Vol 27, No 5 April 1998

Seminars in Arthritis and Rheumatism

Roy D. Altman, MD - Norman L. Gottlieb, MD - David S. Howell, MD
Editors

W. B. Saunders Company
A Division of Harcourt Brace & Company

Seminars in
Arthritis and Rheumatism

ROY D. ALTMAN, MD, NORMAN L. GOTTLIEB, MD,
DAVID S. HOWELL, MD, *Editors*

Seminars in Arthritis and Rheumatism (ISSN 0049-0172) is published bimonthly by W.B. Saunders Company. Corporate and editorial offices: The Curtis Center, Independence Square West, Philadelphia, PA 19106-3399. Accounting and circulation offices: 6277 Sea Harbor Dr, Orlando, FL 32887-4800. Months of issue are February, April, June, August, October, and December. Periodicals postage paid at Orlando, FL 32887-4800, and at additional mailing offices.

POSTMASTER: Send change of address to *Seminars in Arthritis and Rheumatism*, c/o W.B. Saunders Company, Periodicals Department, 6277 Sea Harbor Dr, Orlando, FL 32887-4800.

Editorial correspondence should be addressed to *Seminars in Arthritis and Rheumatism*, c/o Roy D. Altman, MD, PO Box 016960, Miami, FL 33101.

Business correspondence (subscriptions or change of address) should be addressed to W.B. Saunders Company, Periodicals Department, 6277 Sea Harbor Dr, Orlando, FL 32887-4800.

Change of address notices, including both the old and new addresses of the subscriber, should be sent at least one month in advance.

Customer Service: 1-800-654-2452.

Yearly subscription rates: United States and possessions: individual, $145.00; institution, $222.00; single issue, $47.00. All other countries: individual, $243.00; institution, $277.00; single issue, $47.00. For all areas outside the United States and possessions, there is no additional charge for surface delivery. For air mail delivery, add $24.00. Student and resident: United States and possessions, and Canada: $67.00; all other countries: $243.00. Subscriptions are payable in advance. To receive student/resident rate, orders must be accompanied by name of affiliated institution, date of term, and the *signature* of program/residency coordinator on institution letterhead. Orders will be billed at individual rate until proof of status is received. Prices are subject to change without notice. Current prices are in effect for back volumes and back issues. Single issues, both current and back, exist in limited quantities and are offered for sale subject to availability. Back issues sold in conjunction with a subscription are on a prorated basis. *Cumulative Index (1980-1989) price:* $80.00; foreign customers, please add $2.25 for surface delivery, or $8.00 for air mail delivery. *Checks* should be made payable to W.B. Saunders Company and sent to *Seminars in Arthritis and Rheumatism*, W.B. Saunders Company, Periodicals Department, 6277 Sea Harbor Dr, Orlando, FL 32887-4800.

Agents for the United Kingdom, Ireland, and Europe: Harcourt Brace & Company Limited, 24-28 Oval Rd, London NW1 7DX, England.

The contents of *Seminars in Arthritis and Rheumatism* are indexed and abstracted in *Index Medicus, Excerpta Medica, Current Contents/Clinical Medicine, Science Citation Index, SCISEARCH, Automated Subject Citation Alert,* and *ISI/BIOMED.*

W.B. Saunders Company *Philadelphia, PA*

A Division of Harcourt Brace & Company

Methotrexate in Rheumatoid Arthritis: An Update With Focus on Mechanisms Involved in Toxicity

Annelies E. van Ede, Roland F.J.M. Laan, Henk J. Blom,
Ronney A. De Abreu, and Leo B.A. van de Putte

Objectives: To provide an update of the current knowledge of the mechanism of action of low-dose methotrexate (MTX) in the treatment of patients with rheumatoid arthritis (RA), with an emphasis on the mechanisms involved in toxicity. We also considered strategies currently used to prevent or decrease toxicity of MTX.
Methods: We reviewed the literature dealing with the subjects of MTX treatment of RA, the mechanisms of action of low-dose MTX regarding efficacy and toxicity, and strategies used to prevent or decrease MTX toxicity.
Results: MTX is a fast working and effective second-line antirheumatic agent (SLA). Its use is limited mainly because of side effects. The mechanisms of action regarding efficacy and toxicity are probably determined by different metabolic pathways. Recent data indicate that the antiinflammatory effect of MTX is mediated by adenosine. However, MTX side effects can only partly be explained by folate antagonism and may also depend on its action on other related metabolic pathways. The latter include the homocysteine-methionine-polyamine pathway and purine metabolism. Variants in these metabolic routes (ie, the C677T mutation in the methylene-tetrahydrofolate reductase [MTHFR] gene), may predispose to the development of side effects. Currently the most promising strategy to decrease or prevent toxicity of MTX is concomitant prescription of folic acid or folinic acid. Other strategies are currently under investigation.
Conclusions: MTX benefits a majority of RA patients. Approximately 30% of patients, however, abandon treatment because of drug-related side effects. Folic acid or folinic acid likely reduces MTX toxicity. More data, however, are needed to evaluate a potential detrimental effect on the antirheumatic efficacy of MTX.

