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 Vileck Foundation.

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

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Key words: adenosine, methotrexate, inflammation, purine metabolism

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 anaemia, 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 and meta-analyses of biochemical reactions required for inflammation 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 tetrahydrofolic acid, 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...
that although the agent inhibits trans-
methylation 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 ac-
culates intracellularly as methotrex-
ate polyglutamate, inhibits aminoimi-
dazole carboxamidoboronic ester (AICAR)
transformylase more potently than other enzymes involved in de novo
purine biosynthesis (14). This enzyme
inhibition leads to accumulation of AICAR intracellularly and AICAR, by
competitively inhibiting AMP deami-
nase, leads to accumulation of AMP
which is released and converted extra-
cellularly to adenosine by the action of
ecto-5'-nucleotidase (CD73, (13, 15)).
Studies with adenosine receptor antag-
onists and in murine models of inflam-
amation in which adenosine receptors
are blocked or deleted provide strong
evidence that the anti-inflammatory ef-
facts of methotrexate are mediated by
adenosine (15-19). Moreover, resist-
ance 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 se-
lective adenosine receptor antagonist,
blocks the anti-inflammatory effects of
adenosine in vitro and in animal models
of arthritis (16) it is possible that drink-
ing 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 re-
rospective 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 ad-
enosine 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 re-
lease since adenosine, acting at A1 and
A2B receptors stimulates hepatic stea-
tosis (33) and adenosine, acting at A2A
receptors, plays a role in the develop-
ment 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 re-
lease which leads to sleep and somno-
ence (36-41). Indeed, in children who
develop coma after administration of
high doses of methotrexate administr-
ation of an adenosine receptor antagonist,
aminoxyline reverses the somnolence
(42).
Thus, the most likely explanation of
methotrexate’s actions in the therapy of
RA is that methotrexate stimulates adeno-
sine release and adenosine suppresses the
inflammatory functions of neutrophils,
macrophage/monoocytes, dendritic cells
and lymphocytes in the pathogenesis of
joint inflammation (43, 44).

References
1. GUBNER R, AUGUST S, GINSBERG V: Thera-
petic suppression of tissue reactivity. II. Effect of aminopterin in rheumatoid arthritis
and psoriasis. Am J Med Sci 1951; 221: 176-
82.
2. VOSSEN K, KATCHAMART W, LOZA E et al.: Multinational evidence-based recommenda-
tions for the use of methotrexate in rheumatic
disorders with a focus on rheumatoid arthri-
tis: integrating systematic literature research
and expert opinion of a broad international
panel of rheumatologists in the 3E Initiative.
3. MATHERLY LH, GOLDMAN DI: Membrane
transport of folates. Vitam Horm 2003; 66:
403-36.
4. JANSEN G, VAN DER HEIJDEN J, OERLEMANS
R et al.: Sulfasalazine is a potent inhibitor of
the reduced folate carrier: implications for
combination therapies with methotrexate in
rheumatoid arthritis. Arthritis Rheum 2004;
5. PHILLIPS DC, WOOLLARD KJ, GRIFFITHS
HR: The anti-inflammatory actions of meth-
otrexate are critically dependent upon the
production of reactive oxygen species. Br J
6. NESHER G, MOORE TL, DORNER RW: In
vitro effects of methotrexate on peripheral
blood monocytes: modulation by folic acid
and S-adenosylmethionine. Ann Rheum Dis
7. NESHER G, MOORE TM: The in vitro effects
of methotrexate on peripheral blood mono-
nuclear cells: modulation by methyl donors
and spermidine. Arthritis Rheum 1990; 33:
954-9.
8. NESHER G, OSBORN TG, MOORE TL: In vitro
effects of methotrexate on polyamine levels
in lymphocytes from rheumatoid arthritis pa-
9. SMITH DM, JOHNSON IA, TURNER RA: Bio-
chemical perturbations of BW 91Y (3-dea-
zaadenosine) on human neutrophil chemo-
tactic potential and lipid metabolism. Int J
10. RIKSEN NP, BARRERA P, VAN DEN BROEK PH
et al.: Methotrexate modulates the kinetics of

Fig. 1. Methotrexate and its effects on cellular metabolism. MTX, methotrexate; MTXglu, methotrex-
ate polyglutamate; CD73, ecto-5'-nucleotidase; RFC1, reduced folate carrier 1; DHFR, dihydrofolate
reductase; THF, tetrahydrofolate; MTHFR, methylene tetrahydrofolate reductase; FPGS, folyl poly-
glutamate synthase; AICAR, aminoimidazole carboxamidoboronic ester; AICAR T'ase; AICAR
transformylase; FAICAR, formyl-AICAR.
Mechanism of action of methotrexate  /  B. Cronstein


