Original Article:

Correlation of pharmacokinetic disposition of methotrexate and serum cytokine levels in rheumatoid arthritis patients

Raisa Aringazina1*, Leysan Myasoutova2, Liudmila Babaskina3, Olga Pashanova4

Abstract

Objective: Methotrexate is the principal basic anti-inflammatory medication used in the rheumatoid arthritis treatment. Therefore, research into its effect on cytokine concentrations may help improve the effectiveness of disease treatment. Study aim: was to investigate the effect of methotrexate therapy on the blood content of proinflammatory cytokines in RA patients, as well as define the relation of proinflammatory cytokine blood concentration to clinical and laboratory disease features and methotrexate pharmacokinetics. Material and methods: Sixty-five patients with rheumatoid arthritis (46 women and 19 men; average age 50.83±8.91 years) underwent an examination. Thirty-two patients (26 women and 6 men) participated in studying methotrexate pharmacokinetics. Results and Discussion: The course of rheumatoid arthritis is accompanied by a significant increase of TNF-α (40.36±4.01 pg/ml), IL-1β (17.68±1.74 pg/ml), and IL-6 (8.39±0.46 pg/ml) in the blood. Oral administration of methotrexate (MT) for patients with rheumatoid arthritis promotes persistent elimination of clinical disease symptoms and reduced production of proinflammatory cytokines: TNF-α drops by 2.08 times (p<0.05) compared to baseline, that of IL-1β and IL-6 – by 2.86 (p<0.05) and 2.84 times (p<0.05), correspondingly. Blood levels for TNF-α and IL-6 are consistent with clinical markers for rheumatoid arthritis, such as joint pain, joint swelling, and morning stiffness in extremities. Blood cytokine concentrations were not correlated with AUC, Cmax, and Tmax. There was an inverse correlation between the blood rates of TNF-α and the weekly dose of MT (r = -0.34, p<0.05) and IL-6 and the weekly dose of MT (r = -0.32, p<0.05). Conclusion: Rheumatoid arthritis is closely associated with increasing proinflammatory cytokine blood levels, which affects the clinical course of the disease. The dosage of methotrexate applied affects the blood concentrations of IL-6 and TNF-α.

Keywords: clinical markers of inflammation; cytokine profile; methotrexate in rheumatoid arthritis; pharmacokinetics of methotrexate; rheumatoid arthritis.

Introduction

Nowadays, the problem of rheumatic disorders (RD) has become especially important in modern medical science.1 This is because RDs are among the most common non-infectious diseases worldwide (third place after cardiovascular and digestive system diseases), which lead to patients' disability.1,2 Rheumatoid arthritis (RA) is the most common type of arthritis for people with RDs. The RA is common to all countries of the world, irrespective of climatic conditions and geographical features.3 As reported by the 2017 Global Burden of Disease study, there were nearly 20 million RA patients in 195 countries, with the highest prevalence in the United Kingdom.

1. Raisa Aringazina, Department of Internal Diseases № 1, Non-Commercial joint-stock Society “West Kazakhstan Marat Ospanov Medical University”, Aktobe, Kazakhstan
2. Leysan Myasoutova, Department of Hospital Therapy, Kazan State Medical University, Kazan, Russian Federation.
3. Liudmila Babaskina, Department of Pharmacy, Sechenov First Moscow State Medical University, Moscow, Russian Federation.
4. Olga Pashanova, Department of Economics and Organization of Pharmacy, Sechenov First Moscow State Medical University, Moscow, Russian Federation.

