REVIEW ON REPORTED ACTIVITIES OF METHOTREXATE AS ANTINEOPLASTIC AGENT

[1]Varun S. Pawar, [2]Gopinath D. Katkade, [3]Vrushali V. Ujagare, [4]Divya A. Kadam, [5]Ashish N. Bhingare

[1]Student, [2]Student, [3]Student, [4]Student, [5]Student,

[1]Department of Pharmacology,

[1]IVM's Indrayani Institute Of Pharmaceutical Education And Research, Talegaon Dabhade, Maval, Pune, India – 410507

Abstract: Methotrexate remains a cornerstone in the treatment of rheumatoid arthritis and other rheumatic diseases. Folate antagonism is known to contribute to the antiproliferative effects that are important in the action of methotrexate against malignant diseases, but concomitant administration of folic or folinic acid does not diminish the anti-inflammatory potential of this agent, which suggests that other mechanisms of action might be operative. Although no single mechanism is sufficient to account for all the anti-inflammatory activities of methotrexate, the release of adenosine from cells has been demonstrated both *in vitro* and *in vivo*. Methotrexate might also confer anti-inflammatory properties through the inhibition of polyamines.

Keywords-**Methotrexate, Renal damage, Oxidative stress, Neutrophil infiltration, Myeloperoxidase**

I. INTRODUCTION

The biological effects on inflammation associated with adenosine release have provided insight into how methotrexate exerts its effects against inflammatory diseases and at the same time causes some of its well-known adverse effects. These activities contribute to the complex and multifaceted mechanisms that make methotrexate efficacious in the treatment of inflammatory disorders.[1]

Methotrexate (MTX) is a folate antagonist first developed for the treatment of malignancies (Farber et al., 1956) and subsequently used in non-neoplastic diseases as an anti-inflammatory and/or immunosuppressive drug. MTX is currently used in the treatment of refractory rheumatoid arthritis (RA) Weinblatt et al., 1985, Williams et al., 1985, and chronic inflammatory disorders including psoriasis, primary biliary cirrhosis and intrinsic asthma. MTX is also effective in the prophylaxis of acute graft-vs.-host disease (GVHD) used either alone or with cyclosporin A and/or prednisone Storb et al., 1986b, Sullivan et al., 1989, Nash et al., 1992, Chao et al., 1993 or FK506 (Nash et al., 1996). MTX has also been used as an adjunct therapy for persistent mild cardiac allograft rejection (Olsen et al., 1990). Most pharmacological studies have addressed

the use of MTX in cancer chemotherapy, where doses could be escalated up to 30 g/m^2 by subsequent administration of the antidote leucovorin (folinic acid, citrovorum factor).

In autoimmune diseases and allografts, however, MTX dosage is usually in the range of 7–15 mg/week, given as a single dose or in two or three divided doses 12 h apart, orally or by intramuscular injections. It was not known whether the antiproliferative and cytotoxic activities of MTX demonstrated in cancer cells apply to low-dose treatments and MTX is mostly regarded as an anti-inflammatory drug. However, we recently reported that MTX selectively induces apoptosis of activated, but not resting lymphocytes, even after short-term exposure to MTX and subsequent activation in drug-free medium. Those data provided the first evidence for an immunosuppressive activity at low-dose intermittent MTX administration (Genestier et al., 1998).

This review does not intend to be exhaustive. It will focus on the most recent or significant mechanisms of action of MTX as an anti-inflammatory and an immunosuppressive drug.[2]

To assess the impact of certolizumab pegol (CZP), a novel PEGylated anti–tumor necrosis factor, in combination with methotrexate (MTX) on productivity outside and within the home, and on participation in family, social, and leisure activities in adult patients with rheumatoid arthritis (RA).[3]

The aim of this experimental study was to investigate the possible role of nitric oxide (NO) levels, and activities of adenosine deaminase (ADA) and xanthine oxidase (XO) in the pathogenesis of methotrexate-induced nephrotoxicity, and was the effect of caffeic acid phenethyl ester (CAPE), the potent free radical scavenger, in decreasing the toxicity. A total of 19 adult male rats were divided into three experimental groups, as follows: control group, MTX-treated group, and MTX+CAPE treated group. MTX were administered intraperitoneally (i.p.) with 20 mg/kg for single dose. CAPE was administered i.p. with a dose of 10 μmol//kg once daily for 7 days. The injection of MTX induced a significant increase in the activities of ADA and XO, and NO levels in renal tissue of rats ($p < 0.0001$). Co-treatment with CAPE caused a significantly decrease activities of ADA and XO, and the levels of NO in renal tissue ($p < 0.0001$). The results of this study revealed that NO, XO and ADA may play an important role in the pathogenesis of MTX-induced oxidative renal damage. CAPE may have protective potential in this process and it will become a promising drug in the prevention of this undesired side effect of MTX.[4]

