ISSN 2664-4142 (Print) & ISSN 2664-6749 (Online)
South Asian Research Journal of Pharmaceutical Sciences
Abbreviated Key Title: South Asian Res J Pharm Sci

| Volume-6 | Issue-2 | Mar-Apr 2024 | **DOI: 10.36346/sarjps.2024.v06i02.001**

Review Article

A Review of Some the Physiological Effects of Methotrexate on Rheumatoid Arthritis

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Article History
Received: 02.02.2024
Accepted: 11.02.2024
Published: 14.03.2024

Abstract: Rheumatoid arthritis is one of the most prevalent autoimmune diseases at the present time, and it affects both sexes and is characterized by the incidence of women more than men about 1:3. Arthritis causes many negative effects in the various body systems, represented by the lymphatic, respiratory, muscular and blood systems, in addition to its effect on the heart, blood vessels and kidneys. Cytokines have an important role in stimulating the occurrence of rheumatoid arthritis, and these cytokines (IL-1, TNF-α, IL-8) attract inflammatory cells to the synovial membrane due to the erosion of bones and joints. Amethopterin, 4-amino 4-deoxy-N10-methylpteroyl-glutamic acid is a folate antagonist) developed more than 50 years ago in combination with several antifolate therapies used to fight malignant tumors. This review study Further elucidation of the mechanism of methotrexate to treat RA in the patients Methotrexate remains the mainstay of RA treatment and it important to identify the patients in whom the drug is effective and those in which toxicity may be an issue. Genetic and biomarkers may help to stratify patients and possibly shed light on the mechanism for efficacy and for toxicity of methotrexate for RA.

Keywords: Rheumatoid arthritis, cytokines, Methotrexate.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammation that primarily affects the synovial ligaments. The English physician Alfred Garrod first coined the term rheumatoid arthritis in 1859, when he described the widespread incidence of arthritis associated with pain and joint stiffness (Storey, 2009).

Rheumatoid arthritis is one of the most prevalent autoimmune diseases at the present time, and it affects both sexes and is characterized by the incidence of women more than men about 1:3 (Tripathi, 2008). Arthritis causes many negative effects in the various body systems, represented by the lymphatic, respiratory, muscular and blood systems, in addition to its effect on the heart, blood vessels and kidneys (Nicola *et al*., 2006). The pathogenesis of osteoarthritis is still unknown, as genetic predisposition interferes with environmental factors, which are all possible conditions for the emergence of this disease (Buch & Emery, 2002; Ribbhammar, 2005). Among the factors that play an important role in the emergence of arthritis are heat shock protein-70, smoking, smocking, the production of rheumatoid factor, stress and cytokines, in particular the tumor necrosis factor, cellular immunity and autoantibodies (Moseley *et al*., 2003; Costanbader *et al*., 2008). Arthritis is a disease condition that occurs at any age and peaks between the ages of 35 and 50 years, and there is recent evidence indicating that inflammatory factors such as tumor necrosis factor-α and interleukins such as IL-1β, IL-6, IL-17, IL- 18, IL-23 and inflammatory enzymes such as Inducible Nitric Oxide Synthase (INOS) and Cycloxygenase-2 (COX-2) produced in T-cells and macrophages play an important role in the pathogenesis of rheumatoid arthritis (Ganesan *et al*., 2016). There are many medications that are used to treat rheumatoid arthritis, such as corticosteroids, non-steroidal anti-inflammatory drugs (NSAIDS), and disease modifying anti rheumatic drugs (DMARDS). It cannot inhibit the progression of the disease, although the use of this class of drugs for short periods does not significantly affect the body, but its use for long periods causes undesirable effects such as gastric perforation,

complications in the cardiovascular system, nephropathy and gastro-) intestinal ulcers (Ganesan *et al*., 2016). Corticosteroids such as prednisolone are potent inhibitors of inflammatory receptors for many diseases, but they cause many side effects, including thinning of the skin, cataracts, osteoporosis as well as high blood pressure (Kalaiselvan *et al*., 2015). Diabetes, glaucoma, and obesity increase susceptibility to disease (Kuncha *et al*., 2014).