Correspondence: Raisa Aringazina, Department of Internal Diseases № 1, Non-Commercial joint-stock Society “West Kazakhstan Marat Ospanov Medical University”, Aktobe, Kazakhstan. Email: raringazina@rambler.ru
(471.8 cases per 100,000 population), Trinidad and Tobago (404.4 cases per 100,000 population), Barbados (40.6 cases per 100,000 population). The lowest rates were reported in Indonesia (91.1 cases per 100,000 population), Timor-Leste (91.4 per 100,000 population), and Sri Lanka (97.2 per 100,000 population). The maximum incidence of this nosology occurs between the ages of 60 and 65, and females are 2 to 4 times more vulnerable than males. In the US, the prevalence of RA among females is 0.78%, while among males—only 0.31%. The disease results in a significant deterioration in the quality of life, and lack of proper and timely treatment leads to disability in almost half of patients within the first five to seven years of illness. Among patients with RA the mortality rate is 54% higher than in the general population. At that, it is higher for the cardiovascular system, the musculoskeletal system, the respiratory and gastrointestinal organs. The five-year survival rate for serious diseases with visceral symptoms is less than 50%. A recent systematic review of 72 studies evaluating the costs of treating patients with RA reported that the annual direct and indirect costs associated with handling this disease ranged from USD 2,408 to USD 8,845. Initially, the total cost of RA treatment is about EUR 2 billion per year, of which 45% represent indirect costs, 45% are direct medical expenses, and 1% accounts for direct non-medical costs.

By its nature, rheumatoid arthritis is a chronically evolving autoimmune systemic disease that affects peripheral joints with symmetrical erosive-destructive lesions. The etiology of the disease is still unknown, and various infectious agents (Epstein-Barr virus, retroviruses, parvovirus B19, bacterial stress proteins, superantigens), endogenous factors (citrullinated peptides and proteins), genetic factors, and harmful habits (smoking) are considered as possible triggers. The pathogenesis of RA is based on autoimmune inflammation, which is systemic, but primarily affecting the joints, i.e., the synovial membrane. The uncontrolled progressive inflammatory process in the synovial membrane of the joints is a key characteristic of RA, distinguishing it from other rheumatic and non-rheumatic inflammatory diseases. Thus, the RA is characterized by the formation of an ectopic focus of hyperplasia (with active proliferative fibroblasts, macrophages, and lymphocytes) in the synovial tissue, i.e., pannus, which is a pathognomonic sign of the disease. Pannus is distinguished by invasive growth that causes the joint cartilage destruction with its subsequent disappearance, the proliferation of granulation tissues, and, therefore, the development of ankyloses.

Over the past few years, scientists have increasingly focused on the pathogenesis of RA in various cytokines. During RA, proinflammatory cytokines of macrophage origin are being excessively produced in synovial tissue. These are interleukin-1 (IL-1) and IL-6, tumor necrosis factor-alpha (TNF-α), and granulocyte-macrophage colony-stimulating factor. The CKs produced within the pannus induce the enzymes that destroy cartilage. That is, major clinical symptoms of RA (chronic synovitis, joint destruction) are associated with proinflammatory CKs, and TNF-α plays a key role in this process. This cytokine demonstrates its biological activity by interacting with specific receptors expressed on fibroblasts, neutrophil leukocytes, keratinocytes, endothelial cells, and others. The binding of TNFα to these receptors activates such transcription factors as NF-kB, JNK, and AP-1, directly regulating the activity of the genes responsible for the biosynthesis of inflammatory mediators, primarily cytokines, and inducing cell apoptosis, i.e., their programmed death.

Over the past few decades, the concept of RA therapy has changed slightly. Induction of persistent remissions is the primary goal of treatment, and the basic anti-inflammatory drug used to achieve this goal is methotrexate (MT). Moreover, an intense MT therapy should begin as soon as possible after the disease onset (best in the first 3 months) with a rapid escalation of the drug dose (up to 20-25 mg/week). By the chemical structure, TM is similar to folic acid and belongs to antimetabolic medications. The mechanism of action of the medicine is based on its antifolates properties. In particular, MT inactivates dihydrofolate reductase, an enzyme that splits folic acid in the human body, deactivating thus the formation of its active metabolites, namely tetrahydrofolate and dihydrofolic acids. They are necessary for converting homocysteine into methionine and for the synthesis of thymidylate and purines, essential substrates for DNA synthesis. It is worth noting that this effect of MT (dihydrofolate reductase inhibition) results in a decrease of DNA biosynthesis and is manifested during therapy at drug doses from 100 to 1,000 mg/m2, which are used in the treatment of cancerous diseases. At lower doses, the mechanism of MT action is somewhat different: MT
metabolites formed due to its polyglutamation in the cell directly inhibit such folate-dependent enzymes as 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) transaminase and thymidylate synthetase. Therefore, polyglutamine metabolites of MT induce adenosine synthesis, which is the most important endogenous anti-inflammatory mediator.  

