Low-Dose Methotrexate: A Mainstay in the Treatment of Rheumatoid Arthritis

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Abstract—Methotrexate administered weekly in low doses is a mainstay in the therapy of rheumatoid arthritis. Although originally developed as a folate antagonist for the treatment of cancer, its mechanism of action in the therapy of rheumatoid arthritis remains less clear. Several mechanisms have been proposed including inhibition of T cell proliferation via its effects on purine and pyrimidine metabolism, inhibition of transmethylation reactions required for the prevention of T cell cytotoxicity, interference with glutathione metabolism leading to alterations in recruitment of monocytes and other cells to the inflamed joint, and promotion of the release of the endogenous anti-inflammatory mediator adenosine. These mechanisms of action and the role of methotrexate in the suppression of rheumatoid arthritis are reviewed.

I. The Use of Methotrexate in the Therapy of Rheumatoid Arthritis

Although the first reported use of methotrexate in the treatment of rheumatoid arthritis was in the early 1950s, soon after its development (Gubner et al., 1951) it did not come into common use in the treatment of rheumatoid arthritis until over 30 years later (Weinblatt et al., 1985; Williams et al., 1985). As an anti-rheumatic agent, methotrexate is administered intermittently (weekly) in doses two or three log orders lower than those required for the treatment of malignancy (5–25 mg/week versus 5000 mg/week). Surprisingly, started early in the course of the disease, methotrexate is nearly as effective as the biologic agents recently introduced for the treatment of rheumatoid arthritis (see Bathon et al., 2000) and is commonly administered in combination with either biological agents or other small molecule antirheumatic drugs. As currently used, low-dose methotrexate is safe and well tolerated. Because of its efficacy and safety, low-dose methotrexate is now first-line therapy for the treatment of rheumatoid arthritis not responsive to nonsteroidal anti-inflammatory drugs alone (American College of Rheumatology Subcommittee on Rheumatoid Arthritis Guidelines, 2002).
A. Pharmacology of Low-Dose Methotrexate

Methotrexate is generally administered to patients with rheumatoid arthritis as a single weekly dose given either intramuscularly or orally. In the treatment of rheumatoid arthritis, the usual dose of methotrexate is in the range of 15 to 17.5 mg/week, although early studies utilized much lower doses and others have reported using higher doses. At the doses commonly used for the treatment of rheumatoid arthritis, the bioavailability of oral methotrexate varies considerably between individuals, but in general is in the range of 70%, and food does not significantly affect uptake of the drug (Fossa et al., 1988; Kozloski et al., 1992; Oguey et al., 1992; Jundt et al., 1993; Lebbe et al., 1994; Bannwarth et al., 1996). There is some evidence that at higher doses oral bioavailability declines, a phenomenon most likely due to the fact that uptake of methotrexate from the gastrointestinal tract is mediated by a saturable transporter, reduced folate carrier 1 (RFC1) (Matherly and Goldman, 2003). A modest fraction of the methotrexate dose is converted to 7-hydroxymethotrexate in the liver. Both methotrexate and 7-hydroxymethotrexate are primarily excreted in the urine, although there is some biliary excretion. The half-life of methotrexate in the serum is in the range of 6 to 8 h after administration of the drug and is undetectable in the serum by 24 h (Fossa et al., 1988; Kozloski et al., 1992; Oguey et al., 1992; Jundt et al., 1993; Lebbe et al., 1994; Bannwarth et al., 1996). By decreasing glomerular filtration rate, nonsteroidal anti-inflammatory drugs may increase the time required to eliminate methotrexate, although this interaction is of little clinical significance (Furst, 1995). Methotrexate is taken up by cells and polyglutamated and methotrexate polyglutamates, likely the active compounds, are long-lived (days to months) in the tissues (Kremer et al., 1986; Dervieux et al., 2003). Indeed, the concentration of methotrexate polyglutamates in erythrocytes roughly correlates with the therapeutic efficacy of the drug (Dervieux et al., 2004).

