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Anti-inflammatory and antiresorptive effects of acyl substitution chromone derivatives in experimental model of rheumatoid arthritis

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Rheumatoid arthritis (RA) is a systemic inflammatory disease affecting mainly small and major joints. The development for new drugs for the treatment of RAs is constantly underway, while the purposeful synthesis of multi-targeted small molecules can be considered a promising direction for the synthesis of new anti-rheumatic drugs.

The aim. To evaluate the anti-inflammatory and antiresorptive effects of acyl substituted chromone derivatives in experimental animal model for rheumatoid arthritis.

Materials and methods. RA was modeled in rats by injection of a suspension of human type II collagen and a complete Freunds adjuvant (in a ratio of 1:1) under plantar aponeurosis of the hind limb of the animal. The analyzed substances under ciphers X3A7 and X3A9 at a dose of 20 mg/kg and the reference drug dexamethasone at a dose of 3 mg/kg were administered intraperitoneally for 28 days from the moment of RA modeling. On the 7th, 14th, 21st and 28th days of the experiment, the severity of the clinical manifestations of RA was determined. After 28 days, changes in the content of cytokines in the rats blood serum were assessed: tumor necrosis factor-α (TNF-α), interleukins (IL-1, IL-6, IL-10 and IL-12). Changes of myeloperoxidase activity and concentrations of matrix metalloproteinases (MMPs) of type 2 and 9 were determined in synovial tissues.

Results. During the study, it was shown that the use of the tested compounds X3A7 and X3A9, as well as the reference, contributed to a decrease in the severity of clinical manifestations of RA, starting from the 14th day of the experiment. Subsequently, it was demonstrated that in animals treated with dexamethasone, the cytokine content in blood serum decreased in relation to untreated animals: TNF-α – by 57.8% (p <0.05), IL-1 – 64.1% (p <0.05), IL-6 – 59.1% (p <0.05) and IL-12 – 72.3% (p <0.05), with an increase in the level of IL-10 – by 75.4% (p <0.05). The cytokine profile of the blood serum changed similarly when the studied compounds were administered to animals. It worth be noting that against the background of the administration of dexamethasone, X3A7 and X3A9 substances, the activity of myeloperoxidase decreased by 41.7 (p <0.05), 61.7 (p <0.05) and 65.0% (p <0.05), respectively, while the concentration of MMP2 decreased by 24.0 (p <0.05), 38.5 (p <0.05) and 34.4% (p <0.05), respectively, and MMP9 – by 13.5 (p <0.05), 37.9 (p <0.05) and 35.6% (p <0.05).

Conclusion. The study showed that the administration of the analyzed chromone derivatives X3A7 and X3A9 suppresses inflammatory reactions and resorptive processes in synovial tissues, which can serve as a basis for their further study as antirheumatic agents.

Keywords: rheumatoid arthritis; chromone derivatives; cytokines; matrix metalloproteinases; myeloperoxidase

Abbreviations: RA – rheumatoid arthritis; FLSs – fibroblast-like synoviocytes; MLSs – macrophage–like synoviocytes; TNF – tumor necrosis factor; IL – interleukin; INs – intact animals; NC – negative control; MP – myeloperoxidase; MMP – matrix metalloproteinase; Nfkb – nuclear factor kappa beta; MAPK – mitogen-activated protein kinase; ANOVA – analysis of variance; ROS – reactive oxygen species.

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Противовоспалительные и антирезорбтивные эффекты ацилзамещенных производных хромона в условиях экспериментального ревматоидного артрита

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Ревматоидный артрит (РА) – системное воспалительное заболевание, поражающее преимущественно мелкие и крупные суставы. Поиск новых лекарственных средств для лечения РА ведется постоянно, при этом перспективным направлением для создания новых противоревматических средств можно считать целенаправленный синтез политаргетных малых молекул.

Цель. Оценить противовоспалительное и антирезорбтивное действие ацилзамещенных производных хромона в условиях экспериментального ревматоидного артрита у крыс.

