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Methotroxate: A friend or a foe? Review article

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Abstract

Methotroxate is one of the first chemotherapeutic drugs used in the early 1950s. Due to a high structural similarity between methotroxate and dihydrofolic acid, it is strongly postulated that the drug acts by competitive inhibition of dihydrofolate reductase reducing the rate of tetrahydrofolate synthesis. It, however, acts by a different mechanism in treatment of rheumatoid arthritis. Despite its various considerable uses in treatment of many diseases, it possesses an important drawback being side effects that may become life threatening in certain circumstance. In this review, the structure, mechanism of action, efficacy, application, metabolism and excertion of the drug have been investigated under the "introduction". In the next section entitled "methods", the most important side effects of methotroxate have been reviewed. Among the various side effect, hepatotoxicity and acute hepatitis is considered as the most common side effects of the methotroxate. Therefore, it has been explained in more detail and a newly established method for the early detection is also explained. Then based on the reviewed literature, in "conclusion" section the most important remarks have been pointed out and summarized.

Key words: Methotroxate, liver fibrosis, FibroScan®, side effects

Introduction

Although other analogues of folic acid were in development during early 1950s, methotrexate known as *amethopterin* at the time, was discovered as an effective drug for treatment of leukemia [1]. This was followed by a research that reported the use of methotroxate in solid tumors and they showed that the drug caused remission in breast cancer [2].

Later in 1956, animal studies revealed that the therapeutic index of methotrexate was better than that of aminopterin, and clinical use of aminopterin was thus abandoned in favor of methotrexate. The research on the activity of methotrexate against various types of cancer was promising. In 1960s Wright et al produced remissions in mycosis fungoides [2, 3].

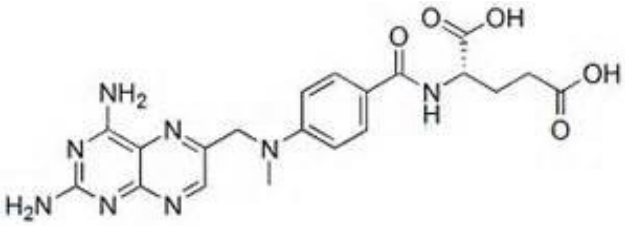
The drug was then recommended for treatment of many other cancers, alone or in combination with other drugs, and was studied for other, noncancer indications in the 1970s. In 1988, it was approved by the U.S. Food and Drug Administration (FDA) to treat rheumatoid arthritis [4].

In 2011, Ben Venue Laboratories shut down their production of injectable preservative-free methotrexate, leading to a shortage of the form of the drug commonly used to treat childhood acute lymphoblastic leukemia [5].

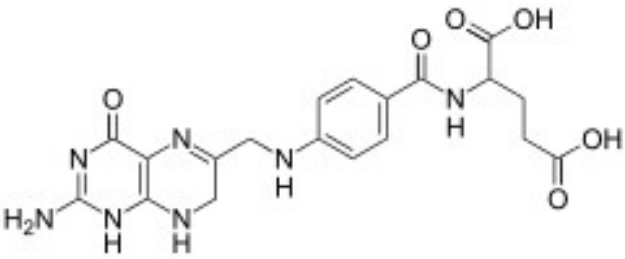
Methotrexate (MTX) is an antimetabolite used in the treatment of adult rheumatoid arthritis, certain neoplastic diseases, severe psoriasis and many other diseases. It is one of the first chemotherapeutic drugs used in the early 1950s [6]. From chemistry point of view, it is a glutamic acid derivative with molecular weight of 454.45 ($C_{20}H_{22}N_8O_5$). Methotrexate was originally developed for chemotherapy either alone or in combination with other agents. It is effective for the treatment of a number of cancers including: breast, head and neck, leukemia, lymphoma, lung, osteosarcoma, bladder, and trophoblastic neoplasms [7].

Also known as amethopterin, MTX is an analog of folic acid and it was the first drug successfully used for treatment of leukemia and choriocarcinoma. MTX is a nonspecific inhibitor of the dihydrofolate reductase (DHFR) of bacteria and cancerous cells as well as normal cells. DHFR can reduce dihydrofolate to tetrahydrofolate, in the presence of NADPH as electron donor. The enzyme mechanism is based on the transfer of a hydride from NADPH to dihydrofolate. The process is continued by protonation of dihydrofolate leading to the formation of tetrahydrofolate and $NADP^+$ [8]. The high flexibility of methionine at position 20 (Met_{20}) and other loops near the active site of DHFR play a key role in promoting the release of the product, tetrahydrofolate. In particular the Met_{20} loop helps stabilize the nicotinamide ring of the NADPH to promote the transfer of the hydride from NADPH to dihydrofolate [8].

Since tetrahydrofolate (vitamine B_9) is an essential precursor of both purines and pyrimidines, the building blocks of DNA and RNA, dihydrofolate reductase could be a the aimimg point for designing of drugs to prevent nucleic acid synthesis. Inhibition of the DHFR, therefore, disturbs the synthesis of both purine (adenine and guanine) and pyrimidine (thymidine) bases.



