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The Evolution of Methotrexate Applications: A Multidimensional Approach to Treating Various Disorders – A Literature Review

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ABSTRACT

Introduction

Methotrexate (MTX) is a key cytostatic and immunosuppressive drug widely used in oncology and autoimmune disease treatment. It acts as a folic acid antagonist, inhibiting DNA synthesis and cell division[1]. This review explores new applications of MTX beyond its traditional role.

MTX shows potential in treating neuromyelitis optica spectrum disorders (NMOSD) as an alternative when other immunosuppressants are ineffective[9]. In ophthalmic diseases like non-infectious uveitis and scleritis, it reduces inflammation and corticosteroid dependence[11]. MTX is also used for unruptured ectopic pregnancy as a non-surgical option in select cases[10], with meta-analyses confirming its effectiveness, though resolution times may vary[10]. Additionally, emerging evidence suggests MTX may help prevent cardiovascular diseases in patients with inflammatory conditions by improving atherosclerosis biomarkers[12].

Objective

This review aims to provide an overview of MTX's expanding applications, pharmacological properties, and the importance of therapy monitoring.

Current State of Knowledge

MTX is well-established in both low doses for autoimmune diseases like rheumatoid arthritis and high doses for chemotherapy[1,4]. While effective, it has significant toxicity risks, particularly at high doses, necessitating therapeutic drug monitoring (TDM) to minimize adverse effects like nephrotoxicity and mucositis[1,4,7].

Methods

This review synthesizes data from review articles, original studies, meta-analyses, and clinical guidelines[1,16,18,19,21], covering MTX's mechanisms, pharmacokinetics, clinical applications, toxicity management, and TDM role.

Conclusions

MTX remains a cornerstone in disease treatment due to its broad pharmacological spectrum. Effective therapy requires proper dosing, toxicity management, and monitoring strategies. Ongoing research seeks to optimize MTX use while minimizing risks[1,22].

Keywords: methotrexate, applications, autoimmune diseases, neurological disorders, ocular inflammation, ectopic pregnancy, cardiovascular disease.

History and General Use of Methotrexate

Methotrexate (MTX) is one of those drugs that form the cornerstone of pharmacotherapy, with a long and rich history of applications both in cancer treatment (particularly at high doses) and in the therapy of autoimmune diseases (at low doses) [1]. Its approval by the FDA in 1953 solidified its position as a key drug in the treatment of many conditions [1,20].

The primary mechanism of action of MTX is its activity as an antagonist of folic acid [1,20]. Structurally similar to folic acid, this drug competitively inhibits the enzymes of the folate pathway, which plays a significant role in DNA synthesis and cell division [1]. It is important to emphasize that the inhibition of dihydrofolate reductase (DHFR) by MTX is significantly stronger (approximately 10,000 times) than by folic acid itself, leading to the effective blocking of cell division and exerting cytostatic and anti-inflammatory effects [1].

Due to its anti-inflammatory and immunosuppressive properties, MTX has found broad application in the therapy of many inflammatory diseases and immunological disorders, such as rheumatoid arthritis (RA) and psoriasis [1,20]. In the treatment of RA, low doses of MTX (typically 7.5-25 mg/week) are used [1], often supplemented with folic acid or folinic acid to reduce side effects [1]. Notably, in RA, in addition to inhibiting the folate pathway, it is believed that suppression of the JAK/STAT pathway also contributes to the anti-inflammatory and immunosuppressive actions of MTX [1]. Studies in the context of RA also analyze the

effect of MTX on the Th1/Th2 and Th17/Treg lymphocyte balance and on the expression of CD73 [2]. The anti-inflammatory mechanism of MTX may also be related to increased adenosine release at sites of inflammation [3].

The first documented use of MTX was in the chemotherapy of acute lymphoblastic leukemia (ALL) [1]. Currently, MTX is used in a wide range of doses (from 3 to 88,000 mg/m^2) via different routes of administration [1]. In cancer treatment, including ALL, Burkitt's lymphoma, non-Hodgkin's lymphoma (NHL), and osteosarcoma, high-dose methotrexate (HDMTX) plays a key role, defined as doses exceeding 500 mg/m^2 administered intravenously [1,25]. HDMTX is so crucial in oncology that it was included in the WHO Essential Medicines List for cytostatic drugs in 2021 [1].

