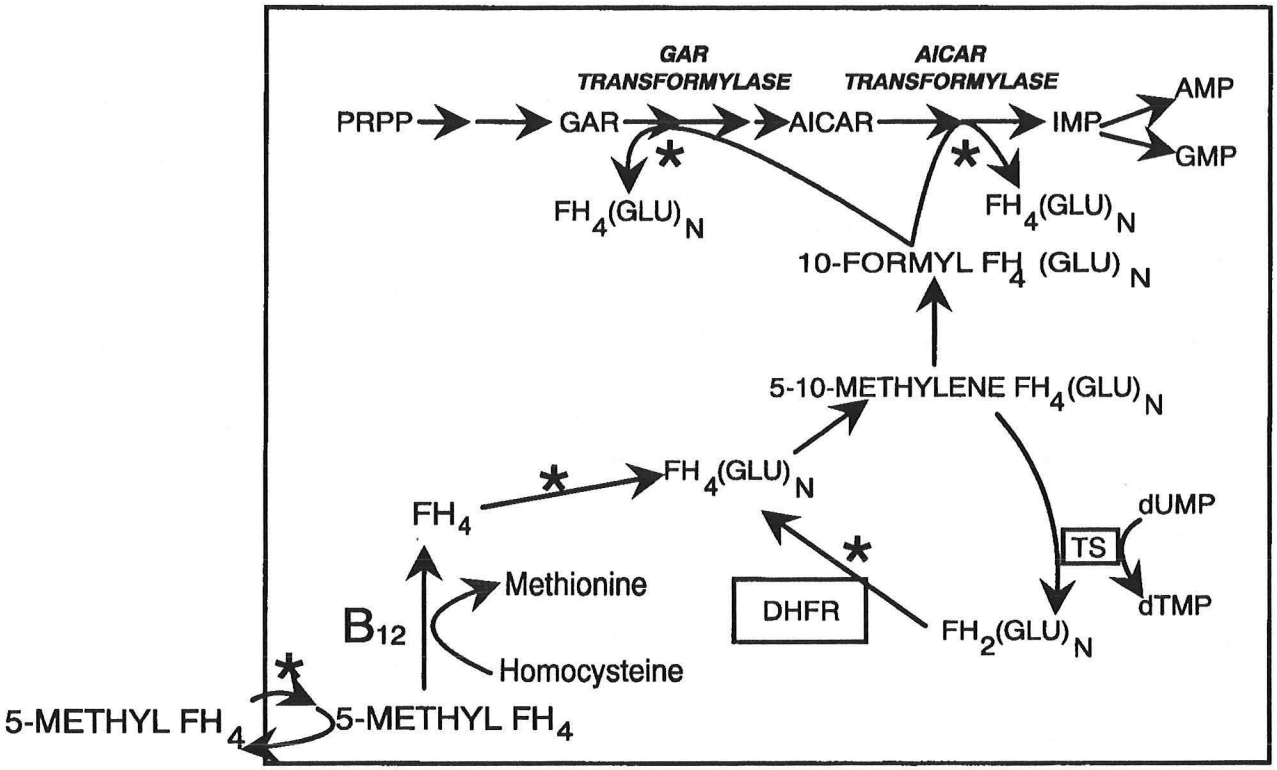


Figure 2

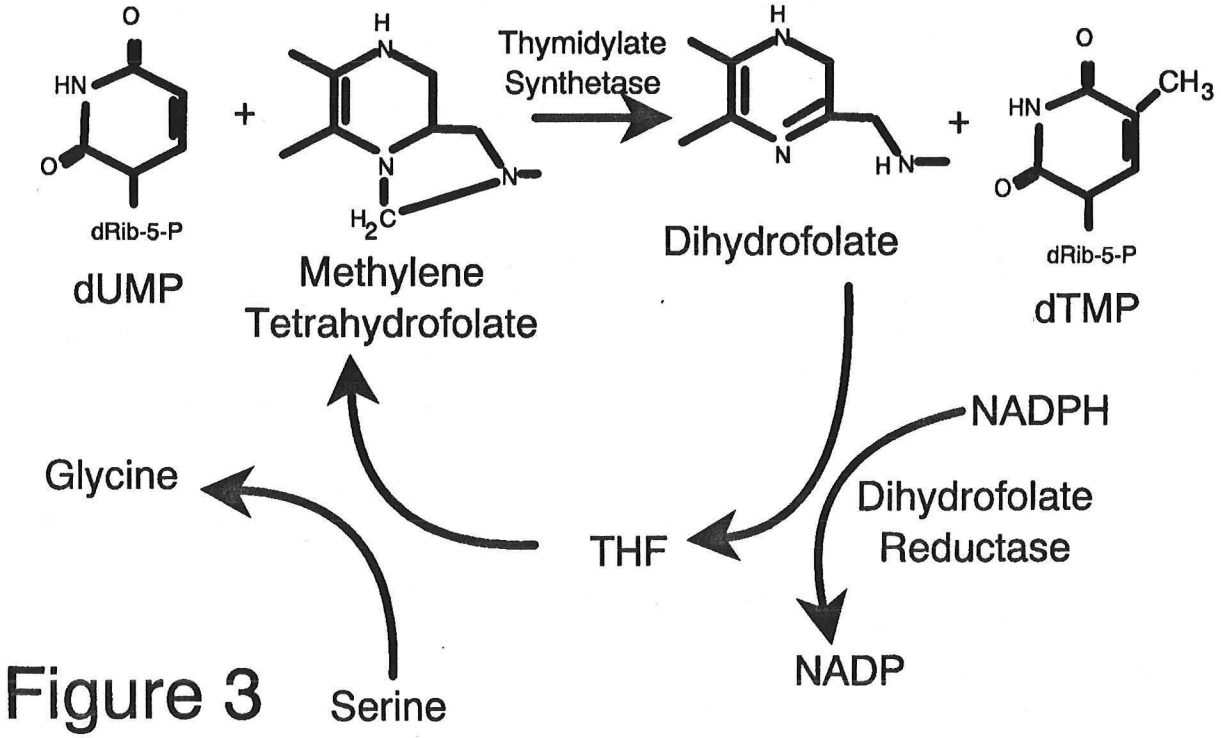


Figure 3

(119). Food slows its absorption slightly, but does not effect the overall bioavailability (89). Once absorbed it distributes in total body water (22). The drug is loosely bound to albumin and drugs which bind albumin may displace methotrexate increasing its effective concentration. Once peak drug concentration have been reached, the drug has a plasma half-life of 2-3 hours (22). Elimination is largely determined by renal excretion (22). In patients with normal renal function plasma half-life increases with age.

Methotrexate is believed to be excreted by glomerular filtration and tubular excretion. The later mechanism is blocked by probenecid. Thus, probenecid prolongs the plasma half-life of methotrexate and increases its effect.

Intracellular accumulation depends on transport into cells via potocytosis as described earlier. Once in cells polyglutamate is added making it impermeable. Polyglutamated forms of methotrexate have been shown to persist in the liver long after the drug has been cleared from the circulation. Moreover, polyglutamated forms of methotrexate inhibit some enzymes not inhibited by methotrexate itself such as AICAR transformlyase (see below).

CLINICAL APPLICATIONS OF "LOW DOSE" METHOTREXATE

Psoriasis

In 1951 Gubner noted fortuitously that when patients with psoriasis took the folate antagonist, aminopterin, their skin lesions rapidly disappeared. Clinical experienced developed with aminopterin, but this drug was later replaced by the less toxic and more stable folate antagonist methotrexate. The drug was initially thought to work by inhibiting proliferation of keratinocytes, but later studies demonstrated that topical application of methotrexate did not improve the lesions. Moreover, the doses used in psoriasis did not seem to inhibit cell growth in other locations (ie most patients did not develop mucositis or pancytopenia). Recent evidence demonstrating that cyclosporin is effective in patients with psoriasis has focused attention on the anti-inflammatory properties of methotrexate. The drug is widely used by dermatologists to treat acute pustular psoriasis of von Zumbusch's type, and extensive psoriasis.

Rheumatoid arthritis