Methotrexate (MTX), IUPAC name: N-[4-{[(2,4-diamino-6-pteridinyl)methyl]methylamino} benzoyl]-L-glutamic acid



Dihydrofolic acid , IUPAC name: N-[4-{[(2-amino-4-oxo-1,4,7,8-tetrahydropteridin-6-yl)methylamino} benzoyl]-L-glutamic acid

Figure 1. The structural similarity of methotroxate (MTX) with dihydrofolic acid

The structural similarity of dihydrofolic acid and MTX (Fig. 1), suggests that the drug acts as a competitive inhibitor of DHFR. The mechanism of action is based on chemical formation of an inactive ternary complex with the enzyme and its co-enzyme, NADPH.

The Bioavailability of Methotrexate

MTX enters the cells through an active transport mechanism. However, a passive type of diffusion is also possible at its very high concentration. Within the cells, MTX binds one or more glutamate residues to form polyglutamate derivatives by the action of folyl-polyglutamate synthetase. This process caused its longer time remaining inside the cells. It is known that MTX especially affects the cells in fast division including cancerous cells, normal cells of the digestive epithelium tract and bone marrow cells [6]. Folinic acid, 5-formyl tetrahydrofolate (Fig. 2) is the active form of folic acid. It is naturally present in some fruits and food products. However, the toxicity of methotrexate for normal tissues can be reduced by prescription of folinic acid which is active in the absence of the DHFR. Care must be taken when using this compound, as it may reduce the efficacy of MTX.

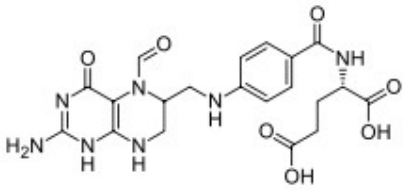


Figure 2. Chemical structure of folinic acid, (2S)-2-{[4-[(2-amino-5-formyl-4-oxo-5,6,7,8-tetrahydro-1*H*-pteridin-6yl)methylamino]benzoyl]amino} pentanedioic acid.

The bioavailability of methotrexate taken by oral route is approximately 75%. It binds to plasma proteins at 35% and penetrates into the brain and the cerebral spinal fluid. The treatments by very high doses of methotrexate, as those used in certain lymphoma and osteosarcoma, necessitate the maintenance of a high and alkaline diuresis and the administration of high doses of folinic acid, 24 or 36 hours after the discontinuation of the infusion of methotrexate, to thwart the toxicity of methotrexate. High-doses of methotrexate are necessary to be used in a variety of disorders including treatment of choriocarcinoma, leukemia, lymphoma, osteosarcoma, head and neck cancers and breast cancer, where it is used in combination with other antineoplastics. Due to its anti-inflammatory effect, low doses are also used in the treatment of psoriasis, rheumatoid arthritis, inflammatory diseases of the digestive tract, of the liver, certain severe asthmas.

Pharmacokinetics

Methotrexate is a weak dicarboxylic acid with pKa 4.8 and 5.5, and thus it is mostly ionized at physiologic pH. When administrated orally, methotroxate could cause its saturation which is dose-dependent, i.e. doses less than 40 mg/m^2 show 42% bioavailability and doses greater than 40 mg/m^2 only 18%. However, its mean oral bioavailability is 33%, and there is no clear benefit to subdividing an oral dose. On the other hand, mean intramuscular bioavailability is 76%. It is worth reminding methotrexate is metabolized by intestinal bacteria to an inactive metabolite 4-amino-4-deoxy-N-methylpteroic acid (DAMPA). The rate of its absorption is dependent on the transit time through gastrointestinal tract (GI).

Methotrexate is almost completely absorbed from parenteral routes of injection. On the other hand, after intramuscular injection, peak serum could be reached in a period of 30 to 60 minutes.

Methotrexate competes with reduced folates for active transport across cell membranes using a single carrier-mediated active transport process. Followed by intravenous administration, the initial volume of distribution is approximately 0.18 L/kg with a steady-state distribution volume of about 0.4-0.8 L/kg. When its concentrations in serum reaches higher than 100 micromolar, passive diffusion becomes a major pathway by which effective intracellular concentrations can be achieved. Methotrexate in serum is approximately 50% protein bound. Laboratory studies demonstrate that it may be displaced from plasma albumin by various compounds including sulfonamides, salicylates,

tetracyclines, chloramphenicol, and phenytoin.

If the therapeutic doses of the drug are administrated through oral or parenteral routs, methotrexate does not penetrate the blood-cerebrospinal fluid (CSF) barrier. High CSF concentrations of the drug may be attained by intrathecal administration.

Mechanism of Action

The mechanism by which methotrexate reacts in treatment of various disorders is usually different depending on the type of disease. Firstly, in the case of cancer chemotherapy, the drug inhibits biological function of dihydrofolic acid reductase. This is the enzyme responsible to catalyse the reductive conversion of dihydrofolates to tetrahydrofolates. These products can then be utilized as carriers of one-carbon groups in the synthesis of purine nucleotides and thymidylate. Based in this mechanism, methotrexate can effectively interfere with DNA synthesis, repair, and cellular replication. Generally, this effect of methotrexate is more important in actively proliferating tissues such as malignant cells, bone marrow, fetal cells, buccal and intestinal mucosa, and cells of the urinary bladder. In malignant tissues, where cellular proliferation is greater than in most normal tissues, methotrexate can seriously impair malignant growth with a little damage to normal tissues.