Even though MT is the reference standard for AR treatment with an undisputed basis of proof of its efficacy,  the present problem is that there are differences in its bioavailability when taken orally, which limits the efficacy of the treatment. Also, there is no clear relationship between the clinical response to MT therapy and its pharmacokinetics. The effectiveness of MT is assumed to be dependent upon its intracellular concentration. Moreover, the influence of MT pharmacokinetic endpoints on proinflammatory cytokine levels in the blood of RA patients has not been sufficiently studied. Given that cytokines are important in disease pathogenesis, the development of such knowledge can help improve the effectiveness of RA patients’ treatment.

The aim of the study – to investigate the effect of methotrexate therapy on the blood content of proinflammatory cytokines in RA patients, as well as define the relation of proinflammatory cytokine blood concentration to clinical and laboratory disease features and methotrexate pharmacokinetics.

Material and methods

Sixty-five patients with AR were examined: 45 (69.2 %) females and 19 (30.8%) males. The age of included patients was from 18 to 75 (the average age was 50.83 ± 8.91). 20 apparently healthy humans (AHH) formed a control group. Verification of the diagnosis RA was carried out according to recommendations of the American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) 2010,  and EULAR recommendations for the management of early arthritis 2016.

Study inclusion criteria: verified diagnosis of RA; duration of RA at least 6 months; moderate to a high degree of RA severity; age over 18 years; no history of MT intake; intake of a stable dose of the nonsteroidal anti-inflammatory drug (NSAID) or prednisolone < 10 mg/day for at least 1 month before the study; body mass index of 20.00-29.99 kg/m2; signed informed consent for participating in the study.

Patients with at least one of the following exclusion criteria were not included in the study: age less than 18 years; history of MT administration; intra-articular injections of anti-inflammatory medicines less than a month prior to the study; treatment history with biologics; ACR functional class of IV RA; the presence of concomitant chronic diseases in the acute or sub/decompensation stage; infectious pathology; body mass index of 30.00 kg/m2 or above or less than 20.00 kg/m2; oncopathology of any localization; mental illness; pregnancy, lactation; alcohol and drug dependence. Patients were treated according to EULAR (2016), and ACR 2015 recommendations. During the study period, patients were taking either previously prescribed oral NSAIDs or prednisolone (at a dose of less than 10 mg/day). Additionally, a steady dose of prednisolone was maintained for four weeks before being included in the study. The initial dose of MT amounted to 7.5 mg/week and was gradually increased thereafter for maximum clinical efficacy. In case of any pronounced toxicity or adverse reactions, the dose of MT was reduced.

Each patient was monitored over 24 months. The assessment of complaints, clinical status, laboratory and instrumental methods was completed before inclusion in the study and thereafter 14 days, 1 month, and every 6 months for the entire duration of the study.

The patient examination complex included detailed study of complaints and collection of medical history, physical examination, laboratory and instrumental assessment methods.

In accordance with the 2010 ACR/EULAR criteria, a detailed examination of the patients’ joints was performed, indicating the number of painful and swollen joints, the presence of morning joint stiffness, and its duration (in minutes). ‘Small’ and ‘large’ joints have been assessed as well. The intensity of joint pain was estimated using a 100 mm visual analog scale (VAS).