Материалы и методы. РА моделировали у крыс путем введения суспензии человеческого коллагена II типа и полного адъюванта Фрейнда (в соотношении 1:1) под подошвенный апоневроз задней конечности животного. Анализируемые вещества с шифрами Х3А7 и Х3А9 в дозе 20 мг/кг и препарат сравнения дексаметазон в дозе 3 мг/кг вводили внутрибрюшинно на протяжении 28-ми дней с момента воспроизведения РА. На 7-й, 14-й, 21-й и 28-й день эксперимента определяли выраженность клинических проявлений РА. По истечении 28-ми дней у крыс в сыворотке крови оценивали изменение содержания цитокинов: фактора некроза опухоли альфа (ФНО-α), интерлейкинов (ИЛ-1, ИЛ-6, ИЛ-10 и ИЛ-12). В синовиальных тканях определяли изменение активности миелопероксидазы и концентрации матриксных металлопротеназ (ММП) 2-го и 9-го типа.

Результаты. В ходе исследования было показано, что применение анализируемых соединений Х3А7 и Х3А9, а также препарата сравнения способствовало уменьшению выраженности клинических проявлений РА, начиная с 14-го дня эксперимента. В дальнейшем было продемонстрировано, что у животных, получавших дексаметазон, содержание цитокинов в сыворотке крови уменьшилось по отношению к нелеченым животным: ФНО-α – на 57,8% (p <0,05), ИЛ-1 – 64,1% (p <0,05), ИЛ-6 – 59,1% (p <0,05) и ИЛ-12 – 72,3% (p <0,05), при повышении уровня ИЛ-10 – на 75,4% (p <0,05). Аналогично изменялся цитокиновый профиль сыворотки крови при введении животным исследуемых соединений. Также стоит отметить, что на фоне введения дексаметазона, веществ Х3А7 и Х3А9 активность миелопероксидазы снизилась на 41,7 (p <0,05), 61,7 (p <0,05) и 65,0% (p <0,05) соответственно, тогда как концентрация ММП2 уменьшилась на 24,0 (p <0,05), 38,5 (p <0,05) и 34,4% (p <0,05) соответственно, а ММП9 – на 13,5 (p <0,05), 37,9 (p <0,05) и 35,6% (p <0,05).

Заключение. Проведенное исследование показало, что введение анализируемых производных хромона Х3А7 и Х3А9 подавляет реакции воспаления и резорбтивные процессы в синовиальных тканях, что может служить основанием для их дальнейшего изучения в качестве противоревматических средств.

Ключевые слова: ревматоидный артрит; производные хромона; цитокины; матриксные металлопротеиназы; миелопероксидаза

Список сокращений: РА – ревматоидный артрит; ФПС – фибробластоподобные синовиоциты; МПС – макрофагальноподобные синовиоциты; ФНО – фактор некроза опухоли; ИЛ – интерлейкин; ИЖ – интактные животные; НК – негативный контроль; МПО – миелопероксидаза; ММП – матриксная металлопротеиназа; Nf-kb – ядерный фактор каппа бета; МАРК – митоген-активируемая протеинкиназа; ANOVA – однофакторный дисперсионный анализ; АФК – активные формы кислорода.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic autoimmune joint disease with a high risk of systemic complications, as well as a significant reduction in the quality of life and early disability. Epidemiological studies show that the prevalence of RA in the general population is 0.5–1%, and the presence of clearly defined gender and genetic correlates is noted [1]. So, Ngo ST et al., in their work demonstrated, that the risk of developing RA during life is higher in women than in men (3.6 *vs*. 1.7%) [2]. Genetic determinants are noted in more than 60% of RA cases, as indicated by the study by Wen YP and Yu ZG [3]. In the Russian Federation, the incidence of RA reaches 610 cases per 100 000, which is the second most important epidemiological indicator among rheumatic diseases, give way only to osteoarthritis (13 000 per 100 000 humans) [4]. It should be noted that the cases of RA increase every year, which is probably mediated by the influence of environmental and social factors, such as smoking, dust and smoke in urban agglomerations, stress and periodontal diseases [5].