Arthritis

The term Arthritis consists of two parts: Arth, meaning joint, and ritis, meaning inflammation. This term is used to describe many pains that affect joints and bones. The development in civil life played a major role in the spread of many diseases, including diabetes, high cholesterol, cardiovascular diseases, and rheumatoid arthritis. And osteoporosis and tooth decay and this was the result of following incorrect lifestyles and lack of movement and lack of exercise, and arthritis is one of the most prevalent diseases at the present time and affects both sexes and at different ages. and jaw and spine (Hafstorm *et al*., 2001).

Etiology

There are many triggers that lead to the emergence of rheumatoid arthritis, including genetic predisposition, many viruses and bacteria, including (Epstein-Barr virus and Mycobacterium tuberculosis), disorders of the immune system, in addition to psychological factors that lead to a weak immune system of a person, which plays a role It is important in contracting the disease, and people with less culture and a low economic and social level are among the problems that lead to rheumatoid arthritis (Majithia & Geraci, 2007). Rheumatoid arthritis begins with inflammation of the synovial membrane of the joints, especially in the fingers and toes, where the accumulation of inflammatory cells in appropriate numbers leads to the breakdown of body tissues, and the accumulation of synovial fluid, swelling and thickening of the joints, leading to the extension of the layer of fibrovascular tissue over the bone and cartilage and from Then the erosion of the bone and cartilage, and in rare cases, rheumatoid arthritis leads to inflammation in the spine and some organs such as the skin, heart and lungs (Hewlett *et al*., 2005).

The Role of Cells and Molecules Inflammatory Mediator in the Progress of Rheumatoid Arthritis

Cytokines have an important role in stimulating the occurrence of rheumatoid arthritis, and these cytokines (IL-1, TNF-α, IL-8) attract inflammatory cells to the synovial membrane due to the erosion of bones and joints (Karray *et al*., 2011). Cytokines cause the proliferation of synovial tissue and the breakdown of cartilage, which causes an imbalance in the balance of inflammatory cytokines within cartilage, including (IL- 4, IL-10, IL-13) (Golbach &Lipsky, 2003).

Studies have indicated that genetic factors are among the causative factors of rheumatoid arthritis, where genetic factors contribute to the occurrence of the disease by more than 60-50% (MacGregor *et al*., 2000), and the presence of anti-citrullinated protein bodies (ACPA) in the blood is a sign of rheumatoid arthritis. Biological diagnosis of rheumatoid arthritis, as it has sensitivity and specialization to the disease and its presence indicates the progression of the disease (Steiner & Smolen, 2002; Farragher *et al*., 2008). Smoking is also a risk factor for rheumatoid arthritis, as there is a close association between smoking and people with rheumatoid arthritis (Wolfe, 2000). Inflammatory factors such as TNF-α and interleukins such as IL-1β, IL-6, IL-17, IL- 18, IL-23 and inflammatory enzymes such as Inducible Nitric Oxide Synthase (INOS) and Cycloxygenase-2 (COX-2) An important role in the pathogenesis of rheumatoid arthritis (Ganesan *et al*., 2016). Among the complex mechanisms that lead to inflammatory reactions in arthritis is that inflammatory mediators such as nitric oxide (NO) and prostaglandins, which act on special receptors located on the cell surface, may participate in inflammatory reactions (Cuzocera *et al*., 2002), where Cytokines such as IL-1β and TNF stimulate the Nitric Oxide Synthase pathway in bone cells, since NO derived from this pathway potentiates cytokines and induces inflammation in bone loss. An elevated level of prostaglandins involved in these complex interactions leads to the deterioration of articular cartilage and articular bone (Trebino *et al*., 2003). Arthritis is also one of the most important serious diseases, and it affects humans and animals, and there are many types of arthritis, which are characterized by pain in the joints, high temperature, inability to walk, and the appearance of stiffness and redness around the joint (Hafstorm *et al*., 2001).

Physiology of Rheumatoid Arthritis Disease

The immunopathological mechanisms of rheumatoid arthritis are not clear. In the presence of pathogens of unknown etiology, a non-specific immune response occurs, as the (immunopathologic) joint in this disease begins with an inflammatory response within the synovial membrane, which is an enlargement of the synovial tissue with infiltration of mononuclear cells and lymphocytes. T invades this tissue and destroys the cartilage and bony surface of the joint, leading to erosions in it, and then invades other tissues such as tendons (Bresnhan, 1999).