Severity of RA was measured using the DAS28 index, including 28 joints: 2 shoulders, 2 elbows, 2 wrists, 2 metacarpophalangeal of 1st-5th fingers, 2 interphalangeal of the 1st finger, 2 proximal interphalangeal of 2nd-5th fingers, and 2 knee joints. For the DAS28 index measurement, the calculator http://www.das-score.nl/das28/en was used. A DAS28 index below 2.6 points indicated an RA remission, 2.6–3.2 points – low RA activity, 3.2–5.1 – moderate severity, and over 5.1 – high RA severity.
The range of laboratory tests included general clinical blood and urine tests, thymol test, the measurement of blood glucose, urea, creatinine, and total bilirubin content, aspartate, and alanineaminotransferase activity, gamma-glutamyltranspeptidase, alkaline phosphatase, ion-, protein-, lipid- and coagulogram.

The range of rheumatic tests involved the determination of C-reactive protein (CRP), rheumatoid factor, antistreptolysin O, which was performed by an immunoturbidimetric method using standard reagent kits from Vital Diagnostic (Russia).

Blood levels of IL-1β, IL-6, and TNF-α were determined by immunoassay using IL-1 beta Human ELISA Kit (USA), Human IL-6 ELISA Kit (USA), and TNF alpha Human ELISA Kit (USA) on a StatFax 2100 immunoassay analyzer (Awareness Technology Inc., USA).

The instrumental examination consisted of electrocardiography (ECG) and X-ray of the joints on the hands and feet.

Pharmacokinetics of MT was studied during patient visits: before treatment, 14 days after MT initiation, and after 1, 6, 12, 18, and 24 months. The pharmacokinetic disposition was examined in 32 patients, including 26 (81.3%) females and 6 (17.7%) males. Blood samples were taken before MT injection (after an 8-hour night fast), 30 min after MT injection, and 1, 2, 4, 6, 8, 12, and 24 h after MT administration. At similar intervals, blood samples were collected from these patients to assess the blood content of the cytokines under study. Urine was also taken within 24 hours to determine the quantity of MT. Additionally, blood and urine samples were taken to determine creatinine levels. Creatinine clearance was computed by Cockcroft-Gault formula using an online calculator (available at: https://www.mdcalc.com/creatinine-clearance-cockcroft-gault-equation). Blood and urine were analyzed for TM content by polarizing the fluorescence immunoassay using Beacon 2000 (Panvera, USA) polarizing fluorimeter. Given that the TM dose adjustment was carried out throughout the study (to ensure optimal control of disease symptoms and stable remission), the pharmacokinetics was studied at different doses of the drug. The following indicators were determined for each volunteer: C_max – the patient’s maximum plasma MT concentration; T_Cmax – the time at which C_max was reached; AUC_0-τ – the area under the pharmacokinetic curve from the time of drug administration (timezero) to the collection of a specific blood sample when the MT content in blood was equal to/above the minimum limit at which the drug could be quantified; AUC_0-∞ – area under the pharmacokinetic curve from time zero to infinity; C_max/AUC – relative drug absorption rate; K_el – drug elimination constant; T_1/2 – elimination half-life; MRT – mean resident time (time of drug retention in the blood).

The findings were statistically processed using non-parametric Wilcoxon and Mann-Whitney tests, as well as the Spearman’s rank correlation coefficient. The values were presented as mean and standard deviation (M±SD). Differences were considered statistically significant at p < 0.05. For comparison of the frequency of symptoms we used the Fisher’s exact test. The AUC value was calculated using the trapezoid method; AUC from 8 to 24 h was estimated using the logarithmic trapezoidal rule, AUC_0-∞ was defined as the ratio of final concentration ok α to the k_el was determined by non-linear regression analysis. Systemic clearance was estimated by the ratio of the MTDose to AUC_0.

**Ethical Standards**

The study was conducted according to the provisions of ICH Good Clinical Practice (1996), International Ethical Guidelines for Biomedical Research Involving Human Subjects of the Council for International Organization of Medical Sciences, Rules of ethical principles for scientific medical research with human participation approved by the Helsinki Declaration (1964-2013).

