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Potential Effects of Methotrexate on Health: A Review

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Abstract: Methotrexate (MTX) is a metabolite that functions as an antifolate, impeding DNA synthesis, repair, and cell replication. Its clinical uses include rheumatoid arthritis treatment and cancer treatments due to its anti-folate mechanism of action. This medication serves as the primary disease-modifying antirheumatic drug (DMARD) for individuals afflicted with dermatological, inflammatory, and autoimmune conditions. Methotrexate has a unique mode of action, both in terms of its usage as a chemotherapeutic drug and as an immunosuppressant, in the management of autoimmune disorders. It can be combined with anti-TNF (Tumour Necrosis Factor) drugs and used as an off-label treatment for graft-versus-host disease. However, there are limitations to its usage, such as increased toxicity problems and brief plasma life. Methotrexate can be used as a nano-drug to decrease administration frequency while maximizing pharmacological effects and minimizing systemic side effects. The utilization of nano-drugs has been found to be more efficacious in comparison to intra-articular or oral delivery methods. Additionally, it has been determined that the co-loading of MTX-SPIONs (Superparamagnetic iron oxide nanoparticles) in NLC (Nanostructured lipid carriers) has been found to be a viable carrier for the delivery of MTX. The present review focuses on potential effects of methotrexate on health.

Keywords: Methotrexate, Immunosuppressant, Rheumatoid arthritis, Cancer, Chemotherapy, Nanodrug

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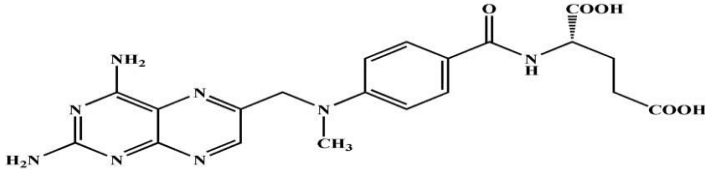
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Introduction

Methotrexate, also known as 4-amino-10-methyl folic acid or MTX is a drug that acts as both a folic acid analogue and antagonist. It is employed in therapy for a variety of cancers in addition to other diseases (Zhao *et al.*, 2022). MTX is a metabolite that acts as an antifolate, thereby hindering the synthesis, repair, and proliferation

of DNA. It was initially implemented as one of the primary medications for children who were diagnosed with leukemia (Kloos *et al.*, 2019). The World Health Organization (WHO) considers MTX to be an essential medication, and it is unquestionably one of the pharmaceutical industry's best achievements, as it has indications

Table 1: Details of Methotrexate

Methotrexate Trade Names	Metoject, Nordimet, Trexall®, Xatmep, Rasuvo, Reditrex, Jylamvo, Methotrexate LPF sodium, Otrexup, Rheumatrex
Molecular Formula	C ₂₀ H ₂₂ N ₈ O ₅
IUPAC Name	2,4-diamino-N10-methyl propyl glutamic acid
Synonym	Amethopterin
Drug Class	Antimetabolite, Immunosuppressants, Antineoplastics, DMARDs
Generic Status	Lower cost
Dosage forms available	Oral tablet, Injectable solution, Injectable powder, Oral paediatric solution
Chemical structure	

that are vastly different from its original intent (Alqarni and Zeidler, 2020). Methotrexate (MTX) has the potential to serve as both a monotherapy and a combination therapy for DMARD (Disease-modifying antirheumatic drugs)-naïve patients (Emery *et al.*, 2013), additionally, the utilisation of this drug as a foundational therapy for individuals who do not respond adequately to MTX, in combination with other conventional or biologic DMARDs, may lead to improved therapeutic results (Singh *et al.*, 2012).

Methotrexate (MTX):

Clinical uses for methotrexate (MTX) include rheumatoid arthritis treatment and cancer treatments. The medication has traditionally been used to treat neoplastic (lymphoblastic) diseases due to its anti-folate mechanism of action (Misra *et al.*, 2020).

Methotrexate (Table 1), formerly referred to as amethopterin, is classified as Class III of the Biopharmaceutical Classification System. This medication serves as the initial disease-modifying antirheumatic drug (DMARD) for individuals experiencing dermatological, inflammatory, and autoimmune conditions (Pivovarov and Zipursky, 2019). MTX is a folate antagonist that is presently

utilised either in isolation or in conjunction with other anticancer agents to manage a variety of malignancies, including osteosarcoma, acute lymphoblastic leukemia (ALL), malignancies of the head and neck, breast cancer, bladder cancer, and non-lymphoma Hodgkin's disease (Kozmiński *et al.*, 2020).

