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Review Article

FOLATES BENEFICIAL? IN RA TREATMENT WITH METHOTREXATE – A REVIEW

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ABSTRACT

Folates, MTX gets metabolised to polyglutamate residues (inhibitors of AICARase) and accumulate in the cytosol of various cells. Folic acid and Folinic acid (N5- Formyl THFA) are essential for the synthesis of precursors of DNA and RNA. RA treatment involves a low dose (< 20mg/week) of MTX is administered along with folic acid supplements in order to reduce the toxic effects of the drug. The direction of current therapies is to treat RA aggressively with early use of MTX as the drug of choice in treatment RA. Role of folic acid / folates which is also used along with the therapy of MTX does not have any conclusive evidences yet it is used in therapy. This article focused on the efficacy role in RA patients treated with MTX along with folic acid /folinic acid.

Keywords: Rheumatoid arthritis (RA), Methotrexate (MTX)

INTRODUCTION

Folates are essential nutrients for DNA synthesis in cell. From a nutritional perspective, a high folate status may have a beneficial/protective effect against cancer, birth defects and age-related diseases. This article provides evidence that a high folate status can also have a differential impact on the therapeutic efficacy of both folate-based and other widely used RA agents. The classical example is Methotrexate (MTX), a folic acid antagonist, which is an essential part in the treatment of RA. Methotrexate (MTX) is the most commonly prescribed disease-modifying anti-rheumatic drug (DMARD) for the treatment of rheumatoid arthritis (RA). Despite its acceptable safety profile, up to 30% of patients discontinue the medication due to toxicity. Folate supplementation has been shown to reduce some of the adverse events of MTX therapy. However, at present, there is no consensus regarding whether all patients on MTX should receive folate supplementation or if it should be initiated once adverse events occur[1].

Furthermore, the optimum dose of folic acid supplementation remains unclear. The current practice in the United States is to start folic acid to all patients receiving MTX therapy and is considered a valid quality measure for RA.

Effect of Folic Acid Supplementation On Efficacy Of MTX

There continues to be uncertainty whether folate supplementation reduces the efficacy of MTX. Most studies including the recent prospective study and the meta-analysis did not find a decrease in efficacy of MTX with folate supplementation. The prospective study of folic acid, higher mean doses of MTX were required in the groups on folic acid and folinic acid supplementation (18 and 16.4mg/week respectively) when compared to the placebo group (14.5 mg/ week). However, there was no evidence of decreased effectiveness of MTX from folate supplementation as measured by the Disease Activity Score (DAS) values. The suggested possibility for the difference in doses noted above is that folate supplementation allows for higher doses of MTX for effective therapy by minimizing the adverse events that lead to its discontinuation[2].

Evidence For Folate Supplementation With MTX Therapy In RA

Given that some of the adverse events from MTX can be mimicked by folate deficiency, there have been studies investigating the role of folic acid in minimizing adverse events from low-dose MTX therapy in patients with RA. A meta-analysis by Ortiz and colleagues included seven randomized controlled trials with a total of 307 patients. 80 patients were on folinic acid (leucovorin) supplementation and 67 patients on folic acid. The study looked at the effects of folic and folinic acid supplementation in reducing gastrointestinal, mucosal and hematologic side effects of low-dose MTX therapy. The meta-analysis concluded that folic acid was effective and resulted in a 79% reduction in mucosal and GI side effects which was statistically significant. For folinic acid a non-statistically significant reduction of 43% was found. Overall, there was no decrease in the efficacy of MTX therapy. The authors concluded that folate supplementation reduced GI and mucosal side effects from low-dose MTX therapy[3].

The effect of folic acid supplementation in reducing hematologic toxicity from MTX therapy remains unclear. In a retrospective review over 5 years, 25 cases of pancytopenia in patients with RA on low-dose MTX were identified. Of the 10 patients with severe pancytopenia, 4 were on folate supplementation while 8 of 15 with nonsevere pancytopenia were on folic acid. 18 of the 25 patients had hypoalbuminemia and 8 had renal impairment. There were 7 fatalities in this study, 4 of which were on folate supplementation. The authors recommended close attention to nutritional status and consideration for folate supplementation in all patients taking MTX. Although it appears folate supplementation may reduce the incidence of severe hematologic toxicity, no definite conclusions can be made based on currently available data[4].

