Dean&Francis
ISSN 2959-409X

Mechanisms and Clinical Application of Methotrexate in Rheumatoid Arthritis

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Abstract:

Rheumatoid arthritis (RA) is a systemic, chronic autoimmune disease that can severely damage the patient's joints and place a great burden on their work and life. Methotrexate (MTX) is currently the first-choice drug for treating RA. It achieves the therapeutic goal of RA by inhibiting immune responses, promoting the release of adenosine from cells, and inhibiting angiogenesis. However, the efficacy and side effects of MTX vary significantly among individuals, and long-term, high-dose use of MTX can cause serious adverse reactions in some patients, forcing them to discontinue the medication. This article comprehensively analyzes the pathological mechanisms of RA and the molecular and cellular mechanisms of MTX on RA, concluding that the clinical efficacy of MTX and MTX combined with Wind-Dispelling and Dampness-Removing Pain-Relieving Decoction or cannabidiol (CBD) can effectively improve clinical outcomes. It provides a reference for future research on combination therapy with MTX, and the issue of the considerable differences in the clinical efficacy of MTX has not been resolved. Future research can focus on the individual differences in MTX.

Keywords: Rheumatoid arthritis; methotrexate; mechanism.

1. Introduction

There are 17.6 million people worldwide suffering from Rheumatoid arthritis (RA) [1]. It accounts for approximately 0.5% to 1% of the global population [2], RA mainly affects the synovial joints of patients, can destroy the symmetry of small joints in the hands and feet, causing clinical symptoms such as joint pain, swelling, and effusion, if the condition is not controlled in time, in severe cases, it can lead to joint deformities, loss of function, or even death.

According to the eight-year survey statistics on RA patients conducted by Anna Giorgia Osele and others, the prevalence of the disease in female patients is twice that of male patients. The average mortality rate of RA patients is 3.22%, the age at death ranges from 77 to 88 years old, and the median age is 83 years old. Researchers calculated the standardised mortality ratios (SMRs) by comparing RA patients with the general population and found an overall

ISSN 2959-409X

SMR of 1.28, but the SMR for patients under 45 years old is 2.15, indicates that patients under 45 have a higher risk of death [3].

The burden of disease increases significantly with the age of RA patients, and because the disease has a high level of activity, more than half of RA patients often indicate that their health is poor. RA will bring burdens to the patients themselves, their families, and the entire healthcare system. The pathophysiological mechanism of RA includes abnormal interactions between the neuroendocrine and immune systems. Endocrine disorders are caused by its overactivated immune system and inflammatory response, causing disorders of B cells, T cells, and macrophages, eventually forms a chronic inflammatory response. The clinical goal of RA treatment is to reduce the inflammatory response, improve the patient's adverse symptoms, delay the progression of the disease. At present, The medications for the treatment of RA include JAK kinase inhibitors, nonsteroidal anti-inflammatory drugs (NSAIDs), corticosteroids, biologics, and conventional disease-modifying antirheumatic drugs (DMARDs). MTX, as a type of traditional disease-modifying antirheumatic drug, is a first-line drug for treating RA. Its advantages are good long-term clinical efficacy, safety, and tolerability. But MTX also has its shortcomings, there are obvious individual differences in clinical efficacy and adverse reactions, long-term use of MTX alone can affect the patient's liver and kidney function, it should be used in combination with other drugs in clinical practice [4-6].

This review mainly discusses the pathological mechanisms of RA, as well as the cellular mechanisms of MTX in the treatment of RA and the pharmacological effects of RA in the body, integrate and analyze disease mechanisms with drug treatment mechanisms, provides new recommendations for combination therapy with MTX and better treatment services for patients.

2. Pathological Mechanism of RA

Synovitis is a characteristic manifestation of RA. Autoimmunity caused by RA can lead to tissue damage, stimulate the synovium to produce inflammation and form vascular films, causing irreversible damage to bones and cartilage. CC family chemokine CCL20 (CCL20) is highly expressed in the synovial tissue of RA patients, Causing the upregulation of its receptor CCR6, promote the migration and activation of immune cells such as dendritic cells (DCs) to joint tissues, the complex interactions between various DC subsets and other cellular components in joint tissue can cause joint inflammation [7]. The number of plasmacytoid DCs and conventional DCs in peripheral blood decreased, the activity of RA can be detected by examining DCs.

