ANTIRHEUMATIC ACTIVITY OF 3-IMIDAZOLE-SUBSTITUTED-4,5-DIARYLOISOXAZOL-3-CARBOXYLIC ACID AMIDE DERIVATIVE, A PROTEINASE INHIBITOR-ACTIVATED TYPE II RECEPTOR

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Treatment of rheumatoid arthritis is a complex and time-consuming process that does not always lead to significant results both due to poor effectiveness of drugs and drug toxicity. It means we need to search for new pharmacological targets to influence the pathological process, one of which is inhibition of proteinase-activated receptors 2 (PAR2 receptors) activity. In 2016–2019, synthesis of low-molecular-weight antagonists of PAR2 receptors belonging to 4,5-dihydroisoxazole-5-carboxamide derivatives was carried out, and in 2023 their anti-inflammatory efficacy was examined using the formaldehyde edema model. The most effective laboratory R004 compound was tested on a model of autoimmune pristane-induced inflammation in rats. During treatment of chronic inflammation in rats, R004 inhibited significant development of edema of feet, damage to small joints, and specific changes in the formula of white blood, and according to biochemical blood test led to normalization of liver and kidney functions and energy metabolism. R004 turned out to be more effective and safer than the comparator drugs such as diclofenac sodium and dexamethasone.

Keywords: rheumatoid arthritis, PAR2-receptors, pristane, autoimmune inflammation, 4,5-dihydroisoxazole-5-carboxamide amide derivative

Compliance with ethical standards: the study was carried out in compliance with all ethical standards recommended in the Russian Federation. Rats were selected as a test system, as animals with a minimum set of characteristics that make it possible to conduct an experiment: a sufficient size of paws for convenient measurements and possibility of taking the volume of blood necessary for the study. The animals were kept in cages of sufficient area and with timely bedding change (2 times a week). Animals are provided with free access to water and food, a 12-hour lighting cycle, optimal temperature and humidity, and supervision by a licensed veterinarian. Although the research protocol did not allow to use painkillers that could distort the results of experiments, all procedures were carried out by qualified and experienced personnel, which ensured minimization of stress and pain. The animal study was preceded by *in vitro* studies of the drug. The power of the statistical tests used was evaluated, which made it possible to form samples of an optimal size. The animal study was approved by the Independent Ethical Committee of the Federal State Budgetary Educational Institution of Higher Education Yaroslavl State Medical University of the Ministry of Health of the Russian Federation, Protocol No. 6 dated 09/14/2023.

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ПРОТИВОРЕВМАТИЧЕСКАЯ АКТИВНОСТЬ ПРОИЗВОДНОГО АМИДА 3-ИМИДАЗОЛ-ЗАМЕЩЕННОЙ-4,5-ДИГИДРОИЗОКСАЗОЛКАРБОНОВОЙ КИСЛОТЫ — ИНГИБИТОРА ПРОТЕИНАЗА-АКТИВИРОВАННОГО РЕЦЕПТОРА II ТИПА

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Лечение ревматоидного артрита является сложным и длительным процессом, не всегда приводящим к значимым результатам как в следствие недостаточной эффективности препаратов, так и их лекарственной токсичности для человека. Это диктует поиск новых фармакологических мишеней воздействия на патологический процесс, одной из которых является блокада активности протеиназа-активированных рецепторов 2 типа (proteinase-activated receptors, II) — PAR2-рецепторов. В течение 2016–2019 гг. был осуществлен синтез низкомолекулярных антагонистов PAR2-рецепторов, относящихся к производным 4,5-дигидроизоксазол-5-карбоксамида, а в 2023 г. была доказана их противовоспалительная эффективность на модели формалинового отека. Наиболее эффективное соединение с лабораторным шифром R004 было испытано на модели аутоиммунного воспаления у крыс, вызванного пристаном. Применение соединения R004 в терапии хронического воспаления у крыс препятствовало значимому развитию отека стоп и поражению мелких суставов, предупреждало специфические изменения формулы белой крови, а по данным биохимического исследования крови приводило к нормализации функций печени и почек и энергетического обмена организма. Соединение R004 оказалось более эффективно и безопасно, чем препараты сравнения диклофенак натрия и дексаметазон.