Pharmacokinetics of MTX:

Pharmacokinetics could aid in comprehending the distribution of toxicity and determining the optimal dose of a drug in order to maximize the therapeutic effect (Wang *et al.*, 2018). Methotrexate (MTX) can be administered through multiple routes for the treatment of rheumatoid arthritis. While oral and subcutaneous administration are viable options, intramuscular administration is considered the most clinically effective with minimal adverse effects (Yang *et al.*, 2020). Absorption of oral MTX is primarily mediated by a membrane transporter that is partially shared with folic acid. Intravenous administration increases plasma concentrations of the drug (Onoue *et al.*, 2014). Most anticancer medications are administered intravenously. The maximum plasma concentration of MTX is achieved in roughly 1.3 hours following intravenous administration, while it takes

approximately 1.5 hours after oral administration. The average oral bioavailability of MTX is between 50 and 80 % but varies between individuals (Maksimovic *et al.*, 2020). Lower dose administration increases bioavailability, whereas higher dose administration decreases bioavailability. The range of elimination half-life for MTX is between 3 to 10 h, with higher doses being associated with longer elimination half-lives (Maksimovic *et al.*, 2020). MTX is primarily excreted via glomerular filtration, active tubular secretion, and biliary secretion (Friedman and Cronstein, 2019). The pharmacokinetics of MTX can be altered by certain drugs such as NSAIDs (Non-steroidal anti-inflammatory drugs), which can reduce renal excretion and result in increased methotrexate toxicity. Similarly, certain antibiotics and sulfonamides can displace methotrexate from protein-binding sites, resulting in increased free drug concentration and toxicity (Zinner and Mayer, 2015).

Overall, MTX is rapidly absorbed after oral administration and widely distributed throughout the body. The drug is primarily eliminated by renal excretion, and its pharmacokinetics can be affected by various factors such as urine pH, protein binding, and drug interactions.

Mechanism of action of MTX:

MTX has a distinct mechanism of action, both in terms of its usage as a chemotherapeutic drug and in the therapy of autoimmune diseases as an immunosuppressant (Cipriani *et al.*, 2014). MTX functions as an antifolate antimetabolite in the treatment of cancer. The transport of methotrexate into the cell is facilitated by the human-reduced folate carriers (Nogueira *et al.*, 2018). Methotrexate reacts with glutamate upon entry, resulting in the formation of methotrexate-polyglutamate (Morrow *et al.*, 2021). Both methotrexate and methotrexate-polyglutamate possess the capability to impede the dihydrofolate reductase enzyme. This enzyme catalyses the conversion of dihydrofolate to tetrahydrofolate, the biologically active form of folic acid. Further inhibition of DNA synthesis is achieved by the

addition of methotrexate-polyglutamate, which acts on purine and thymidylate synthase to prevent *de novo* purine synthesis. This has a cytotoxic effect, and therefore this mechanism is used in cancer therapy (Cronstein and Aune, 2020).

In the context of autoimmune disorders, multiple mechanisms contribute to the decision to use methotrexate as the primary therapeutic option. The compound inhibits the enzyme AICAR transformylase (amino-imidazole-carbox-amido-ribonucleotide), inhibiting adenosine and guanine metabolism and accumulation of adenosine. Due to the anti-inflammatory effect of adenosine, this results in the inhibition of T-cell activation, the downregulation of B-cells, and the enhancement of the sensitivity of activated CD-95 T cells. Additionally, the compound represses methyltransferase activity and inhibits beta-1 interleukin binding to its receptor on the cell surface (Brown *et al.*, 2016).