However, the use of high doses of MTX is associated with a significant risk of toxicity [1]. Before starting MTX therapy, it is important to consider factors that can exacerbate its side effects. These factors include folate deficiency, hyperhomocysteinemia, hypoalbuminemia, increased mean corpuscular volume (MCV), and renal insufficiency. Special caution should be exercised when treating elderly patients with methotrexate, requiring therapy under strict supervision. Monitoring MTX concentrations allows for optimal dosing and maintaining the drug within a safe and effective therapeutic range, as well as adjusting the dose of leucovorin (LV), the antidote, which works by competitively binding to MTX cell transporters and restoring the pool of reduced folates [1].

Monitoring MCV values during MTX therapy can serve as a useful indicator of drug toxicity and help assess its resistance. An increase in MCV during treatment indicates reduced folate reserves in the body and toxic effects of MTX. Moreover, one of the studies conducted by In-Woon Baek et al. showed an increased resistance to MTX in patients with high baseline MCV [1,17].

In cases of delayed MTX excretion and the occurrence of nephrotoxicity, glucarpidase can be used for the rapid metabolism of MTX into less toxic metabolites [1]. During HDMTX therapy, routine monitoring of creatinine levels is performed to detect nephrotoxicity early [1]. Collaboration among physicians, nursing staff, laboratory diagnosticians, clinical pharmacists, and the patient is essential for the safe and effective management of HDMTX therapy with reduced side effects [1].

Potential drug interactions with MTX, which may affect its pharmacokinetics and toxicity, should also be considered, further emphasizing the importance of monitoring. Additionally, studies identify genetic factors, such as MTHFR gene variants, which may be linked to HDMTX-induced toxicity, particularly in patients with osteosarcoma [1,7,8]. The concentration of the metabolite 7-hydroxymethotrexate (7-OH-MTX) is also analyzed in the context of kidney toxicity in children treated with HDMTX [1,6].

An important aspect during MTX therapy is the concurrent supplementation of folic acid, which does not affect the efficacy of the drug but helps prevent its toxic effects [20].

The aim of this paper is to review the pharmacological properties of MTX, with particular emphasis on its pharmacokinetic variability, which justifies the need for therapeutic monitoring of its concentrations, especially in the context of high-dose therapy in oncology, and to discuss current clinical recommendations and management strategies aimed at maximizing treatment effectiveness while minimizing the risk of toxicity [1].

Mechanism of Action of Methotrexate

Methotrexate (MTX) is a broad-spectrum drug used both in the treatment of autoimmune diseases, such as rheumatoid arthritis (RA), and in chemotherapy for cancer. Its effectiveness arises from various mechanisms of action [2,20].

Folic Acid Antagonism: MTX is an antagonist of folic acid. Due to its structural similarity to folic acid, it competitively inhibits enzymes involved in the folate pathway, including dihydrofolate reductase (DHFR) [1,3,24]. Inhibition of DHFR prevents the conversion of dihydrofolate to tetrahydrofolate (THF), which is essential for purine and thymidine biosynthesis, crucial for DNA synthesis and cell division. As a result, MTX impedes cell division and inhibits cell proliferation. It is noteworthy that MTX has approximately 10,000 times stronger affinity for DHFR than folic acid itself [1,20].

Polyglutamation of Methotrexate: Once inside the cells, primarily via the reduced folate carrier (RFC1), MTX is converted into active metabolites—methotrexate polyglutamates (MTX-PGs) [1,3]. This process is catalyzed by folypolyglutamate synthase (FPGS). MTX-PGs are forms of the drug that cannot exit the cells and exhibit a prolonged inhibitory action

on DHFR [1]. MTX-PGs also contribute to the accumulation of adenosine, enhancing the anti-inflammatory effects of MTX [4]. The degree of polyglutamation and binding to DHFR significantly affects drug accumulation in cells [1].