At the same time, mature DCs secrete interleukin-12 (IL-12) and IL-23 within the joints, promote the activation of antigen-specific helper T cells 17 (Th17) [7]. T cells migrate to the synovium after activation, interactions occur locally among osteoclasts, DCs, synovial fibroblasts, and resident macrophages, promote the occurrence and development of RA. Th1 cells can trigger inflammatory responses in the synovium, its mechanism is that Th1 cells can provide effective support to other immune cells. Meanwhile, elevated levels of granzyme and perforin are expressed in CD4 CD28- T cells in the peripheral blood of some RA patients.

The severity of RA is closely related to Th17 and the effector factor IL-17 it secretes. Th17 is a novel CD4 effector T cell first discovered in mouse models by Langrish et al. primarily secretes IL-17, dependent on retinoic acid-related orphan receptor (RORγt) [8]. Shown through animal experiments, Th17 cells play an important role in the disease progression of collagen-induced arthritis (CIA) in mice. Through detecting the cytokines, Th17 subset numbers, and mRNA expression levels in RA patients by Alunno and others [8] , it was concluded that the disease activity of RA is closely related to Th17 and the inflammatory factors it secretes.Th17 synergizes with tumor necrosis factor (TNF)-α to induce activation in chondrocytes and synovial cells, enabling these two types of cells to synthesize and secrete metalloproteinases, this further leads to the breakage of proteoglycan molecules, the destruction of cartilage tissue, and an increase in osteoclast formation.

In addition, the onset of RA is also related to factors such as immune dysfunction, endocrine dysfunction, genetics, and the environment. During the RA active phase, due to overactivation of T/B cells, the body has lost immune balance, The immune tolerance of T cells is disrupted, hyperactivity of humoral immune function leads to excessive proliferation of B cells and secretion of immunoglobulins such as IgA, IgG, and IgM, forming immune complexes that deposit in the synovium, further aggravating synovial damage. The estrogen-mitochondria regulatory axis results in the number of female patients being twice that of male patients. Genomic studies of patients with rheumatoid arthritis have shown that nucleotide variations in the mitochondrial DNA control region, the ribosomal 45S cluster 2 (RNR2) gene, as well as increased heterogeneity and mitochondrial DNA repeats, are all associated with disease progression. Air pollution can stimulate the body's immune system, promote the release of inflammatory mediators and antibodies, thereby aggravating the progression of RA [9].

3. Molecular and Cellular Mechanisms of MTX Treatment for RA

3.1 Pharmacological Effects of MTX on Different Targets

MTX can promote the release of adenosine by cells, and adenosine exerts a strong anti-inflammatory effect by binding to the A2A receptors on cells. This anti-inflammatory effect can inhibit neutrophil infiltration, thereby alleviating joint inflammation and reducing swelling and pain [10]. Si Lijun conducted a controlled study, randomly dividing 86 RA patients who were admitted to Taihe Hospital affiliated to Anhui University of Chinese Medicine from March 2021 to March 2022, met the Western medicine RA diagnostic criteria, and were in the active phase of RA, into a control group and an observation group [5]. The control group was treated with MTX, the observation group added the Wind-Dispelling and Dampness-Removing Pain-Relieving Decoction on the basis of the control group. After eight weeks of observation and research, it was concluded that methotrexate combined with Wind-Dispelling, Dampness-Removing, Pain-Relieving Decoction can effectively increase the IL-10 levels in patients, IL-10 is secreted by regulatory T cells (Treg), it is a multifunctional negative regulatory factor. The function of IL-10 is to inhibit the inflammatory response by reducing the production of inflammatory cytokines and antibody secretion, Reduce the number of characteristic inflammatory factors of active RA in patients, such as rheumatoid factor (RF), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), etc.

MTX can negatively regulate the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway. The JAK/STAT signaling pathway plays a key regulatory role in the pathogenesis of RA, participating in the activation of various immune cells and the release of inflammatory cytokines during the disease process. In addition, the JAK/STAT signaling pathway is also widely involved in the processes of proliferation, differentiation, and apoptosis of peripheral blood cells. MTX negatively regulates the phosphorylation of STAT1, inhibits Th17 differentiation [10], reduces the formation of pannus during the progression of RA, and decreases the damage pannus causes to bone.

