Mechanisms of Action of Methotrexate

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Abstract
As one of the most utilized disease-modifying anti-rheumatic drugs, methotrexate (MTX) has revolutionized the treatment of rheumatoid arthritis as well as many other non-rheumatic chronic inflammatory diseases. Far from a simple anti-proliferative agent as was once thought, our understanding of how it exerts its anti-inflammatory effects has grown over the years. The mechanisms of action of MTX are reviewed here, and we look at how this knowledge helps to explain some of its most common side effects.

Now widely regarded as a gold standard in the therapy of rheumatoid arthritis and many other inflammatory diseases, a yardstick against which the efficacy of newer disease-modifying antirheumatic drugs and biologic agents are judged, our understanding of the mechanisms of action of methotrexate (MTX) has also broadened over its many years in clinical use. These insights have not only allowed us to pursue new avenues in the design of novel therapeutic agents but also appreciate the mechanisms behind the generation of common drug side effects with the hope that these may be minimized or eradicated in future therapeutic modalities.

Oncologic Origins
Methotrexate is a drug that has seen wide applications in the treatment of malignant diseases. Its effectiveness is attributed to its ability to inhibit key enzymes in the biosynthesis of purines and pyrimidines, thereby attenuating malignant cell proliferation and turnover. As a potent inhibitor of dihydrofolate reductase, the rate-limiting enzyme in the production of tetrahydrofolate, it decreases the de novo production of purines and pyrimidines and interferes with DNA synthesis. It is, therefore, not surprising that it may find application in inflammatory diseases where a high turnover of inflammatory cells, such as T lymphocytes, in target tissues is rampant. The effectiveness of MTX in this regard is undisputed, but the inhibition of cellular turnover is also the culprit behind many of the side effects of MTX, such as bone marrow suppression and stomatitis. The widespread use of folic acid or folinic acid as a means of reducing these side effects are, however, not associated with any reduction in anti-inflammatory efficacy, thus raising a dilemma towards the putative anti-inflammatory mechanism of action by folate antagonism alone.1 Folinic acid in high doses, on the other hand, competes with the gastrointestinal uptake of MTX by its transporter protein, RFC-1, and thereby interferes with its anti-inflammatory actions. Folate pathway polymorphisms though associated with variations in red cell folate concentrations are, however, not predictive of disease activity in patients with rheumatoid arthritis on MTX.2

From Folates to Polyamines
The ability of MTX to inhibit dihydrofolate reductase gives rise to reduced production of tetrahydrofolate and methyltetrahydrofolate, which are methyl donors in chemical reactions resulting in the production of methionine and S-adenosylmethionine and ultimately polyamines. These polyamines, including spermine and spermidine, have been shown to accumulate in the synovium and urine of patients with rheumatoid arthritis.3 Mononuclear cells, in turn, convert these into toxic products, such as ammonia and hydrogen peroxide that inhibit stimulated T lymphocyte function.

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Thus, MTX may confer some of its anti-inflammatory effects by virtue of its ability to inhibit the generation of these lymphotoxic products. Although MTX indeed suppresses polyamine levels in lymphocytes of rheumatoid arthritis patients, this effect is also reversed by folic acid.\(^4\) Furthermore, 3-deazaadenosine, an inhibitor of transmethylation, had no effects in rheumatoid arthritis.\(^5\)

### The Adenosine Connection

Important metabolites of MTX include MTX polyglutamates, derived from MTX and 7-hydroxymethotrexate, and which reside in a variety of tissues, such as liver, erythrocytes and adipose tissue, persisting for weeks to months. The existence of these active polyglutamates and their requirement in the mechanism of action of MTX explains the slow onset of anti-inflammatory action, possibility of infrequent (weekly) dosing and prolonged duration of anti-inflammatory activity of MTX despite its short serum half-life in the region of 8 hours. Methotrexate itself is in fact almost undetectable in the serum 24 hours after administration. Methotrexate polyglutamates are potent inhibitors of the enzyme aminoimidazole carboxamide ribonucleotide (AICAR) transformylase, among others.\(^1,5\)

Levels of AICAR metabolites are elevated in the urine of rheumatoid arthritis patients treated with MTX. The net effect of this inhibition is the accumulation of AICAR and its metabolites, which are inhibitors of adenosine deaminase and AMP deaminase.\(^2,6\) By reducing the catabolism of adenosine and adenine nucleotides, adenosine levels increase directly and indirectly from dephosphorylation of AMP, particularly by 5'-ectonucleotidase. Adenosine accumulation following MTX administration has been demonstrated in animal models.\(^6\) The released adenosine, acting through one of four cell receptors, induces a variety of anti-inflammatory effects.

The effect of MTX-induced adenosine release has been studied in a number of in vivo models. The development of adjuvant-induced arthritis is prevented by direct infusion of adenosine into the knees of rats. In a carrageenan-induced air pouch model of inflammation, the anti-inflammatory adenosine into the knees of rats. In a carrageenan-induced air pouch model of inflammation, the anti-inflammatory adenosine antagonists is caffeine, a non-selective antagonist that binds to all known adenosine receptors. In a rat adjuvant arthritis model, caffeine as well as theophylline (another non-selective adenosine receptor antagonist) both reversed the beneficial effects of MTX treatment.\(^8\)

Methotrexate has also been shown to induce adenosine release in humans.\(^9\) While the direct effects of adenosine on inflammatory parameters are difficult to assess in humans owing to its short serum half-life (less than 10 seconds), the effects of adenosine antagonism by caffeine has been studied despite difficulties in the precise quantification of dosing. Some have found that caffeine, at least in high doses of consumption, interferes with the anti-inflammatory effectiveness of MTX therapy in patients with rheumatoid arthritis,\(^10\) while others have observed no differences.\(^11\)

The proposed mechanisms of action of MTX are depicted in Figure 1.