A clinical manifestation of RA is symmetrical polyarthritis affecting small and major joints, with the development of periarticular, articular and systemic complications. Pathophysiology of RA is mediated by a synovial tissue dysfunction. Now it is known that the activation of macrophage-like (MLSs) and fibroblast-like synoviocytes (FLSs), which are primary sources of cytokines and immunoglobulins, is observed in RA. In addition to their cytokine-synthetic functions, MLS and FLS activate proteases and other resorption enzymes. At the same time, there is a pronounced pathogenetic variability of synoviocytes: FLSs produce various pro-inflammatory cytokines, for example, interleukin (IL)-1b, IL-6, a tumor necrosis factor alpha (TNF-α), while MLSs, in addition to cytokine synthesis, provide the activation of matrix metalloproteinases (MMPs), mainly the 2nd and 9th subtypes [6]. The above changes lead to the formation of a specific "rheumatoid pannus", which provides the formation of erosions in the late stages of RA.

Taking into account the peculiarities of the RA pathogenesis, i. e. the autoaggression of the immune system against the synovial tissue, drugs with a predominant anti-inflammatory and immunosuppressive effect are used in the treatment of this condition, e.g., corticosteroids, leflunomide, methotrexate, monoclonal antibody medications (sarilumab, canakinumab, adalimumab, certolizumab, infliximab, tocilizumab, and secukinumab) [7]. However, in most cases, the clinical response to the standard therapy is insufficient, which determines the need for a combined use of anti-rheumatic drugs.

According to Smolen JS et al., to reach an optimal effect, it is necessary to use 3 anti-rheumatic medications, herewith, the most rational combinations include corticosteroid+methotrexate+tocilizumab; corticosteroid+methotrexate+rituximab; corticosteroid+methotrexate+tofacitinib. However, even when using this approach, a number of patients fail to reach the necessary clinical effectiveness [8].

In this regard, the scientific and practical medical community is studying new biomarkers and pharmacologically active molecules, the further incorporation of which will expand the range of medicines for the RA treatment. In the context of a targeted approach to the development of anti-rheumatic drugs, several promising targets including macromolecular and low-molecular complexes, are identified. Macromolecular compartments are usually represented by protein enzymatic targets (MMP, janus kinases, and cytokines), whereas low-molecular complexes include prostaglandins, lipoxins, nitric oxide, and reactive oxygen species (ROS) [9].

In the previous studies, it was shown that some chromone derivatives have a complex pharmacological action, including anti-cytokine and antioxidant properties, which makes this compounds promising for studying as possible medications for the RA correction. At the same time, the highest level of activity was found in acyl-substituted compounds, which had been selected as the analyzed objects in this study [10].

THE AIM of the work was to evaluate the anti-inflammatory and antiresorptive effects of acyl substituted chromone derivatives under experimental rheumatoid arthritis conditions in rats.

MATERIALS AND METHODS

Experimental animals

The study was performed on 50 adult male Wistar rats with a body weight 220–240 g, obtained from the laboratory animal nursery "Rappolovo" (Russia). During the experiment, the animals were kept in standard conditions of the vivarium of Pyatigorsk Medical and Pharmaceutical Institute – branch of Volgograd State Medical University (PMPI) in polypropylene boxes by 5 individuals in each. The admission of rats to complete extruded food and water was not restricted. The litter material (granulated hardwood fraction) was changed at least once every 3 days. The conditions of keeping excluded the animal stress: ambient temperature is 22±2°C, relative humidity is 55–65% and a 12-hour

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daily cycle. All manipulations were performed under chloral hydrate anesthesia (an intraperitoneal injection of chloral hydrate (PanReac Applichem, Spain) at a dose of 350 mg/kg). The euthanasia of the animals was performed after the anesthesia and removal of biomaterials by a cervical dislocation. The work concept was approved by the Local Ethics Committee of PMPI (Protocol No. 7 dated June 1, 2023) and was in the line with the provisions of Directives of EU 2010/63[1] and the principles of ARRIVE 2.0 [11].

Experimental model of RA

RA was modeled in rats by the administration of 2 mg/ml (Sigma-Aldrich, Germany) human type II collagen and an incomplete Freund's adjuvant (Sigma-Aldrich, Germany) in the 1:1 ratio. The components were mixed in a phosphate buffer solution with a pH=7.4 to form a stable suspension and administered into the soft part of the pad of the rat's hind limb in a volume of 0.2 ml. After 7 days, the injection was repeated, and the volume administered was reduced to 0.1 ml [12].