Transport

The transmembrane transport of folate, and antifolate analogs that share structural similarity with the folate compounds, occurs by at least two different mechanisms. These mechanisms are energy-dependent, suggesting an active transport of MTX and reduced folates. The mechanism involves a relatively low-affinity reduced folate transmembrane carrier that is capable of transporting reduced folate analogues such as leucovorin and MTX with approximately the same efficiency. Although its affinity for reduced folates and MTX is in the micro molar range, this system transports folic acid poorly. Intracellular transport of MTX by this carrier occurs in association with an anionic gradient, which acts as a co-factor for this carrier[5, 6].

Another mechanism of transport is by transporter receptor a membrane- associated folate-binding protein (FBP). That has nanomolar affinity for both the reduced folates and folic acid. It is found in multiple cell lines and its expression increases when cells are adapted to low folate concentrations[7, 8]. This folate receptor is a 40 kDa glycoprotein and in cell lines appears to be responsible for internalization of folates and MTX. MTX is a relatively poor substrate for the FBP, with affinities that are 10 to 30 fold lower than that of the reduced folates. The relative expression of each of these transport system appears to depend, to a large extent, on the extracellular folate concentration.

At high concentration, MTX uses passive diffusion as well as the low-affinity transporter. This passive diffusion is the most important route used in mutant cells that do not possess the active transporters[5].

(Anti)Folate Transport

MTX has to be transported across the cellular membrane into the cell and the drug has to be retained in the cell. MTX can enter the cell via several processes that may operate exclusively or simultaneously in mammalian cells[9]. The reduced folate carrier (RFC) is an integral membrane protein that mediates inward transport of folates and antifolates in most neoplastic cells. RFC displays a characteristic profile of high affinity transport for reduced folate cofactors and MTX but a poor affinity for transport of folic acid[10, 11, and 12]. In general, RFC activity is relatively high in undifferentiated neoplastic and fetal tissues, whereas its activity declines upon cellular maturation/differentiation. In addition, functional RFC activity can be subject for metabolic regulation as changes in the cellular folate and purine status can down-regulate transport activity[13].

Folic acid can up regulate the expression and function of various efflux pumps, such as that of the multidrug resistance associated efflux pump MRP1 and of BCRP. The Membrane Folate Receptor (MFR)/membrane associated Folate Binding Protein (mFBP) is structurally different from RFC, since it resides in the outer layer of the plasma membrane via a glycosylphosphatidyl inositol (GPI) anchor. MFRs are glycosylated membrane proteins (molecular weight 38–44 kD) with a high affinity for folic acid; but approximately 3-fold and 100-fold lower, for reduced folate cofactors and MTX[14, 15,16].

Mechanism of Folic Acid Supplementation

After being taken up in the cell, folic acid enters the folate cycle via DHFR and is subsequently converted to CH2-THF and CH3-THF. The later serves as a methyl donor for synthesis of methionine from homocysteine, a reaction that is catalyzed by methionine synthase and vitamin B12 dependent. Folic acid supplementation leads to a depletion of homocysteine, along with an increase of S-adenosyl-methionine, a substrate for methylation reactions[17].

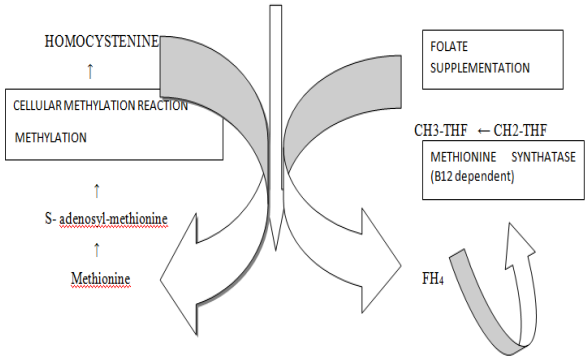

Fig 1: Mechanism of folic acid supplementation. After being taken up in the cell, folic acid enters the folate cycle via DHFR and is subsequently converted to CH2-THF and CH3-THF. The latter serves as a methyl donor for synthesis of methionine from homocysteine, a vitamin B12 dependent reaction that is catalyzed by methionine synthase. Folic acid supplementation leads to a depletion of homocysteine, along with an increase of S-adenosyl-methionine, a substrate for methylation reactions.

Enzymatic Targets of MTX

Dihydrofolate reductase

Formation of thymidylate (dTMP) from deoxyuridylate(dUTP), catalyzed by TS, involves both one-carbon transfer and the oxidation of the reduced folate cofactor, 5,10 methylenetetrahydrofolate(5,10-methylene FH4), resulting in the generation of dihydrofolate (FH2) Fig: 2. Normally, the FH2 so formed is reduced by DHFR in the presence of NADPH to tetrahydrofolate, which equilibrates the active reduced folate cofactor pool (Fig: 2). MTX is a potent inhibitor of the enzyme DHFR. Blockade of this enzyme prevents regeneration of FH4 from FH2 produced in the de novo synthesis of dTMP from dUTP by the TS [18].