Benefits of MTX:

Methotrexate (MTX) is a frequently utilised medication that has demonstrated both safety and efficacy in the treatment of patients afflicted with psoriasis, systemic lupus erythematosus (SLE), inflammatory bowel disease (IBD), vasculitis, and various other connective tissue diseases. It is also one of the primary chemotherapeutic options for the treatment of a wide variety of cancers (Bedoui *et al.*, 2019). It is a well-known anticancer medication that is also frequently utilized as an immunosuppressant in the treatment of autoimmune diseases. MTX is widely considered to be one of the most effective chemotherapeutic options for treating a variety of malignancies. However, the drug's efficacy and safety in individuals who have blood disorders have not been proven, and the use of the drug during pregnancy is not suggested. As a result of the medication's anti-inflammatory and immunomodulatory effects, it is also useful in the treatment of patients who have undergone organ transplantation (Chan and Cronstein, 2010). Moreover, the combination of MTX and

anti-TNF (Tumor Necrosis Factor) medications has demonstrated therapeutic advantages in managing various medical conditions such as ulcerative colitis, non-lymphoma, breast carcinoma, and malignancies of the head and neck. In case of graft-versus-host disease, MTX produces the same results as cyclosporin. Methotrexate is utilised as an off-label therapeutic intervention for various medical conditions.

Some of the benefits of MTX are:

1. **Cancer treatment:** MTX is a pharmacological agent used in chemotherapy that is effective against several malignancies. This medication works by slowing or halting cancer cell growth.
2. **Rheumatoid arthritis treatment:** The therapeutic application of this treatment is prevalent in the management of rheumatoid arthritis, a persistent autoimmune disorder characterised by inflammation of the joints. MTX alleviates RA symptoms such as joint discomfort, swelling, and stiffness. Furthermore, methotrexate may slow the development of joint damage in RA.
3. **Psoriasis treatment:** MTX is effective in treating psoriasis, a skin condition that causes red, scaly patches. It works by reducing the growth of skin cells and suppressing the immune system.
4. **Other autoimmune disorder treatment:** MTX is utilised for the treatment of additional autoimmune conditions, such as lupus, vasculitis, and juvenile idiopathic arthritis. The medication helps to reduce inflammation, pain, and other symptoms by suppressing the immune system.
5. **Low cost:** It is a low-cost medication, which makes it accessible to a wide range of people who may not be able to afford other more expensive treatments.

Though MTX has a range of benefits, it has been observed that the administration of this treatment may result in adverse reactions, which can range from nausea, vomiting, fatigue, alopecia, and decreased haematological parameters. Some of these side effects can be managed with medications or other interventions. Therefore, it is

essential to work with a healthcare professional who can monitor treatment progress and manage side effects.

Side effects of MTX:

MTX is associated with a significant number of side effects, even at low doses, despite its widespread use. Among the most common adverse effects are nausea, mucosal ulcers, and decreased appetite (Chande *et al.*, 2014). MTX in high doses may induce many serious adverse effects, however, moderate doses may be tolerated by the patient. MTX's most significant unintended effect is hepatotoxicity (Conway and Carey, 2017b). MTX is a teratogenic drug that can lead to congenital malformations if taken during pregnancy (Affleck and Walker, 2008; Dawson *et al.*, 2014).

According to the IARC, MTX is in group 3 (IARC, 2018) and belongs to a class of medications called antimetabolites (Olsen, 1991). Several potentially hazardous side effects have been associated with it, including pericardial serositis, gastrointestinal problems, myelosuppression, hepatotoxicity, and lymphoproliferative diseases (Albrecht and Müller-Ladner, 2010)

According to the reports, approximately 95% of pregnancies were terminated when a single high dose of MTX was administered before 8 weeks of gestation (Hausknecht, 1995). MTX has been shown to cause significant teratogenic effects in fetuses, including cranium and limb deformities (Lloyd *et al.*, 1999), decreases reactive nitrogen species (RNS) levels, and increases caspase-3 activity, both of which are required for inducing apoptosis via oxidative stress (Elango *et al.*, 2014). Methotrexate's potential to cause nephrotoxicity may be attributed to its ability to induce oxidative stress in rat renal tissues (Devrim *et al.*, 2005).

Additional adverse effects encompass gastrointestinal manifestations such as nausea, emesis, and diarrhoea (Wang *et al.*, 2018); painful mouth sores making eating and drinking difficult (Chamorro-Petronacci *et al.*, 2019); hair loss or thinning; can make patients more sensitive to sunlight, increasing the risk of sunburn and other

skin irritation (Panda *et al.*, 2021). Furthermore, MTX can harm the liver, especially at higher doses or with long-term use therefore, patients taking the drug need to have their blood levels and liver function monitored regularly (Conway and Carey, 2017c). It can also cause interstitial pneumonitis, which can cause shortness of breath and coughing (Conway and Carey, 2017a). MTX can induce blood cell abnormalities by suppressing bone marrow function leading to low blood counts and increasing the risk of infection, anemia, bleeding, and bruising. Overall, methotrexate is an effective medication for treating a variety of medical conditions, but its use can cause serious side effects.