The first report of the beneficial effect of methotrexate in rheumatoid arthritis patients was a manuscript by Gubner et al in 1951 (42). Methotrexate was used because it was though to inhibit the proliferation of connective tissue. The uncontrolled study demonstrated that six of seven patients with rheumatoid arthritis improved on methotrexate. The literature did not mention the use of methotrexate in rheumatoid arthritis again until the 1980's. In 1983, Hoffmeister reported the response of 78 patients with refractory rheumatoid arthritis to methotrexate (48). They found that "complete remission" occurred in 28 patients and that there was a marked improvement in 45 patients. In 1985, four large randomized placebo-controlled trials were published (8,122,131,140). All four trial demonstrated that methotrexate decreased joint swelling, and tenderness and improved a number of subjective assessments of clinical condition. Meta-analysis of these four studies demonstrated that methotrexate treatment improved joint tenderness and swelling, pain, overall well-being as assessed by both the patient and physician, the length of morning stiffness, grip strength and erythrocyte sedimentation

rate (124). Therefore, the data clearly demonstrate that methotrexate ameliorates the symptoms of rheumatoid arthritis over the first 6 months of therapy. Several long term studies have demonstrated that these symptomatic improvements last up to 84 months (72,133,134).

The dosage of methotrexate used in rheumatoid arthritis varies with efficacy and tolerance of the patient from 7.5 to 15 mg/week. These doses are similar to those used in patients with psoriasis and substantially less than those used in patients with malignancies. Recent studies have demonstrated that, within this range, higher doses work better than lower doses (40). Therefore, it is recommended that the dose be pushed up to 15 mg unless the patient enters a remission or develops side effects.

The efficacy of methotrexate has been compared with other more tradition disease modifying agents (DMARDs) such as auranofin (oral gold), myochrysine (IM Gold) and azathioprine (43,52,53,81,96,117,139). The data demonstrate that methotrexate may work faster than these agents. Moreover, they demonstrate that methotrexate is at least as effective as these three drugs and probably better tolerated over the short term than either IM gold or azathioprine.