This study deals with individual and species variations in the converting activity of methotrexate (MTX) to 7-hydroxymethotrexate in animals and humans. When MTX 7-hydroxylase was assayed in six human liver cytosols, a 48-fold range of intersubject variation of the activity was observed. The variations were correlated to the concentrations of aldehyde oxidase activity in human subjects assayed with benzaldehyde as a substrate. Species differences of liver MTX 7-hydroxylase activity were also observed. The activity was highest in rabbits, followed by rats, hamsters, and monkeys but was undetectable in dogs. Strain differences of MTX 7-hydroxylase activity based on aldehyde oxidase activity were also observed in rats and mice. The results suggest that aldehyde oxidase functions as MTX 7-hydroxylase in livers of animals and humans, and

the observed differences of MTX 7-hydroxylase activity are due to variations in the amount of aldehyde oxidase present.[5]

Nephrotoxicity is one of the adverse side effects of methotrexate (MTX) chemotherapy. The mechanism of renotoxicity of MTX is not fully understood. It is essential to understand the mechanism of nephrotoxicity of MTX in order to diminish the side effects and hence maximize the benefits of chemotherapy. *Objectives:* The aim of the study was to verify whether oxidative stress and neutrophil infiltration play a role in MTX-induced renal damage using a rat model. *Methods:* Adult male rats were administered MTX at the dose of 7 mg/kg body weight intraperitoneally for 3 consecutive days and sacrificed 12 or 24 h after the last dose. Vehicle-treated rats served as controls. The kidneys were removed and used for light microscopic and biochemical studies. Myeloperoxidase activity, a marker of neutrophil infiltration was measured in kidney homogenates along with the markers of oxidative damage including protein carbonyl content, protein thiol and malondialdehyde. The activities of the antioxidant enzymes, namely glutathione peroxidase, glutathione S-transferase, superoxide dismutase and catalase, were also assayed. *Results:* MTX treatment induced damage to the glomeruli and tubules. Plasma creatinine levels in the MTX-treated rats were significantly elevated compared with controls. A significant increase in myeloperoxidase activity ($p < 0.05$) was observed in the kidneys of MTX-treated rats. Protein carbonyl content and malondialdehyde, sensitive and reliable markers of oxidative damage to proteins and lipids, respectively, were significantly elevated ($p < 0.01$) in the kidneys of MTX-treated rats compared with controls. The activities of the antioxidant enzymes, namely, superoxide dismutase and glutathione peroxidase, were significantly elevated ($p < 0.01$ and $p < 0.05$, respectively) in kidneys of rats following MTX treatment. *Conclusion:* The results of the present study provide evidence for the role of neutrophil infiltration and oxidative stress in MTX-induced renal damage. Administration of inhibitors of myeloperoxidase or scavenging hypochlorous acid, the product of myeloperoxidase, by supplementation with antioxidants as an adjuvant therapy may be promising in alleviating the renal side effect of MTX.[6]

Methotrexate (MTX), a folic acid antagonist, is widely used as a cytotoxic chemotherapeutic agent. MTX-associated neurotoxicity is an important clinical problem. The aim of this study was to investigate the role of caffeic acid phenethyl ester (CAPE) on cerebellar oxidative stress induced by MTX in rats. A total of 19 adult male rats were divided into three experimental groups as follows: MTX group (MTX treated), MTX+CAPE group (MTX+CAPE treated), and control group. MTX was administered intraperitoneally (i.p.) with a single dose of 20 mg kg^{-1} on the second day of experiment. CAPE was administered i.p. with a dose of 10 µmol kg^{-1} day^{-1} for 7 days. Malondialdehyde (MDA) levels and activities of superoxide dismutase (SOD) and catalase (CAT) were determined in cerebellar tissue of rats. MTX caused to significant increase in MDA levels (an important marker of lipid peroxidation) in the MTX group compared with the controls ($p = 0.006$). CAPE significantly reduced the MTX induced lipid peroxidation in the MTX+CAPE group compared to the MTX ($p = 0.007$). The activities of SOD and CAT were significantly increased in the MTX group when compared with the control group ($p = 0.0001$, $p = 0.004$, respectively). The increased activities of these enzymes were significantly reduced by CAPE treatment ($p = 0.004$, $p = $

