How does methotrexate suppress inflammation?

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This work was supported by grants from the National Institutes of Health (AR54897, AR56672), OSI Pharmaceuticals and the Vilcek Foundation.

Received on June 28, 2010; accepted in revised form on July 22, 2010.

Clin Exp Rheumatol 2010; 28 (Suppl. 61): S21-S23.

Key words: adenosine, methotrexate, inflammation, purine metabolism

Conflict of interest: Dr. Cronstein holds or has filed applications for patents on the use of adenosine A_{2A} receptor agonists to promote wound healing and use of A_{2A} receptor antagonists to inhibit fibrosis; 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 loosening. He has been a consultant (within the past two years) for King Pharmaceutical (licensee of patents on wound healing and fibrosis above). CanFite Biopharmaceuticals, Savient Pharmaceuticals, Bristol-Myers Squibb, Roche Pharmaceuticals, Cellzome, Tap (Takeda) Pharmaceuticals, Prometheus Laboratories, Regeneron (Westat, DSMB), Sepracor, Amgen, Endocyte, Protalex, Allos, Inc., Combinatorx, Kyowa Hakka; has received honoraria from Tap (Takeda) Pharmaceuticals; and has been received for membership in the Scientific Advisory Board of CanFite Biopharmaceuticals.

ABSTRACT

Methotrexate remains the most widely used agent for the treatment of rheumatoid arthritis and other chronic inflammatory diseases. Although introduced as a chemotherapeutic agent for the treatment of malignancies, it is clear that, in the doses used, the mechanism of action in the suppression of inflammation differs from simply suppression of purine and pyrimidine metabolism, resulting in inhibition of proliferation. Here we review the proposed mechanisms of action of methotrexate.

The first report on the use of methotrexate's closely related analogue aminopterin for the treatment of rheumatoid arthritis (RA) was in 1951 (1). Several decades passed before the agent was again used to treat RA at which time aminopterin was no longer manufactured but methotrexate (amethopterin) remained available. Both of these drugs were the products of a rational drug design process in which antagonistic analogues of folic acid, known to be required for purine and pyrimidine synthesis, were developed to prevent cell proliferation for the treatment of cancer. Although originally applied to patients with RA in doses commonly utilised for the treatment of cancer, methotrexate is now used at doses that are up to two log orders lower than its use to treat tumours.

The disparity between the methotrexate doses required to inhibit rapid cellular proliferation and those used to treat RA and other inflammatory diseases raises a question as to whether the mechanisms are the same. Indeed, it is likely that although many of the typical side effects of methotrexate, as used to treat RA, are due to inhibition of cellular proliferation (*e.g.* leucopenia and anemia, stomatitis and GI ulcerations, alopecia) the doses of methotrexate used to treat RA may affect different physiologic or pharmacologic reactions. Further evidence against the antiproliferative effects of methotrexate mediating the anti-inflammatory effects of the drug in the treatment of RA was recently reviewed by Visser and colleagues (2) who noted that in multiple individual studies and meta-analyses folic acid doses greater than 5mg/week diminished GI and hepatic toxicity without affecting efficacy. In contrast, high doses of folinic acid reversed the anti-inflammatory effects of methotrexate therapy (2), a phenomenon most likely explained by competition by folinic acid, but not folic acid, for cellular uptake of methotrexate (3, 4). Thus, it is difficult to ascribe the anti-inflammatory effects of methotrexate to its anti-proliferative effects.

One mechanism that has been invoked to explain the anti-inflammatory effects of methotrexate is that it induces production of reactive oxygen species (ROS) with support from *in vitro* studies of rapidly dividing cell lines (5). In these cells methotrexate induced increased concentrations of cytosolic peroxide levels and inhibited proliferation and stimulated cellular function in a manner that was reduced by the addition of antioxidants. Although these studies are consistent they were never carried out in primary cells and monocyte/macrophages do not generally undergo cellular division, unlike the cultured cell lines studied. No evidence from primary cells, animal models or patient material has been adduced to support this hypothesis.

Another hypothesis invoked to explain the anti-inflammatory effects of methotrexate is that, by inhibiting the generation of tetrahydrofolate, a donor of methyl groups required in a large number of biochemical reactions, methotrexate inhibits transmethylation reactions required for inflammation (6-8). Although this is an attractive hypothesis supported by *in vitro* results, it is not supported by clinical data. Prior studies of a selective transmethylation inhibitor, 3-deaza-adenosine, indicate