The effect of methotrexate and other DMARDs on the long term course of rheumatoid arthritis is less clear. Since these agents clearly improve patient well-being, it is not ethical to place patients into a long-term placebo-controlled trial. Thus, there are no placebo controlled trials that demonstrate that methotrexate prevents joint destruction in rheumatoid arthritis. In contrast, there are a number of reports demonstrating radiographic progression despite methotrexate therapy even in patients that clinically respond to methotrexate (4,45,72,77,103). Whether this progression is slowed by methotrexate is less clear. Two double blind controlled trials found that radiologic progression was slower in patients taking methotrexate than in patients taking auranofin (77,132) whereas another study demonstrated that methotrexate slowed radiologic progression when compared with azathioprine (53). In another study, the rate of radiographical deterioration seemed to slow while patients were on methotrexate when compared to deterioration prior to starting methotrexate (97). This finding conflicted with a similar study done several years earlier which showed that methotrexate had no effect on the radiographic progression of rheumatoid arthritis (85). Finally, a meta-analysis of 355 methotrexate-treated and 205 non-methotrexate treated patients (Table I) suggested that methotrexate treatment slowed radiographic progression more than azathioprine, but not more than gold (4). In a these studies the difference were not great. Moreover, the natural history of rheumatoid arthritis has not been drastically altered by any of these agents.

Another way to look at the long term effect of methotrexate on rheumatoid arthritis is to look at the number of patients on methotrexate after several years. Table II illustrates the data from several series. It is clear that about 50% patients remain on methotrexate for more than 2 years. Withdrawal occurs because of side effects, lack of complicance and lack of efficacy.

Table I
META-ANALYSIS OF THE RATES OF RADIOGRAPHIC PROGRESSION IN MTX-TREATED AND NON-MTX DMARD TREATED PATIENTS WITH RA

	Treatment Group			
	Non-MTX		MTX	
	n	Rate	n	Rate
Gold Salts	153	0.008	186	0.008
Azathioprine	52	0.012	48	0.004*

*p = 0.049

Table II
WITHDRAWALS FROM METHOTREXATE THERAPY: LONG TERM FOLLOWUP

			Number of patients withdrawn (%)			
Investigator	Duration of Followup	Number of Patients	Side effects	Lack of efficacy	Miscellaneous	Total
Weinblatt	84 Months	26	3	1	10	14
Kremer	90 Months	29	4	2	5	11
Fehlauer	24 Months	124	38	15	11	64
Total		179	45 (25%)	18 (10%)	26 (14%)	89 (49%)

Methotrexate and Felty's syndrome

Patients with rheumatoid arthritis may develop neutropenia and splenomegaly. The degree of leukopenia can be significant and lead to opportunistic infections. This complication of rheumatoid arthritis is called Felty's syndrome. Because leukopenia is a complication of methotrexate, investigators have been reluctant to use methotrexate to treat Felty's syndrome. However, a number of recent reports have suggested that methotrexate can increase leukocyte counts in patients with Felty's syndrome resistent to other forms of treatment (6,37,50,51,118). Low dose methotrexate should be viewed as a therapeutic option in Felty's syndrome.

The use of folate/folinic acid to minimize toxicity in rheumatoid arthritis

A number of investigators have attempted to reduce the "minor" side effects of methotrexate by giving folate or folinic acid (20,44,58,111,123). The goal of this therapy is to rescue the cells from pharmacologically induced folate deficiency. Since folinic acid is already reduced it maight be more effective than folate at rescuing the cells. A number of studies have confirmed that both folate and folinic acid decrease the toxicity of "low dose" methotrexate (58,82,111,115,123) although one trial reported that the effect was not statistically significant (20). The more important question is whether these agents also decrease efficacy. Their effects on efficacy seem to depend on the agent used and its dosage. In each case where folate was used, no decrease in efficacy was observed (82,115). The effect of folinic acid on the efficacy of methotrexate therapy in RA has been more controversial. Several trials have indicated that it did alter efficacy, but one prospective trial and several small uncontrolled series indicated that efficacy was diminished (58,123). Since folate is cheaper than folinic acid most investigators favor its use. Moreover, it is thought that the range of doses that decrease toxicity without altering efficacy might be narrower for folinic acid than for folate. In practice rheumatologist frequently use folate when patients have difficulty tolerating methotrexate before giving up and going on to other drugs.

Systemic Lupus Erythematosus

In a retrospective uncontrolled analysis of 17 patients treated with a mean weekly dose of methotrexate of 8.5 mg/week, methotrexate appeared to decrease steroid requirements and ameliorate symptoms of arthritis, rash and/or pleuritis (138). Methotrexate was also used in another trial in 5 patients with childhood SLE that had disease refractory to steroids and/or had side effects from Cytoxan and prednisone that necessitated reducing therapy (1). In all patients, methotrexate allowed the physicians to decrease the steroids and eliminate Cytoxan. In 3 patients, renal disease appeared to respond to methotrexate therapy, although the effects of methotrexate on renal disease in SLE have not been well studied.