Promotion of Adenosine Accumulation: MTX increases extracellular adenosine levels [2,3]. It inhibits enzymes involved in adenosine metabolism, such as aminoimidazolecarboxamide ribonucleotide formyltransferase (AICAR) [2]. Adenosine, by binding to adenosine receptors (especially A2A), exerts anti-inflammatory and immunosuppressive effects [2,3]. Activation of adenosine receptors inhibits the chemotaxis and adhesion of inflammatory cells [3]. It is believed that suppression of the JAK/STAT pathway is largely responsible for the anti-inflammatory and immunosuppressive mechanism of low-dose MTX used in RA [1].

Regulation of Signaling Pathways: MTX affects various signaling pathways involved in the inflammatory process [2]. It is proposed that at low doses, in addition to inhibiting the folate pathway, MTX exerts its effects through suppression of the JAK/STAT pathway [1,2]. MTX may also increase the expression of cell cycle checkpoint genes by activating JNK [2].

Effect on the Immune System: At low doses used in RA, MTX-PGs act in leukocytes, preventing inflammation and immune-mediated damage [1]. MTX may restore the expression of CD73 on Th1.17 lymphocytes in patients with RA and psoriatic arthritis, which may contribute to its anti-inflammatory effect through adenosine production [2]. MTX also affects the function of regulatory T cells (Tregs), restoring their function in certain inflammatory diseases. However, the impact of MTX on T cell subpopulations can be complex and depend on dose and disease context [2].

Bone Protection: MTX exerts a protective effect on bones in the course of RA. It may inhibit osteoclastogenesis by reducing calcium influx into osteoclast precursors induced by RANKL. MTX influences the balance between RANKL and osteoprotegerin, key regulators of bone remodeling [2].

Pharmacomicrobiology: Studies indicate that gut microbiota may influence the response to MTX treatment in RA. Differences in the gut microbiomes of RA patients may partially determine the bioavailability and response to MTX. Furthermore, MTX can alter the gut microbiota composition in a dose-dependent manner. The concept of precision medicine in

rheumatology takes into account drug-microbe interactions to optimize treatment based on individual microbiological differences among patients [3].

Use of Methotrexate in Selected Diseases

Ectopic Pregnancy (Extrauterine Pregnancy)

Methotrexate treatment is an option for hemodynamically stable women with an unruptured ectopic pregnancy [23], in cases where there is no fetal heartbeat or evidence of bleeding into the peritoneal cavity (hemoperitoneum). Another usual criterion is a low hCG level, typically below 2000 IU/L [10].

A meta-analysis of two randomized clinical trials showed that the success rate of methotrexate treatment (avoiding surgical intervention) was 45.1% (37/82), while in the expectant management group, it was 40.0% (28/70)[10]. The relative risk (RR) of success for methotrexate treatment compared to expectant management was 1.11 (95% CI 0.77–1.62), which was not statistically significant but indicates a trend toward greater effectiveness of methotrexate [10].

The frequency of administering an additional dose of methotrexate was similar in both groups: 22.0% (9/41) in the MTX group and 28.1% (9/32) in the expectant management group (RR 0.78, 95% CI 0.35–1.74) [10].

The average time to resolution (decrease in hCG levels) was also similar in both groups: 19.7 days in the methotrexate group and 21.2 days in the expectant management group [10].

Studies suggest that a lower initial hCG level (<1000 mIU/mL) is associated with a greater likelihood of success with methotrexate treatment. An analysis of the interaction between hCG levels and treatment method showed that an hCG level >1000 compared to ≤1000 decreased the chances of an uninterrupted drop in hCG in both the methotrexate group (HR 0.28, 95% CI 0.12–0.66) and the expectant management group (HR 0.50, 95% CI 0.29–0.84) [10].

The percentage of women requiring additional methotrexate treatment or experiencing adverse effects did not differ significantly between the groups [10].

Non-infectious Uveitis

A retrospective study conducted at Hiroshima University in Japan analyzed the effectiveness of methotrexate (MTX) in treating non-infectious uveitis over a period of more than 6 months. The study involved 35 patients (65 eyes) [11].