On the other hand, its mechanism of action in rheumatoid arthritis is almost unknown. It may affect immune function in order to ease the painful conditions. In vitro studies have revealed that methotrexate could possibly inhibit DNA precursor uptake by stimulated mononuclear cells. On the other hand, another possibility describes the suppression that is caused in production of interleukin 2 (IL2).

It is suggested that beneficial effects of methotrexate on articular swelling and tenderness is normally observed as early as 3 to 6 weeks. Most studies of methotrexate in patients with rheumatoid arthritis are relatively short-term (3 to 6 months).

In psoriasis, the rate of production of epithelial cells in the skin is greatly increased over normal skin. This differential in proliferation rates is the basis for the use of methotrexate to control the psoriatic process.

Cancer

In the case of cancer treatment, the mechanism of methotrexate action is through competitive inhibition of dihydrofolate reductase (DHFR), an enzyme that participates in the tetrahydrofolate synthesis [9]. The structural similarity of methtroxate and dihydrofolate (Fig. 1), the enzyme natural substrate, leads to the entrance of the drug into the active site causing preventation of substrate entry (Fig. 3).

The affinity of methotrexate for DHFR is about one thousand-fold that of folate. DHFR catalyses *de novo* conversion of dihydrofolate to active tetrahydrofolate. Folic acid is needed for the *de novo* synthesis of the nucleoside thymidine, required for DNA synthesis. On the other hand, folate importantly participates in synthesis of purine bases. Therefore, the bio-synthesis of all purines would be considerably retarded as a result of enzyme inhibition.

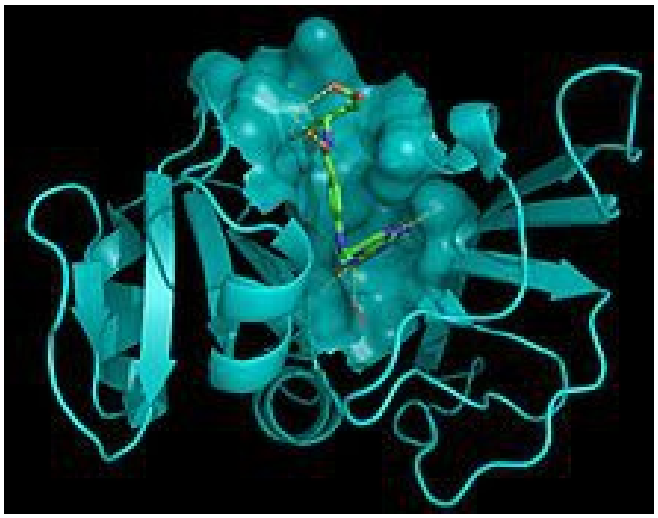


Figure 3. The competitive type of inhibition by methotrexate of DHFR. The drug (green) is complexed into the active site of DHFR (blue) inhibiting the entrance of the actual substrate.

In this way, methotrexate, could inhibit the synthesis of DNA, RNA, thymidylates, and proteins.

As methotroxate is a cytotoxic agent during the S-phase of the cell cycle, it could exhibit a high toxic effect on rapidly dividing cells including malignant and myeloid cells as well as gastrointestinal and oral mucosa, causing a wide range of side effects. On the other hand, due to a scarcity of dTMP, cell death occurs via thymineless death in a number of rapidly dividing cancerous cells.

Oral doses of methotrexate are prescribed for treatment of gestational choriocarcinoma, chorioadenoma destruens and hydatidiform mole. This type of administration is usually used for maintenance therapy in combination with other chemotherapeutic agents. Methotrexate is consumed either alone or in combination with other anticancer agents in the treatment of breast cancer, epidermoid cancers of the head and neck, advanced mycosis fungoides (cutaneous T cell lymphoma), and lung cancer, particularly squamous cell and small cell types. Methotrexate tablets are also used in combination with other chemotherapeutic agents in the treatment of advanced stage non-Hodgkin's lymphomas.

On some cases, non-Hodgkin's lymphoma and other tumors may be observed in patients receiving low-dose oral methotrexate. This type of side effect is regressed partially or completely after reducing or withdrawal of Methotrexate and it does not require active anti-lymphoma treatment. It is wise to weight benefits against the potential risks before using methotrexate alone or in combination with other drugs, especially in pediatric patients or young adults.

Psoriasis

Following diagnosis of severe, recalcitrant, disabling psoriasis and when it is not adequately responsive to other forms of therapy, then methotrexate is prescribed for symptomatic control. It is important to ensure that a psoriasis "flare" is not due to an undiagnosed concomitant disease affecting immune responses.