Inflammatory Bowel Disease

In an uncontrolled prospective 12 week trial of methotrexate (25 mg/week) in 21 patients with refractory IBD (14 Crohn disease, 7 UC), methotrexate improved Crohn's disease and UC activity indices and decreased steroid use (67). In another similar trial methotrexate decreased the steroid requirements in patients with both UC and Crohn's (15).

Primary Biliary Cirrhosis (PBC)

The efficacy of methotrexate in PBC was evaluated in an uncontrolled short term trial of 9 patients. The data demonstrated a decrease in alkaline phosphatase, bilirubin, serum alanine aminotransferase, fatigue and pruritus (49,60-62). Important questions remain about its long term safety and efficacy and it is not yet recommended for use outside of controlled trials. Controlled trial is now underway at UT Southwestern.

Primary Sclerosing Cholangitis (PSC)

Several uncontrolled trials have suggested that methotrexate improves liver enzyme values and reduces pruritus in patients with PSC (60-62,65). Unfortunately a more recent

double blind placebo controlled trial found that it had no effect on liver histology over a 2 year period of time despite decreasing the alkaline phosphatase level (66). This result underscores the importance of controlled trials for all the disorders in which methotrexate is thought to be beneficial.

Vasculitis

Methotrexate was used in combination with steroids in one uncontrolled trial involving 29 patients with Wegener's granulomatosis (47). The majority of these patients were apparently refractory to other treatments and all had "life threatening involvement". The data demonstrated that the therapy improved 79% of patients and induced complete remission in 69% of patients. The authors concluded that methotrexate was a reasonable alternative treatment for patients unable to tolerate Cytoxan.

There are also a number of case reports suggesting that methotrexate might be beneficial in patients with cutaneous polyarteritis nodosa (57), cutaneous manifestations of Bechet's disease (57), and Takayasu's arteritis (76).

Juvenile Rheumatoid Arthritis

As in adult Rheumatoid Arthritis, methotrexate provides symptomatic improvement in patients with JRA. The most convincing trial was a randomized placebo controlled multicenter trial that demonstrated that methotrexate reduced joint pain and swelling and the sedimentation rate compared with placebo (137). A recent uncontrolled trial suggested that methotrexate might improve measures of carpal deterioration in children who respond clinically to methotrexate (46).

Psoriatic Arthritis

The efficacy of methotrexate in psoriatic arthritis was assessed in one retrospective uncontrolled study of 40 patients (36). Patients received a mean dose of 11.2 mg/week. 15 percent of the patients experienced a complete remission in this short term trial, whereas 58% of the patients had an subjective improvement in joint pain and an objective improvement in the number of involved joints.

Adult onset Still's disease

In one uncontrolled trial, 6 patients with adult onset Still's disease who were refractory to more traditional therapy (NSAIDs, steroids) were given methotrexate (12). 3 patients experienced a complete remission and 1 had a partial response. The authors concluded that it may be a useful drug in this disorder. In a similar trial (68), methotrexate reduced fever and joint complaints in 4
patients that had failed to response to NSAIAs and prednisone.

Dermato/polymyositis

Dermatomyositis and polymyositis were frequently fatal disorders prior to the use of corticosteroids. Currently, the vast majority of patients respond well to corticosteroids. However, there remains a small group of patients that are refractory to corticosteroids or whom require high doses of corticosteroids for prolonged periods of time to control their

disease. These patients are traditionally treated with immunosuppressants including methotrexate. The efficacy of methotrexate is based on uncontrolled trials, because it does not seem ethical to place these patients on placebo. For example, in 1974, Metzger et al. treated 22 steroid refractory patients with polymyositis or dermatomyositis with prednisone and intravenous methotrexate (79). Clinical improvement was noted in 77% of the patients. Those patients responding normalized their CPK after a mean period of 10 weeks and muscle strength improved after an average of 13 weeks. Similarly, Joffe et al treated 55 patients with steroid resistant myositis with methotrexate and prednisone and noted a response rate of approximately 75% (54). The later investigators noted that male patients with myositis responded to methotrexate better than female patients and that methotrexate was superior to prednisone and azathioprine in certain subgroups of patients.

Acute Graft-vs-Host Disease

The efficacy of methotrexate in graft-versus-host disease was examined in a large prospective trial involving 179 patients who received HLA-identical marrow grafts for the treatment of AML or CML (116). The investigators compared Cytoxan therapy with methotrexate in preventing GVH and recurrent leukemia. Over the nearly 10 year followup no statistically important difference was observed between these therapies. The authors concluded the methotrexate and Cytoxan are comparable with regards their ability to prevent acute and chronic GVH.

Ocular inflammatory disease

In an uncontrolled trial (107), methotrexate was used to treat 22 patients with steroid resistant ocular inflammatory disease [vitreitis (9), scleritis (4), inflammatory pseudotumor (3), orbital myositis (3) and retinal vasculitis (3)]. 16 of the 22 had a reduction in inflammatory activity and 14 were able to decrease or discontinue steroids. 6 patients had no response. The authors concluded that methotrexate appears to be effective for steroid resistant ocular inflammatory disease.