B. Efficacy of Methotrexate in the Therapy of Rheumatoid Arthritis

The first report of the use of methotrexate in the therapy of rheumatoid arthritis was in 1951 (Gubner et al., 1951), although over the next 30 years there were only scattered reports of methotrexate's use for the treatment of RA. Results of open label trials performed in the early 1980s suggested that methotrexate could be useful in the treatment of rheumatoid arthritis refractory to other available agents (Wilke et al., 1980; Willkens et al., 1980; Hoffmeister, 1983). During the last half of the 1980s, placebo-controlled trials were carried out and demonstrated that low dose, weekly pulse methotrexate was an effective therapy for rheumatoid arthritis, although the criteria for response varied considerably among the studies (Thompson et al., 1984; Andersen et al., 1985; Weinblatt et al., 1985; Williams et al., 1985; Willkens, 1985; Furst et al., 1989). As experience with methotrexate in the therapy of rheumatoid arthritis increased, it quickly became apparent that this drug was more effective and better tolerated than the other agents then available for the treatment of RA. A greater percentage of patients continued to take methotrexate for their rheumatoid arthritis for longer than any other second-line agent (Kremer and Lee, 1986; Weinblatt et al., 1988, 1992, 1994, 1998; Alarcon et al., 1989; Hanrahan et al., 1989; Mielants et al., 1991; Sany et al., 1991; Kremer and Phelps, 1992; Bologna et al., 1997; Rau et al., 1997). Indeed, some of these studies lasted as long as 11 years. More recent studies in patients with early rheumatoid arthritis indicate that methotrexate compares favorably to biological agents, a finding that clearly surprised many, although longer follow-up of the patients enrolled in these trials suggests that the biologic agents may better prevent bone destruction than methotrexate in rheumatoid arthritis (Bathon et al., 2000; Genovese et al., 2002; Bathon and Genovese, 2003; Baumgartner et al., 2004).

In all of the trials with methotrexate, the drug has been well tolerated, although toxicities were encountered. Gastrointestinal toxicity, stomatitis, alopecia, marrow suppression, and liver function abnormalities were commonly encountered, although more recently folic acid or folinic acid supplementation has diminished the frequency of liver function test abnormalities, stomatitis, and marrow suppression (see below). Interstitial pulmonary inflammation and fibrosis occur in as many as 1% of the patients studied and necessitates termination of the drug. The frequency with which low-dose methotrexate causes clinically significant liver fibrosis has been debated but does not appear to be a great risk (Lanse et al., 1985; Weinstein et al., 1985; Kevat et al., 1988; Shergy et al., 1988; Brick et al., 1989; Kremer et al., 1989; Furst et al., 1990; Whiting-O'Keefe et al., 1991; Bjorkman et al., 1993; Ruderman et al., 1997; Richard et al., 2000). Although the use of methotrexate in patients with pre-existing liver disease is not recommended and patients are advised not to drink alcoholic beverages while taking the drug, it is not currently recommended that patients undergo regular liver biopsies while taking methotrexate for rheumatoid arthritis.

C. Concomitant Use of Methotrexate with Other Anti-Inflammatory Drugs

It has probably been more than 50 years since patients with rheumatoid arthritis were treated with only a single drug. Aspirin, nonsteroidal anti-inflammatory drugs, corticosteroids, and various other second-line agents are generally taken by almost all patients with active RA. More recently, combinations of methotrexate

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1Abbreviations: RFC1, reduced folate carrier 1; RA, rheumatoid arthritis; AICAR, 5-aminoimidazole-4-carboxamide ribonucleotide; MTHFR, methylene tetrahydrofolate reductase.
with other second-line agents (sulfasalazine, hydroxychloroquine, anti-tumor necrosis factor agents, and other biologicals) have been reported to have greater efficacy than methotrexate alone without greater toxicity (O’Dell et al., 1996, 2002; Maini et al., 1998; Lipsky et al., 2000; Ferraccioli et al., 2002; Hochberg et al., 2003; Schroder et al., 2004; St Clair et al., 2004). Nonsteroidal anti-inflammatory agents modestly diminish renal clearance of methotrexate and its major metabolite 7-hydroxymethotrexate, although this interaction is generally not clinically significant (Ahern et al., 1988; Weinblatt, 1989; Stewart et al., 1990, 1991; Tracy et al., 1992, 1994; Kremer and Hamilton, 1995; Kremer et al., 1995). Hydroxychloroquine alters the pharmacokinetics of methotrexate; there is slower clearance and uptake with a greater area under the curve for methotrexate in patients taking the combination (Carmichael et al., 2002), and this interaction may account for the greater efficacy of the combination of hydroxychloroquine and methotrexate than methotrexate alone (O’Dell et al., 2002). Leflunomide, a second-line small molecule therapy for rheumatoid arthritis which inhibits pyrimidine synthesis, has been safely used in combination with methotrexate, although severe liver and bone marrow toxicity have been reported with the combination (Mroczkowski et al., 1999; Weinblatt et al., 1999, 2000; Kremer et al., 2002, 2004; Hill et al., 2003).