Ключевые слова: ревматоидный артрит, PAR2-рецепторы, пристан, аутоиммунное воспаление, производное 4,5-дигидроизоксазол-5-карбоксамида

Соблюдение этических стандартов: исследование выполнено с соблюдением всех этических стандартов, рекомендованных в Российской Федерации. В качестве тест-системы были выбраны крысы, как минимально удовлетворяющие по своим характеристикам животные для возможности проведения эксперимента: достаточный размер лап для удобства измерений и возможность забора необходимого для исследования объема крови. Животные содержались в клетках достаточной площади и с своевременной сменой подстила (2 раза в неделю). Животным обеспечен свободный

доступ к воде и пище, 12-часовой цикл смены освещения, оптимальные температура и влажность, наблюдение лицензированного ветеринара. Хотя протокол исследования не позволял применения обезболивающих препаратов, способных исказить результаты экспериментов, все процедуры проводились квалифицированным и опытным персоналом, что обеспечило минимизацию стресса и болезненных ощущений. Настоящему исследованию на животных предшествовали исследования препарата *in vitro.* Проведена оценка мощности используемых статистических тестов, что позволило сформировать оптимальные по размеру выборки. Исследование на животных одобрено независимым этическим комитетом Федерального государственного бюджетного образовательного учреждения высшего образования «Ярославский государственный медицинский университет» Министерства здравоохранения Российской Федерации, протокол от 14.09.2023 № 6.

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Rheumatoid arthritis (RA) is an incurable immune-mediated inflammatory disease with a multifactorial etiology, which affects up to 1.0% of the world's population [1], with its prevalence being increased with age. According to official statistics, more than 300 thousand patients with RA were registered in Russia in 2017 [2]. Its progression leads to joint deformities, destruction of cartilage and bone tissue and subsequent disability.

Currently, treat-to-target (T2T) has been established as a guiding principle for the treatment of RA [2, 3]. It does not recommend any specific drugs, but only gives general treatment recommendations. The primary therapeutic goal for RA is to achieve remission [2]. For this purpose, several classes of drugs are used such as disease-modifying anti-rheumatic drug (DMARDs), which constitute an extensive group of synthetic and biological drugs that have a common ability to influence the pathogenetic mechanisms of RA; glucocorticoids, which are recommended to be used in combination with DMARDs, and nonsteroidal anti-inflammatory drugs used to relieve acute and chronic pain [4].

Treatment of rheumatoid arthritis is a long process, and the drugs used to treat it are far from being safe and significantly effective. In average, 20–50% of patients are forced to discontinue therapy due to lack of efficacy or intolerance to prescribed medications [5]. Therefore, there is an urgent need to develop new pharmacological targets, which would increase the effectiveness and reduce the toxicity of the drugs used.

One of the promising therapeutic targets includes proteinase-activated receptors (PARs). They belong to the class of G-protein-coupled receptors (GPCRs). Irreversible activation by proteases is the specific feature of these receptors. Discovered in the 1990s, 4 forms of PARs (PAR1, PAR2, PAR3 and PAR4) are expressed on the cell membrane of almost all organs and systems and regulate numerous physiological functions, including contraction of smooth muscle cells, sensitivity to pain, release of lipid mediators, cytokines, and neuropeptides. Activation of PAR2 is associated with clinical manifestations in the form of inflammation, swelling, and pain. They are expressed in immune cells of both the innate and adaptive immune systems and play an important role in the development of a wide range of diseases [6]. Their activation contributes to the occurrence of inflammation, fibrosis and proliferation of connective tissue. Experiments have demonstrated that inhibition of PAR2 activity prevents the development of RA pathogenesis and positively modifies the course of the disease [6]. The drugs that inhibit PAR2 are searched in the following directions: indirect blockade of PAR2 activity; creation of monoclonal antibodies; search for PAR2 inhibitors among peptide compounds; and synthesis of low molecular weight inhibitory compounds. The last direction is the most perspective one [7].

During 2016–2019, synthesis of low molecular weight PAR-2 antagonists related to 4,5-dihydroisoxazole-5-carboxamide amide derivatives was done. The work was based on preliminary mathematical prediction of pharmacologically significant properties of a large number of multinucleated derivatives of imidazole, isoxazole and oxazole with a wide structural diversity. The antagonistic activity of the compounds with respect to PAR-2 was evaluated in *vitro* using the CHO cell line with high expression of human PAR-2 [8]. Five most active P AR-2 antagonists, derivatives of 4.5- dihydroisoxazol-5-carboxamide, the structure of which is shown in the figure, were investigated in *vivo* on a formaldehyde edema model in various dosages. It would allow to select the most active compound for testing on a model of chronic autoimmune inflammation [9].