Sarcoidosis

Sarcoidosis is chronic granulomatous disorder of unknown etiology that either spontaneously regresses or responds to steroid therapy. A small percentage of patients exhibit a chronic relapsing course that requires steroid therapy for many years. As chronic steroid therapy is associated with a number of complications, there has been a desire to develop non-steroidal agents that are effective in this disorder. In 1990, Baughman and Lower (18) treated 22 patients with prednisone or methotrexate. The decision to use methotrexate was non-random, but the characteristics of the patients in both groups at entry appeared quite similar. The study found that methotrexate and steroids were comparable with regard to their ability to improve the vital capacity, percentage of lymphocytes in the bronchial lavage, and the activation state of the alveolar macrophages.

Asthma

Asthma is frequently treated with anti-inflammatory agents. In many cases this can be accomplished with inhaled steroids, but a small percentage of patients required chronic

systemic steroid therapy. To avoid the complication of chronic steroid use, investigators have used a variety of immunosuppressant agents including methotrexate. Several randomized placebo-controlled trials (see table III) have demonstrated that methotrexate has steroid sparing effects on these patients (33,83,109). One small randomized placebo-controlled trial failed to show a statistically significant effect of methotrexate (35), but this appears to be due the small size of the study and to the ability of the placebo to decrease the steroid requirement by 40%. The main concern among investigators is whether the reduced steroid dose provided by methotrexate outweighs the complications of long term methotrexate use. Moreover, there is at least one report of methotrexate inducing asthma. If methotrexate works by inducing adenosine release at inflammatory sites (see below), it is not surprising that it might induce bronchoconstriction.

Table III

RESULTS OF RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED TRIALS OF METHOTREXATE IN STEROID DEPENDENT ASTHMA

Investigator	No. of Patients	Study Length	Mean MTX Dosage	Results
Mullarkey et al.	14	11.7 Months	12.5 mg/week	Decreased prednisone dose in MTX group only by 36% ($P < 0.01$)
Shiner et al.	60	24 week	15 mg/week	Decreased prednisone dose in MTX group only ($P < 0.005$)
Dyer et al.	10	24 week	15 mg/week	Decreased prednisone dose in MTX group only by 30% ($P < 0.01$)
Erzurum et al.	18	13 week	15 mg/week	Decreased prednisone dose in both groups.

MECHANISM UNDERLYING THE ANTI-INFLAMMATORY EFFECTS OF METHOTREXATE

Although methotrexate clearly blocks DNA synthesis and is cytotoxic when high dose regimens are used, low dose regimens do not appear to be anti-proliferative. Moreover, the effect of methotrexate appear to be more dramatic and more rapid than other agents that are more cytotoxic. These findings have suggested that methotrexate therapy might have anti-inflammatory properties that may be unrelated to its anti-proliferative effect. It is clear that treating patients with methotrexate reduces a number of inflammatory signs such as the sedimentation rate (71,134), the serum levels of soluble cytokine receptors (16), the level of IL1 in the synovial fluid (121), the spontaneous production of IgM-RF by peripheral blood mononuclear cells (90), and spontaneous DNA synthesis by peripheral blood mononuclear cells isolated from the blood (55). It seems likely that these findings represent the result of decreased

disease activity rather than providing clues as to its mechanism of action.

Several investigators have identified changes in cellular physiology induced by in vivo methotrexate therapy that might explain its anti-inflammatory properties. Thus, several laboratories have demonstrated that polymorphonuclear cells removed from patients on methotrexate are defective in assays of chemotaxis (87,120). Another group demonstrated that neutrophil migration into the dermis was diminished in patients on methotrexate (128). Finally, a group of investigators demonstrated that neutrophil counts in the synovial fluid decrease with methotrexate therapy (121). These data suggest the hypothesis that methotrexate reduces the ability of polymorphonuclear cells to migrate into the synovium.

Other investigators have demonstrated that monocytes removed from rheumatoid arthritis patients on methotrexate have a diminished ability to produce IL1 (23). Since IL1 is pro-inflammatory, this findings has led to the hypothesis that methotrexate works by inhibiting monocyte IL1 release. Unfortunately, a separate study did not confirm this finding (106).

Finally, a third group of investigators have demonstrated that neutrophils isolated from patients on methotrexate have a reduced ability to produce leukotriene B_4 (LTB_4) in response to calcium ionophore (112,113) LTB_4 increases neutrophil and monocyte chemotaxis, neutrophil adherence to endothelial cell surfaces, aggregation and degranulation of neutrophils, and vascular permeability. The data suggest yet a third mechanism for the anti-inflammatory effects of methotrexate.

Recently, Cronstein et al. suggested that methotrexate might mediate it anti-inflammatory effects by stimulating the release of adenosine (28,30-32). This hypothesis which appears to explain all the observed phenomena was based on the following findings. First, a number of investigators have demonstrated that adenosine has anti-inflammatory properties. Thus, adenosine blocks polymorphonuclear cell superoxide production stimulated by N-formyl-methionyl-leucyl-phenyl-alanine and adhesion to endothelium induced by platelet activating factor (29,31,87,101,120). Adenosine also inhibits the production of pro-inflammatory cytokines such as TNF and IL1 by macrophages (23,74,91). Second, methotrexate causes the release of adenosine by connective tissue cells. The mechanism by which methotrexate stimulates adenosine release is not yet clear, but it is though to be through it ability to increase intracellular concentration of 5-aminoimidazole-4-carboxaminide ribonucleotide (AICAR) (13,30,32). Methotrexate polyglutamate causes in increase in AICAR concentration by inhibiting the folate dependent enzyme, 5-aminoimidazole-4-carboxaminide ribonucleotide transformylase (13). This enzyme is responsible for metabolizing AICAR. AICAR is thought to increase intracellular adenosine concentrations in part by inhibiting adenosine deaminase (13).