D. Use of Folic Acid to Prevent Methotrexate-Induced Toxicity

Methotrexate, the product of one of the first attempts at rational drug design, was originally developed as an antagonist of folic acid. At the doses commonly used to treat patients with cancer, methotrexate blocks folic acid-dependent steps in the synthesis of purines and pyrimidines and thereby blocks the proliferation of malignant cells (Fig. 1). This effect on purine and pyrimidine biosynthesis is also responsible for many of the drug’s toxicities, including bone marrow suppression and stomatitis. Based on the literature detailing methotrexate use in the therapy of cancer and leukemia, most patients were advised not to take concomitant folic acid while taking methotrexate. Indeed, an early study (Tishler et al., 1988) suggested that folic acid, when administered concomitantly with methotrexate at 3-fold higher doses than the methotrexate, reversed the anti-inflammatory effects of the drug. However, blinded and controlled trials of the concomitant administration of either folic acid or folic acid to patients with rheumatoid arthritis taking methotrexate demonstrated no difference in therapeutic efficacy of the methotrexate and prevention of methotrexate-mediated toxicity (Morgan et al., 1990, 1994, 1998; Morgan et al., 1993; Dijkmans, 1995; Cooper, 1996; Kavanaugh and Kavanaugh, 1996; Shiroky, 1996, 1997; Hunt et al., 1997; Ortiz et al., 1998, 2000; Pincus, 1998; Ravelli et al., 1999; Endresen and Husbry, 2001; van Ede et al., 2001b). Indeed, although regular use of folic acid or folinic acid supplements during methotrexate therapy are not explicitly recommended, the most recent guidelines issued by the American College of Rheumatology for the therapy of rheumatoid arthritis include the suggestion that folic acid or folinic acid may be useful in the prevention of complications of methotrexate therapy (American College of Rheumatology Subcommittee on Rheumatoid Arthritis Guidelines, 2002). Because folic acid and methotrexate compete for the same transporter for absorption from the gastrointestinal tract and for cellular uptake (Matherly and Goldman, 2003), it is likely that the reversal of methotrexate’s anti-inflammatory effects by high doses of folic acid results from diminished methotrexate uptake in those patients, so patients taking folic acid are advised to skip their folic acid doses for a period around the day that they take their methotrexate.

II. Mechanism of Action of Methotrexate As an Anti-Inflammatory Agent

Methotrexate was introduced for the therapy of rheumatoid arthritis without any clear understanding of its mechanism of action. Thus, initial efforts at dissecting methotrexate’s pharmacologic effects on inflammation were carried out in patients taking the drug, and interpretation of these clinically derived observations are difficult. Although there is a clear change in the levels of many of the mediators of inflammation measurable in the serum or synovial fluid in patients with rheumatoid arthritis, the absence of any evidence for a link between the metabolic effects of methotrexate and the observed changes raises the possibility that the observed changes in inflammatory mediators are not primary but due to some other more basic effect of the drug.

Because of the difficulty of interpreting the clinical observations in vitro and in vivo, experiments have been performed to dissect out the biochemical mechanisms responsible for methotrexate’s effects. Extrapolation
from studies performed in cell culture is always difficult because of the differences among cell lines and the questionable relevance of a particular measure to the pathogenesis of the disease under study. With methotrexate, these studies are even more difficult to interpret because the effects of methotrexate are observed over weeks to months in patients but over hours to days in tissue culture or days to weeks in animals. Similarly, studies in animals may be misleading because the doses of the drug used are not similar to those used in patients. Thus, for a drug such as methotrexate in which different doses of the drug have very different clinical uses, high doses to prevent proliferation of malignant cells and very low doses over prolonged periods to treat inflammatory diseases, the concentration of the drug used in a given in vitro experiment or the dose of the drug administered in vivo is critical. These considerations would suggest that many of the publications cited in favor of one or another mechanism may not be relevant to the effects of methotrexate in patients with rheumatoid arthritis.

