Therapeutic Applications and Challenges of a Widely Used Antifolate Drug

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Abstract

Introduction and purpose: Methotrexate (MTX) is an antifolate metabolite that interferes with the synthesis and repair of DNA and with cell replication. It has anti-inflammatory and immunomodulatory properties and was first used in 1951 for the treatment of RA and psoriasis. Methotrexate (4-amino-10-methylfolic acid, MTX) is a popular disease-modifying drug for the treatment of rheumatoid arthritis, psoriasis and leukaemia. MTX is an antagonist of folic acid, primarily through inhibition of the proliferative effect. MTX and its metabolites are associated with many serious adverse effects, including gastrointestinal and hepatic toxicity, myelosuppression, stomatitis, etc. Taking methotrexate may contribute to a reduction in the quality of life for patients. Additionally, long-term use of methotrexate requires regular monitoring, which can be burdensome and stressful for patients. In this context, different approaches have been used to improve the therapeutic properties of MTX and to reduce its adverse effects. The long history of the drug has made it possible to assess its efficacy and to collect data on adverse events, including potentially life-threatening adverse events. This article aims to provide a literature-based overview of current knowledge about methotrexate in clinical practice.

Materials and Methods: Review and summary of research studies available in open-source format on Google Scholar, PubMed.

Conclusion: The result is a picture of methotrexate as a drug that is used for many conditions, but is associated with many side effects that can be a significant problem for patients and healthcare professionals.

Keywords: methotrexate; overdose; rheumatoid arthritis; toxicity; patient challenges;

Introduction

As a folate antagonist with unique anti-inflammatory, anti-proliferative and anti-metabolic effects, MTX can significantly reduce the levels of pro-inflammatory cytokines by regulating the infiltration of a variety of immune and other inflammatory cells, which include
the neutrophils, as well as monocytes, mastocytes, Th (helper) cells and B cells in the RA synovium [1]. MTX inhibits the synthesis of purines and pyrimidines by inhibiting dihydrofolate reductase (DHFR) and thymidylate synthase (TYMS), which are involved in catalysing the formation of thymine residues, thereby inhibiting the formation of deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and protein, resulting in the suppression of the aforementioned inflammatory cell proliferation. It is therefore used in many diseases such as RA, psoriasis, juvenile idiopathic arthritis, pharmacological treatment of ectopic pregnancy, multiple sclerosis, systemic lupus erythematosus and many other connective tissue diseases. In the treatment of autoimmune inflammatory diseases, MTX is usually given orally as a single weekly dose. In clinical practice, therapy is started at a dose of 10 mg/week and escalated by 5 mg every 2-4 weeks to a maximum dose of 20-30 mg/week, depending on clinical response or intolerance [1, 2]. The use of parenteral MTX, especially in the form of a subcutaneous (SC) injection, has gained a great amount of interest lately and is of superior benefit when compared to the oral administration.

**Application of Methotrexate**

Methotrexate is a medication with immunosuppressive and cell proliferation-inhibiting properties. Low doses of methotrexate, 10-25mg per week, are used in the treatment of autoimmune diseases. High-dose therapies above 500mg/m² body surface area are used in the treatment of cancerous diseases [3]. Methotrexate is also used in the treatment of ectopic pregnancy. The medication is available in oral form or as a solution for injections, which can be administered subcutaneously, intramuscularly, intravenously, intra-arterially, and intrathecally. *For patients with renal impairment, it is necessary to adjust the dose based on their creatinine clearance. The medication should not be administered when this value is below 30% [4]. In patients with liver function disorders, methotrexate should not be used when the bilirubin concentration exceeds 5 mg/dl [5].

Patients undergoing methotrexate therapy should be closely monitored for the occurrence of life-threatening complications. The performed tests should include blood morphology, aminotransferase levels, and creatinine clearance. Initially, monitoring should be conducted weekly for the first 4 weeks of treatment, after which a reduction in frequency may be considered [6].

Examples of therapy using methotrexate include:

**Rheumatoid arthritis:** Usually, doses of 7.5-25mg per week orally or intravenously 15-25mg per week are used. The recommended approach is to use the lowest effective dose [1].

**Juvenile idiopathic arthritis:** The medication can be used in children over 3 years of age. It is recommended to administer 10-15mg/m² of body surface area per week subcutaneously or intramuscularly [7].