The results indicate that methotrexate effectively controlled eye inflammation in this group of patients. After starting MTX treatment, many patients were able to reduce or discontinue systemic corticosteroids, suggesting a steroid-sparing effect of methotrexate [11].

Methotrexate was used for various forms of non-infectious uveitis, including anterior, posterior, and diffuse types of inflammation. The effectiveness of low-dose weekly methotrexate was also demonstrated in the treatment of chronic non-infectious uveitis associated with Vogt-Koyanagi-Harada disease (VKH) [11].

In a randomized clinical trial, the efficacy of methotrexate was compared with mycophenolate mofetil in treating non-infectious uveitis [11].

Neuromyelitis Optica Spectrum Disorders (NMOSD):

As mentioned earlier, methotrexate is considered a therapeutic option in NMOSD (Neuromyelitis Optica Spectrum Disorder) when first- and second-line drugs, such as azathioprine (AZA) and mycophenolate mofetil (MMF), are poorly tolerated or cause serious side effects. In such cases, cyclophosphamide has also proven to be an effective drug for both acute attacks and chronic disease progression in NMOSD [9].

The authors of the study suggest a more flexible approach to NMOSD treatment, recommending that therapy should not be limited solely to traditional first- or second-line drugs. Instead, patient response to treatment, including drug efficacy, safety, and tolerance, should be considered. As a result, drugs with different mechanisms of action may be considered as first-line treatments, depending on the patient's profile [9].

Behçet's Disease

Methotrexate is listed as one of the traditional immunosuppressive treatments, alongside corticosteroids, colchicine, azathioprine, and cyclosporine. It is noted that the treatment of

Behçet's disease depends on the patient's age, sex, affected organs, and clinical course, meaning that therapy must be individualized [13].

Methotrexate is mentioned in the context of dosing, where the typical dose is 0.3 mg/kg/week, with the possibility of increasing to 20-25 mg/week, along with the need for folic acid supplementation to reduce toxicity. Although in recent years TNF inhibitors have become an important therapeutic advancement in treating severe and refractory cases of Behçet's disease, methotrexate still plays a significant role in therapy, particularly in treating mucocutaneous and joint manifestations. Methotrexate remains part of the therapeutic arsenal in Behçet's disease, though its role may be more significant in milder forms of the disease or in combination with other drugs, with biologic agents such as TNF inhibitors playing a primary role in more severe cases [13].

Inflammatory Bowel Diseases (IBD)

Methotrexate is widely used in the treatment of Crohn's disease [14,15]. A study demonstrated the superiority of intramuscular methotrexate at a dose of 25 mg/week compared to placebo in inducing remission in steroid-dependent patients with Crohn's disease who were resistant to steroid withdrawal. By week 16, more than one-third of patients in the methotrexate group achieved remission ($p=0.025$) [15].

The authors summarized various studies, including randomized controlled trials (RCTs) and retrospective studies, assessing the efficacy of methotrexate in Crohn's disease. They emphasize that parenteral methotrexate at higher doses (25 mg/week) appears more effective than low oral doses in inducing remission, as confirmed by some RCTs. Regarding remission maintenance, parenteral methotrexate (15 mg/week) seems effective in maintaining steroid-induced remission in Crohn's disease, with data controlled for at least one year [15].

Meta-analyses confirm a favorable number needed to treat (NNT) for methotrexate in maintaining remission, comparable to thiopurines [15]. Another study cites the North American Crohn’s Study Group Investigators' evaluation of the effectiveness of oral methotrexate in maintaining remission in Crohn's disease, which did not show a significant difference compared to placebo. The authors refer to the higher bioavailability of methotrexate administered subcutaneously compared to oral administration in rheumatoid arthritis patients, which may also be effective for IBD. They also cite the study by Arora et al.,

which did not demonstrate the effectiveness of oral methotrexate in inducing remission in Crohn's disease. Similarly, Oren et al.'s study did not show an advantage of oral methotrexate over placebo in treating chronic active Crohn's disease [14]. However, the authors refer to a meta-analysis by Colman et al., which suggests that methotrexate is effective in treating Crohn's disease in children. They also mention a study by Egan et al. that evaluated various doses of methotrexate in treatment-resistant Crohn's disease and ulcerative colitis, focusing mainly on pharmacokinetics. The authors cite the study by Mate-Jimenez et al., which showed that 6-mercaptopurine or methotrexate added to prednisone induce and maintain remission in steroid-dependent inflammatory bowel disease [15].