Despite these caveats, there are currently several proposed mechanisms for the anti-inflammatory effects of methotrexate. The first hypothesis, based on methotrexate’s known antifolate properties, posits that methotrexate inhibits proliferation of the cells responsible for synovial inflammation in RA. The second biochemical explanation is that methotrexate inhibits the synthesis of potentially toxic compounds (the transmethylation products spermine and spermidine) that accumulate in chronically inflamed tissues. A third proposed mechanism, most recently propounded, is that methotrexate reduces intracellular glutathione levels by an oxidant-associated mechanism leading to diminished macrophage recruitment and function. A fourth mechanism has been proposed, supported by in vitro, in vivo, and clinical data, in which adenosine, released in high concentrations from cells and tissues after treatment with methotrexate, mediates the anti-inflammatory effects of methotrexate. It is most likely that some combination of these mechanisms is responsible for the potent anti-inflammatory effects of methotrexate.

A. Folate Antagonism

The initial rationale for the use of low-dose methotrexate for the treatment of inflammatory arthritis was that methotrexate, by preventing synthesis of purines and pyrimidines required for cellular proliferation, inhibits proliferation of the most rapidly dividing lymphocytes or other cells responsible for the synovial inflammation. Thus, some workers have reported that methotrexate diminishes pyrimidine synthesis by T cells and prevents antigen-dependent proliferation (Genestier et al., 1998; Paillot et al., 1998; Izeradjene et al., 2001; Quemeneur et al., 2003). Indeed, T cells taken from patients taking methotrexate exhibit diminished antigen-dependent proliferation of T cells that is folate-reversible (Genestier et al., 1998), although this effect is only detectable for 24 h after a dose of methotrexate. Despite the in vitro and clinical data, the observation that neither folic acid nor folinic acid reverses the anti-inflammatory effects of methotrexate in patients with rheumatoid arthritis (see above) is strong evidence that other mechanisms must account for the anti-inflammatory effects of the drug (Morgan et al., 1990, 1993, 1994, 1998; Dijkmans, 1995; Cooper, 1996; Kavaugh and Kavanaugh, 1996; Shiroky, 1996, 1997; Hunt et al., 1997; Ortiz et al., 1998, 2000; Pincus, 1998; Ravelli et al., 1999; Endresen and Husby, 2001; van Ede et al., 2001b).

B. Inhibition of Spermine and Spermidine Production

Increased polyamine concentrations are found in urine, synovial fluid, synovial tissue, and peripheral blood mononuclear cells from patients with rheumatoid arthritis (Hawkes et al., 1994), and the accumulated polyamines may be metabolized by monocytes to form, among other molecules, NH₃ and H₂O₂, which may be toxic to lymphocytes (Talal et al., 1988; Flescher et al., 1989, 1992; Nesher and Moore, 1990; Yukioka et al., 1992; Furumitsu et al., 1993; Nesher et al., 1996). By inhibiting dihydrofolate reductase, methotrexate inhibits the formation of tetrahydrofolate which donates a methyl group during the synthesis of methionine from homocysteine (Fig. 1). Methionine can be further converted to S-adenosyl-methionine, which serves as a methyl donor in a large number of cellular reactions, including the synthesis of the polyamines spermine and spermidine. Thus, methotrexate may inhibit the accumulation of polyamines that contribute to tissue injury in rheumatoid arthritis. The hypothesis that inhibition of transmethylation reactions is anti-inflammatory in the treatment of rheumatoid arthritis has been tested in patients; an agent that inhibits transmethylation reactions, 3-deazaadenosine, showed some promise as an anti-inflammatory drug in vitro and in vivo studies (Leonard et al., 1978; Zimmerman et al., 1978; Medzihradszky, 1984; Sung and Silverstein, 1985; Krenitsky et al., 1986; Yagawa et al., 1986; Fantone et al., 1989; Jurgensen et al., 1989, 1990; Prus et al., 1989; Prytz et al., 1989; Smith et al., 1991; Jeong et al., 1996). The drug was administered to patients with rheumatoid arthritis, and despite inhibition of transmethylation reactions measured in the cells of the patients taking the drug (Smith et al., 1991), 3-deazaadenosine did not affect the course of rheumatoid arthritis (M. Weinblatt, personal communication).