**Severe psoriasis, Psoriatic arthritis, Systemic lupus erythematosus:** The therapy starts with a dose of 15mg once a week orally, up to a maximum of 25mg once a week [8].

**Crohn's disease:** A dose of 15mg per week is used intramuscularly to maintain disease remission [9].

**Ectopic pregnancy:** Single-dose protocol - administration of 50 mg/m² body surface area intramuscularly, assessment of the effect in terms of a decrease in beta-hCG concentration in the blood at 4 and 7 days after administration of the medication; if there is no decrease of 15%,
a second dose is administered. This protocol does not require folic acid supplementation, and the risk of adverse effects is lower. Multidose protocol - administration of 1 mg/kg body weight on days 1, 3, 5, 7 of therapy alternately with administration of folic acid 0.1 mg/kg body weight on days 2, 4, 6, 8. This protocol shows higher efficacy in studies but is less tolerated and currently less frequently used.

Acute lymphoblastic leukemia: doses are calculated per square meter of the patient's body surface area. An example treatment protocol involves administering 20-40mg/m^2 of methotrexate per week in combination with 6-mercaptopurine. The MTX dose should be gradually titrated under the control of white blood cell count or neutrophil count in a peripheral blood smear, to determine the lowest effective dose allowing achievement of the desired bone marrow suppression [10].

**Pulmonary toxicity**

An estimated 0.3-11.6% of patients treated with methotrexate develop pulmonary complications [11]. Early diagnosis of interstitial lung disease is essential to prevent the development of irreversible pulmonary fibrosis. MTX pneumonitis is thought to be caused by a variety of mechanisms, including hypersensitivity, direct drug toxicity to lung tissue, or immunosuppressive effects leading to recurrent viral or other infections. There is evidence that MTX activates MAPK pathways and modulates the expression of cytokines that can trigger the lung inflammatory response [12]. MT pneumonia usually has an acute or subacute course and occurs in the first year of treatment, although cases of later presentation have been described [13,14]. Men over 60 years of age and cigarette smokers are more likely to develop interstitial pneumonia associated with methotrexate treatment. MT-related interstitial lung disease (ILD) is more often diagnosed in groups of patients with diabetes, chronic kidney disease and hypoalbuminemia [15]. Common pulmonary symptoms include dry cough, shortness of breath and pleuritic pain, while systemic symptoms include fatigue, fever and malaise [16]. Other diseases need to be excluded before MTX pulmonary toxicity is diagnosed. The criteria established by Carson are valuable for diagnosis. Treatment includes stopping methotrexate and starting systemic corticosteroids [15]. In most cases, pulmonary complications are transitory, but some patients may develop pulmonary fibrosis, which causes a permanent reduction in respiratory parameters [11].

**Nephrotoxicity**

Methotrexate can accumulate metabolites in the renal tubules, which is the basis of its renal toxicity [17]. Approximately 90% of the dose administered to is in the form of urinary excretion. Serum methotrexate concentrations increase with renal impairment [6]. In general, older age, male gender, higher doses of MTX, lower baseline renal function and the use of certain antibiotics have been associated with a higher risk of MTX-mediated nephrotoxicity. Kidney damage caused by methotrexate is manifested as non-oliguric acute renal failure with a sudden rise in the plasma creatinine level. Acute kidney injury (AKI) occurred in 2%-13% of patients treated with MTX [18]. Intravenous fluid rehydration, urine alkalinisation and leucovorin rescue are the most common and current treatments for methotrexate-induced nephrotoxicity. Most importantly, leucovorin counteracts methotrexate by promoting the continued production of folate, which mitigates potential adverse effects such as bone marrow suppression, liver toxicity and gastrointestinal side effects [19].

**Haematological toxicity**
Patients treated with MTX, especially at higher doses (above 500mg/m2), may suffer from haematological damage such as myelosuppression, leukopenia, neutropenia and megaloblastic anaemia [6]. The kidneys are responsible for eliminating MTX from the body. Higher doses of the drug may increase the risk of side effects, although they can provide more benefit in therapy. More than 90% of the tubules in the kidney excrete MTX. Any renal insufficiency may lead to poor removal of MTX, increasing toxicity to the bone marrow - as was shown in a case study published in 2009 [20]. Patients may be afraid of the side effects, which can be fatal; and up to 25% of treatments are discontinued [21]. One of the side effects of MTX is pancytopenia, which occurs in up to 5% of patients on low-dose MTX treatment. It happens because MTX damages bone marrow, which is then unable to produce blood cells [22]. Pancytopenia is difficult to predict, because can occur unexpectedly during treatment. Thrombocytopenia develops in about 0,3-0,7% of RA patients treated with MTX [23]. Many studies have been carried out to explain this MTX-induced bone marrow defect. Some studies have shown that toxicity is caused by excessive unbound extracellular MTX. Another source showed an association between MTX-related neutropenia and patients' socio-cultural status, cognitive abilities and distress [24]. Recent research has shown that MTX-induced thrombocytopenia is caused by increased mitochondrial dysfunction resulting in platelet apoptosis [25]. However, the actual mechanism of MTX-induced damage to bone marrow cells is not known.