Consequently, generation of 5-10-methylene FH4 is also inhibited with the result that pathways that utilize this cofactor are blocked, providing that exogenous FH4 is not available or that its transport into the cell is competitively blocked by MTX. The inhibition of DHFR by MTX is stoichiometric and competitive with FH2.[19]

Folic and folinic acids antagonize the inhibiting effect of MTX by increasing the intracellular level of FH2 and FH4, respectively. Indeed, when the ratio of FH2 to MTX increases, for instance, by addition of folic acid, there is competition between these compounds for enzyme binding with the displacement of MTX from the critical few DHFR binding sites that are sufficient to maintain normal levels of FH4 synthesis. Folinic acid (N5-formyl-FH4) does not compete with MTX for binding to DHFR but directly overcomes MTX blockade of this enzyme by increasing FH4.

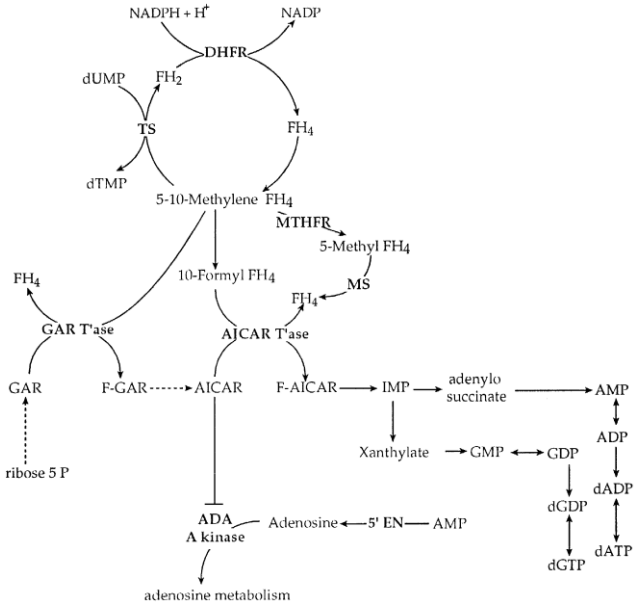

Fig. 2: Cycle and pathway of de novo synthesis of purine nucleotides from ribose 5-phosphate. Direct and indirect enzyme targets of MTX are represented in bold. Abbreviations used: AICAR: 5-amino 4 carboxamide ribonucleotide; AICARTase: AICAR transformylase; F-AICAR: formyl AICAR; DHFR: dihydrofolate reductase; 5-EN: 5X ectonucleotidase; GAR: glycinamide ribonucleotide; GAR Tase: GAR transformylase; F-GAR: formyl GAR; MS: methionine synthetase; MTHFR: methylene tetrahydrofolate reductase; TS: thymidilate synthase.

Adenosine as Anti-Inflammatory Molecule

Biochemical studies demonstrated that MTX inhibition of AICAR transformylase by MTX resulted in increased release of adenosine by fibroblasts, endothelial cells and other cell types [20]. Adenosine is a potent endogenous anti-inflammatory purine nucleotide that inhibits superoxide anion generation in neutrophil-mediated injury to endothelial cells. [21,22] Similarly, adenosine inhibits adhesion and phagocytosis of immunoglobulin-coated particles by neutrophils [23,24]. Moreover, MTX-mediated reduction of leukocyte accumulation in the inflamed hamster air pouch model is partially reversed by injection of ADA into the air pouch, and completely reversed by the specific adenosine receptor A2 antagonist, 3,7-di-methyl-1 propargylxanthine(DMPX)[25]. These observations provide converging evidence that the anti-inflammatory effects of MTX are mediated by adenosine and its A2-receptors. While these data document the anti-inflammatory properties of MTX, they do not exclude an immunosuppressive activity. Indeed, deficiency in ADA is associated with a severe combined immunodeficiency disease. The A2 receptors are expressed on various subsets of lymphocytes depending on their activation status, and adenosine has been shown to increase cyclic AMP content and to inhibit several lymphocyte responses reviewed in [24].