C. Methotrexate Alters Cellular Redox State

Based on in vitro studies, it has recently been reported that methotrexate reduces intracellular glutathione concentrations, and this leads to reversible inhibition of macrophage and lymphocyte function (Phillips et al., 2003). Although this is an interesting hypothesis and the in vitro data reported in this article generally sup-
port this conclusion, it is unlikely to explain the anti-inflammatory actions of methotrexate in patients with rheumatoid arthritis since intracellular glutathione levels are reduced to well below the methotrexate-induced levels already in the synovial cells of these patients (Maurice et al., 1997). In addition, the inflamed synovium is filled with cells that generate reactive oxygen metabolites (neutrophils and macrophages), and prior studies have clearly shown evidence of oxygen radical-mediated injury in synovial cells from patients with rheumatoid arthritis before any therapy; methotrexate has been shown to suppress, either directly or indirectly, the generation of toxic oxygen metabolites (Sung et al., 2000).

D. Methotrexate Increases Extracellular Adenosine Concentrations

Methotrexate and its major metabolite 7-hydroxymethotrexate are taken up by cells and polyglutamated (Chabner et al., 1985). Methotrexate polyglutamates have been shown to be even more active than the parent drug as inhibitors of a variety of folate-dependent enzymes, but the enzyme inhibited most effectively by methotrexate polyglutamates is AICAR (5-aminoimidazole-4-carboxamido ribonucleotide) transformylase (Allegra et al., 1985; Baggott et al., 1986). The inhibition of AICAR transformylase by methotrexate would be expected to lead to intracellular AICAR accumulation (Fig. 2). Because AICAR inhibits AMP deaminase and AICAR’s dephosphorylated metabolite AICARiboside directly inhibits adenosine deaminase, AICAR accumulation could lead to the release of AMP (which may be dephosphorylated to adenosine) and/or adenosine (Barankiewicz et al., 1990; Vincent et al., 1996) which is a potent endogenous anti-inflammatory mediator (reviewed in (Hasko and Cronstein, 2004).

There is clear evidence that methotrexate treatment leads to AICAR accumulation; patients taking large doses of methotrexate for the treatment of cancer excrete increased concentrations of aminoimidazole carboxamide, a metabolite of AICAR, in their urine, consistent with the notion that AICAR accumulates intracellularly in patients treated with even high-dose methotrexate (Luhby and Cooperman, 1962). More relevant to its anti-inflammatory action, patients taking low-dose methotrexate for psoriasis also excrete increased aminoimidazole carboxamide in their urine, further confirming the hypothesis that methotrexate promotes AICAR accumulation (Baggott et al., 1999).

AICAR accumulation causing adenosine release was first suggested by Gruber and colleagues (Gruber et al., 1989) who showed that intracellular AICAR accumulation (induced by infusion of AICARiboside) is associated with increased adenosine release in vivo.

The initial studies to test the hypothesis that adenosine release mediates the anti-inflammatory effects of methotrexate were performed in vitro. In these studies, methotrexate treatment increased adenosine release from cultured endothelial cells and fibroblasts and the adenosine released diminished stimulated neutrophil adhesion to the monolayers of cultured cells (Cronstein et al., 1991). Subsequent in vivo studies confirmed the hypothesis that adenosine mediates the anti-inflammatory effects of methotrexate; pharmacologically relevant doses of methotrexate induce intracellular AICAR accumulation in splenocytes, increase adenosine concentrations in inflammatory exudates, and diminish leukocyte accumulation at an inflamed site (Cronstein et al., 1993). Moreover, the increase in exudate adenosine concentration was responsible for the anti-inflammatory effects of the drug since adenosine receptor antagonists or adenosine deaminase, an enzyme which converts adenosine to the receptor-inactive nucleoside inosine, completely reversed the effect of methotrexate on leukocyte accumulation (Cronstein et al., 1993). Identical observations on the effects of methotrexate-induced aden-
ossine release on leukocyte extravasation were made by Asako and colleagues in a different animal model of acute inflammation (Asako et al., 1993). More recent studies in an animal model of rheumatoid arthritis further support the role of adenosine, acting at its receptors, as the mediator of the anti-inflammatory effects of methotrexate (Montesinos et al., 2000). In these studies, adenosine receptor antagonists theophylline and caffeine reverse the effects of methotrexate on the development of adjuvant arthritis (Montesinos et al., 2000). Recent studies using adenosine receptor knockout mice provide further corroborative evidence in support of the hypothesis that adenosine, acting at A2A and possibly A3 receptors, mediates the anti-inflammatory effects of methotrexate and a methotrexate analog (Montesinos et al., 2003). Perhaps of greatest clinical significance are the recent reports that the antirheumatic effects of methotrexate are diminished in patients ingesting an adenosine receptor antagonist caffeine in coffee (Silke et al., 2001; Nesher et al., 2003). Thus, the evidence strongly supports the hypothesis that the anti-inflammatory effects of methotrexate are mediated by adenosine acting at adenosine receptors on inflammatory cells in vivo models of both acute and chronic inflammation and, most likely, in patients with rheumatoid arthritis.