Autoimmune Disorders

Among various cases of the autoimmune disorders, a few mostly widely speared include rheumatoid arthritis, Juvenile dermatomyositis, psoriasis, psoriatic arthritis, lupus and Crohn's disease. It has been experienced that certain low doses of methotrexate is safe and comparably tolerated by patient for treatment of certain autoimmune diseases [10]. Low-dose methotrexate is the drug of choice in treatment of rheumatoid arthritis and has been shown the highest effectiveness [11]. The drug is among the most effective and commonly used medicines in the treatment of various forms of arthritis and other rheumatic conditions. It is known as a disease-modifying anti-rheumatic drug (DMARD), because it not only decreases the pain and swelling of arthritis, but it also can decrease damage to joints and long-term disability. A careful monitoring of the drug is needed in order to reduce risk of liver injury and infections. However, although the doses taken for autoimmune diseases is much lower than what is prescribed for cancer therapy, its common side effects including hair loss, nausea, headaches, and skin pigmentation still presist [10]. It has been experienced that majority of patients receiving methotrexate for up to one year had less pain, functioned better, with less swollen and tender joints, and moderate disease activity. The progress of the disease has been followed by x-ray diffraction method and the patterns have shown that the disease in considerably slowed down and even stopped in many patients receiving methotrexate [11]. Unlike the drug mechanism for cancer treatment, when used for rheumatoid arthritis treatment, inhibition of DHFR is not thought to be the main mechanism. In this case, the inhibition is through its interfrance with the purine metabolism. Retardation in purine metabolism could end to accumulation of adenosine, or the inhibition of T cell activation followed by suppression of intercellular adhesion molecule expression by T cells [12]. In most of such cases, diet supplementation with folic acid is helpful and recommended. However, in its low-dose regimen methotrexate blocks the binding of interleukin 1 beta to the interleukin 1 receptor on target cells [13].

Management of selected adults with severe, active rheumatoid arthritis (ACR), or children with

active polyarticular-course juvenile rheumatoid arthritis could be managed partly by methotrexate. These are generally patients who have had an insufficient therapeutic response to, or are intolerant of, an adequate trial of first-line therapy including full dose non-steroidal anti-inflammatory agents (NSAIDs). However, aspirin, NSAIDs and steroids is usually continued, although the possibility of increased toxicity with concomitant use of NSAIDs. It is has been shown that gradually reduced steroids after the patient responded to methotrexate is effective in reducing the toxicity effects.

Metabolism of Methotrexate

Metabolism of methotrexate started in liver and intracellular media, after it is completely absorbed. The product of its metabolism is resulted to polyglutamated forms which can then be converted back to methotrexate by hydrolase enzymes. The products of methabolism, polyglutamates, could act as inhibitors of dihydrofolate reductase and thymidylate synthetase. In most cases, a small amount of methotrexate polyglutamates may remain in tissues to be used later in extended periods. The retention and prolonged drug action of these active metabolites vary among different cells, tissues and tumors. On the other hand, at doses commonly prescribed, a small proportion of the drug may be metabolized to 7-hydroxy methotrexate. However, at the high doses used in osteogenic sarcoma, ccumulation of these metabolites may become significant. The aqueous solubility of 7-hydroxy methotrexate is 3 to 5 fold lower than the parent compound. Low doses of methotrexate used through oral routs could be partially metabolized by intestinal flora.

Final Excretion of Methotrexate

Renal excretion is the primary route of elimination and is dependent upon dosage and route of administration. There is limited biliary excretion amounting to 10% or less of the administered dose. Enterohepatic recirculation of methotrexate has been proposed. Renal excretion occurs by glomerular filtration and active tubular secretion. Nonlinear elimination due to saturation of renal tubular reabsorption has been observed in psoriatic patients at doses between 7.5 mg and 30 mg. Impaired renal function, as well as concurrent use of drugs such as weak organic acids that also undergo tubular secretion, can markedly increase Methotrexate serum levels. A significant correlation could be observed between clearance of methotrexate and endogenous creatinine clearance.

However, the clearance rate may vary widely at different cases and normally it decreases at elevates amounts of administrated methotrexate. Methotrexate toxicity is the result of delayed drug clearance. It has been postulated that the toxicity of methotrexate for normal tissues is more dependent upon the duration of exposure to the drug rather than the peak level achieved. When a patient has delayed drug elimination due to compromised renal function, a third space effusion, or other causes, methotrexate serum concentrations may remain elevated for prolonged periods.

The potential for toxicity from high dose regimens or delayed excretion is reduced by the administration of leucovorin calcium during the final phase of methotrexate plasma elimination.

Pharmacokinetic monitoring of methotrexate serum concentrations may help identify those patients at high risk for Methotrexate toxicity and aid in proper adjustment of leucovorin dosing. Methotrexate has been detected in human breast milk. The highest breast milk to plasma concentration ratio reached was 0.08:1.

Half-Life of Methotrexate

The terminal half-life reported for methotrexate is approximately 3 to 10 hours for patients receiving treatment for psoriasis, or rheumatoid arthritis or low dose antineoplastic therapy (less than 30 mg/m2). For patients receiving high doses of methotrexate, the terminal half-life is 8 to 15 hours.

Contraindications

Methotrexate could fetal death or teratogenic effects when administered to a pregnant woman. Methotrexate is contraindicated in pregnant women with psoriasis or rheumatoid arthritis and

should be used in the treatment of neoplastic diseases only when the potential benefit outweighs the risk to the fetus. Because of the potential for serious adverse reactions from methotrexate in breast fed infants, it is contraindicated in nursing mothers.

Patients with psoriasis or rheumatoid arthritis with alcoholism, alcoholic liver disease or other chronic liver disease should not receive Methotrexate.

Patients with psoriasis or rheumatoid arthritis who have overt or laboratory evidence of immunodeficiency syndromes should not receive Methotrexate.

Patients with psoriasis or rheumatoid arthritis who have preexisting blood dyscrasias, such as bone marrow hypoplasia, leukopenia, thrombocytopenia or significant anemia, should not receive Methotrexate.