Analyzed compounds

The studied compounds 3-formyl-4-oxo-4H-1-benzopyran-7-yl acetate (cipher X3A7) and 3-formyl-4-oxo-4H-1-benzopyran-6-yl acetate (cipher X3A9) were obtained at the Department of Organic Chemistry of PMPI. The structure of the analyzed substances was identified by IR, UV, and NMR spectroscopy [13]. The studied compounds were administered intraperitoneally at a dose of 20 mg/kg in the form of a fine-dispersed suspension prepared *ex tempore* on the basis of a phosphate buffer solution with a pH=7.4 [14]. Dexamethasone at a dose of 3 mg/kg, intraperitoneally (KRKA, Slovenia) was used as a reference drug [15]. The duration of the administration of the studied substances and the reference agent was similar and amounted to 28 days from the moment of RA modeling.

Experimental groups

All animals were randomized by body weight (no more than a 10% deviation in the group and between the groups) into 5 equal groups of 10 individuals in each: intact animals (IA); negative control (NC); a group of the animals, receiving the reference drug (dexamethasone); a group of the animals receiving the analyzed compound under the X3A7code; a group of the animals that received the analyzed compound under the X3A9 code. The rats of all groups, with the exception of IA, according to the method described above RA, were modeled. The IA group received an equivalent volume of a phosphate buffer solution instead of the RA inducer.

Evaluation of RA clinical manifestations

Clinical manifestations of RA in animals were studied according to the score scale presented by E. Moases Ghaffary and S.M. Abtahi Froushani [16]. According to this procedure, the severity of RA clinical manifestations is judged by the sum of points, where each point corresponds to one of the RA symptoms: 0 points – a paw without edema and redness; 1 point – a paw with hyperemia and mild edema; 2 points – a paw with moderate edema; 3 points – a paw with severe edema, the joint mobility is limited; 4 points – a paw with severe edema and a complete lack of the joint mobility. The clinical severity of RA in rats was assessed on the 7th, 14th, 21st, and 28th days of the study [16].

Biomaterial sampling

On the 28th day of the experiment, under chloral hydrate anesthesia, the blood was collected from the abdominal part of the aorta of rats in test tubes with sodium ethylenediaminetetraacetate. Next, the blood was centrifuged (Armed LC-04A Centrifuge, Russia), 1000 g for 10 min), and the serum was obtained, in which the changes in the level of cytokines: TNF-α, IL-1, IL-6, IL-10, and IL-12 were evaluated. After the blood sampling, the hind limb joint was isolated, dissected and the synovial tissue was taken. The synovial tissues were homogenized in a phosphate buffer with pH=7.4. The homogenate was centrifuged at 10 000 g for 20 min. The changes in the concentration of MMP2, MMP9, and myeloperoxidase (MPO) were evaluated in the obtained supernatant.

MPO activity evaluation

The MPO activity was evaluated spectrophotometrically at 450 nm using an Infinite F50 microplate reader (Tecan, Austria). The course of the analysis was as follows: 80 µl of a hydrogen peroxide solution (0.75 mM), 40 µl of 2.9 mM tetramethylbenzidine solution, 10 µl of a 14.5% dimethyl sulfoxide solution, 60 µl of a phosphate buffer solution with pH=7.4 were added to 10 µl of the analyzed sample. The samples were incubated at 37°C for 15 min, then 50 µl of a sulfuric acid solution was added (2 mM). The MPO activity was evaluated according to the standard curve "optical density–enzyme activity" and expressed in IU×10^{-3}/ml [17].

[1] Directive 2010/63/EU of the European Parliament and of the Council European Union for the Protection of Animals Used in scientific purposes. Krasilshchikova MS, Belozertseva IV editors. St. Petersburg; 2012. 48 p. Russian

Determination of cytokine level in the blood and MMPs in synovial tissues

The content of cytokines in the blood serum and MMPs in synovial tissues was determined by a solid-phase enzyme-linked immunoassay using specific reagent kits. The kits had been provided by Cloud Clone Corp. (USA). The analysis procedures followed the manufacturer's recommendations. The analytical signal was read using an Infinite F50 microplate reader (Tecan, Austria).

Statistical analysis

The results were processed by methods of variational statistics using the capabilities of the software package "StatPlus 7.0" (AnalystSoft Inc., USA, License 16887385). The obtained data were checked for the normality of the distribution according to the Shapiro–Wilk test. Parametric ANOVA methods with the Newman–Keuls post-test and nonparametriccstatic analysis methods – the Kruskal–Wallis test were used to compare the groups. The differences were considered statistically significant at p <0.05.