The pathological mechanisms can be divided into three main stages, the first is the onset stage, in which some cells migrate to the synovial tissue, and this stage is characterized as non-specific and dependent on various environmental factors related to RA in the late stages of non-specific cases. Treatment This type of inflammation may, in many cases, lead to complete recovery in a short or long time, or lead to chronic inflammation of low intensity responsible for the second stage, which is characterized by inflammation of the synovial tissue and cellular infiltration with the development

of inflammation and its transfer to the synovial tissue Cartilage This condition is similar to chronic rheumatoid arthritis (RA), but it is a non-specific inflammation. The third stage is very important, and includes the stimulation of immune proteins by cellular activities, after which the development and spread of inflammation of the entire synovium and destruction of the joints. This stage causes the destruction of the cartilage and bone directly, all of these manifestations are the hallmark symptoms of RA (Combe & Dougdos, 2001). The damage that occurs at the level of the synovial membrane is caused by four types of mechanisms that are represented in non-specific enzymatic mechanisms by releasing a large amount of protein-degrading enzymes, metalloproteases, collagenases that degrade cartilage, and humoral mechanisms with the production of rheumatic factor (FR) (globulins). Anti-IgG immunoreactivity, autoantibodies to a number of antigens (antibodies, anti-nuclear, anti-cytoplasm) and cellular-mediated with overactivity of helper lymphocytes in the synovial tissue membrane, as well as mechanisms by various cytokines notably IL-1, IL-1 -6 and TNFα by its inflammatory and IL-8 effect on polymorphonuclear neutrophil cells (Raissauni *et al*., 2005), and stimulating cytokines play an essential pathogenic role in inflammatory processes, synovitis and cartilage destruction, where an imbalance occurs within inflamed joints Among the stimulatory cytokines such as IL-1, IL-6 and TNF-α and the anti- inflammatory cytokines such as IL-13, IL-4 and IL-10, soluble TNF-α receptors are also present. And IL-1, which is presented in a small amount, is insufficient due to the competitive action of (IL-1-RA) on the IL-1 receptor and thus cannot stop the inflammatory action (Golbach & Lipsky, 2003). At the level of the synovial membrane, the immune mechanisms of RA are characterized by They are complex, as there is significant overlap between B and T lymphocytes, macrophages, and cytokines. It is believed that the first mechanism begins with the activation or activation of CD+4 lymphocytes and this activation is carried out by the APCs (Denarie *et al*., 2017). At the synovial fluid (SF) level, neutrophils are dominant and are attracted by a number of chemoattractants. These cells release activating enzymes such as collagenases that play a role in the erosion of surface cartilage and bone (Ferrara *et al*., 1991). In the synovial fluid, there are immunoglobulins consisting mainly of the Rheumatoid Factor (RF) of the type IgG and IgM that form immune complexes and lead to the activation of the complement system that increases the inflammatory state, from which RA is released starting with the activity of CD4+, which doubles the immune response. By stimulating other immune cells, which are monocytes, fibroblasts, chondrocytes and osteoclasts, the release of special cytokines (IL-1, IL-6 and TNFα) causes inflammation at the level of the synovial tissue (Weyand, 2000).

Disease-Modifying Anti Rheumatic Drugs (DMARDs

There are other ways to treat RA, which are represented by a group of antirheumatic agents (DMARDs) such as methotrexate, infliximab and rituximab. DMARDs are able to slow disease progression and reduce the devastating effects of untreated disease progression. These medications are often prescribed when there are symptoms or signs of active inflammatory condition. DMARDs do not have direct anti-inflammatory effects, but they improve joint pain. It also reduces systemic symptoms and rheumatic factor within several months of its use. The early use of these drugs in combination with each other has strong effects in relieving inflammation through its high inhibitory ability to many inducers of inflammation, thus improving disease management. Many DMARDs have been applied in the treatment of rheumatoid arthritis, some alone and others combined (Kremer *et al*., 2004).