Liver toxicity

The toxic effect of methotrexate on the liver was first observed in 1971 during a clinical study of patients with psoriasis treated with this medication [26]. Most of the current researches show, that there exists an increased risk of elevated liver enzymes activity in patients taking methotrexate therapy. However, most often that does not lead to hepatic damage. Long-term therapy, especially involving high doses, may have an adverse effect on the organ. Patients treated for RA or psoriasis and those with coexisting liver disease are at particularly high risk of organ damage. Alcohol, obesity and diabetes are also significant factors in the progression of liver damage [27].

Currently used dosing and monitoring regimens during methotrexate therapy have significantly reduced the toxic impact of the drug. Recently used treatment regimens have allowed a reduction in the hepatic risk - the most up to date studies of transaminase activity during methotrexate therapy showed increased levels in 22%, but two times the upper limit in only 1% of participants [28]. Serious liver degeneration caused by methotrexate therapy is much rarer in patients on modern, properly administered therapy. It has been estimated that 5-year liver damage does not occur in more than 1/1000 people treated with methotrexate [29]. Mild and severe hepatic fibrosis and cirrhosis have been reported.

Attempts to explain the pathogenesis of the toxic effect of methotrexate on the liver are hampered by the lack of detailed knowledge of the exact mechanisms of methotrexate action. The statement that this drug is a dihydrofolate reductase inhibitor does not allow a precise description of the therapeutic and toxic effects of this substance. One possible cause of toxicity is folate deficiency as a result of enzyme inhibition. Another possibility is the activation of Ito cells by methotrexate; if the cells are stimulated for a long time, they will convert into myofibroblasts, which leads to liver fibrosis. These proposed mechanisms have not been confirmed by scientific research. The use of folic acid is recommended during therapy, it prevents a higher increase in liver enzymes [30]. Patients treated with methotrexate have a follow-up liver biopsy. The Roenigk scale is used to assess organ changes.
**Gastrointestinal toxicity**

Adverse effects related to the digestive system are the most common group of side effects that occur during methotrexate therapy. The principal symptoms are: nausea, vomiting, painful abdominal cramps and diarrhea; later, malabsorption of food and medication may develop, resulting in weight loss. These factors have a major impact on patients during treatment and can occur with both low and high doses of methotrexate [31]. A study in 2016 showed an increased frequency of diarrhea in RA patients and an increased number of oral ulcers in patients infected with the EBV virus [32]. The limiting factor in increasing the dose is the impact of methotrexate on the digestive tract. The use of the drug may cause an adverse effect in the form of inflammation of the intestinal mucosa or even spread to the entire digestive system. As the exact mechanisms causing damage to the digestive organs are not known, there is currently no method of preventing them. Many studies have been carried out to better understand the toxic effects of the drug on the gastrointestinal mucosa and changes in the phagocyte system. In one of them*, eight-week-old BALB/c mice were injected intraperitoneally with 1 mg/kg MTX every 3 days for 14 days. In the control group mice were administered with PBS. At the end of the study cycle, samples of the intestinal mucosa were taken for histological examination. It was found that the group of animals treated with MTX showed atrophy of the villi, accumulation of goblet cells and defects in the mucosa of the jejunum and colon with infiltration of inflammatory cells. No intestinal mucosal changes were observed in the control group [33].

Some studies suggest that methotrexate may cause DNA breaks in proliferating intestinal epithelial cells and induce oxidative stress in the cells, leading to the development of inflammatory processes in the mucosa and submucosa [34,35]. Repeated inflammatory processes during therapy lead to dysregulation of the intestinal mononuclear phagocyte system [33].