Recent in vivo evidence has supported this hypothesis. First, Asako et al demonstrated that topical application of both adenosine and methotrexate blocked leukocyte binding to rat mesenteric vessels induced by platelet activating factor (11). This effect was blocked by the addition of adenosine receptor antagonists (A_2) or adenosine deaminase. Similarly, Cronstein et al. found that methotrexate injected intraperitoneally at doses designed to induce serum levels comparable to those acheived by low dose oral methotrexate therapy in humans suppressed inflammation in the murine air pouch model of inflammation (32). They found that methotrexate treatment markedly increased AICAR concentrations in the spleen and in

carrageenan-inflamed air pouches and increased the adenosine concentration in the inflammatory fluid. The anti-inflammatory effect of methotrexate was blocked by adenosine deaminase and adenosine A_2 receptor antagonists. The authors concluded that methotrexate is an nonsteroidal anti-inflammatory agent that works by stimulating the release of adenosine at inflammatory sites.

DRUG INTERACTIONS

Although trimethoprim inhibits mammalian dihydrofolate reductase less well than it inhibits the bacterial enzyme it still inhibits mammalian DHFR. This inhibitory effect synergies with methotrexate to induce a severe macrocytic anemia and pancytopenia (41). This combination is, therefore, not recommended.

Probenecid decreases the excretion of methotrexate and prolongs its serum half-life. At least one case of severe pancytopenia has resulted from the co-administration (17).

Concern has been raised over the use of methotrexate with nonsteroidal anti-inflammatory agents. NSAID's frequently decrease renal function and might prolong the half-life of methotrexate. Moreover, many nonsteroidal inflammatory agents, including aspirin, bind to albumin and might displace methotrexate. This fear has been confirmed by several case reports of severe pancytopenia and renal failure developing in patients given both drugs. However, one study examined the effect of NSAID's on methotrexate pharmacokinetics and found no effect (2). Moreover, the combination is now routinely used by rheumatologists throughout the world with only a handful of reports of adverse drug interactions. In practice, as long as the patients are closely followed for toxicity when any change in medication is made, NSAID's and methotrexate can be used together safely.

TOXICITY

Liver

The possibility that methotrexate might injure the liver was first raised when oncologists using methotrexate to treat leukemias noted elevated transaminases associated with methotrexate therapy and one study demonstrated a high incidence of liver fibrosis on followup biopsies (25). The possibility that "low dose" methotrexate therapy caused serious life threatening liver injury was introduced by dermatologists who began reporting the occurrence of cirrhosis in psoriasis patients receiving methotrexate. This stimulated a large number of studies examining the relationship of methotrexate therapy to cirrhosis in patients with psoriasis (24,78,84,86,98,99,125,126,135,141). The data demonstrate that psoriasis patients taking methotrexate have a higher incidence of cirrhosis than patients not on methotrexate. Moreover, there are a number patients who had pretreatment biopsies that were considered normal that then developed cirrhosis on methotrexate. The incidence of cirrhosis seemed to be associated with alcohol intake and the cumulative dose of methotrexate. While none of these studies rigorously eliminated other causes of cirrhosis such as hepatitis C, the data suggested strongly that methotrexate might cause liver disease in psoriasis patients. The data also found that cirrhosis could not be predicted by LFT's taken just prior to the biopsy. These findings led to guidelines in which patients with psoriasis that are placed on methotrexate

receive liver biopsies before therapy and at various intervals after initiation of therapy based on the cumulative dose of methotrexate. In addition, alcohol consumptions is strongly discouraged.

Whether methotrexate causes cirrhosis in other diseases is less clear. It is clear, however, that low dose methotrexate causes elevated transaminases in all patients (Table IV) and that the incidence of fibrosis is higher in patients on methotrexate (39,69,72,134). In patients with rheumatoid arthritis, elevations in transaminases do not correlate with fibrosis unless you omit the values over the first 2 years of therapy (39,69,72,134). It now appears that a large number of individuals get elevated transaminases early in their course of methotrexate, but that these levels decrease over time (69). Those individual who continue to have elevated LFT's after 2 years of therapy are more likely to develop fibrosis. No correlation was observed between the alkaline phosphatase or ALT and subsequent liver fibrosis. The increase in transaminases is worsened by co-administration of salicylates and improved by the co-administration of hydroxychloroquine (39). Recent studies have demonstrated that hydroxychloroquine decreases the bioavailability of methotrexate and it has been suggested that this is the mechanism whereby it decreases liver function tests. Whether these effects alter the course of the liver disease is not yet known.