INDEX WORDS: Methotrexate; rheumatoid arthritis; mechanism of action; toxicity.

METHOTREXATE (MTX, amethopterin) is a folic acid antagonist that was introduced into the treatment of childhood leukaemia in 1947. In 1951 Gubner et al reported a favorable effect in patients with psoriasis, psoriatic arthritis, and rheumatoid arthritis (RA) (1). Low-dose MTX has been commonly used in the treatment of psoriasis since the 1960s and was approved for this purpose by the Food and Drug Administration in 1971. However, its use in the treatment of RA only begun during the 1980s and it was not approved by the Food and Drug Administration for this indication until 1988 (2-5).

From the Department of Rheumatology and the Laboratory of Pediatrics and Neurology, University of Nijmegen, The Netherlands.
Address reprint requests to Annelies E. van Ede, MD, Department of Rheumatology, University of Nijmegen, PO Box 9101, 6500 HB Nijmegen, The Netherlands.
Annelies E. van Ede, MD: Department of Rheumatology, University of Nijmegen; Roland F.J.M. Laan, MD, PhD: Department of Rheumatology, University of Nijmegen; Henk J. Blom, PhD: Laboratory of Pediatrics and Neurology, University of Nijmegen; Ronney A. De Abreu, PhD: Laboratory of Pediatrics and Neurology, University of Nijmegen; Leo B.A. van de Putte, MD, PhD: Professor, Head of Department, Department of Rheumatology, University of Nijmegen.

0049-0172/98/2705-0003$8.00/0

Currently, MTX is prescribed by rheumatologists all over the world. MTX has proved to be a very effective, fast-working, second-line antirheumatic agent (SLA) with the best efficacy-toxicity ratio (6-8). Nevertheless, the main reason for discontinuation of MTX is not inefficacy but toxicity (9-12). Because of its clear-cut and long-lasting efficacy, much effort is currently being made to develop strategies to decrease or prevent its toxicity. In approximately 30% of RA patients, toxicity leads to discontinuation of MTX therapy. Therefore, diminishing of toxicity may result in better treatment of this group of RA patients (9-22).

MTX acts as a folate antagonist, but this does not directly explain the full spectrum of side effects. However, MTX exerts its action also on other related metabolic pathways. Recently, a gene mutation was discovered in one of these metabolic routes that may predispose to the development of side effects (23-25). Other promising research focuses on the homocysteine-methionine-polyamine pathway and on purine metabolism.

To better understand the possible mechanisms operative in both efficacy and toxicity of MTX in the treatment of RA patients, we describe folate metabolism and folate dependent metabolic pathways in which MTX is involved. Subsequently, we then discuss how these may relate to efficacy and side effects. Finally, we discuss strategies to prevent or decrease toxicity of MTX.

FOLATE METABOLISM

Figure 1 gives an outline of folate metabolism and folate dependent pathways. Because humans are not capable of forming folic acid from their basic constituents, dietary intake is essential (26). Dihydrofolate-reductase (DHFR) catalyzes the conversion of dihydrofolate (DHF) into tetrahydrofolate (THF), which serves as a central component of folate dependent pathways (27,28). THF is also formed from 5-methyl-THF during the conversion of homocysteine (the methyl group acceptor) into methionine, and in the steps involved in purine synthesis. The biologically active folates are derivatives of tetrahydrofolates and participate in one carbon transfer reactions. In the cells, these folates are converted into polyglutamated forms and thereby retained intracellularly. Serum folate consists mainly of 5-methyl-THF, which is the predominant circulating form of folate. In rapidly dividing tissues, 5-methyl-THF and 10-formyl-THF are equally predominant. 5-Methyl-THF is more susceptible to both intra- and extracellular changes in folate status (29).

Folates are involved in several important metabolic routes: 5,10-methenyl-THF/10-formyl-THF, 5,10-methylene-THF, and 5-methyl-THF deliver one-carbon units for the synthesis of purines, pyrimidines and methionine respectively.

Purine-Metabolism

Purines are necessary for the synthesis of the nucleic acids adenosine monophosphate (AMP) and guanosine monophosphate (GMP), and finally deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). The purine-metabolism is composed of a de novo synthesis and a salvage route (Fig 2). Complete purine de novo synthesis (PDNS) exists only in proliferating cells such as of bone marrow and liver and is partly performed in lymphocytes and mononuclear cells (30). The central enzyme is 5-phosphoribosyl-1-pyrophosphate (PRPP) synthetase. In PDNS the 5,10-methenyl-THF dependent enzyme glycinamide ribosyl-5-phosphate (GAR) formyltransferase and the 10-formyl-THF dependent enzyme amino-imidazolcarboxamide ribosyl-5-phosphate (AICAR) formyltransferase are involved. Salvage probably takes place in all body cells, but measurements have been done mainly in lymphocytes and erythrocytes. In the purine salvage route, the enzymes hypoxanthine-guanine phosphoribosyl transferase (HGPRT), purine nucleoside phosphorylase (PNP), adenosine deaminase (ADA), and purine-5'nucleotidase (5'NT) are involved.