The study design and form of informed consent for patients participating were approved during a meeting of the University’s bioethics committee. Before being included in the study, patients had the opportunity and sufficient time to read all elements of the Informed Consent to Voluntary Participation form with a detailed description of the patient’s rights, goals, objectives, and research methods, expected results, etc. Patients who had questions were provided with comprehensive responses.

**Results**

The design of the study is presented in Figure 1.

A total of 65 patients took part in the study, with female predominance. The mean disease duration among the patients studied was 9.86±2.04 years. At that, 9 (13.9%) patients showed X-ray stage II according to Steinbrocker, 51 (78.5%) patients had stage III, and stage IV was observed in 5 (7.6%) patients. Among the patients included in the research,
12 (18.5%) had the I functional class of the disease, 49 (75.4%) – the II functional class, and another 4 (6.1%) – the I functional class. The average degree of RA severity during the calculation of the DAS28 index was high, representing 5.18 ± 0.73. Rheumatoid factor (RF) was detected in the blood of 46 patients (70.8%), meaning they were seropositive; no RF was found in another 19 (29.2%) patients, meaning they were seronegative.

The main clinical symptoms observed in all RA patients under study were joint swelling and pain with the average number of swollen joints being 13 (3.0 to 28.0), painful joints being 19 (4 to 28), and the duration of morning joint stiffness being 96.40 ± 11.25 minutes. All patients (100.0%) had overall weakness, leucocytosis, and increased erythrocyte sedimentation rate (ESR). The average white blood cell count in patients with RA was 13.2 ± 1.85 × 10⁹/L and ESR was 61.20 × 10.37 mm/h.

In addition to joint lesions, the patients under study had systemic manifestations of the disease: lymphadenopathy – in 32 (49.3%) patients, amyotrophy – in 27 (41.5%) patients, anemia of chronic disease – in 20 (30.8%) patients, weight loss – in 20 (30.8%) patients, rheumatoid nodules – in 14 (21.5%) patients, fever – in 13 (20.0%) patients, diffuse interstitial fibrosis – in 6 (9.2%) patients, and rheumatoid vasculitis – in 2 (3.1%) patients.

Therapy with oral MT administration reduced the intensity of the main clinical and laboratory RA symptoms already on the 30th day (the end of 1st month) of the treatment. Thus, on the 30th day of the treatment, only 19 (29.2%) patients complained of joint pain versus 65 (100.0%) before treatment (p < 0.001); joints swelling was noted in 16 (24.6%) versus 65 (100.0%) patients before treatment (p < 0.001); 22 patients (33.8%) reported morning stiffness compared to 65 (100.0%) before therapy (p < 0.001). The DAS28 index decreased from (5.18 ± 0.73) to (3.12 ± 0.16) scores (p < 0.05). In the general blood analysis, the white blood cell count decreased from (13.2 ± 1.85) × 10⁹/L to (5.93 ± 0.72) × 10⁹/L (p < 0.05), and the ESR dropped from (61.20 ± 10.37) mm/h to (22.47 ± 3.26) mm/h (p < 0.05). The 6th month of treatment ended in obtaining a stable regression of the disease symptoms (clinical and laboratory).
Investigation of cytokine profile showed that patients with RA had a significant increasing of pro-inflammatory cytokines compared to AHHs (Table 1). The blood content of TNF-α in RA patients before treatment was 2.03 times higher compared to AHHs (p < 0.05), IL-1β was 2.86 times higher (p < 0.05), and the level of IL-6 exceeded reference values by 3.08 times (p < 0.05).