In a large survey of rheumatologist nationwide it was estimated that the incidence of cirrhosis is about 1/1000 patients [Table V; (127)]. The number of these cases that can be attributed soley to methotrexate is not known. As yet there is no data to suggest that transaminase elevation correlates with cirrhosis. There is at least one patient who presented with severe liver disease and normal transaminases over the year prior to presentation (94). This patient also had a normal liver biopsy prior to beginning methotrexate. This is the only rheumatoid arthritis patient I was able to find that had a normal liver biopsy prior to beginning methotrexate and went on to develop cirrhosis. The data indicate, therefore, that whereas methotrexate clearly causes cirrhosis in patients with psoriasis it only rarely if ever causes it in patients with rheumatoid arthritis.

Table IV

PREVALENCE OF ABNORMAL LABORATORY FINDINGS IN THE YEAR PRECEDING THE ONSET OF LIVER DISEASE IN RA PATIENTS TREATED WITH METHOTREXATE

	AST	ALT	Alkaline Ptase	Albumin	Total Bilirubin
	% of test that are abnormal				
Matched cases	45	22	45	64	20
Controls	7	15	23	9	0

Walker et al.

Table V
CUMULATIVE INCIDENCE OF CIRRHOSIS AMONG RHEUMATOID ARTHRITIS PATIENTS RECEIVING METHOTREXATE

Respondents	2,228
No. of patients receiving MTX > 5 years	16,600
No. of patients reported to have serious liver disease	142
No. of patients documented to have cirrhosis	18
CUMULATIVE INCIDENCE	1/1,000

Table VI
RECOMMENDATIONS FOR MONITORING FOR HEPATIC SAFETY IN RHEUMATOID ARTHRITIS PATIENTS RECEIVING METHOREXATE

A. BASELINE
   1. Tests for all patients
      a. LFTs (AST, ALT, Alk Phos., Bilirubin, albumin, hepatitis serology,
      b. CBC and serum creatinine
   2. Pretreatment liver biopsy if:
      a. Excessive alcohol intake
      b. Persistent abnormal LFTs
      c. Hepatitis B or C infection.
B. Monitor AST, ALT, albumin at 4-8 week intervals
C. Perform liver biopsy if:
   1. 5 of 9 determinations of AST within a 12 month period are abnormal.
   2. There is a decrease in serum albumin below the normal range.
D. If results of liver biopsy are:
   1. Grade I-IIIa, resume MTX.
   2. Grade IIIb or cirrhosis, D/C MTX.
E. Discontinue MTX if patients has persistent LFT abnormalities and refuses liver biopsy.

methotrexate is controversial. Recently, a number of investigators in the field met to establish guidelines for monitoring liver toxicity in rheumatoid arthritis (69). They decided that the weak correlation between AST values after 2 years of methotrexate therapy and fibrosis on liver biopsy was sufficient evidence to warrant liver function testing and biopsies if the AST values were persistently elevated. They state that they are unaware of any rheumatoid arthritis patient taking methotrexate that developed cirrhosis with normal LFT's. The guidelines are included in Table VI. Most rheumatologists attempt to normalize the AST by decreasing the dose before they perform a liver biopsy.

Nodulosis

A number of investigators have reported that patients with rheumatoid arthritis develop an increase in the number and size of their rheumatoid nodules when treated with methotrexate (63,70,105,133). This appears to occur in 4-12% of the patients. In many cases they appear over the hands, but they can occur throughout the body. In several cases, patients have developed serious problems from the nodules. In one case, the nodules involved cardiac tissue and lead to congestive heart failure and cardiac arrest (19). It has been suggested that plaquenil may decrease the size and number of these nodules (26).

Hypersensitivity Pneumonitis

Pneumonitis has been in infrequent complication of both low and high dose methotrexate therapy. It has been reported after treatment with methotrexate for leukemia, cancer, psoriasis, polymyositis, and rheumatoid arthritis. The incidence of pneumonitis in rheumatoid arthritis patients treated with methotrexate ranged from 4-7% (5,21,72,114). Methotrexate induced pneumonitis is characterized by dyspnea, cough, fever, tachypnea, and bibasilar rales (104). Typically, investigations reveal a normal or slightly elevated WBC count with occasional eosinophilia, a PO_2 of less than 55 mm Hg on room air and a chest radiograph demonstrating interstitial infiltrates and in some cases diffuse alveolar infiltrates (104). Cultures for pathogens are generally negative and biopsies reveal nonspecific interstitial infiltrates, bronchiolitis obliterans with lymphocytic infiltrates and granuloma formation in some specimens. The criteria for methotrexate pneumonitis as proposed by Searles and McKendy is provided in Table VII. Bronchoalveolar lavage reveals a lymphocytic alveolitis with a predominance of CD4+ T cells and a relative deficiency of CD8+ T cells (136). The reaction seems to occur independent of the cumulative dose of methotrexate as it has been reported in individuals that have taken as little as 90 mg of methotrexate (104). In some cases methotrexate has been reinstituted after recovery without a recurrence of symptoms (27), but in others it recurred (64). Methotrexate induced pneumonitis is generally treated by withdrawing methotrexate and treating with steroids and antibiotics. There is no data suggesting that steroids are helpful. Most patients recover within days of stopping the methotrexate therapy, but deaths have been reported. It is important to consider the possibility of opportunistic infection in these patients. There is at least one case of pulmonary cryptococcoses that was initially thought to have methotrexate induced pneumonitis.