RESULTS

Effect of analyzed compounds and reference material on changes in clinical severity of RA in rats

During the study, it was shown that the NC group of rats showed a progressive deterioration of the course of RA, which was reflected in an increase in the score scale for assessing the severity of RA (Fig. 1) in comparison with IA on the 7th day of the study by 13.0 times (p <0.05); on the 14th day – by 12.0 times (p <0,05) on the 21st day – by 21.3 times (p <0.05) and on the 28th day – by 14.3 times (p <0.05).

Against the background of the dexamethasone use, starting from the 14th day of the experiment, a decrease in the severity of clinical RA symptoms was observed in animals, while the total score of the scale used in this study was lower than in the NC group on the 14th, 21st and 28th days of the study by 33.3, 46.9, and 36.4%, respectively (p <0.05 for all). A similar trend was observed when the analyzed compounds were used. In the animals treated with compound X3A7, the clinical severity of RA decreased by 25.0% (p <0.05) on day 14, by 34.4% (p <0.05) on day 21, and by 33.3% (p <0.05) on day 28 in relation to the NC group of rats. When using compound X3A9 on the 14th, 21st, and 28th days of the experiment, the total score of the RA clinical symptoms assessment scale was lower than that of the NC group by 27.1 (p <0.05), 35.3 (p <0.05), and 32.1% (p <0.05), respectively. It should be noted that there were no significant differences between the groups of the animals that had received dexamethasone and the studied compounds.

Effect of analyzed compounds and reference agent on changes in concentration of cytokines in the blood serum of RA rats

The analysis of changes in the concentration of cytokines in the blood of rats with RA (Table 1) allowed us to establish that the content of TNF-α, IL-1, IL-6 and IL-12 in the NC group of the animals increased relative to the IA group by 2.3 (p <0.05), 3.1 (p <0.05), 3.0 (p <0.05) and 2.6 (p <0.05) times, respectively, while the level of IL-10, on the contrary, decreased by 35.4% (p <0.05). When dexamethasone was used, the following decrease in the concentration of pro-inflammatory cytokines in the rat blood serum was observed: TNF-α-by 57.8% (p <0.05), IL-1-by 64.1% (p <0.05), IL-6-by 59.1% (p <0.05) and IL-12-by 72.3% (p <0.05), with an increase in the level of IL-10 – by 75.4% (p <0.05). In the rats treated with compounds X3A7 and X3A9, the TNF-α content decreased by 20.8 (p <0.05) and 19.6% (p <0.05); IL-1 decreased by 45.2 (p <0.05) and 38.4% (p <0.05); IL-6 decreased – by 28.3 (p <0.05) and 22.0% (p <0.05); IL-12 – by 27.9 (p <0.05) and 31.3% (p <0.05), respectively. At the same time, the IL-10 concentration increased by 51.5 (p <0.05) and 56.2% (p <0.05) in comparison with the untreated rats after the administration of X3A7 and X3A9, respectively.

Effect of analyzed compounds and reference agent on changes in MMPs concentration in synovial tissues of rats with RA

In the course of this study part, it was found that in the NC group of rats in synovial tissues, the concentration of MMP2 and MMP9 (Fig. 2) exceeded similar indicators of the IA group by 6.8 (p <0.05) and 6.9 (p <0.05) times, respectively. When dexamethasone was used, the concentration of MMP2 decreased by 24.0% (p <0.05) and MMP9 decreased by 13.5% (p <0.05) relative to the NC group of rats. At the same time, in the animals treated with compound X3A7, the content of MMP2 and MMP9 was significantly lower than that in the NC group of rats by 38.5 (p <0.05) and 37.9% (p <0.05), respectively, whereas with the administration of X3A9 substance, the abatement was 34.4 (p <0.05) and 35.6% (p <0.05), respectively. It should be noted that the concentration of MMP9 in the rats treated with X3A7 and X3A9 was 28.3 (p <0.05) and 25.5% (p <0.05) lower than in the group of the animals treated with dexamethasone (Fig.2).