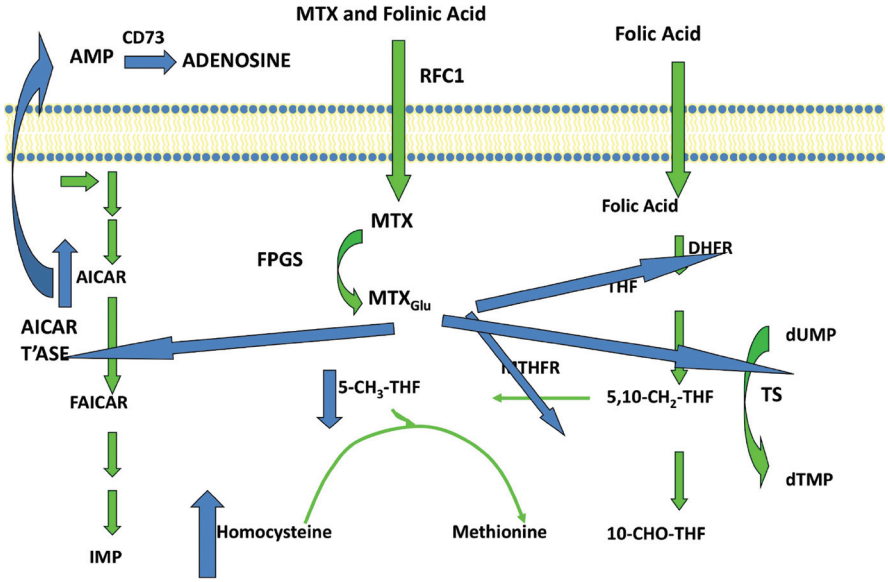

Fig. 1. Methotrexate and its effects on cellular metabolism. MTX, methotrexate; MTX_{glu}, methotrexate polyglutamate; CD73, ecto-5'nucleotidase; RFC1, reduced folate carrier 1; DHFR, dihydrofolate reductase; THF, tetrahydrofolate; MTHFR, methylene tetrahydrofolate reductase; FPGS, folyl polyglutamate synthase; AICAR, aminoimidazole carboxamidoribonucleotide; AICAR T'Ase, AICAR transformylase; FAICAR, formyl-AICAR.

that although the agent inhibits transmethylation reactions in patients there is no effect on disease activity (9).

Finally, methotrexate has previously been shown to induce adenosine release both *in vitro* and *in vivo* in both animal models of inflammation and in patients with RA (10-13) and adenosine, acting at its receptors, is a potent inhibitor of inflammation. Methotrexate, which accumulates intracellularly as methotrexate polyglutamate, inhibits aminoimidazole carboxamide ribonucleotide (AICAR) transformylase more potently than other enzymes involved in *de novo* purine biosynthesis (14). This enyme inhibition leads to accumulation of AICAR intracellularly and AICAR, by competitively inhibiting AMP deaminase, leads to accumulation of AMP which is released and converted extracellularly to adenosine by the action of ecto-5'-nucleotidase (CD73, (13, 15)). Studies with adenosine receptor antagonists and in murine models of inflammation in which adenosine receptors are blocked or deleted provide strong evidence that the anti-inflammatory effects of methotrexate are mediated by adenosine (15-19). Moreover, resistance to the anti-inflammatory effects of methotrexate correlate with poor adenosine release following methotrexate treatment in different strains of mice (20). Because caffeine, a poorly selective adenosine receptor antagonist, blocks the anti-inflammatory effects of adenosine *in vitro* and in animal models of arthritis (16) it is possible that drinking coffee or other caffeinated drinks might interfere with the therapeutic actions of methotrexate. A prospective study and a case-control study (21, 22) support this hypothesis although a retrospective study of RA patients does not support reversal of the therapeutic effects of methotrexate by coffee (23). Other pharmacogenetic studies provide further support for the role of the adenosine pathway in the mechanism of action of methotrexate (24-32).

Adenosine release also may help explain some of the toxicities of methotrexate. Clearly the anti-proliferative effects of methotrexate explain the stomatitis, anemia, leucopenia and alopecia that occasionally accompanies methotrexate therapy for RA or psoriasis. In contrast, the hepatic toxicity may result from methotrexate-mediated adenosine release since adenosine, acting at A_1 and A_{2B} receptors stimulates hepatic steatosis (33) and adenosine, acting at A_{2A} receptors, plays a role in the development of hepatic fibrosis (34, 35). Some patients suffer from severe fatigue on the day they take their methotrexate and this is likely due to CNS adenosine release which leads to sleep and somnolence (36-41). Indeed, in children who develop coma after administration of high doses of methotrexate administration of an adenosine receptor antagonist, aminophylline reverses the somnolence (42).

Thus, the most likely explanation of methotrexate's actions in the therapy of RA is that methotrexate stimulates adenosine release and adenosine suppresses the inflammatory functions of neutrophils, macrophage/monocytes, dendritic cells and lymphocytes in the pathogenesis of joint inflammation (43, 44).

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