Role of Folate Supplementation

In India, most patients with RA reach a rheumatologist relatively late in the course of their illness after having tried a variety of remedies. By this stage most of them have developed deformities and the early window of opportunity during which treatment could potentially alter disease course has long since closed. The rheumatologist is then left with the unrewarding task of attempting to achieve tight disease control while focusing on rehabilitation as a mainstay. At the same time a fair share of blame also rests with physicians who are not sensitized to the need for establishing an early diagnosis and starting therapy early. The use of early diagnostic tests like anti-CCP antibodies continues to be very low. It is not uncommon to see patients with evident disease who are either not being given DMARDs or being prescribed suboptimal doses. Steroids, long considered the panacea for most illnesses by local practitioners, continue to be abused, being used in high doses, for prolonged periods and in treating burnt out disease.

After a long period of dependence on NSAIDs, an exciting era has dawned. Newer agents are being discovered regularly and DMARD combinations are being fine tuned. We can offer more hope to our patients today than ever before. Words such as "cure" and "remission" are more often being encountered in the literature. Failure to translate clinical research findings into clinical practice can prove to be very expensive for our patients. The need of the hour is to educate both patients and physicians about the importance of an early diagnosis and early goal-directed treatment for rheumatoid arthritis. Failing this, we shall continue to demonstrate to our students patients with multiple classical deformities, the result of having missed the "window of opportunity"!

CONCLUSIONS

Folate supplementation was given to prevent drug-associated toxicity. However, this also enabled to increase the dose (or prevent dose de-escalation), ultimately resulting in increased antitumor activity of the antifolate based combination. Since in several countries food is fortified with folic acid or patients take food and/or vitamin-like supplements this may affect drug, especially antifolate, efficacy. Notably, we have also observed that a high folate status will increase not only the expression but also the functional capacity of various ABC transporters such as MRP and BCRP. It is therefore unlikely that folic acid decrease MTX-mediated immunosuppressive activity at the doses used in RA. Furthermore, folic acid may not interfere with the adenosine production and the anti-inflammatory properties of MTX, while minimize some side effects through as yet unknown metabolic pathways.

In conclusion, MTX, like other antimetabolites that interfere with purine and pyrimidine nucleotides synthesis, is a potent immunosuppressor that may delete T cell clones that undergo antigenic activation during drug treatment. In addition, MTX, through the induction of adenosine synthesis, and possibly other mechanisms, has anti-inflammatory activities. The beneficial effects of MTX treatment observed in autoimmune and chronic inflammatory disorders, and in bone marrow transplantation, may be attributed to both the anti-inflammatory and the immunosuppressive effects of the drug.

Folic acid can be given daily; except in patients with a very low baseline folate, there is no need to administer loading doses of folate. We found it easier to ask patients to take folic acid every day than to ask them to take it only on the days MTX is not administered; this procedure is safe given the fact that folic acid does not alter the efficacy of MTX.

During the first few months of MTX administration, or when the dose of the compound is being escalated, it is recommended to obtain hematologic and biochemical profiles every month; once the dose is stable, the interval can be increased to 6–8 weeks.