Toxic effects may be related in frequency and severity to dose or frequency of administration but have been seen at all doses. Because they can occur at any time during therapy, it is necessary to follow patients on methotrexate closely. Most adverse reactions are reversible if detected early. When such reactions do occur, the drug should be reduced in dosage or discontinued and appropriate corrective measures should be taken.

The clinical pharmacology of methotrexate has not been well studied in older individuals. Due to diminished hepatic and renal function as well as decreased folate stores in this population, relatively low doses should be considered, and these patients should be closely monitored for early signs of toxicity.

Overdosage

Monitoring of the serum methotrexate concentration is essential in determining the optimal dose and duration of treatment with a medication to decrease its adverse toxicity.

Leucovorin is a usual drug that is able to diminish the toxicity and counteract the effect of inadvertently administered overdosages of methotrexate. It is worth indicating that its administration should begin as promptly as possible. As the time interval between methotrexate administration and leucovorin initiation increases, the effectiveness of leucovorin in counteracting toxicity decreases.

In cases of massive overdosage, hydration and urinary alkalinization may be necessary to prevent the precipitation of methotrexate and/or its metabolites in the renal tubules. In general, neither hemodialysis nor peritoneal dialysis has been shown to improve methotrexate elimination. However, effective clearance of Methotrexate has been reported with acute, intermittent hemodialysis using a high-flux dialyzer [14].

Methods

Methotrexate Side Effects

In common with other known cytotoxic substances, methotrexate posses a broad range of, adverse effects. The most highly reported instant side effects of methotrexate include fever, pneumonia, nausea, joint pain, weakness, fatigue, rashes and diarrhea. However, fever among females aged 60+ years old has the highest reports and pneumonia among children aged 2-9 comprises the lowest incidence. Interestingly, most of its reported side effects have much lower incident in men than women [15]. Some serious side effects of the drug have been reported in central nervous system. It this case, adverse reactions to methotrexate is especially observed when given via the intrathecal route at high doses [16].

Methotrexate may cause cirrhosis (scarring) of the liver, but this side effect is rare and most likely to occur in patients who already have liver problems or are taking other drugs that are toxic to the liver.

Lung problems can occur when taking methotrexate. These side effects are more common in people with poor lung function. However, persistent cough or shortness of breath should are rarely

reported.

It is worth indicating that most patients do not experience side effects, and for those who do, many of the minor side effects will be improved with time.

Methotrexate can cause serious birth defects due to its teratogenic effect and it has been recommended that the drug is not used during pregnancy, when planning to become pregnant or while breastfeeding. However, in a single dose or in combination with misoprostol, it is prescribed as an abortifacient for termination of pregnancies during the early stages when abortion is the treatment of choice.

Consumptions of large doses and long term could lead to blood disorders including leukopenia, thrombocytopenia, agranulocytosis; digestive disorders such as nausea, vomiting, anorexia, stomach pains; increase in sensitivity to infections; damage to spermatozoa and oligospermia.

Resistance of cancer cells to MTX is also due to the side effect of long term use which is explained by different mechanisms including decrease of its entry into cells, production of resistant DHFR and reduction in the synthesis of MTX polyglutamate.

It is worth to notice that despite its various side effects, methotrexate is still the medication of choice for many disorders which are briefly reviewed in following sections. In this review, the side effects are classified according to the type of diseases for which the drug is used for its treatment.

Hepatic

Although methotrexate is the most effective medication in the treatment of a number of chronic disorders, but due to hepatotoxicity caused in long-term and high doses, its use is considerably limited. In general, its administration is stopped when an increase in concentration of serum aminotransferase is observed. On the other hand, there is little information about the nature or seriousness of the elevation of these enzymes and little, if any, correlation between them and liver histology.

Hepatotoxicity and acute hepatitis could be considered as the most common side effects of the drug. These are followed by chronic fibrosis and cirrhosis, decreased serum albumin, and liver enzyme elevations. Therefore, baseline liver biopsy would be needed to investigate the depth of damage. Monitoring hepatic enzymes every 4 to 8 weeks is helpful to guide if discontinuing MTX is needed. This condition usually occurs when serum transaminase levels exceeded 2- 3 times above the baseline. This type of investigation could be helpful as an alternative to the invasive biopsy. It has been investigated that when methotrexate is administered in conjunction with either nicotinamide or methionine, the rise in the death rate and in glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) activities associated with methotrexate application is markedly reduced [17].

Persistent abnormalities in liver function tests may precede appearance of fibrosis or cirrhosis in the rheumatoid arthritis population. This is beneficial to the liver function as, by frequent monitoring activities of liver enzymes, the dosage could be altered in order to minimize its adverse side effects on liver without the need for the invasive check up by liver biopsy.

Immunologic

Immune suppression induced during or after therapy with methotrexate could raise new and/or opportunistic infections. Even in low-dose therapies such as in RA, infections during MTX therapy may occur in more than 50% of patients during low-dose therapy (as in RA). Immunologic side effects including case reports of sometimes fatal opportunistic infections including pneumocystis carinii pneumonia has been reported most frequently. Infections associated with severe immunosuppression, such as disseminated herpes zoster, Listeria meningitis, Mycobacterium avium intracellulare pneumonia, and systemic fungal infections are among the most important immunological side effects.