The aim of the study was to determine the potential therapeutic efficacy of a promising compound from a number of 3-imidazole-substituted-4,5-dihydroisoxazole carboxylic acid amide derivatives on a model of autoimmune inflammation in rats.

MATERIALS AND METHODS

A single subcutaneous injection of pristane into the base of the rat tail in a volume of 0.1 ml [10], caused chronic inflammation followed by pathological changes seen in white rats at early stages of RA: a chronic recurrent process, typical histological signs of joint damage, as well as development of specific serological immune abnormalities can be observed [10].

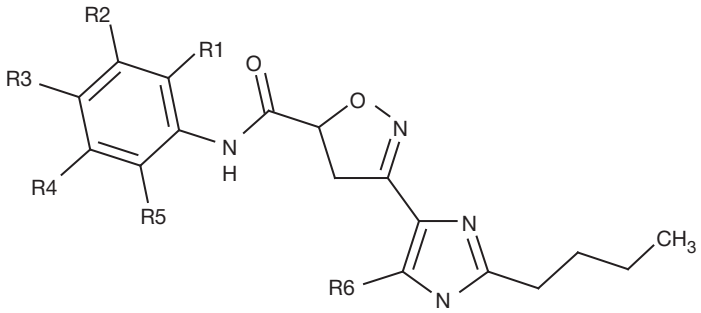

Fig. The general formula of synthesized derivatives of 4,5-dihydroisoxazole-5-carboxamide

Table 1. Changes in paw volume in rats with pristane inflammation

Intervals	Paws	Intact animals	Control animals	Diclofenac sodium	Dexamethasone	R004
Initially	Right	193.6±9.9	194.3±8.3	203.2±13.0	196.7±7.9	188.0±8.1
	Left	196.5±9.7	198.6±9.9	201.7±9.2	201.0±5.9	194.8±7,.9
	Average value	195.1±5.9	197.4±6.7	202.4±7.7	198.8±4.9	191.4±5.8
At 7 days	Right	194.1±8.7	202.0±7.2	196.4±11.9	174.3±5.9*/***	185.0±9.1
	Left	196.0±8.0	203.1±5.8	207.0±11.5	186.7±5.4***	189.8±6.1
	Average value	195.0±5.1	202.7±4.6	201.7±9.1	180.5±4.8*/**/***	187.4±5.2***
At 14 days	Right	184.3±7.0	205.0±4.1*/**	202.8±13.7	170.2±5.2*/***	168.0±6.0***
	Left	193.3±7.3	204.8±7.5	205.0±14.0	180.2±5.6*/***	178.2±7.7***
	Average value	188.8±4.8	204.9±5.1*/**	203.9±8.1	175.1±4.5*/**/***	173.6±6.0***
At 21 days	Right	191.3±5.0	209.0±4.6*/**	214.5±9.1*	177.4±5.0*/***	170.0±7.0***
	Left	195.1±6.6	207.8±5.5*	209.3±11.0	189.4±4.4*/***	182.8±7.7***
	Average value	193.2±4.3	208.5±4.4*/**	211.9±8.0**	183.4±4.2*/***	176.4±6.0***
At 28 days	Right	186.3±5.5	204.4±4.5*/**	208.6±8.8**	184.5±6.2*/***	175.0±6.0***
	Left	190.3±5.3	200.6±6.5	203.8±8.8	194.1±5.4	185.2±7.0
	Average value	188.3±4.7	202.7±4.8*/**	206.2±7.2	189.5±4.6***	180.1±5.6***

*) significant difference from baseline (in dynamics)
**) significant difference from intact ones
***) significant difference from control one

The experiment was carried out on 50 white mongrel rats with a mean weight of 180–220 g, kept at a temperature of 22 ± 2 °C, humidity of 55 ± 5%, in a 12/12-hour light cycle, with unlimited access to food and water. All animals were divided into 5 groups with 10 rats in every group: intact animals in the first group; control animals (rats were injected with pristane and saline solution) in the second group; rats of the third group were injected pristane + diclofenac sodium at a dose of 5 mg/kg [11]; rats of the fourth group were injected with pristane + dexamethasone 1 mg/kg [10]; animals of the fifth group were injected with pristane + R004 10 mg/kg. The duration of the experiment was 28 days; on a daily basis, dexamethasone and physiological solution, diclofenac sodium and R004 were administered s/c and intragastrically respectively. Dynamic examination of the animals was carried out 5 times: at baseline and on the 7th, 14th, 21st and 28th days of the experiment. The following parameters were studied: the volume of the affected foot using digital anhydrous plethysmometer PH1901 (Russia), the width of the articular slits using digital X-ray of the lower extremities (on day 28 only) [12], rectal temperature, general blood test, biochemical values (total protein, albumins, globulins, glucose, triglycerides, total cholesterol, AlAT, AsAT, total bilirubin, GGT, alkaline phosphatase, creatinine, urea).