Interestingly, it was originally assumed, based on mechanistic considerations, that methotrexate induced direct release of adenosine. However, studies by Morabito and colleagues demonstrate that the adenosine released from methotrexate-treated cells is derived from adenine nucleotides converted extracellularly to adenosine by the action of the enzyme ecto-5’-nucleotidase (Morabito et al., 1998). Thus, the relatively specific inhibitor of ecto-5’-nucleotidase, a,β-methyleneadenosine-5-diphosphate completely abrogated the methotrexate-induced increase in extracellular adenosine in supernates of cultured endothelial cells exposed to a noxious stimulus (stimulated neutrophils). Strikingly, cells deficient in ecto-5’-nucleotidase did not release adenosine, whether treated with methotrexate or exposed to a noxious stimulus (H2O2), but transfection and expression of ecto-5’-nucleotidase in the deficient cells permitted methotrexate to induce adenosine release following a noxious stimulus. When studied in an in vivo model of inflammation, the inhibitor of ecto-5’-nucleotidase completely blocked the methotrexate-induced increment in exudate adenosine and reversed the anti-inflammatory effect of methotrexate. Thus, methotrexate, presumably via its capacity to increase intracellular AICAR concentrations, promotes release of adenine nucleotides that are converted at inflamed sites to adenosine.

Corroborating evidence that methotrexate therapy induces adenosine release in patients and that adenosine mediates some of the anti-inflammatory effects of methotrexate have been published by other groups as well. As noted above, patients treated with high-dose methotrexate excrete increased quantities of aminomimidazole carboxamide in their urine, a finding that is consistent with the notion that AICAR accumulates after methotrexate therapy (Luhby and Cooperman, 1962). Bernini et al. (1995) have reported that children treated with methotrexate for leukemia had higher adenosine concentrations in their cerebrospinal fluid, and the highest adenosine concentrations were observed in those patients who exhibited central nervous system toxicity (lethargy and coma). In those patients with central nervous system toxicity, administration of an adenosine receptor antagonist rapidly reversed the toxicity, indicating that the adenosine released into the central nervous system is sufficient to occupy adenosine receptors and dramatically alter central nervous system function. Two studies in patients provide evidence that methotrexate increases adenosine release (Laghi Pasini et al., 1997; Baggott et al., 1999).

Evidence against the hypothesis that adenosine mediates the anti-inflammatory effects of methotrexate was first provided by Andersson and colleagues (Andersson et al., 2000) who found that adenosine receptor antagonists do not reverse the anti-inflammatory effects of methotrexate in a model of arthritis in rats. The variance between the results of this study and the prior studies is most likely due to the very high (3- to 5-fold higher) dose of methotrexate required to inhibit arthritis in the model studied. Moreover, methotrexate-mediated suppression of inflammation was completely reversed by folic acid supplementation in the rats studied by Anderson and colleagues, whereas folic acid administration does not affect the therapeutic effects of methotrexate in patients with rheumatoid arthritis (see above).