AICAR and its metabolites inhibit adenosine kinase (AK) and ADA with the consequent increase of adenosine, which has antiinflammatory properties (31-33).

Pyrimidine-Metabolism

The 5,10-methylene-THF dependent enzyme thymidylate synthetase (TS), catalyzing the conversion of deoxyuridylate (dUMP) to deoxythymidylate (dTMP), forms a rate limiting step in DNA synthesis (26).

Homocysteine-Methionine-Polyamine Pathway

Homocysteine and folate status are inversely related. Homocysteine is probably an even better

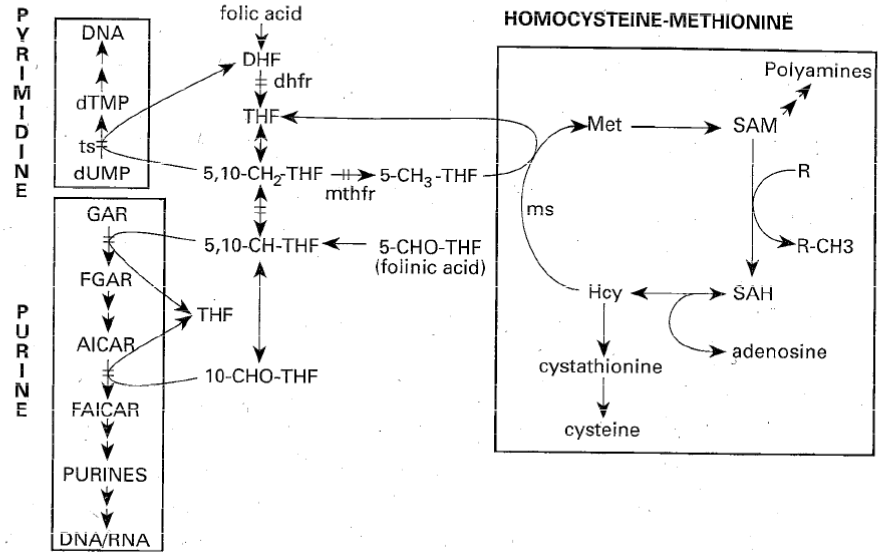

Fig 1. Simplified metabolic scheme illustrating folate metabolism and its relation to purine, pyrimidine, and homocysteine-methionine metabolism. Known inhibition of enzymes by methotrexate is indicated by —‖— Abbreviations: DNA, deoxyribonucleic acid; dTMP, deoxythymidylate; ts, thymidilate synthetase; dUMP, deoxyuridylate; GAR, glycinamide ribosyl-5-phosphate; FGAR, form-glycinamide ribosyl-5-phosphate; AICAR, amino-imidazolcarboxamide ribosyl-5-phosphate; FAICAR, form-amino-imidazolcarboxamide ribosyl-5-phosphate; RNA, ribonucleic acid; DHF, dihydrofolate; dhfr, dihydrofolate-reductase; THF, tetrahydrofolate; 5,10-CH_2-THF, 5,10-methylene tetrahydrofolate; 5,10-CH-THF, 5,10-methenyl tetrahydrofolate; 10-CHO-THF, 10-formyl tetrahydrofolate; mthfr, methylene-tetrahydrofolate reductase; 5-CH_3-THF, 5-methyl tetrahydrofolate; 5-CHO-THF, 5-formyl tetrahydrofolate (folinic acid); Met, methionine; SAM, S-adenosyl-L-methionine; ms, methionine synthetase; Hcy, homocysteine; SAH, S-adenosyl-L-homocysteine; R, methyl-acceptor; R-CH3.

parameter for measuring the effective capacity of folate metabolism than folate blood levels (34-38).

Homocysteine is not derived from the diet but from transmethylation of methionine. It is reused by remethylation to methionine and transsulfuration to cysteine, regulated by methylene-tetrahydrofolate reductase (MTHFR) and cystathionine-β-synthase (CS) respectively (39). 5-Methyl-THF is necessary as a methyl donor for the conversion of homocysteine to methionine, which in turn can be converted into S-adenosyl-L-methionine (SAM) by adenosine triphosphate (ATP). SAM is the most important methyl group donor in the cell; during these methyltransferase reactions, SAM is converted into S-adenosyl-L-homocysteine (SAH). SAH is degraded by SAH-hydrolase into homocysteine and adenosine. Homocysteine is an inhibitor of methyltransferases. Therefore, the ratio between SAM and SAH is an important determinant of intracellular transmethylation capacity (40,41).

SAM is not only converted into SAH, but also into decarboxy-SAM, which is the substrate for the synthesis of polyamines. In polyamine synthesis, the decarboxylation of SAM by SAM-decarboxylase is a rate-limiting step (41,42). The polyamines putrescine, spermidine, and spermine are essential for cell functions including proliferation, differen-

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.