The results were statistically processed using the Biostat program. Every value determined in various experiments was 6–10. The Student's t-test (in the presence of a normal distribution) and the Mann-Whitney U-test was applied to the comparison of the values between groups, whereas the Student's T-test with a Bonferroni adjustment was used in case of multiple testing. Reliability of intra-group differences was determined by the Student's paired *t*-test. The differences were considered significant at $p < 0.05$ [13].

THE RESULTS OF THE SURVEY AND THEIR DISCUSSION

Changes of rectal temperature and paw volume of animals, as well as the width of the interarticular gap of their feet are the investigated values that most specifically reflect the process of inflammation in rats.

In the intact group, there was an unreliable change in the volume of feet throughout the experiment (Tab. 1). At 14 days after pristane injection to white rats, the volume of the right foot and the average value of both feet were significantly increased from baseline by 5.5% and 3.8% respectively. On the 21st day of the experiment, the volume of both feet significantly increased from baseline: by 7.6% for the right one, by 3.1% for the left one; on the 28th day, the volume of the right foot and both feet remained significantly increased by 5.2% and 2.7%, respectively. In relation to healthy animals, foot volume in rats was significantly increased by 6.5–11.2% on days 14–28 of pristane inflammation.

When treating rats with diclofenac sodium, a significant increase in foot volume (right and average) by 5.6% and 4.7% from baseline occurred only on the 21st day of the experiment.

Table 2. Width of the interarticular foot slits in rats with pristane inflammation

Group	Tarsometatarsal	Metatarsal-phalangeal	Interphalangeal
Intact animals	0.266±0.008	0.283±0.011	0.228±0.018
Control animals	0.236±0.004*	0.251±0.007*	0.204±0.008
R004	0.240±0.004*/***	0.256±0.011	0.226±0.007**/****
Diclofenac	0.213±0.010*/**	0.247±0.006*	0.198±0.014
Dexamethasone	0.223±0.010*	0.230±0.014*	0.202±0.09

*) significant difference with the intact ones
**) significant difference with the control one
***) significant difference with Diclofenac
****) significant difference with Dexamethasone

On day 28, the volume of feet in rats became normal from baseline, but the right foot became significantly more voluminous (+12%) than in intact animals. No increased foot volume was seen in rats treated with dexamethasone. Starting from the 7th day of the experiment and until the end, there was a significant decrease in foot volume by 9.4–13.4% from baseline and by 9.3–16.6% in relation to the control group. No significant increase in foot volume was seen when R004 was administered to animals, and, moreover, the paw volume of treated rats was significantly lower by 7.5–18.7% than in the control group and, at the same time, did not differ from those in the intact group (Tab. 2).

The experiment showed that when administered to animals, chronic inflammation is developed, which is accompanied by swelling of paws. Diclofenac sodium reduced (but did not completely prevent) severity of the inflammatory process; its therapeutic effect was more pronounced in the first three weeks of the developing process, and then slightly decreased (against the background of its administration, there was a significant increase in the volume of the rat's right paw on day 21 and a significant increase in its volume relative to intact animals on day 28). Dexamethasone completely blocked the inflammatory edema significantly reducing the volume of rat paws both in comparison with the baseline and in relation to intact control on days 4 and 14 of the experiment. This may be evidence of dexamethasone myopathy due to impaired synthesis of muscle proteins. Treatment of rates with R004 was optimal: in animals, there was no significant increase of foot edema, both when compared with baseline and in relation to healthy rats.

Modeling of autoimmune inflammation with pristane caused damage to the joint tissue of white rats, one of the manifestations of which was an X-ray change in the width of the articular gap (Tab. 2). It significantly decreased by 11.1% in the prematarsal-metatarsal joints and by 11.3% in the metatarsal-phalangeal joints; in the interphalangeal joint, the narrowing was observed as a trend (–11.5%). In this case, diclofenac sodium did not exert any therapeutic activity: the width of the articular gap in the metatarsal-phalangeal and metatarsal-phalangeal joints changed almost to the same extent as in the control group (–12.7% and –13.2%), and in the premaxillary-metatarsal joint, there was even a deterioration: the width of the interarticular gap was significantly decreased not only in relation to intact animals (–20%), but also in relation to control ones as well (–9.7%). Dexamethasone also had no therapeutic activity: the width of the articular gap significantly decreased by 16.4% in the prematarsal-metatarsal joint and by 18.7% in the metatarsal-phalangeal joints, and in the interphalangeal joint, narrowing of the articular gap was observed as a trend