Methotrexate as nanodrug:

Conventional drugs have poor solubility and lack of selectivity after injection, and most of them tend to build up in non-target regions of patients' bodies and can cause undesirable side effects (Patra *et al.*, 2018). Only a small fraction of drugs actually reaches their intended targets (cells or tissues). In recent years, nano-drugs have gained a great deal of popularity due to the fact that they can be used as delivery vehicles (Li *et al.*, 2019). By encapsulating or attaching therapeutic medications to nanostructures they can be delivered to target tissues more precisely and released in a controlled manner (Mu *et al.*, 2020). Nanodrug delivery systems have the potential to reduce the number of times a drug needs to be taken while simultaneously maximising its pharmacological effects and minimising its systemic adverse effects. This could result in improved therapeutic compliance and clinical outcomes (Sahu *et al.*, 2021).

Certain barriers, such as increased toxicity issues and a short plasma half-life, have limited its application. In order to mitigate adverse effects and enhance the pharmacokinetic profile of the administered medication, MTX was encapsulated in a Zn/PEG nano-composite (Ma, 2020). The delivery of MTX through lipid nanovesicles may be more efficient than either intra-articular or oral administration (Prabhu *et al.*, 2012). The co-

loading of MTX-superparamagnetic iron oxide nanoparticles with NLC (Nanostructured lipid carrier) is a suitable method for delivering MTX. They undergo rapid internalisation by cancer cells, which results in an increase in apoptotic-mediated cell death when an alternating magnetic field is present (Ong *et al.*, 2020).

Some potential benefits of methotrexate as a nano-drug are:

1. Improved efficacy: It has been discovered that nano-drug delivery of methotrexate is more efficacious. The application of a nano-sized drug delivery system facilitates the direct delivery of the drug to the intended site of action, leading to an improvement in the drug's bioavailability and ultimately enhancing its therapeutic effectiveness (Rana and Sharma, 2019).

2. Reduced toxicity: Using methotrexate as a nano drug can possibly decrease the toxicity of the drug because it enables the drug to be delivered in lower doses and in a targeted manner (Kopeckova *et al.*, 2019). The nano-sized delivery system can help protect healthy cells from exposure or damage, reducing side effects.

3. Controlled release: Nano drug delivery systems offer a controlled release of the therapeutic agent, allowing for sustained drug levels over a longer period. This controlled release leads to optimum drug concentrations in the target location, which results in improved clinical outcomes (Adepu and Ramakrishna, 2021).

4. Enhancing solubility: MTX is a hydrophobic drug (Cerqueira *et al.*, 2019), which presents a challenge in its formulation. The utilisation of nanoscale drug delivery systems enhances the solubility of methotrexate, allowing it to dissolve in water, which enhances its bioavailability (Suresh *et al.*, 2022).

5. Targeted therapy: As a nanodrug, methotrexate enables targeted therapy, as the drug can be delivered directly to the disease site. This system can decrease drug distribution to non-targeted areas of the body, resulting in fewer adverse

effects, reduced drug dosage, and enhanced efficacy (Bahrami *et al.*, 2017).

Conclusion

Methotrexate is an antifolate metabolite used to treat neoplastic diseases due to its anti-folate mechanism of action. Methotrexate is an antifolate antimetabolite used as a chemotherapeutic drug and immunosuppressant in autoimmune diseases. It is an effective chemotherapeutic option for treating a variety of cancers, autoimmune diseases, and graft-versus-host diseases. Besides so many uses MTX is known for many side effects. It is a teratogenic drug with potentially dangerous side effects, including hepatotoxicity, pericardial serositis, gastrointestinal side effects, and lymphoproliferative diseases. Thus, it can be inferred that in order to mitigate the adverse effects of MTX and enhance its effectiveness, nanodrug formulations can be used which will also help to reduce the frequency of administration and minimize negative systemic impacts, which in turn will improve adherence to treatment and patient outcomes.

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