### Methotrexate Effects and Side Effects: A Mechanistic Perspective

As has been mentioned, the anti-proliferative effects of MTX are sufficient to explain many side effects of MTX, such as bone marrow suppression, alopecia, and stomatitis. Has our deeper understanding of the molecular mechanisms of action of MTX helped to explain some of its other effects—both desired and undesired?

As may occur in untreated rheumatoid arthritis patients, MTX treatment itself can induce the formation of nodules, an accumulation of multinucleated cells derived from differentiated mononuclear cells. Methotrexate enhances the generation of multinucleated giant cells in vitro, as does adenosine A1 receptor occupancy, while an A1 receptor antagonist reverses these effects. Thus, MTX-induced nodulosis in rheumatoid arthritis patients may be a consequence of the localized effects of released adenosine on monocyte differentiation.\(^6\)

One of the most severe side effects of MTX treatment is the development of hepatic fibrosis or cirrhosis. We have previously shown that MTX, in pharmacologically relevant concentrations, induces the release of adenosine from hepatocytes. Ethanol, indeed, has the same effect on hepatocyte release of adenosine. The released adenosine, in turn, binds to the adenosine A\(_{2A}\) receptor on hepatic stellate cells, the principal fibrogenic cell type in the liver, and promotes the production of matrix proteins, including collagen types I and III, suppressing the production of some matrix metalloproteinases. This hypothesis has been tested in pharmacologic as well as gene-deficient models of both reversible and non-reversible chemical induction models of hepatic fibrosis, where the profibrogenic effects have been attributed to ligation of the adenosine A\(_{2A}\) receptor.\(^13,14\) These findings may have come as no surprise since it has previously been shown that adenosine, also acting at the A\(_{2A}\) receptor, increases the rate of wound healing in both normal and diabetic in vivo models, again suggesting an enhancing effect of matrix protein production following the release of adenosine as a result of injury.\(^15\) Indeed, in a large retrospective study of over 120,000 subjects, coffee consumption has been noted...
to be protective against death from hepatic cirrhosis, where the relative risk has been worked out to be 0.77 per cup of coffee per day,\(^{16}\) and others have directly attributed the beneficial effects of coffee to caffeine alone.\(^{17-19}\)

Adenosine, acting at the A\(_1\) receptor, also acts on the perifornical lateral hypothalamus and has a regulating effect on wakefulness and somnolence, thus potentially explaining the sleepiness some patients experience after MTX dosing.\(^{20}\) Indeed, in children receiving high doses of MTX, severe sleepiness and coma are often encountered, and Bernini and colleagues reported that theophylline, another non-selective adenosine receptor antagonist, could reverse the CNS toxicity of MTX in children treated with high doses of MTX.\(^{21}\)

On a more positive note, MTX protects against the development of atherosclerotic heart disease, the occurrence of which is known to be increased in rheumatoid arthritis patients and not accounted for by traditional cardiovascular risk factors alone. Immune reactants such as interferon-\(\gamma\) (IFN-\(\gamma\)) and complement split products disrupt cellular defense against cholesterol loading by interfering with two major proteins responsible for exporting cholesterol out of the cell for excretion, namely cholesterol 27-hydroxylase and ATP binding cassette transporter A1 (ABCA1). The accumulation of cholesterol in macrophages induces their transformation into foam cells, an essential component of the atherosclerotic plaque. Methotrexate treatment increases the expression of cholesterol 27-hydroxylase expression, thereby promotes cholesterol export from macrophages and reduces their tendency to transform into foam cells. COX-2 inhibitors reduce the expression of cholesterol 27-hydroxylase and ABCA1 in macrophages, as do traditional non-selective non-steroidal anti-inflammatory drugs. Methotrexate reverses this propensity to reduce monocyctic cholesterol export. These atheroprotective properties of MTX are mediated by adenosine acting through the A\(_{2A}\) receptor since application of an A\(_{2A}\) receptor antagonist mitigates the beneficial effects of MTX on cholesterol transport.\(^{22}\)

**Conclusions**

Our understanding of the mechanisms of action of MTX has advanced over recent years. This knowledge has helped explain not only the beneficial anti-inflammatory effects of MTX treatment but also the causes of some of its untoward side effects. As this understanding evolves, it is hoped that it will serve as a template for the design of newer disease-modifying anti-rheumatic drugs, capitalizing on our grasp of the valuable assets yet avoiding the adverse effects.

**Conflict of Interest**

Bruce N. Cronstein, holds patents on the use of adenosine A\(_{2A}\) receptor agonists to promote wound healing and the use of A\(_{2A}\) receptor antagonists to inhibit fibrosis, the use of adenosine A\(_1\) receptor antagonists to treat osteoporosis and other diseases of bone, the use of adenosine A\(_1\) and A\(_{2B}\) receptor antagonists to treat fatty liver, and the use of adenosine A\(_{2A}\) receptor agonists to prevent prosthesis loos-
en. Edwin S. L. Chan holds a patent on the use of A2A receptor antagonists to inhibit fibrosis. Bruce N. Cronstein is consultant for Bristol-Myers Squibb, Novartis, Can-Fite Biopharmaceuticals, Cypress Laboratories, Regeneron (We-stat, DSMB), Endocyte, Protalex, Allos, Inc., Savient, Gismo Therapeutics, Antares Pharmaceutical, and Medivector.

References