Table 1. Changes in cytokine content in the blood of RA patients after methotrexate therapy

<table>
<thead>
<tr>
<th>Indicator</th>
<th>AHH (n=20)</th>
<th>Patients with rheumatoid arthritis (n=65)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before treatment</td>
<td>After 14 days</td>
</tr>
<tr>
<td>TNF-α, pg/ml</td>
<td>19.85±1.31</td>
<td>40.36±4.01</td>
</tr>
<tr>
<td>IL-1β, pg/ml</td>
<td>6.19±0.38</td>
<td>17.68±1.74*</td>
</tr>
<tr>
<td>IL-6, pg/ml</td>
<td>2.73±0.13</td>
<td>8.39±0.46*</td>
</tr>
<tr>
<td>Weekly MT dose, mg</td>
<td>-</td>
<td>7.5</td>
</tr>
</tbody>
</table>

Note. * - the difference is statistically significant compared to AHH (p < 0.05); ** - the difference is statistically significant compared to the index before treatment (p < 0.05).

During MT treatment, a positive dynamic was observed with respect to decreasing levels of all proinflammatory cytokines studied. Already in 14 days after MT administration, there was a significant decrease of IL-1β content in the blood by 1.48 times (p < 0.05) and that of IL-6 – by 1.34 times (p < 0.05); as for TNF-α, only attend encytoa decrease of its content in the blood was noted on the 14th day; one month later, its level significantly decreased by 2.58 times (p < 0.05) compared to the pre-treatment levels. At the sixth month of the MT therapy, the blood content of TNF-α, IL-1β, and IL-6 was not statistically different from that of AHH (p > 0.05).

Correlations between proinflammatory cytokine levels in the blood and clinical and laboratory parameters were established well (Table 2).

Table 2. Correlations between proinflammatory cytokine blood levels, clinical disease manifestation and pharmacokinetic endpoints in RA patients

<table>
<thead>
<tr>
<th>Marker</th>
<th>TNF-α</th>
<th>IL-1β</th>
<th>IL-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Joint pain</td>
<td>r=0.42 p&lt;0.05</td>
<td>-</td>
<td>r=0.21 p&lt;0.05</td>
</tr>
<tr>
<td>Swelling in the joints</td>
<td>r=0.54 p&lt;0.05</td>
<td>-</td>
<td>r=0.51 p&lt;0.05</td>
</tr>
<tr>
<td>Morning stiffness</td>
<td>r=0.48 p&lt;0.05</td>
<td>-</td>
<td>r=0.26 p&lt;0.05</td>
</tr>
</tbody>
</table>

Note*: ESR – erythrocyte sedimentation rate; RF – rheumatoid factor; MT – methotrexate; AUC – area under the curve.

The analysis of MT pharmacokinetics did no reveal any correlation between the pharmacokinetic parameters of the drug and blood levels of TNF-α, IL-1β, and IL-6. Only a weak inverse correlation between the weekly dose of MT and blood levels of
IL-6 (r=−0.32 p<0.05) and TNF-α (r=−0.34 p<0.05), as well as a direct correlation between the frequency of adverse MT events and IL-1β blood levels, was reported.

**Discussion of the Results**

It has been established that the course of RA was accompanied by a significant increase of proinflammatory cytokines TNF-α (40.36±4.01 pg/ml in the blood (2.03 times (p<0.05) higher compared to AHH), IL-1β (17.68±1.74 pg/ml or 2.86 times (p<0.05) higher compared to AHH), and IL-6 (8.39±0.46 pg/ml, or 3.08 times (p<0.05) higher in comparison with AHH). In the authors’ opinion, these data confirm the considerable role of TNF-α, IL-1β and IL-6 in the pathogenesis of RA. This is also confirmed by the presence of correlations between blood levels of cytokines under study and some symptoms and laboratory indexes: for TNF-α and joint pain (r=0.42 p<0.05), joint swelling (r=0.54 p<0.05), morning stiffness (r=0.48 p<0.05), fatigue (r=0.33 p<0.05), ESR (r=0.28 p<0.05), white blood cell count (r=0.35 p<0.05), and RF (r=0.39 p<0.05); for IL-6 and joint pain (r=0.21 p<0.05), joint swelling (r=0.51 p<0.05), morning stiffness (r=0.26 p<0.05), fatigue (r=0.24 p<0.05), white blood cell count (r=0.39 p<0.05), ESR (r=0.30 p<0.05), and RF (r=0.25 p<0.05).