Methotrexate

Amethopterin, 4-amino 4-deoxy-N10-methylpteroyl-glutamic acid is a folate antagonist) developed more than 50 years ago in combination with several antifolate therapies used to fight malignant tumors (Bertino *et al*., 1993), targets the (MTX) Dihydrofolate reductase (DHFR) enzyme (Kameda *et al*., 2010). Folic acid is a water- soluble vitamin that cannot be synthesized by the human body and therefore nutritional content is essential to obtain it. It is one of the many forms of folate that is Part of the metabolism within the body is the main form of folate in plasma is 5- methyl tetra-hydrofolate (THF). Folate, as well as antifolates such as methotrexate, can enter cells via reduced folate carrier- RFCs or folate receptors, and the RFCs have been found to have a greater affinity for MTX than for folic acid and vice versa. correct (Anderson *et al*., 2012; Jansen *et al*., 2014). In addition to the inhibition of DHFR by MTX as a result of reducing folate competition in these reactions, methotrexate is responsible for the direct or indirect inhibition of many folic acid- dependent reactions through its effect on other enzymes involved in the DNA synthesis pathway such as Thymidine synthetase, TransformylaseAmidophosphoribosyltransferase (Fairbank *et al*., 1999; Smolen *et al*., 2013). Although many of the enzymes targeted by MTX are known, there is controversy about the mechanism by which MTX works. Studies (Seitz *et al*., 1998; Dolhain *et al*., 1998) indicated an effect of MTX on cytokines, as these studies showed Through the immunological analysis of joint tissue biopsies, the concentrations of both ßlL-1 and TNF-α decrease, and in the bone marrow, the supply of mononuclear cells is increased after using MTX as a treatment for patients with rheumatoid arthritis. MTX enhances the formation of IL-1RA receptors and suppresses ßlL-1 production), as shown by Barrera *et al*., (1994) that a single dose of MTX given to patients with RA leads to a decrease only in the production of ßlL-1 and prepared mononuclear cells by peripheral blood (Peripheral blood derived mononuclear cells-PBMC) and not in the production of α-TNF or other white blood cells when cells are stimulated by lipopolysaccharide- (LPS) 48 hours after administration. It is worth noting that the activity of T-cells can be affected after a period of six weeks of taking doses of MTX, as the production of CD4 + T cells from the cytokine α-TNF decreases dramatically, while the production of IL-10 by these cells increases (Rudwaleit *et al*., 2000).

MTX Effects on Immune/Inflammatory Cell Proliferation and Apoptosis

Recent data have already suggested that the disruption of the cell cycle caused by high dose MTX treatment may be the initial step of the apoptotic sequence of dying cells and may explain the antiproliferative effects of the drug.54 The involvement of the APO-1/Fas (CD95) receptor/ligand system in MTX induced apoptosis has been recently identified in leukaemia cells, with a peak of apoptosis between 24 and 48 hours (Friesen *et al.*, 1996). In addition, MTX was found to inhibit markedly the spontaneous proliferation of U937 monoblastic leukaemia cells in vitro and induce the rapid expression of the apoptosis receptor CD95 also in presence of 1,25-OHcholecalciferol (Seitz *et al*., 1998). Results of another recent investigation, are in agreement with these latter studies and seem to suggest that intermediate MTX concentrations (50 μg/ml), as obtained in serum after low dose treatment, can induce both a significant cell growth inhibition and apoptosis, at least in monocytic immature cells (THP-1cell line) (Cutolo *et al*., 2000). For cell proliferation, the lowest in vitro MTX concentrations (from 5 to 500 ng/ml) were confirmed to be ineffective.54 In that study no significant effects on synovial macrophage proliferation were obtained with an MTX concentration of 50 μg/ml (achievable in the serum with low dose MTX treatment in RA (Cutolo *et al*., 2000). The explanation for the lack of modulatory in vitro potency of MTX on synovial macrophage growth and apoptosis, as already found for cyclosporine A, may be that MTX affects only immature differentiating monocytes and not differentiated cells (that is, tissue infiltrating monocytes and resident macrophages, respectively) (Cutolo *et al*., 1998). Therefore, these findings suggest that MTX might inhibit recruitment of immature and inflammatory monocytes into inflammatory sites and could reduce the survival of these cells in the inflamed synovial tissue (Cutolo *et al.*, 2000). A recent paper investigated whether other immunosuppressive properties of low dose MTX treatment were related to apoptosis. The study showed that activated T cells from human peripheral blood underwent MTX induced apoptosis, which was completely abrogated byaddition of folinic acid (Cronstein *et al*., 1993).