Table VII
CRITERIA FOR THE DIAGNOSIS OF MTX PNEUMONITIS

1	Acute onset of shortness of breath
2	Fever > 38.0 °C
3	Tachypnea > 28/min and nonproductive cough
4	Radiologic evidence of pulmonary interstitial or alveolar infiltrates
5	White blood cell count > 15
6	Negative blood and sputum cultures
7	PFT's revealing restrictive pulmonary function with decrease diffusion capacity.
8	pO_2 < 55 on RA
9	Histopathology consistent with bronchitis or interstitial pneumonitis

Definite Dx = 6 or more criteria

Searles and McKendry

Hematologic abnormalities

Since methotrexate is toxic to growing cells it is not surprising that it causes pancytopenia. Leukopenia or thrombocytopenia occurs in 5-10 percent of patients. Occasionally patients develop a severe pancytopenia. Risk factors for this include renal failure, a rising MCV, folate deficiency, and increasing age (3). All of these problems are dose dependent and respond to decreasing or stopping methotrexate. In severe cases, it may be useful to give leucovorin. This is particularly important when overdose is suspected and should be given within 48 hours of the overdose.

Gastrointestinal side effects

Gastrointestinal side effect develop in about 50% of patients taking "low dose" methotrexate (70,72,130,133). The majority of these patients experience nausea. Anorexia, diarrhea and vomiting are also common side effects. Stomatitis occurs in 6% of patients (130). These symptoms all dose dependent and respond to oral folate supplementation, which as discussed earlier may not inhibit the anti-inflammatory properties of the drug.

Postoperative effects.

Several orthopedic studies have examined the effect of methotrexate therapy on postoperative infection and healing in rheumatoid arthritis patients receiving total hip replacements (102). In one group of patients methotrexate was discontinued 1 week prior to surgery whereas in the other group methotrexate was continued up to surgery and through the postoperative period. No differences in infection or healing were noted. Another 10 year retrospective analysis of patients receiving total hip replacements gave similar results (92). Despite these findings surgeons frequently discontinue methotrexate prior to surgery.

Miscellaneous

Sensitive measurements of renal function have demonstrated that methotrexate therapy can diminish renal function, but these changes did not appear to be clinically relevant. There

are case reports of "methotrexate induced" asthma, Peyronie's disease, and porphyria cutanea tarda (56,88,93).

Malignancy

There have been numerous case reports of malignancy developing in patients on methotrexate. However, Bailin et al reviewed 224 patients treated with methotrexate who had been followed up for 7-8 years and was unable to document an increased incidence of malignancy (14).

There is one report of 2 patients on methotrexate that developed lymphomas associated with Epstein-Barr virus that resolved upon discontinuing methotrexate (59). This appears to caused by the immunosuppressive properties of methotrexate rather than its carcinogenesis.

Immunodeficiency

Patients on low dose methotrexate have developed *pneumocystis carinii* pneumonia, cryptococcoses pneumonia, herpes zooster, and disseminated histoplasmosis (7,10,38,73,75,95,110). These complications occur in less than 1% of patients.

Dermatologic

Alopecia has been reported in less than 1% of patients taking "low dose" methotrexate (130). Other skin eruptions seen with higher doses are very rare with low dose regimes.

Teratogenesis

Methotrexate may cause fetal abnormalities if administered early in pregnancy. Folic acid antagonist have been used to induce abortion in the past. Methotrexate induced fetal anomalies include skeletal abnormalities, cleft lip, meningomyelocele, ear anomalies, and anencephaly (80,129). Methotrexate should not be administered to pregnant women or women of child bearing age not using contraception.

Reproductive

Methotrexate may cause transient oligospermia, but it appears to be relatively uncommon in patients on low dose regimen (34). Methotrexate has little effect on ovarian function (108). There does not appear to be a higher incidence of congenital abnormalities in children of women previously treated with methotrexate (100).

CONCLUSION

Methotrexate has anti-inflammatory properties when administered at doses of 5-15 mg/week by oral, IM or intravenous routes. These properties ameliorate the symptoms of patients with a variety of inflammatory conditions including rheumatoid arthritis, psoriasis, and asthma. Methotrexate has a number of toxicities related to its anti-proliferative effects such as pancytopenia and mucositis. It also causes gastrointestinal symmptoms in many patients. Finally, methotrexate rarely induces liver fibrosis and pneumonitis which can result in death. Methotrexate exerts its anti-inflammatory effect by inhibiting AICAR transformylase leading to an increase in AICAR which indirectly leads to the release of adenosine which has anti-

inflammatory effects. New folate antagonists, such as lometrexol, inhibit AICAR transformylase specifically without inhibiting dihydrofolate reductase and may be more effective or less toxic anti-inflammatory agents than methotrexate.

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