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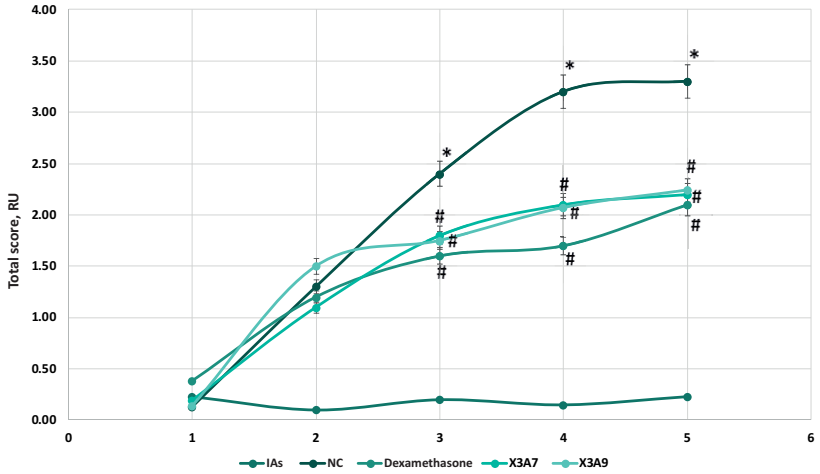

Figure 1 – Influence of analyzed compounds and reference agent on changes in clinical severity of RA in rats

Note: IAs – intact animals; NC – negative control; * – significantly relative to IA (Newman–Keuls test, p <0.05); # – significantly relative to NC (Newman–Keuls test, p <0.05).

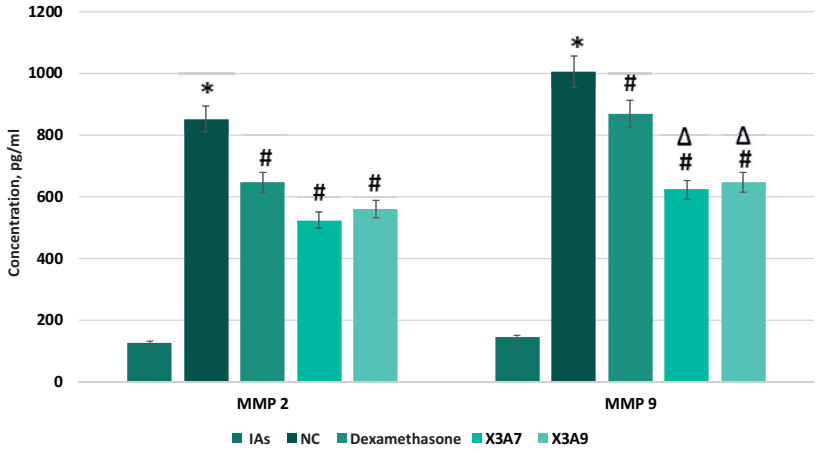

Figure 2 – Influence of analyzed compounds and reference agent on changes in matrix metalloproteinases concentration in synovial tissues of rats with rheumatoid arthritis

Note: IAs – intact animals; NC – negative control; MMP 2, MMP 9 – matrix metalloproteinases 2 and 9; * – significantly relative to the IA group (Newman–Keuls test, p <0.05); # – significantly relative to the NC group (Newman–Keuls test, p <0.05); Δ – significantly relative to the group of animals treated with dexamethasone (Newman–Keuls test, p <0.05).

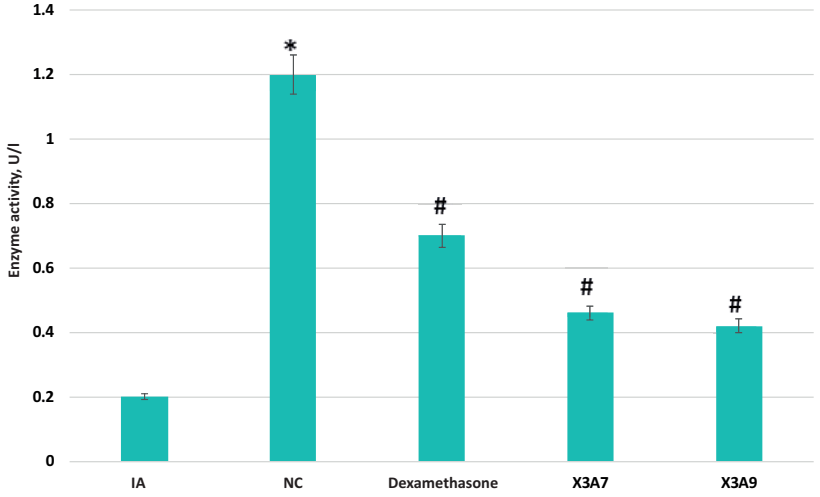

Figure 3 – Influence of analyzed compounds and reference agent on MPO concentration change in synovial tissues of RA rats

Note: IAs – intact animals; NC – negative control; * – significantly relative to IA (Newman–Keuls test, p <0.05); # – significantly relative to NC (Newman–Keuls test, p <0.05).