Other evidence against the hypothesis that methotrexate promotes adenosine release and adenosine mediates the anti-inflammatory effects were provided by two recent clinical studies (Egan et al., 1999; Smolenska et al., 1999). In the work by Smolenska et al. (1999), a single dose of methotrexate significantly reduced purine synthesis for a day without any concomitant increase in erythrocyte AICAR concentration. The observation that purine synthesis is inhibited for a day after a dose of methotrexate is consistent with and probably accounts for the methotrexate-mediated reduction in antigen-induced proliferation of T cells, although the clinical importance of reduced cellular proliferation 1 day a week is unknown. The observation that AICAR does not accumulate in the erythrocytes of these patients 1 day after a single dose of methotrexate is expected, since AICAR accumulation in erythrocytes should not be detectable for weeks after starting methotrexate therapy because red blood cells do not actively synthesize purines, and any intracellular accumulations of AICAR must result from metabolic changes in bone marrow precursors. Because the half-life of erythrocytes is measured in months, only a minuscule percentage of the circulating red cells would have had an opportunity to accumulate
AICAR. The work by Egan and colleagues (Egan et al., 1999) was not designed in such a way that any conclusions could conceivably be drawn; patients were administered a single parenteral dose of methotrexate followed several minutes later by sigmoidoscopic sampling for rectal adenosine. Even when administered parenterally, methotrexate reaches peak serum concentrations 4 to 6 h after the dose, and accumulation of methotrexate polyglutamates (with resulting enhanced adenosine release) takes hours to days.

III. Pharmacogenetics of Methotrexate in the Treatment of Rheumatoid Arthritis

As technology has progressed, it has become possible to pinpoint genetic factors that modulate response to various drugs, and methotrexate has received its share of attention. Identifying a genetic predisposition to a toxic reaction to a drug such as methotrexate is much easier than pinpointing the factors that may predispose to a better response to the drug. Toxic reactions are discrete and easily identifiable, whereas therapeutic responses to methotrexate are often difficult to define; drug response in rheumatoid arthritis is generally a composite measure comprised of findings on physical examination (tender and swollen joints), laboratory results (C-reactive protein or erythrocyte sedimentation rates), and subjective responses (e.g., Modified Health Assessment Questionnaire, Physician’s Global Response). Moreover, response to drug therapy in rheumatoid arthritis is clearly much better when the drug is started early in the course of the disease, regardless of the drug (see Baumgartner et al., 2004). Thus, defining genetic factors that predispose to a better response to the drug is complex, and the genetic contribution may be different depending on when the drug is started in the course of the disease. Because of these problems, there is much more data available on the genetic factors that may predispose to methotrexate’s toxicity than to its efficacy.

A. Genetic Factors Predicting Increased Risk of Drug Toxicity

Methotrexate was developed as an analog of folic acid, and many of the factors governing cellular handling of methotrexate are identical to those involved in folate metabolism. Methotrexate is taken up by specific transporters into the cell where it interferes with the synthesis of purines and pyrimidines as well as blocking the conversion of homocysteine to methionine. Severe deficiency of MTHFR is associated with homocysteinemia and homocysteinuria, neuropathy and encephalopathy, and coagulopathy and vasculopathy. However, there are common variants in the enzyme that are associated with a modest decrease in MTHFR activity; 40% of the population is heterozygous for the C677T polymorphism and 8 to 10% are homozygous for this polymorphism. Heterozygosity for the C677T polymorphism leads to a 30 to 40% reduction in enzyme activity, and homozygosity is associated with a 70% reduction in activity. The A1298C polymorphism is in linkage disequilibrium with the C677T polymorphism so that 50 to 100% of those with the minority polymorphism at position 677 will also have the minority polymorphism at position 1298. Recent studies have elucidated the role of genetic polymorphisms in the enzyme involved in the conversion of homocysteine to methionine, MTHFR, in excess methotrexate marrow toxicity in patients with rheumatoid arthritis (van Ede et al., 2001a; Urano et al., 2002; Kumagai et al., 2003). Thus, the results of these studies demonstrate that the C677T polymorphism is associated with enhanced methotrexate-mediated marrow toxicity.

B. Genetic Factors Predicting Increased Drug Efficacy

Clearly, a genetic test that predicted response to methotrexate would be greatly welcome in the rheumatology community. One recent report has indicated that the A1298C polymorphism is associated with diminished efficacy of methotrexate (defined as requiring >10 mg/week methotrexate), although this was not confirmed in a subsequent study (Urano et al., 2002; Kumagai et al., 2003). In another recent study, an additive effect on methotrexate efficacy was demonstrated for polymorphisms in thymidylate synthase (involved in folate-dependent pyrimidine synthesis), AICAR transformylase, and RFC1 (the protein that transports methotrexate into the cell). Individuals with a polymorphism in more than one of these genes had a better response to methotrexate than those with none. This was a small study, however, and the role of these polymorphisms in methotrexate response requires further study (Dervieux et al., 2003).

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