**Dermatological side effects of methotrexate use**

Less common complications of methotrexate therapy include various types of skin changes. The mechanism of their occurrence is not fully elucidated. The most frequently observed manifestation is oral cavity inflammation in the form of ulcers. This condition is observed in 11-17% of patients undergoing methotrexate therapy, especially commonly seen with high doses used in cancer treatment, but it can also occur with low doses [36,37]. After administration 14% patients may develop nonspecific changes such as rashes resembling measles, localized around the neck and torso [38]. Patients may develop alopecia. Subcutaneous nodulosis is also described, resembling those seen in rheumatoid arthritis, but typically these nodules resulting from methotrexate therapy appear more abruptly, are located on the hands and feet, are smaller, and persist despite good control of the underlying disease. This complication occurs in about 8% of patients undergoing treatment [39]. Photodermatoses after sun exposure are very rarely observed [38]. The majority of skin changes accompanying methotrexate therapy subside after discontinuation of treatment.

**Ectopic pregnancy:**

Ectopic pregnancy (EP), also called extraterine pregnancy, is the implantation of a growing blastocyst outside the endometrial cavity of the uterus. The frequency of ectopic pregnancy is approximately 20 per 1000 confirmed pregnancies [40]. The risk factors are: having multiparity, a previous episode of EP, the insertion of an intrauterine device (IUD) before pregnancy, abdominal operations and the use of assisted-reproduction methods [41].
Approximately 90% of EPs are situated in the fallopian tube; the remaining 10% occur in the peritoneal cavity, cervix, ovary, interstitial part of the oviduct, broad ligament, uterine cornea. Pharmacological treatment of ectopic pregnancy is primarily based on the administration of methotrexate [42]. The mechanism of action of methotrexate is inhibition of DNA synthesis at different stages of the cell cycle, which results in the death of fast-dividing cells, including the trophoblasts. This is the mechanism that led to the treatment of ectopic pregnancy with methotrexate. While the effectiveness of methotrexate in the treat of ectopic pregnancy is about 70 to 90%, according to the type of regimen that is applied, the use of methotrexate requires specific circumstances, both in terms of the overall condition of the patient and the features of the ectopic pregnancy. In this case, the patient requires neither symptoms of haemodynamic failure as well as a number of other symptoms related to fallopian tube rupture [42,43] to be able to address the possibility of developing side-effects [42,43].

**Neurotoxicity**

Methotrexate (MTX) has the potential to cause acute, sub-acute or long-term neurotoxicity. The mechanisms by which toxicity occurs have not yet been fully elucidated. Various hypotheses have been put forward to explain this neurotoxicity. For instance, one hypothesis is that MTX impairs transmethylation reactions, which are required for the production of proteins, lipids and myelin. MTX lowers the levels of methionine and S-adenosylmethionine in the fluid while elevating the levels of S-adenosyl homocysteine and homocysteine [44]. It has been established that increased levels of homocysteine may be involved in the vascular events of MTX neurotoxicity. During the first 2 weeks of therapy, 1% to 4.5% of patients receiving high-dose MTX (HD-MTX) experience neurological symptoms [45]. Leukoencephalopathy is the most common type of acute neurological presentation [46]. This condition can only be diagnosed subclinically through magnetic resonance imaging or autopsy with symptoms such as confusion, insomnia, seizures, agitation, and coma. Vomiting, nausea, headache and aseptic meningitis have been reported in association with intrathecal administration of MTX. Side effects are usually mild and last for 12 to 72 hours. Subacute neurotoxicity has been reported several weeks after beginning methotrexate treatment, with epilepsy, cerebellar symptoms and paraplegia. Chronic encephalopathy develops gradually and may lead to lasting neurological impairments [47].

**Conclusion**

Our article discusses the challenges that clinicians face when dealing with complications related to methotrexate therapy. Methotrexate is a crucial component in the treatment of various diseases, including psoriasis, rheumatoid arthritis, and malignant tumors. When initiating and continuing treatment, it is important to consider factors that may increase drug toxicity to the organism of patients. Patients with chronic kidney disease or liver dysfunction are particularly sensitive to the toxic effects of methotrexate. The drug should not be administered to patients with immunodeficiencies due to its immunosuppressive effect. Knowledge of the therapeutic and toxic mechanisms of activity of MTX may be of additional interest in the discovery of new therapeutic targets to treat inflammatory and immune-mediated pathologies while reducing adverse toxic effects.

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Author's contribution

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