Hematologic Side Effects

Myelosuppression is one of the primary toxic effects of methotrexate. Suppresstion of hematopoiesis leads to anemia, aplastic anemia, pancytopenia, leukopenia, neutropenia, thrombocytopenia, lymphadenopathy, and lymphoproliferative disorders. In these cases, folate therapy and/or leucovorin rescue is recommended which may be preventive or palliative.

Cytopenia occurs in 5% to 25% of patients with rheumatoid arthritis (RA) who receive long-term therapy.

Methotrexate can suppress hematopoiesis and cause anemia, aplastic anemia, pancytopenia, leukopenia, neutropenia, and/or thrombocytopenia. In patients with malignancy and preexisting hematopoietic impairment, the drug should be used with caution and controlled monitoring for hematologic side effects.

Gastrointestinal (GI)

Extremely rare cases of colitis and toxic megacolon have been associated with the use of MTX. Gastrointestinal side effects are usually controlled by folate supplementation if on low dose therapy, as in rheumatoid arthritis. However, gastrointestinal side effects, with high-dose administration, are expected. Serious nausea, vomiting, diarrhea, or stomatitis may result in symptomatic dehydration. GI symptoms could also be eliminated by supplementation with folate which does not affect efficacy of MTX. In cases where appearance of diarrhea and ulcerative stomatitis is observed, it may be required to withdraw the therapy. However, if the treatment with the drug is not stopped, hemorrhagic enteritis and death from intestinal perforation may occur in some cases.

Nervous System

Some serious side effects of the drug have been reported in central nervous system. It this case, adverse reactions to methotrexate is especially observed when given via the intrathecal route at high doses [16].

Central nervous side effects include headaches, dizziness, drowsiness, blurred vision, subtle cognitive dysfunction, moodiness, tinnitus or unusual cranial sensations. Serious neurotoxicity has been associated with the use of high-dose MTX after intrathecal or intraventricular administration to patients who have undergone craniospinal irradiation, but has also been described in other patients who have received low-dose oral therapy. In the case of prescribing high doses of the drug to patients who have undergone craniospinal irradiation, severe neurotoxicity is expected.

Respiratory

A special type of hypersensitivity reaction and a toxic reaction with diffuse alveolar damage and nonspecific lung injury are the most frequent respiratory side effects. The hypersensitivity reaction is characterized by interstitial pneumonitis, granuloma formation, and the development of bronchopneumonia. Pulmonary function tests in affected patients reflect a restrictive ventilatory defect and decreased oxygen diffusing capacities. Respiratory side effects including toxicity can occur at any dosage and can mimic infectious pneumonia.

Methotrexate-induced lung disease is a potentially dangerous lesion, which may occur acutely at any time during therapy and which has been reported at doses as low as 7.5 mg/week. Pulmonary symptoms are not always fully reversible and stop of treatment together with careful investigation are necessary.

Renal

Renal side effects are associated with high-doses of MTX which include renal insufficiency. The renal defect leads to the concentration of a major circulating metabolite, 7-OH MTX resulting in its precipitation in the renal tubule. Aggressive and adequate hydration and urinary alkalinization helps minimize the risk of MTX-induced nephropathy, cystitis, and hematuria.

Dermatologic

Cases of severe, sometimes fatal, dermatologic

reactions, such as toxic epidermal necrolysis, Stevens-Johnson syndrome, exfoliative dermatitis, skin necrosis, and erythema multiforme, have been reported in children and adults, within days of oral, intramuscular, intravenous, or intrathecal methotrexate administration. Reactions have been observed after single or multiple, low, intermediate or high doses of methotrexate for the treatment of neoplastic and non-neoplastic diseases. Dermatologic side effects include erythematous rashes, desquamation, epidermal necrosis, pruritus, urticaria, photosensitivity, pigmentary changes, alopecia, ecchymosis, telangiectasia, acne, cutaneous vasculitis, furunculosis, and alopecia.

Severe, occasionally fatal, skin reactions have been reported following single or multiple doses of methotrexate. However, discontinuation of therapy would generally ease the occurred symptoms.

Oncologic Side Effects

In rare circumstances, methotrexate could be oncogenic, leading to development of some lymphomas and leukemias. However, hematologic malignancies are uncommon in RA patients receiving MTX. Underlying rheumatoid arthritis (RA) or Sjogren's syndrome are independent risk factors for the development of non-Hodgkin's lymphoma.

Malignant lymphomas may occur even in patients receiving low-dose methotrexate. However, its siscontinuation may cause regression of lymphoma does not regress. Methotrexate may induce "tumor lysis syndrome" in patients with rapidly growing tumors. Appropriate supportive and pharmacologic measures may prevent the syndrome.

Cardiovascular

The MTX side effects on cardiovascular system may include pericarditis, pericardial effusion, myocardial ischemia, hypotension and ventricular arrhythmias. However, most of these symptoms are very rare.

Other

Many other side effects are also associated with ling time and high doses of MTX. However, these are rarely observed and have not remained for long. They included rare cases of bone and soft tissue necrosis following radiation therapy in patients receiving MTX, gynecomastia in patients with rheumatoid arthritis, conjunctivitis and serious visual changes of unknown origin.