(–11.4%). Administration of R004 reduced the degree of narrowing of the articular gap significantly by 9.8% in relation to healthy animals, this occurred in the pre-metatarsal joint only. At the same time, changes in the width of the articular gap in the metatarsophalangeal joint did not reach the level of reliability (–9.5%), and they were absent in the interphalangeal joint. It was significantly wider by 10.8% in relation to the control group and by 11.9% in relation to the dexamethasone group.

Thus, experience has shown that with pristane inflammation, damage to the articular surfaces occurs. Diclofenac sodium and dexamethasone proved to be ineffective; R004 partially prevented the damaging effect of pristane on articular tissue.

Measurement of rectal temperature in white rats turned out to be a less informative indicator: its changes in all the studied groups did not actually differ from those of intact animals.

Blood test in animals allowed measurement in dynamics: before the initial injection of pristane and on the 28th day of the experiment. In the intact control group, an elevation in the white blood cell count was observed during the experiment, but only eosinophils and monocytes were significantly increased by 51% and 80% respectively. After administration of pristane, the qualitative picture did not change for most of the studied values, only two values significantly differed from those in the intact control group: there was a 2.5-fold increase in neutrophil count from baseline (p 0.05), and lymphocyte count decreased by 35% (Tab. 3).

While using diclofenac sodium, there was an even greater increase in neutrophil count (4 times compared to baseline, p 0.05), whereas lymphocyte count in blood decreased almost twice (p 0.05). Additionally, basophils were no longer detected in blood. When using dexamethasone, there was a tendency to a low number of neutrophils (their content increased in other groups), the content of neutrophils increased 4 times, but the level of lymphocytes dropped 20 times. Basophils and eosinophils were not detected in blood any longer. R004 leveled drug-induced blood changes: none of the investigated values was changed in a reliable way. Qualitative differences between animals of this group and intact rats consisted in a tendency to a decreased number of basophils and lymphocytes, and significant quantitative differences were determined for lymphocytes only: WBC content in R004 group in relation to intact animals was 36.2% lower though the baseline content was almost the same (Tab. 3).

Thus, a significantly high neutrophils and low lymphocytes in the control group of rats indicate that the immune system was affected. Unlike with reference drugs, therapeutic use of R004 neutralizes these processes to some extent. With diclofenac sodium, these changes were aggravated (monocytes and basophils were added to the drop in lymphocyte content), and dexamethasone itself had a significant immunosuppressive and lymphotoxic activity, contributing to an even greater change in the investigated parameters.

Analyzing the data obtained with respect to red blood, it can be noted that the administration of pristane produced practically no negative effect on its values.

Metabolism of proteins, fats and carbohydrates. Pristane causes a disturbed balance of protein metabolism.

Table 3. Changes in white blood values (x10^9/l) against the background of pristane inflammation

Values	Intervals	Intact animals	Control animals	Diclofenac sodium	Dexamethasone	R004
WBC	initially	7.40±0.71	8.08±0.77	7.41±1.52	8.51±0.91	8.29±1.01
	28 days	9.32±1.12	9.20±1.67	9.80±1.82	7.88±1.92	9.01±1.12
Basophils	initially	0.008±0.001	0.015±0.007	0.018±0.006	0.017±0.008	0.024±0.010
	28 days	0.032±0.014	0.022±0.008	0*/**/***	0*/**/***	0.006±0.006
Eosinophils	initially	0.098±0.016	0.137±0.046	0.114±0.036	0.067±0.031	0.144±0.048
	28 days	0.148±0.016*	0.152±0.031	0.178±0.038	0*/**/***	0.188±0.042
Neutrophils	initially	1.62±0.45	1.95±0.45	1.63±0.25	1.76±0.21	2.34±0.55
	28 days	2.12±0.43	4.80±1.02*/**	6.48±1.75*/**	7.05±1.57*/**	3.96±0.81
Lymphocytes	initially	5.32±0.85	5.48±0.89	5.30±1.49	6.05±1.19	5.30±0.56
	28 days	6.22±0.88	3.55±0.36**	2.88±0.56*/**	0.33±0.02 */**/***	3.97±0.69**
Monocytes	initially	0.368±0.105	0.493±0.135	0.348±0.088	0.620±0.105	0.486±0.125
	28 days	0.663±0.110*	0.610±0.216	0.260±0.84**/***	0.500±0.146	0.590±0.136