0.034, respectively). As a result, CAPE may protect from oxidative damage caused by MTX treatment in rat cerebellum.[7]

Inefficient polyglutamylation is a mechanism of resistance to methotrexate (MTX) in childhood T-lineage acute lymphoblastic leukemia (T-ALL) and in acute myeloid leukemia (AML) in comparison with childhood c/preB-ALL. We analyzed the profile of MTX polyglutamylation in childhood c/preB-ALL, T-ALL, and AML (n = 45, 15, and 14, respectively), the activity of the MTX-polyglutamate synthesizing enzyme folylpolyglutamate synthetase (FPGS) (n = 39, 11, and 19, respectively) and of the MTX-polyglutamate breakdown enzyme folylpolyglutamate hydrolase (FPGH) (n = 98, 25, and 34, respectively). MTX-Glu$_{4-6}$ accumulation after 24 hours exposure to 1 μmol/L [^{3}H]-MTX in vitro was lower in T-ALL (threefold) and AML (fourfold) compared with c/preB-ALL ($P \leq .001$). The FPGS activity was twofold lower in T-ALL and AML than in c/preB-ALL samples ($P < .01$). FPGH activity was not different between c/preB-ALL and T-ALL, but threefold higher in AML ($P < .001$). FPGS, FPGH, and the ratio FPGS/FPGH were correlated with MTX-Glu$_{4-6}$ accumulation ($r = .49$, $r = -.34$ and $r = .61$, respectively). Multivariate analysis showed that FPGS, but not FPGH, was an independent contributor for MTX-Glu$_{1-6}$ accumulation, but not for MTX-Glu$_{4-6}$ accumulation. In conclusion, low FPGS activity is associated with low accumulation of MTX-Glu$_{4-6}$ in T-ALL and AML. For the group of AML as compared with the group of ALL, a high FPGH activity can play an additional role.

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NOWADAYS, CHILDHOOD acute lymphoblastic leukemia (ALL) has an event-free survival of 70%, which is in striking contrast to that for childhood acute myeloid leukemia (AML), which is approximately 40%.1 Methotrexate (MTX) is an important drug in the treatment of childhood ALL,2 but clinical trials have shown that AML patients have low response rates to MTX, similar to the response rates of clinically resistant relapsed ALL patients (summarized by Bender3). Although in these trials, the dosages of MTX administered were low and the number of patients was limited, MTX is not included in standard therapy protocols for AML.

Little is known about the mechanisms of MTX resistance in pediatric AML. Studies in a limited number of samples from mainly adult AML patients have suggested that intrinsic MTX resistance can be ascribed to lower polyglutamylation capacities of AML cells compared with ALL cells.4-7 Inefficient polyglutamylation will result in a decrease of MTX-polyglutamates, especially MTX-Glu$_{4-6}$, which are preferentially retained intracellularly and provoke inhibition of thymidylate synthase and enzymes involved in purine nucleotide synthesis.8

For childhood ALL, accumulation of MTX and MTX-polyglutamates was correlated with event-free survival9 and with short-term antileukemic effect.10 Less efficient polyglutamylation of MTX was observed in leukemic blasts from children with T-ALL compared with c/preB-ALL, both in vitro11 and in vivo.12,13

The polyglutamylation defect in T-ALL and AML cells was associated with a lower activity of folylpolyglutamate synthetase (FPGS), the enzyme that catalyzes the polyglutamate chain formation, as compared with c/preB-ALL cells.14 The difference in FPGS activity was not associated with a decreased FPGS mRNA expression,15 but with a lower affinity of FPGS for MTX in AML cells.16

Besides decreased synthesis of the glutamate side chain, polyglutamylation defects may also be caused by increased breakdown of polyglutamates by folylpolyglutamate hydrolase (FPGH).17Recently, a number of reports have demonstrated a possible role for FPGH in contributing to MTX resistance in experimental model systems. In human soft tissue sarcoma cell lines, intrinsic MTX resistance resulting from impaired polyglutamylation18 could be explained by a higher FPGH activity compared with MTX responsive cell lines.19 H35 rat hepatoma and human CCRF-CEM leukemia cell lines have recently been reported to acquire MTX resistance by increasing FPGH activity compared with the MTX sensitive parental cell lines.20,21

A role for FPGH in clinical resistance to MTX has not been established, although in a recent report including eight ALL and seven AML samples, Longo et al.22 reported that the ratio FPGS/FPGH was better at predicting the amount of MTX-polyglutamates accumulated to determine either activity alone. In the present study, we examined the FPGS and FPGH activities as well as the polyglutamylation profile in childhood leukemia samples and found evidence that inefficient polyglutamylation was associated with a high FPGH activity in AML, but not in T-ALL. [8]