Methotrexate (MTX) has been assigned to pregnancy category X by the FDA. It can cause fetal death or teratogenic effects when administered to a pregnant woman. Therefore, pregnancy should be avoided if either partner is receiving MTX, during and for a minimum of 3 months after therapy for male patients, and during and for at least 1 ovulatory cycle for female patients. However, the drug has been successfully used to terminate tubal pregnancy.

As MTX could be excreted into human milk in low concentrations, it must be contraindicated during breast-feeding because of several potential problems, including immune suppression, neutropenia, adverse effects on growth, and carcinogenesis.

In a retrospective study of patients who received methotrexate as part of their treatment for ectopic pregnancy, it has been found that routine measurement of serum aspartate aminotransferase and creatinine levels may not be necessary before instituting a single-dose methotrexate treatment regimen for the management of ectopic pregnancy [18].

On the other hand, experts consider the use of MTX to be contraindicated during breast-feeding because of the unknown risk of this concentration of MTX to the nursing infant, the possibility of accumulation of the drug in neonatal tissues, and because of the known adverse effects of MTX in breast-fed infants.

It is suggested that the occurrence of bone marrow suppression, thrombocytopenia, and pneumonitis may increase with age. Therefore, elderly patients should be closely monitored for early signs of hepatic, bone marrow and renal toxicity. In chronic use situations, certain toxicities may be reduced by folate supplementation.

Slow hair loss is reported in some patients, but in most cases it grows back after stopping the medication. Methotrexate can also increase the sensitivity of the skin to sunlight, so limiting sun exposure and the use of sunscreen is advised.

Discussions

Interactions of Methotroxate with Medications

Many synthetic and natural drugs including prescription and over-the-counter medicines, vitamins, and herbal products can interact with methotrexate. Intrestingly, the mechanism responsible for the drug action can alter effect of methotroxate and it may vary according to the type of altering agent.

Some of the most common drugs that interact with methotrexate include azathioprine; leucovorin; phenytoin; probenecid; theophylline; antibiotics or sulfa drugs; isotretinoin, retinol, tretinoin; non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen, naproxen, celecoxib, diclofenac, indomethacin, meloxicam or salicylates such as aspirin. It must be emphasized that methotrexate elimination is reduced in patients with impaired renal function, ascites, or pleural effusions. Such patients require especially careful monitoring for toxicity, dose reduction or, in some cases, discontinuation of methotrexate administration.

To mention a few examples in more detail, penicillins can increase the risk of methotroxate toxicity by a decrease in the elimination of methotrexate [7]. Probenecid could also increase the toxicity risk of the drug through a similar mechanism, i.e. inhibiting it exertion. Additionally, neurotoxicity of methotrexate is reported to be induced by phenobarbital and carbamazepine [19]. These are antiepileptic drugs that can reduce transport of methotrexate in choroid plexus by down-regulation of the reduced folate carrier [16]. However, it is known that their effects can be reversed by folinic acid (leucovorin) through a process known as "leucovorin rescue."

Nimesulide is a non-steroidal anti-inflammatory drug which is frequently used as adjuvant therapy for symptomatic alleviation of rheumatoid arthritis. Using a collagen-induced arthritis model in mice, influence of nimesulide on the disease modifying anti-rheumatic properties of methotrexate has been studied. It was observed that methotrexate alone showed modest but significant analgesic and anti-inflammatory effects, and the effects of single dose of nimesulide were comparable. However, when used synergistically, nimesulide could significantly improve the profile of methotrexate in terms of arthritic index and joint mobility [20].

The anti-arthritic effect of single MTX and its combination with human serum albumin (HAS) in a special form named albumin-coupled methotrexate (MTX-HSA) have been studied in murine model of collagen-induced arthritis (CIA). It was shown that MTX-HSA was considerably more effective than MTX in CIA. They concluded that both drugs could act synergistically and albumin was taken up by peripheral blood cells. The researchers suggested that they might be one of the potential target cells of this novel anti-arthritic treatment approach [21].

When the drug is administrated concomitantly with radiotherapy, methotrexate could lead to an increase in the risk of soft tissue necrosis and osteonecrosis.

Laboratory Tests

Methotrexate therapy should be seriously accompanied with close monitoring the patient response to promptly detect toxic side effects. The most commonly used assessments include a complete blood count with differential and platelet counts, hepatic enzymes, renal function tests, and a chest X-ray. In the cases of rheumatoid arthritis and psoriasis, monitoring of these parameters is most useful: hematology at least monthly, renal function and liver function every 1 to 2 months.

Transient liver function test abnormalities are observed frequently after methotrexate administration and are usually not cause for modification of methotrexate therapy. Persistent liver function test

abnormalities, and depression of serum albumin are generally indicators of serious liver toxicity and require evaluation.

A relationship between abnormal liver function tests and fibrosis or cirrhosis of the liver has not been established for patients with psoriasis. Persistent abnormalities in liver function tests may precede appearance of fibrosis or cirrhosis in the rheumatoid arthritis population.

Pulmonary function tests are also useful if methotrexate-induced lung disease is suspected, especially if baseline measurements are available.

Screening the Adverse Effect of Methotroxate

Liver fibrosis is the most important side effects occurred in patients receiving methotroxate as a long term high dose therapy. Although liver biopsy is still the gold standard method to find out the extent of damage occurred to liver due to methoroxate therapy, but it could only show1/50,000 of the liver volume [22].