*) significant difference from baseline
**) significant difference from intact ones
***) significant difference from control one

Table 4. Effect of pristane inflammation on blood biochemical parameters

Values	Intact animals	Control animals	Diclofenac sodium	Dexamethasone	R004
Total protein g/l	70.63±1.05	70.50±0.45	57.90±2.64*	66.67±2.16	70.20±0.71
Albumins g/l	36.25±0.31	34.00±0.90	30.20±0.31*	31.33±2.31	35.40±0.90
Globulins g/l	34.38±0.81	36.50±0.55	27.00±0.81*	35.34±2.01	34.80±0.78
Albumins/globulins	1.08±0.04	0.93±0.04*	1.19±0.06**	0.87±0.07*	1.02±0.04
Glucose mmol/l	9.10±0.09	8.22±0.15*	8.81±0.13**	9.73±0.61**	9.05±0.30**
Triglycerides	0.44±0.05	0.57±0.07	0.68±0.17	2.30±0.25*/**	0.51±0.06
Glucose/triglycerides	20.70±1.41	14.42±1.12*	12.96±1.56*	4.23±1.02*/**	17.75±1.18**
Total cholesterol	2.86±0.09	2.19±0.08*	2.57±0.12**	2.50±0.29	2.39±0.26
AlAT	43.25±5.30	43.08±3.32	64.30±10.30	80.50±15.30*/**	47.75±2.80
AsAt	93.25±5.07	126.58±8.03*	117.50±11.07	150.17±18.96*	105.12±1.64**
Total bilirubin	1.93±0.07	1.88±0.06	2.07±0.09	1.95±0.04	2.14±0.09
GGT	4.00±0.00	4.00±0.00	4.00±0.00	7.33±1.20*	4.00±0.00
Creatinine	30.75±0.30	34.83±0.80*	33.80±1.00*	26.00±2.30**	33.75±0.60*
Urea	3.43±0.18	3.46±0.11	4.30±0.16*	4.45±0.18*	3.81±0.08
Alkaline phosphatase	74.75±17.65	73.17±8.62	103.40±16.60	225.00±76.85	97.40±3.67

*) significant difference with the intact ones
**) significant difference with the control one

Despite the fact that the change in blood albumin and globulin is trending, a significant decrease in the albumin/globulin index (AHI) by 13.9% indicates a shift in protein synthesis towards globulins, which structurally include antibodies that are essential in autoimmune inflammation. A significant decrease in glucose by 9.7% and cholesterol by 23.6% (decrease in the rate of synthesis of steroid hormones and repair of cell membranes) was observed as well. Against the background of a decrease in glucose concentration and a tendency to increase the TG content, the carbohydrate-fat index (CFI) significantly decreased by 30% (the ratio of glucose and triglycerides). It shows the body's shift to "fatty" energy, which is more consumable in terms of oxygen and energy substrates (Tab. 4).

Diclofenac sodium caused a uniform decrease in the synthesis of both albumins and globulins, which may be associated with the impaired liver function. A further decrease in the CFI by 37.4% compared to intact animals occurred, demonstrating an increase in the lipid component of energy balance. With dexamethasone, dissonance increased in favor of globulin synthesis (a significant decrease in AHI by 19.5% in relation to healthy animals) and a sharp decrease in CFI by almost 5 times, showing the body's shift to "lipid" energy (the effect of high doses of glucocorticoids). The therapeutic administration of R004 stops changes in metabolism of proteins, fats and carbohydrates that occur against the background of pristane inflammation.

Functional biochemistry of the liver and kidneys. When pristane was administered, there was a significant increase of AsAT activity by 35.7% and creatinine content by 14% (Tab. 4). It means that the liver and kidneys display an interest in developing inflammatory process. There was only a significant increase in blood creatinine (+9.8%) with R004, but to a lesser extent than for the control group. When using diclofenac sodium in sick animals, only blood creatinine (+9.9%) and urea (+25.4%) significantly increased, indicating at a possible kidney damage. The tendency to an increased activity of blood transaminases and alkaline phosphatase, as well as to an increased concentration of bilirubin, demonstrates liver function strain. Dexamethasone showed a significant increase in the activity of AlAT by almost

2 times, AsAT by 1.6 times, GGT by 1.8 times and alkaline phosphatase by 3.1 times (the latter $p > 0.05$), demonstrating cytolysis of hepatocytes and developing cholestasis. A significant increase of blood levels of urea shows a decrease in the reabsorption function of the kidneys.