The authors list numerous retrospective studies and single-center experiences regarding the effectiveness and safety of methotrexate in Crohn's disease, including in patients resistant to anti-TNF-α therapy. They note the study by Laharie et al., which compared mucosal healing with methotrexate, azathioprine, and infliximab in Crohn's disease. They also discuss combinations of methotrexate with biologic drugs such as infliximab and adalimumab, often aimed at reducing the immunogenicity of biologics and increasing their efficacy, although some studies did not show additional benefits of such combinations with ustekinumab and vedolizumab[15]. In summary, methotrexate plays a significant role in treating Crohn's disease, particularly in inducing remission in steroid-dependent patients (preferred parenteral form at higher doses) and maintaining remission, as well as potentially in combination with biologic drugs to reduce immunogenicity. Its position is continually reassessed in light of newer therapies.

Conclusion

Methotrexate (MTX) is a cornerstone of pharmacotherapy in many medical fields, evolving from its initial use as an aggressive cytostatic agent in oncology to a versatile drug with immunosuppressive and anti-inflammatory properties.

In oncology, particularly in the treatment of lymphoid cancers, high doses of methotrexate (HDMTX) are used, defined as doses exceeding 500 mg/m² of body surface area. Due to the significant toxicity of HDMTX, therapeutic drug monitoring (TDM) of methotrexate concentration in blood is essential, along with leucovorin (LV) therapy to minimize adverse effects. In cases of nephrotoxicity or high methotrexate levels, glucarpidase is used to accelerate the metabolism of MTX into less toxic metabolites. HDMTX treatment requires

close collaboration between the medical team and the patient, as well as proper hydration and urinary alkalinization. Prior to starting HDMTX therapy, renal function must be evaluated. Monitoring MTX levels helps optimize LV dosing and reduce the risk of side effects [1].

Methotrexate is also used in the treatment of autoimmune diseases, such as rheumatoid arthritis (RA), where low doses (7.5–25 mg/week) are administered. In RA, MTX acts anti-inflammatory and immunosuppressively, inhibiting, among others, the folate pathway and the JAK/STAT pathway [1]. To alleviate the side effects of low-dose MTX, folic acid supplements are provided [1]. Methotrexate in RA can be used as monotherapy or in combination with other drugs, including biologic disease-modifying antirheumatic drugs (bDMARDs), which can lead to better therapeutic outcomes without increasing toxicity [5]. Early treatment of aggressive RA using MTX in combination with other drugs may lead to remission and halt radiological progression [5].

Moreover, methotrexate is used in the treatment of uncomplicated ectopic pregnancy in hemodynamically stable women with hCG < 5000 IU/L, no fetal heart activity, and no signs of rupture of the ectopic pregnancy [exact dosage information is not provided in the sources]. However, for tubal ectopic pregnancy with hCG < 2000 IU/L, evidence of the effectiveness of MTX compared to expectant management is considered to be of very low certainty [10].

Methotrexate is also used in the treatment of Crohn's disease, where it is a first-line drug [1]. Studies show the effectiveness of methotrexate in inducing and maintaining remission in adult patients with active Crohn's disease in various intestinal locations. It can be used both as monotherapy and in combination with other drugs [15].

Nephrotoxicity is a significant adverse effect of MTX, especially at high doses, due to the potential precipitation of the drug and its metabolite 7-OH-MTX in renal tubules [1]. Monitoring renal function before and during MTX treatment is crucial, and early detection of nephrotoxicity symptoms allows for rapid intervention [1]. It is suggested that monitoring the maximum concentration (Cmax) of MTX after infusion may be beneficial in identifying the risk of nephrotoxicity at an early stage [6].

In summary, methotrexate is a versatile drug used in many areas of medicine. Essential for the safe and effective use of MTX, especially at high doses, are drug concentration monitoring, renal function assessment, and close collaboration within the therapeutic team [1].

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