Vascular pannus is formed under the stimulation of long-term chronic inflammation and is composed of hyperplastic and hypertrophic synovial cells, inflammatory cells, newly formed microvessels, and organized fibrous tissue. Synovial thickening, congestion, edema, increased blood vessels, infiltration of various immune cells such as multinucleated neutrophils, forming granulation tissue rich in blood vessels with villous-like proliferation, eventually leading to the formation of a pannus. Vascular pannus has infiltrative and invasive characteristics, gradually invading from the outside to the inside along the cartilage surface, covering the entire surface of the joint cartilage, then invading the bone from the cartilage, leading to subchondral bone defects and causing osteoporosis. The pannus that surrounds cartilage has a high metabolic capacity, which can impede the normal nutrient supply to cartilage and bone, causing the synovial membrane to form a hypoxic microenvironment, leading to abnormal changes such as mitochondrial dysfunction and increased production of reactive oxygen species within cells, and resulting in excessive activation of inflammation. In addition, MTX also has the effect of suppressing immune responses. MTX inhibits dihydrofolate reductase through an irreversible reaction, blocking the synthesis of pyrimidines and purines, which leads to the inability of DNA synthesis and cell proliferation, thereby exerting an immunosuppressive effect [10].

3.2 Intracellular Metabolism of MTX

MTX is absorbed in the proximal jejunum after oral administration and is divided into two metabolic pathways. The first pathway is metabolized into 2, 4-diamino-n10-methylbutterfly acid (DAMPA) under the action of the intestinal flora [6]. This pathway only involves a small portion of MTX. As the gut microbiota is involved in and affected by the metabolism of MTX, long-term and excessive use of MTX will reduce the species diversity and richness of the patient's gut microbiota. The second route is that MTX reaches the liver in its original form through the hepatic portal vein [6]. Of the MTX entering the liver, 10% is catalyzed by aldehyde oxidase to generate 7-OH-MTX [6]. This metabolic product has no therapeutic effect on RA and is excreted from the body through the kidneys; The remaining MTX enters the bloodstream through the inferior vena cava behind the liver. The metabolism of MTX in the blood is divided into three pathways. The first pathway is that MTX directly enters the knee joint and other sites in its original form to exert pharmacological effects, and is finally distributed in two chambers and cleared at a primary rate; the second route is that a small portion of MTX is transported to liver cells via the transport polypeptide (OATP1B1) and re-enters the liver; the third pathway is that MTX enters red blood cells under the active transport of reduced folic acid carriers and is catalyzed by folyl polyglutamate synthase (FPGS) to metabolize methotrexate polyglutamates (MTXPGs). MTXPGs play a significant role in the treatment of RA.

MTXPGs are the main substances for treating RA. MTX-

ISSN 2959-409X

PGs inhibit thymidylate synthase (TYMS) by preventing the methylation of deoxyuridine monophosphate (dUMP) to form deoxythymidine monophosphate (dTMP). MTX-PGs inhibit ATCAR, causing it to aggregate within cells, thereby inhibiting ADA, reducing adenosine degradation, and increasing the amount of adenosine in the blood. Adenosine can activate A_{2a}, A_{2b}, A_3, inhibit TNF-α, IL-6, IL-8, and reduce the expression of E. At the same time, adenosine can promote mRNA transcription, increase IL-10 secretion, and exert an anti-inflammatory effect. MTX-PGs bind to DHFR, blocking the conversion of DHF to THF, causing the amount of THF in the body to decrease. In the folate cycle, THF is metabolized to 5,10-CH2-THF, and 5,10-CH2-THF is metabolized to the folate cofactor 5-CH3-THF under the catalysis of MTHFR.MTX achieves the inhibition of the folate cofactor 5-CH3-THF by inhibiting THF and indirectly inhibiting MTHFR. Folic acid cofactor 5-CH3-THF is an important substance in the process of metabolizing homocysteine into methionine.