Thus, the experiment showed that with pristane inflammation, liver and kidney damage is possible. R004 weakens this process, whereas diclofenac sodium and dexamethasone enhance it.

CONCLUSIONS

1. R004 in the treatment of chronic inflammation in rats prevented significant development of edema of feet, damage to small joints, and specific changes in white blood count, and according to biochemical blood test led to normalization of liver and kidney functions and energy metabolism.

2. R004 turned out to be more effective and safer than the comparator drugs such as diclofenac sodium and dexamethasone.

References

1. GBD 2021 Rheumatoid Arthritis Collaborators. Global, regional, and national burden of rheumatoid arthritis, 1990–2020, and projections to 2050: a systematic analysis of the Global Burden of Disease Study 2021. The Lancet Rheumatology. 2023; 5 (10): e594-e610. DOI: 10.1016/S2665-9913(23)00211-4. PMID: 37795020; PMCID: PMC10546867.
2. Klinicheskie rekomendatsii. Revmatoidnyy artrit. 2021–2022–2023 (24.08.2021). Utverzhdeny Minzdravom RF. Russian.
3. Tanaka Y. Current concepts in the management of rheumatoid arthritis. Korean J Intern Med. 2016; 31 (2): 210–218. DOI: 10.3904/kjim.2015.137. Epub 2016 Feb 26. PMID: 26932398; PMCID: PMC4773729.
4. Nasonov EL. Lechenie revmatoidnogo artrita. Klinicheskaya farmakologiya i farmakoterapiya. 2017; 26 (1): 4–10. Russian.
5. Prasad P, Verma S, Surbhi, Ganguly NK, Chaturvedi V, Mittal SA. Rheumatoid arthritis: advances in treatment strategies. Mol Cell Biochem. 2023; 478 (1): 69–88. DOI: 10.1007/s11010-022-04492-3. PMID: 35725992.
6. McCulloch K, McGrath S, Huesa C, Dunning L, Litherland G, Crilly A, et al. Rheumatic Disease: Protease-Activated Receptor-2 in Synovial Joint Pathobiology. Front Endocrinol (Lausanne). 2018; 9: 257. DOI: 10.3389/fendo.2018.00257. PMID: 29875735; PMCID: PMC5974038.
7. Cho NC, Seo SH, Kim D, Shin JS, Ju J, Seong J, et al. Pharmacophore-based virtual screening, biological evaluation and binding mode analysis of a novel protease-activated receptor 2 antagonist. J Comput Aided Mol Des. 2016; 30 (8): 625–37. DOI: 10.1007/s10822-016-9937-9. PMID: 27600555.
8. Abassi YA, Xi B, Zhang W, Ye P, Kirstein SL, Gaylord MR, et al. Kinetic cell-based morphological screening: prediction of mechanism of compound action and off-target effects. Chem Biol. 2009; 16 (7): 712–23. DOI: 10.1016/j.chembiol.2009.05.011. PMID: 19635408; PMCID: PMC2836189.
9. Korsakov MK, Fedorov VN, Smirnov NA, Shetnev AA, Leonova OV, Volkhin NN, et al. Screening of anti-inflammatory activity of 4.5-dihydroisoxazol-5-carboxamide (PAR-2 inhibitors) based on formaldehyde oedema model among white lab rats. Research Results in Pharmacology. 2023; 9 (4): 105–111. DOI: 10.18413/rrpharmacology.9.10061.
10. Tuncel J, Haag S, Hoffmann MH, Yau AC, Hultqvist M, Olofsson P, et al. Animal Models of Rheumatoid Arthritis (I): Pristane-Induced Arthritis in the Rat. PLoS One. 2016 May 26; 11 (5): e0155936. DOI: 10.1371/journal.pone.0155936. PMID: 27227821; PMCID: PMC4881957.
11. Bibik EYu, Yaroshevskaya OG, Devdera AV, Demenko AV, Zakharov VV, Frolov KA, et al. Poisk sredstv s protivovospalitel'noy aktivnost'yu sredi proizvodnykh tetragidropiridо [2,1] [1,3,5] tiadiazina. Khimiko-farmatsevticheskiy zhurnal. 2017; 51 (8): 16–19. Russian.
12. Skupnevskiy SV, Pukhaeva EG, Badtiev AK, Rurua FK, Batagova FE, Farnieva Zh G. Metod neinvazivnoy otsenki razvitiya revmatoidnogo artrita u laboratornykh gryzunov. Vestnik novykh meditsinskikh tekhnologiy. 2022; (4): 69–72. DOI: 10.24412/1609-2163-2022-4-69-72. Russian.
13. Glants S. Mediko-biologicheskaya statistika. M.: Praktika, 1998; 459 p. Russian.