Table 1 – Effect of analyzed compounds and dexamethasone on changes in concentration of cytokines in the blood serum of RA rats

Group	TNF-α, ng/ ml	IL-1, ng/ml	IL-6, ng/ml	IL-12, ng/ml	IL-10, ng/ml
IAs	2.47±0.29	2.4±0.31	2.47±0.24	3.38±0.25	3.67±0.34
NC	5.62±0.18*	7.3±0.53*	7.5±0.6*	8.83±0.69*	1.3±0.14*
Dexamethasone	2.37±1.15#	2.62±0.45#	3.07±0.76#	2.45±0.67#	2.28±0.15#
X3A7	4.45±0.36#	4±0.49#	5.38±0.53#	6.37±0.56#	1.97±0.19#
X3A9	4.52±0.32#	4.5±0.62#	5.85±0.60#	6.07±0.72#	2.03±0.15#

Note: IAs – intact animals; NC – negative control group; TNF-α – tumor necrosis factor alpha; IL – interleukin; * – significantly relative to IA (Newman–Keuls test, p <0.05); # – significantly relative to NC (Newman–Keuls test, p <0.05)..

Effect of analyzed compounds and reference agent on changes in myeloperoxidase activity in synovial tissues of rats with RA

In the course of the study, it was shown that the MPO activity (Fig. 3) in the NC group of rats exceeded that in the IA group by 6.0 times (p <0.05), whereas when using dexamethasone, compounds X3A7 and X3A9, the MPO activity decreased by 41.7 (p *<0.05)* relative to the NC group of the animals by 61.7% (p <0.05) and 65.0% (p <0.05), respectively.

DISCUSSION

Treatment of autoimmune diseases, such as RA, is a complex clinical task, since in some cases, it is necessary to take into account the polyethological nature of the disease and its complex pathophysiology. Despite the presence of a fairly extensive range of drugs for the treatment of RA, the search for new drugs is constantly conducted [18].

This is largely due to the high risk of complications arising from the treatment of RA and a low effectiveness of medicines. For example, according to Wang SS et al., the frequency of therapeutic failures, both in the case of using classical anti-rheumatic drugs and modern targeted drugs, remains quite high [19].

Recently, more attention has been focused on the development of orally active low-molecular compounds that are comparable in effectiveness to biological drugs. Due to their low molecular weight, they easily enter the cell, bind to intracellular signaling molecules, and inhibit key target molecules responsible for the development of RA. The examples of such drugs introduced into the clinical practice, are janus-kinase inhibitors: tofacitinib, peficitinib, and upadacitinib [20]. At the same time, the drugs based on small molecules can affect on other biomarkers that mediate the formation of RA pathogenesis, for example, MMPs [21].

MMPs are a family of calcium-dependent endopeptidases. The active site of enzymes is represented by the zinc-binding domain. Currently, more than 20 different MMPs have been identified, that are, classified depending on specific substrates: collagenases, stromelizines, gelatinases, matrilysins, membrane-type MMPs, etc. In addition to the enzymatic activity of MMPs, growth factors, cytokines, and chemokines are released, and proteinase inhibitors are inactivated. In the human body, MMPs are synthesized as proenzymes in leukocytes, macrophages, endothelial cells, chondrocytes, and synoviocytes [22].

MMPs play a key role in the pathogenesis of RA. FLSs, which have a tumor-like appearance, secrete various proteases, including MMP, which destroy components of the extracellular matrix, mainly proteoglycans and articular cartilage collagen. In the cartilage tissue of RA, the activity of MMP1–3, MMP6, MMP9 and MMP13 increases. MMP3, MMP1 and MMP2 are also able to activate a cartilage resorption by collagenolysis and degradation of synovial proteoglycans [23]. At the same time, MMP2 and MMP9 are identified as potential anti-rheumatic targets, which is reflected in the work by Li N et al. [24].