The use of MT in RA patients is effective since it permanently eliminates the major clinical symptoms of the disease, standardizing thus laboratory parameters. Oral MT treatment contributes to a decrease in pro-inflammatory cytokine production, demonstrated by the reduction dynamics of TNF-α, IL-1β, and IL-6 in the blood of patients studied from the 14th day after the start of MT therapy (Table 1). Such positive effects of MT on the RA course and pro-inflammatory cytokine levels persisted throughout the study. Moreover, from the 6th month of MT treatment, the levels of TNF-α, IL-1β, and IL-6 in RA patients did not differ statistically from AHH (p>0.05). This effect is of high importance, given the systemic effects of IL-6, such as stimulation of B-lymphocytes differentiation, biosynthesis of osteophase proteins in the liver, activation of osteoclast genesis, degradation of cartilage. The detected correlations between the IL-6 blood levels and clinical and laboratory RA symptoms once again confirm that this cytokine plays one of the leading roles in the RA pathogenesis. Similar correlations were found for TNF-α as a key pro-inflammatory cytokine the main clinical symptoms of the disease are associated with. The obtained findings are well consistent with the literature and studies on the involvement of proinflammatory cytokines in the pathogenesis of RA, the MT effectiveness for the treatment of RA patients, as well as its effect on the cytokine profile.

**Conclusions**

The course of rheumatoid arthritis was established to be accompanied by a significant increase in blood concentrations of TNF-α (40.36±4.01 pg/ml in the blood (2.03 times higher (p<0.05) compared to AHH), IL-1β (17.68±1.74 pg/ml, 2.86 times (p<0.05) higher compared to AHH), and IL-6 (8.39±0.46 pg/ml, 3.08 times (p<0.05) higher compared to AHH).
Therapy with oral methotrexate administration in rheumatoid arthritis patients promotes a stable elimination of clinical disease symptoms and reduced proinflammatory cytokine production. Thus, the level of TNF-α decreases by 2.08 times (p<0.05) compared to baseline, that of IL-1β and IL-6 – by 2.82 (p<0.05) and 2.84 times (p<0.05), respectively. Blood levels of TNF-α and IL-6 correlated with clinical markers of rheumatoid arthritis: TNF-α and joint pain (r=0.42 p<0.05), joint swelling (r=0.54 p<0.05), morning stiffness (r=0.48 p<0.05), fatigue (r=0.33 p<0.05), ESR (r=0.28 p<0.05), white blood cell count (r=0.35 p<0.05), and RF (r=0.39 p<0.05); IL-6 and joint pain (r=0.21 p<0.05), joint swelling (r=0.51 p<0.05), morning stiffness (r=0.26 p<0.05), fatigue (r=0.24 p<0.05), white blood cell count (r=0.39 p<0.05), ESR (r=0.30 p<0.05), and RF (r=0.25 p<0.05). These values correspond to the incidence of methotrexate side effects (r=0.36 p<0.05). No correlation between the blood levels of the cytokines studied and AUC, C\text{\textsubscript{max}}, and T\text{\textsubscript{max}} were revealed. However, an inverse correlation between TNF-α (r=-0.34 p<0.05) and IL-6 (r=-0.32 p<0.05) blood levels and MT weekly dose were established.

**Prospects for further research.** Comparison of the effectiveness of oral and subcutaneous methotrexate forms in rheumatoid arthritis treatment.

**Funding.** This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

**Conflict of interests.** Authors declare that they have no conflict of interests.

**Data Availability.** Data will be available on request.

**Authors’ contribution.**

Data gathering and idea owner of this study: Raisa Aringazina and Olga Pashanova

Study design: Leysan Myasoutova and Liudmila Babaskina

Data gathering: Liudmila Babaskina

Writing and submitting manuscript: Raisa Aringazina

Editing and approval of final draft: Leysan Myasoutova

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