Methotrexate is an effective anticancer and immunosuppressive agent. However, nephrotoxicity is one of the complications of its use. On the other hand, curcumin, a naturally occurring polyphenolic compound, is reported to have antioxidant and anti-inflammatory properties. Those two properties are likely to prevent methotrexate-induced nephrotoxicity. The aim of this study is to evaluate the possible protective effect of curcumin against methotrexate-induced nephrotoxicity and delineate various mechanism(s) underlies this effect in rats. Nephrotoxicity was induced in Wistar rats by intraperitoneal administration of methotrexate (7 mg/kg/day) for three consecutive days. Curcumin administration in methotrexate-intoxicated rats resulted in nephroprotective effects as evidenced by the significant decrease in levels of serum creatinine and urea as well as renal malondialdehyde, nitric oxide, and tumor necrosis factor-α with a concurrent increase in renal glutathione peroxidase and superoxide dismutase activities compared to nephrotoxic untreated rats. Additionally, immunohistochemical analysis demonstrated that curcumin treatment markedly reduced cyclooxygenase-2 expression. Histopathological examination confirmed the protective effects of curcumin. In conclusion, curcumin protected rats from methotrexate nephrotoxicity, at least in part, through its antioxidant and anti-inflammatory activities.[9]

Methotrexate (MTX) is an effective antineoplastic drug associated with wide organ toxicity. Accumulating evidence implicates oxidative stress to be a leading underlying mechanism of MTX-induced neurotoxicity. The study explores antioxidant potential of virgin coconut oil (VCO) or *Moringa oleifera* seed oil (MOO) in MTX-induced oxidative stress-mediated cerebral neurotoxicity and inflammation in rats. Rats treated with VCO or MOO (5 ml/kg bw) for 17 days were administered MTX (20 mg/kg, intraperitoneally) on day 14 only. Cerebral activities of acetylcholinesterase, antioxidant enzymes, lipid peroxidation, reduced

glutathione and nitric oxide levels as well as cytokines were evaluated. MTX-induced neurotoxic alterations were significantly abrogated by MOO and VCO supplementation via inhibition of cholinesterase, oxidative stress, and anti-inflammatory mechanisms. VCO and MOO showed comparable antioxidant potentials with the standards in DPPH and FRAP assays. VCO and MOO are promising natural oils for modulating MTX neurotoxicity in cancer patients. Methotrexate chemotherapy induces neurotoxicity in cancer patients, and this is a source of worry for clinicians. This study reports, for the first time, the beneficial health effects of functional food oils, *Moringa oleifera* seed oil, and virgin coconut oil against anticancer drug methotrexate-induced cerebral neurotoxicity. Supplementation of these natural oils may be beneficial in the prevention of cerebral neurotoxic side effect in cancer patients undergoing methotrexate chemotherapy.[10]

This review presents recent data supporting the methotrexate (MTX) mechanisms of action, which are likely to account for its anti-proliferative and immunosuppressive effects in rheumatoid arthritis (RA). The effects of MTX *in vivo* may be mediated by reducing cell proliferation, increasing the rate of apoptosis of T cells, increasing endogenous adenosine release, altering the expression of cellular adhesion molecules, influencing production of cytokines, humoral responses and bone formation. Several reports indicate that the effects of MTX are influenced by genetic variants, specific dynamic processes and micro-environmental elements such as nucleotide deprivation or glutathione levels. The challenge for the future will be linking biological and genetic markers relevant to the response to MTX in RA.[11]

Methotrexate is a folic acid antagonist known to be teratogenic in humans. Several cases of congenital malformations after fetal exposure to methotrexate have been published, resulting in the establishment of the 'fetal methotrexate syndrome'. However, it is unclear which congenital anomalies can truly be attributed to methotrexate exposure. The objective of this review is to delineate a consistent phenotype of the fetal methotrexate syndrome. We performed a systematic review that yielded 29 cases of (congenital) anomalies after *in utero* exposure to methotrexate and compared their malformation pattern to that of children and fetuses with congenital anomalies in general. Statistically significant higher proportions of microcephaly, craniosynostosis, tetralogy of Fallot, pulmonary valve atresia, limb reduction defects and syndactyly were found in the methotrexate group, indicating that these congenital anomalies are truly part of the fetal methotrexate syndrome. These results aid clinicians with diagnosing fetal methotrexate syndrome.[12]

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