The study showed that the use of the analyzed chromone derivatives contributes to a decrease in the concentration of MMP2 and MMP9 in synovial tissues in rats. This fact can be associated with both a direct enzyme-blocking effect and an indirect decrease in the activity due to the presence of the anti-cytokine activity. At the same time, the direct blockade of MPP by chromone derivatives is probably related to the peculiarities of the structure of substances. For example, MMP-blocking properties were established for naringenin, which has a similar scaffold to the tested compounds [25]. A similar activity was investigated by Lim H et al., for apigenin and vogonin.

In this study, apigenin and vogonin were shown to reduce the MMP activity by blocking a c-Fos / protein activator-1 (AP-1) and janus-kinase 2 [26]. It should be emphasized that the effect of the analyzed substances on the activity of MMP9 statistically significantly exceeded the effect of dexamethasone. This fact is especially important in the context of the antiresorptive

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effect of chromone derivatives, since it is MMP9 that is distinctly pathological in RA. So, Itoh T et al., have demonstrated that in the MMP9-knockout mice, RA symptoms can spontaneously decrease, whereas with a complete MMP2 deficiency, the RA symptom can only increase, indicating a higher pathogenetic significance of MPP9 [27].

It is also worth noting the anti-cytokine effects of the analyzed chromone derivatives. Thus, the study showed that the administration of X3A7 and X3A9 compounds contributed to a decrease in the concentration of pro-inflammatory (TNF-α, IL-1, IL-6 and IL-12) and an increase in the content of anti-inflammatory cytokines (IL-10), which suggests that these substances have anti-inflammatory and probable immunosuppressive activities. In the context of this study, the detailed mechanism of these effects remains open, but considering the effect of chromone derivatives on MPO, it can be assumed that the decrease in the level of pro-inflammatory cytokines occurs due to the blockade of this enzyme. MPO is known like a haeme containing peroxidase, expressed mainly in neutrophils and to a lesser extent, in monocytes. In the presence of hydrogen peroxide and halides, MPO catalyzes the formation of ROS, including hypochlorous acid (HOCl), playing a leading role in the immune response mediated by neutrophils. In addition, in RA, the increased MPO activity promotes a cytokine overproduction, acting as an endogenous pro-inflammatory mediator, a decrease in the activity of which can prevent an inflammatory damage of joints [28]. At the same time, the presence of other potential mechanisms of the anti-cytokine action of the studied acyl-substituted chromone derivatives cannot be denied. The analysis of the anti-inflammatory and immunosuppressive activities of the structures related to the studied compounds allowed us to establish that a decrease in the cytokine production may be associated with the effect on a number of intracellular signaling mechanisms, including Nf-kb, janus-kinases, inducible nitric oxide synthase and p38-MAPK [29]. For example, Liu H et al, showed that a diphenol derivative of chromone inhibited inflammatory responses in RAW264.7 cell culture by blocking *toll*-like receptors and reducing the intracellular pro-inflammatory response mediated by Nf-kb and p38-MAPK [30]. Similar results were obtained by Xing T et al., who demonstrated that amide-substituted chromone derivatives reduce the intensity of a lipopolysaccharide-induced inflammation to the one comparable with ibuprofen [31]. However, it should be noted, that the effect of the tested chromone derivatives on intracellular pro-inflammatory signaling pathways requires a separate study.

Study limitations

The study does not provide data on the mechanisms of the anti-inflammatory action of 3-formylchromone derivatives. In the future, it is necessary to compare the effectiveness of the analyzed chromone derivatives with anti-rheumatic drugs of the biological origin.

CONCLUSION

The use of the analyzed chromone derivatives under the experimental RA conditions is accompanied by a complex effect on the synovial tissues of rats, which is expressed in a decrease in the intensity of inflammatory reactions and the concentration of the cartilage matrix resorption enzymes MMP2 and MMP9, which indicates the presence of an antiresorptive effect. The obtained results suggest the relevance of further studies of acyl-substituted chromone derivatives as a drugs of complex pathogenetic therapy of RA with a predominant antiresorptive effect.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

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