REFERENCES

1. Wolfe F, Pincus T, Fries JF. Use of second line "disease modifying" anti-rheumatic drugs (DMARDs) within 5 months of disease onset by 64% of 750 rheumatoid arthritis patients under care of 142 US rheumatologists:An inception cohort study (abstr). Arthritis Rheum. 1997;40:218.
2. Van Ede AE, Laan RFJM, Rood MJ, Huizinga TWJ, van de Laar MAFJ, van Denderen CJ. Effect of folic or folinic acid supplementation on the toxicity and efficacy of Methotrexate in rheumatoid arthritis: a forty-eight week, multicenter, randomized, double-blind, placebo-controlled study. Arthritis Rheum. 2001; 44(7): 1515–24.
3. Ortiz Z, Shea B, Suarez-Almacor ME, Moher D, Wells GA, Tugwell P. The efficacy of folic acid and folinic acid in reducing methotrexate GI toxicity in rheumatoid arthritis: a metaanalysis of randomized controlled trials. J Rheumatol. 1998; 25: 36–43.
4. Vikas Agarwal, Sandeep Chauhan, Ram Singh, Atul Sachdev, Sanjay D'Cruz, Anita Tahlan. Pancytopenia with the first dose of Methotrexate in a patient with psoriasis Arthritis. J Indian Rheumatol. 2005;13:60-61.
5. Goldman ID, Matherly LH. The cellular pharmacology of Methotrexate. Pharmacol Ther. 1985; 28:77.
6. Jansen R, Ledley FD. Heterozygous mutations at the mut locus in fibroblasts with mut0 methylmalonic academia identified by polymerase-chain-reaction cDNA cloning. Am J Hum Genet. 1990 Nov;47(5):808-14.
7. Anthony JC, Le Resche, L Niaz. Limits of the 'Mini Mental State' as a screening test for dementia and delirium among hospital patient. Psychological Medicine 1982;12 :397-408.
8. Janssen Timmen U, Lemaire P, Mattéi MG, Revelant O, Charnay P. Structure, chromosome mapping and regulation of the mouse zinc finger gene *Krox-24*; evidence for a common regulatory pathway for immediate-early serum-response genes. Gene 1989;80:325-336.
9. Westerhof GR, Rijnboutt S, Schornagel JH, Pinedo HM, Peters GJ, Jansen G. Functional activity of the reduced folate carrier in KB, MA104, and IGROV-I cells expressing folate-binding protein. Cancer Res. 1995;55:3795–3802.
10. Andrew B. Bernarda, J. Bradford Jensenb. Exceptional exporter performance: cause, effect, or both? Journal of Inernational Economics 1999; 47:1–25.
11. Dicker AP, Waltham MC, Volkenandt M, Schweitzer BI, Otter GM, Schmid FA, Sirotnak FM, Bertino JR. Methotrexate resistance in an in vivo mouse tumour due to a non-active-site dihydrofolate reductase mutation. Proc Natl Acad Sci U S A. 1993 Dec 15;90(24):11797-801.
12. Zhao R, Goldman ID. Resistance to antifolates. Oncogene 2003;22:7431–7457.
13. Bernard AB, Jensen JB. Exporters, Skill-Upgrading, and the Wage Gap. Journal of International Economics 1997a; (42):3–31.
14. Jansen KLR. Neuroscience and the near-death experience: roles for the NMDA-PCP receptor, the sigma receptor and the endopsychosins. Medical Hypothese 1990b;31:25-29.
15. Wang Y, Nesbitt JE, Fuentes NL, Fuller GM. Molecular cloning and characterization of the rat liver IL-6 signal transducing molecule, gp130. Genomics 1992;14:666–672.
16. Westerhof GR, Rijnboutt S, Schornagel JH, Pinedo HM, Peters GJ, Jansen G. Functional activity of the reduced folate carrier in KB, MA104, and IGROV-I cells expressing folate-binding protein. Cancer Res. 1995;55:3795–3802
17. Jackson RC, Harrap KR. Studies with a mathematical model of folate metabolism. Arch. Biochem. Biophys. 1973;158:827–841.
18. Jackson RC. Kinetic simulation of anticancer drug interactions. International Journal of Bio-Medical Computing 1980 May;11(3):197–224.
19. White JC, Goldman ID. Mechanism of action of methotrexate IV. Free intracellular methotrexate required to supress dihydrofolate reduction to tetrahydrofolate by Ehrlich ascites tumour cells in vitro. Mol. Pharmacol. 1976;12:711.
20. Allegra CJ, Drake JC, Jolivet J, Chabner BA. Inhibition of phosphoribosyl-aminoimidazole-carboxamide transformylase by methotrexate and dihydrofolic acid polyglutamates. Proc. Natl. Acad. Sci. U. S. A. 1985b;82:4881–4885.
21. Cronstein, BN, Rosenstein ED, Kramer SB, Weissmann G, Hirschhorn R. Adenosine; a physiologic modulator of superoxide anion generation by human neutrophils. Adenosine acts via an

A2 receptor on human neutrophils. J. Immunol. 1985;135:1366–1371.

22. Roberts PA, Newby AC, Hallett MB, Campbell AK. Inhibition by adenosine of reactive oxygen metabolite production by human polymorphonuclear leukocytes. Biochem J. 1985;227:669–674.
23. Salmon JE, Cronstein BN. Fc gamma receptor-mediated functions in neutrophils are modulated by adenosine receptor occupancy. A1 receptors are stimulatory and A2 receptors are inhibitory. J. Immunol. 1990;145:2235-2240.
24. Cronstein BN, Hirschhom R. Adenosine and host defense. In: Williams, M. Ed.., Adenosine and Adenosine Receptors. Humana Press, Clifton, NJ, 1990:475–500.
25. Cronstein BN, Naime D, Ostad E. The anti-inflammatory mechanism of Methotrexate- Increased adenosine release at inflamed sites diminishes leukocyte accumulation in an in vivo model of inflammation. J. Clin. Invest. 1993; 92:2675–2682.