Liver Stiffness Measurement

Transient elastography (FibroScan®) is a non-invasive method proposed for the assessment of hepatic fibrosis by measuring liver stiffness. It is a completely no-invasive method that can be easily performed at the bedside or in the out-patients clinic with immediate results and considerable reproducibility. Howevere, as with many other methods of screening, it suffers from some imitations including failure in about 5% of cases, mainly in patients with substantial thoracic fat. However, the method is validated for the diagnosis of significant fibrosis and cirrhosis caused by drug side effects as in the case of methotroxate, or through chronic hepatitis C, recurrence of hepatitis C and after liver transplantation. Liver fibrosis could also happen as co-infections in HIV-HCV patients and chronic cholestatic diseases, but needs further evaluation in other chronic liver diseases.

Fibroscan® is an excellent tool for early detection of cirrhosis and evaluation of portal hypertension, for which it may have prognostic value as well. However, it is worth indicating that more studies are needed using Fibroscan® for the follow-up of patients with and without treatment, and for the screening of patients at risk of liver disease. On the other hand, although FibroScan is a good method for the evaluation of fibrosis in patients with chronic liver disease or damages caused by methotroxate, it has to be borne in mind that FibroScan could only evaluate liver stiffness. Therefore, Fibroscan® values should then be interpreted according to clinical, biological and morphological data.

The accuracy of liver stiffness measurement for the detection of fibrosis and cirrhosis in HIV/hepatitis C virus (HCV)-coinfected patients has been assessed and its accuracy compared with other noninvasive [23]. It was found that liver stiffness measurement is a promising noninvasive method to assess fibrosis in HIV-infected patients with chronic HCV infection.

It has been shown that liver biopsy is only able to show1/50,000 of the liver volume and the resulting error in focally distributed liver disease as CFLD [22]. Alternative attempts to diagnose and follow-up the liver disease range from routine biochemistry and calculated scores to surrogate fibrosis markers in serum, hepatic clearance tests, various imaging techniques and more recently the use of non-invasive transient elastography, Fibroscan®.

On the other hand, it has been studied that Fibroscan® could serve as a screening tool for detection of cystic fibrosis-associated liver disease (CFLD). Using the technique, it has been shown that liver stiffness is significantly increased in clinical CFLD patients [24].

However, more recently, it has been investigated that Fibroscan® could be feasible in CF patients. In contrast to its use in paediatric NASH [25] the investigation is facilitated due to the pulmonary hyperinflation enlarging the intercostal space and the non-obesity of these patients.

Although Fibroscan® is a good method for the evaluation of fibrosis in patients with chronic liver disease, it has to be borne in mind that FibroScan

evaluates liver stiffness. Therefore, Fibroscan® values have to be interpreted according to clinical, biological and morphological data [26].

Conclusions

Since its discovery in 1950s, methotroxate is known as an effective drug for treatment of leukemia, many other types of cancer, rheumatoid arthritis, psoriasis, autoimmune disorders and a range of other serious diseases. Similar to many effective drugs, methotrexate posses a broad range of, toxic and adverse effects. These include instant side effects such as fever, pneumonia, nausea, joint pain, weakness, fatigue, rashes and diarrhea. Some more serious side effects of the drug have been reported in central nervous system, respiratory tract and hepatic cells.

Methotrexate may cause cirrhosis of the liver, but this side effect is rare and most likely to occur in patients who already have liver problems or are taking other drugs that are toxic to the liver

On the other hand, when the drug is administrated concomitantly with radiotherapy, methotrexate it could lead to an increase in the risk of soft tissue necrosis and osteonecrosis. It is strongly recommended that methotrexate therapy be seriously accompanied by close monitoring the patient response to promptly detect toxic side effects. The most commonly used assessments include a complete blood count with differential and platelet counts, hepatic enzymes, renal function tests, a chest X-ray and liver stiffness measurement. FibroScan® is a non-invasive effective method for the evaluation of fibrosis caused due to the long term use of methotroxate. However, it has to be borne in mind that FibroScan® evaluates liver stiffness and its values have to be interpreted according to clinical, biological and morphological data.

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List of Abbrevations

ACR	Active rheumatoid arthritis
ALT	Alanine transaminase
AST	Aspartate transaminase
BMI	Body mass index
CF	Cystic fibrosis
CFLD	Cystic fibrosis associated liver disease
CIA	Collagen-induced arthritis
CSF	Blood-cerebrospinal fluid
DAMPA	4-amino-4-deoxy-N-methylpteroic acid
DHFR	Dihydrofolate Reductase
DMARD	Disease-modifying anti-rheumatic drug
FDA	Food and Drug Administration
GGT	Gamma-glutamyltransferase
GI	Gastrointestinal
HAS	Human serum albumin
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
IL	Interleukin
IUPAC	International Union of Pure and Applied Chemistry
Met	Methionine
MTX	Methotrexate
NADP+	Nicotineamide Adenin Dinucleotide Phosphate (oxidized form)
NADPH	Nicotineamide Adenin Dinucleotide Phosphate (reduced form)
NSAID	None-streoidal anti-inflamarory drugs
PBMC	Peripheral blood mononuclear cells
RA	Rheumatoid arthritis