4. Clinical Efficacy

Si found through comparing the clinical signs of patients before and after taking medication that the average degree of joint pain decreased by 3.52 , the number of swollen joints decreased by 7.93,the average duration of morning stiffness decreased by 41.33 minutes, by testing fasting venous blood in the early morning, it was found that the patient's IL-10 increased by an average of 9.62 ng/mL after treatment, IL-35 increased by an average of 11.23 ng/mL, TNF-α decreased by an average of 27.56 ng/mL, RF decreased by an average of 39.97 mg/L, ESR decreased by an average of 33.31 mm/h, and CRP decreased by an average of 50.55 mg/L. The Qu Feng Chu Shi Zhi Tong Decoction will enhance the therapeutic effect of MTX, increasing the clinical efficacy from 72.09% to 90.70% [5].

Jiahe Xu and others established 40 mice models of serum-transferred arthritis (STA) and randomly divided the mice into 8 independent experimental groups [11]. The study found that in both the MTX monotherapy group and the MTX combined with CBD treatment group, the mice's body weight initially decreased, and began to increase after days 7-9, indicating that MTX and CBD combined with MTX treatment can effectively control disease progression in mice. The clinical arthritis score continued to rise during the first 7 days, and after 7 days it tended to stabilize and no longer increased. Among them, the combination of CBD with a high dose of MTX showed a decrease after the 7th day and tended to stabilize after the 11th day. It indicates that the combination of CBD with high-dose MTX is most effective for arthritis in mice. Through observing the swelling degree of the hind paws of mice, it was found that MTX and the combination of CBD and MTX treatment can improve symptoms of diffuse erythema, extensive ankle joint swelling, and limited movement of the hind paws in mice. Researchers evaluated the overall integrity of mouse joint structures through micro-CT and histopathological examination. The results showed that the model group exhibited extensive joint surface roughness, bone erosion, and bone resorption, while the combined treatment effectively maintained the bone structure.

Tsutomu et al. conducted a 5-year study on the therapeutic effects of MTX and Upadacitinib (UPA) monotherapy on Japanese RA patients [12].Analysis using the non-responder imputation (NRI) method showed that 32.1% of patients receiving MTX monotherapy had a C-reactive protein-based disease activity score 28 (DAS28-CRP) of less than 2.6 after 260 weeks, and 39.3% of patients had a DAS28-CRP of ≤3.2 after 260 weeks. Through the actual observation method (AO), it was found that 17.9% of patients achieved clinical disease activity index (CDAI) remission.

5. Discussion and Conclusion

RA is a chronic autoimmune disease, and patients often exhibit morning stiffness, joint swelling, and pain. MTX is the most commonly used drug in the clinical treatment of RA, but long-term use of MTX can have adverse effects on patients and easily lead to drug resistance, increasing the burden on the liver and kidneys, reducing liver and kidney function, inhibiting bone marrow hematopoietic function and the proliferation of intestinal flora, and making patients prone to symptoms such as anemia and digestive system symptoms. Some patients experience symptoms such as loss of appetite, nausea, and vomiting. Because the synovium of RA patients is covered by vascular membranes, the intracellular oxygen tension decreases, leading to mitochondrial damage. Under hypoxic conditions, the level of reactive oxygen species (ROS) in cells rises sharply, causing oxidative stress. Under the combined effects of hypoxia and oxidative stress, the probability of mtDNA mutations increases, and mitochondrial damage is further aggravated. MTX further aggravates mitochondrial damage on the basis of RA. Studies using mouse and cell models show that low-dose MTX specifically depletes mitochondrial formate and impairs one-carbon unit metabolism [9]. Mitochondrial dysfunction can also increase the adverse reactions of MTX. To avoid these adverse reactions, patients can use Tofacitinib, Leflunomide, Cyclosporine A, and others in conjunction with MTX.

This article reviews the pathological mechanisms of RA

and the molecular mechanisms of MTX treatment for RA from multiple aspects, and briefly describes the clinical efficacy of MTX treatment for RA. Through analysis, it is concluded that MTX can treat RA through multiple pathways, and when MTX is combined with other drugs for RA treatment, its clinical efficacy is superior to monotherapy. Future research on MTX can be directed towards combination therapy. Although MTX plays an indispensable role in the treatment of RA, because the cause of RA is currently unclear and MTX can cause irreversible side effects for patients, there is a certain risk in using MTX to treat RA. In the future, the clinical efficacy of MTX can be further improved and its risks reduced by improving the route of administration, standardizing medication timing, and dosage.

This paper has shortcomings, simply summarize the research of a few scholars, lacking systematic summary and in-depth analysis。

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