Литература

1. GBD 2021 Rheumatoid Arthritis Collaborators. Global, regional, and national burden of rheumatoid arthritis, 1990–2020, and projections to 2050: a systematic analysis of the Global Burden of Disease Study 2021. The Lancet Rheumatology. 2023; 5 (10): e594-e610. DOI: 10.1016/S2665-9913(23)00211-4. PMID: 37795020; PMCID: PMC10546867.
2. Клинические рекомендации. Ревматоидный артрит. 2021–2022–2023 (24.08.2021). Утверждены Минздравом РФ.
3. Tanaka Y. Current concepts in the management of rheumatoid arthritis. Korean J Intern Med. 2016; 31(2): 210–218. DOI: 10.3904/kjim.2015.137. Epub 2016 Feb 26. PMID: 26932398; PMCID: PMC4773729.
4. Насонов Е. Л. Лечение ревматоидного артрита. Клиническая фармакология и фармакотерапия. 2017; 26 (1): 4–10.
5. Prasad P, Verma S, Surbhi, Ganguly NK, Chaturvedi V, Mittal SA. Rheumatoid arthritis: advances in treatment strategies. Mol Cell Biochem. 2023; 478 (1): 69–88. DOI: 10.1007/s11010-022-04492-3. PMID: 35725992.
6. McCulloch K, McGrath S, Huesa C, Dunning L, Litherland G, Crilly A, et al. Rheumatic Disease: Protease-Activated Receptor-2 in Synovial Joint Pathobiology. Front Endocrinol (Lausanne). 2018; 9: 257. DOI: 10.3389/fendo.2018.00257. PMID: 29875735; PMCID: PMC5974038.
7. Cho NC, Seo SH, Kim D, Shin JS, Ju J, Seong J, et al. Pharmacophore-based virtual screening, biological evaluation and binding mode analysis of a novel protease-activated receptor 2 antagonist. J Comput Aided Mol Des. 2016; 30 (8): 625–37. DOI: 10.1007/s10822-016-9937-9. PMID: 27600555.
8. Abassi YA, Xi B, Zhang W, Ye P, Kirstein SL, Gaylord MR, et al. Kinetic cell-based morphological screening: prediction of mechanism of compound action and off-target effects. Chem Biol. 2009; 16 (7): 712–23. DOI: 10.1016/j.chembiol.2009.05.011. PMID: 19635408; PMCID: PMC2836189.
9. Korsakov MK, Fedorov VN, Smirnov NA, Shetnev AA, Leonova OV, Volkhin NN, et al. Screening of anti-inflammatory activity of 4.5-dihydroisoxazol-5-carboxamide (PAR-2 inhibitors) based on formaldehyde oedema model among white lab rats. Research Results in Pharmacology. 2023; 9 (4): 105–111. DOI: 10.18413/rrpharmacology.9.10061.
10. Tuncel J, Haag S, Hoffmann MH, Yau AC, Hultqvist M, Olofsson P, et al. Animal Models of Rheumatoid Arthritis (I): Pristane-Induced Arthritis in the Rat. PLoS One. 2016 May 26; 11 (5): e0155936. DOI: 10.1371/journal.pone.0155936. PMID: 27227821; PMCID: PMC4881957.
11. Бибик Е. Ю., Ярошевская О. Г., Девдера А. В., Деменко А. В., Захаров В. В., Фролов К. А. и др. Поиск средств с противовоспалительной активностью среди производных тетрагидропиридо [2,1] [1,3,5] тиадиазина. Химико-фармацевтический журнал. 2017; 51 (8): 16–19.
12. Скупневский С. В., Пухаева Е. Г., Бадтиев А. К., Руруа Ф. К., Батагова Ф. Э., Фарниева Ж. Г. Метод неинвазивной оценки развития ревматоидного артрита у лабораторных грызунов. Вестник новых медицинских технологий. 2022; (4): 69–72. DOI: 10.24412/1609-2163-2022-4-69-72.
13. Гланц С. Медико-биологическая статистика. М.: Практика, 1998; 459 с.