Finally, in vitro activation of peripheral blood taken from patients with RA after MTX injection resulted in apoptosis. However, several studies have recently shown that low dose MTX may well induce antiproliferative effects on immune cells owing to inhibition of dihydrofolate reductase and folate dependent transmethylations as apoptosis independent mechanisms. A recent study showed that patients with RA, treated with MTX, expressed low concentrations of circulating purines and pyrimidines, with consequent reduced availability for DNA and RNA synthesis and cell proliferation (Smolénska *et al*., 1999).

MTX Effects on Cyclo-Oxygenases and Lipoxygenase

Prostaglandins and leucotrienes are strongly involved in the inflammatory reaction. In particular, prostaglandins are important mediators of joint destruction in RA. A recent study investigated the effects of MTX on cyclooxygenase (COX) metabolism by evaluating the prostaglandin E2 (PGE2) synthesis in cultured human rheumatoid synoviocytes (Vergne *et al*., 1998) The results showed a dose dependent decrease of IL1 induced PGE2 production by cultured RA synoviocytes that was determined by MTX treatment, whereas neither COX-1 nor COX-2 mRNA expression was affected by MTX incubation.77 In a more recent study the effects of MTX on COX-1(thromboxane B2) and COX-2 (PGE2) activity were evaluated in whole blood of patients with RA treated with MTX (Mello *et al.*, 2000) Interestingly, COX-2 activity was found to be reduced in the plasma of patients with RA treated with MTX in comparison with healthy controls. Inhibition of COX-2 activity was also found when blood of normal donors was co-incubated with the serum of MTX treated patients with RA. However, direct action of MTX on either enzyme was excluded (Mello *et al*., 2000). The inhibitory effect exerted by MTX on neutrophil chemotaxis, found in synovial fluid of patients with RA, might further determine decreased COX concentration in inflammatory joints (Kraan *et al*., 2000).

Side Effects of using MTX Treatment

MTX has an effect on the immune system and due to the risk of developing severe side effects, this treatment is provided only for severe cases when the patient has not responded to other previous treatments. MTX may cause severe harm to fetuses, so its use during pregnancy is prohibited, and MTX also harms the fertility of women and men, but this damage is repairable, so it is necessary to refrain from starting pregnancy until three months have passed from the completion of treatment, so it is associated with treatment with MTX Undergo routine tests for blood cell count, liver and kidney function (Ranganathan *et al*., 2006).

Tetrahydrofolic acid - THF Acid, a compound necessary for cell division and building the genetic material of the cell (DNA) The effectiveness of MTX is to prevent these processes from occurring, and it particularly affects rapidly dividing cells, and in this way MTX also harms healthy cells Such as cells in the mucous membranes of the mouth and intestines, as well as in bone marrow cells (Bone Marrow) for this reason in treatments that include high drug doses of MTX, usually treatments containing folic acid, also known as (Leucovorin, which is a derivative of tetrahydro Folic acid in this way can compensate for the deficiency of folic acid, which is being degraded, thus giving healthy cells a chance to recover. MTX, like all other anti-tumor drugs, has an inhibitory effect on the production of different types of blood cells in the bone marrow, which leads to anemia) and increases the body's susceptibility to infections and the occurrence of abnormal blood bleeding, and MTX may lead to changes in lung tissue, which require immediate cessation of treatment,

so it is necessary to inform the to the attending physician if frequent coughing and shortness of breath occur. Other common side effects of MTX include soreness and sores in the mouth, diarrhea, altered sense of taste, hyperpigmentation of the skin, irritation of the skin and eyes, temporary hair loss, cytotoxic effect (when taken in high doses), nausea, vomiting, sensitivity. Sunlight and an allergic reaction to treatment (Bingham *et al*., 2010).

CONCLUSION

This review study Further elucidation of the mechanism of methotrexate to treat RA in the patients Methotrexate remains the mainstay of RA treatment and it important to identify the patients in whom the drug is effective and those in which toxicity may be an issue. Genetic and biomarkers may help to stratify patients and possibly shed light on the mechanism for efficacy and for toxicity of methotrexate for RA.

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