



The effect of derivatives of tetrahydropyrido[2,1-b][1,3,5] thiadiazine on hematologic indices of rats with subacute parotitis

Elena Y. Bybik¹, Oksana G. Yaroshevskaya¹, Andrey V. Demenko¹, Konstantin A. Frolov¹, Viktor V. Dotsenko², Sergey G. Kryvokolysko¹

¹ Lugansk State Medical University, Ig 50-letiya Oborony Luganska St, 91045 Lugansk, Russian Federation

² Kuban State University, 149 Stavropol'skaya St., 350040 Krasnodar, Russian Federation

Corresponding author: Oksana G. Yaroshevskaya (korokina@bsu.edu.ru)

Academic editor: Oleg Gudyrev ♦ Received 11 July 2018 ♦ Accepted 13 July 2018 ♦ Published 10 August 2018

Citation: Bybik EY, Yaroshevskaya OG, Demenko AV, Frolov KA, Dotsenko VV, Kryvokolysko SG (2018) The effect of derivatives of tetrahydropyrido[2,1-b][1,3,5] thiadiazine on hematologic indices of rats with subacute parotitis. *Research Results in Pharmacology* 4(2): 77–83. <https://doi.org/10.3897/rrpharmacology.4.28305>

Abstract

Introduction: Regardless of belonging of NSAIDs to one or another chemical group, they all have common side effects that can occur when using these drugs for a long or short period. One type of toxicity in the spectrum of side effects of modern non-steroidal anti-inflammatory drugs is hematotoxicity.

Objectives: to study the indices characterizing the red and white hematopoiesis in a clinical blood examination in animals with a simulated inflammation process against a background of pharmacocorrection with original thiadiazine derivatives.

Methods: The experiments were carried out on 48 white pedigree mature rats of both sexes weighing 170–210 g. The tetrahydropyrido [2,1-b] [1,3,5] thiadiazine derivatives II, III and V were selected, since they showed the strongest anti-inflammatory, analgesic and antipyretic properties in the previous experiments. The animals were divided into eight groups: intact (rats without the pathology), control (with inflammation), referent 1 (inflammation+diclofenac sodium 0.5 mg/kg), referent 2 (inflammation+analgin 5 mg/kg), referent 3 (inflammation+indomethacin 5 mg / kg) and three test groups (with test substances administered at a dose of 5 mg/kg). Administration of the drugs was carried out for 14 days at the above doses. The standard methods were used to determine the number of erythrocytes, hemoglobin, color index, ESR, leukocytes and neutrophils.

Results: In the analysis of the numerical results of the experiment, the valid ranges of values were determined for most parameters under study, which made it possible to use nonparametric statistical methods, including the Wilcoxon signed-rank test, to evaluate the reliability of differences. The use of tetrahydropyrido [2,1-b] [1,3,5] thiadiazine derivatives II, III, V in animals with experimental parotitis was accompanied by an increase in the number of erythrocytes in comparison with that in the control group.

Conclusion: The studies of three derivatives of tetrahydropyrido [2,1-b] [1,3,5] thiadiazine, which have a high anti-inflammatory activity, proved that the compounds III and V have no hematotoxicity.

Keywords

hematotoxicity, tetrahydropyrido [2,1-b] [1,3,5] thiadiazine derivatives, parotitis.

Introduction

Historically, anti-inflammatory drugs have their origin in the happy discovery of certain plants, and their extracts are used to relieve pain, fever and inflammation. When salicylates were discovered in the mid-19th century to become the active components of willow SPP, this enabled these compounds to be synthesized from this; acetyl-salicylic acid or trademark aspirin was developed (Rainsford 2007). Nonsteroidal anti-inflammatory drugs (NSAIDs), which act by inhibiting cyclooxygenase (COX) isoenzymes, was discovered over 100 years ago. They remain a key component of the pharmacological treatment of acute and chronic pain. COX-1 and COX-2 isozymes have different biological functions; analgesic activity is primarily (though not exclusively) associated with inhibition of COX-2, with the various side effects resulting from inhibition of COX-1 and COX-2 (Brune and Patrignani 2015). More recently, a spinal site of cyclooxygenase activity relevant to pain has been proposed. In animals, prostaglandins are synthesized in the spinal cord (Eisenach et al. 2010). Currently, in the arsenal of a doctor there is a wide range of drugs that can block the development of an inflammatory reaction. They include non-steroid anti-inflammatory drugs, glucocorticoids (GC), anti-cytokinic drugs as well as Symptomatic Slow Acting Drugs for Osteoarthritis (SYSADOA, previously referred to as "chondroprotectors"). Furthermore, there are various drugs for the "point" exposure on individual inflammatory mediators being developed and in the stage of clinical approbation (Karateev et al. 2017). In particular, the potential for gastrointestinal toxicity with traditional non-selective NSAIDs may be a significant limitation to their use. Gastrointestinal bleeding, ulceration and perforation are the most common serious adverse events associated with NSAIDs and often lead to the cessation of NSAID therapy and expensive treatment for gastropathic symptoms themselves (Collantes et al. 2002, Karateev et al. 2015). Currently, a long list of the approved anti-inflammatory drugs contains a very small number of high-performance samples with a minimum of side effects with their long-term use (Karateev et al. 2015, Smolen et al. 2016, Nasonov 2015, Clauw 2015). Therefore, the search for new and safe means of pharmacotherapy for inflammatory diseases is an urgent task of modern pharmacological research (Kroon et al. 2015).

One of the promising directions of this research may be a complex study of partially hydrogenated 1,3,5-thiadiazines, which have a wide spectrum of pharmacodynamic activity (Dotsenko et al. 2015). The screening studies of the new tetrahydropyrido [2,1-b] [1,3,5] thiadiazine derivatives (Dotsenko et al. 2014, Osolodkin et al. 2013) showed that compounds I, II have the most evident analgesic activity. A number of substances showed a high analgesic activity, exceeding that of analgin (Bybik et al. 2016). In addition, on the "formalin paw edema" model, the compounds with evident anti-inflammatory activity were found among the test samples. Compounds III-IV (Fig. 1)

showed the best results, exceeding the sodium diclofenac 3.5 and 2.13 times, respectively (Bybik et al. 2016).

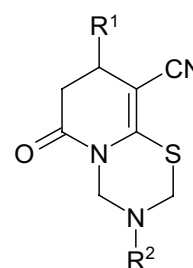


Figure 1. Formulas of test substances. **I:** $R^1 = 3,4,5-(\text{MeO})_3\text{C}_6\text{H}_2$, $R^2 = \text{Bn}$; **II:** 2-MeOC₆H₄, 2-EtC₆H₄; **III:** 2-MeOC₆H₄, 3-Cl-4-MeC₆H₃; **IV:** 3,4,5-(MeO)₃C₆H₂, 2-FurCH₂; **V:** 3-MeO-4-EtOC₆H₃, 4-ClC₆H₄.

Studies of the acute toxicity of tetrahydropyrido [2,1-b] [1,3,5] thiadiazine derivatives with intraperitoneal single administration to rats by V.B.Prozorovsky's method at an ascending dose showed that the samples II, III and V (Fig. Formulas of test substances) referred to toxicity class 3 (moderately toxic compounds), the LD₅₀ value for them was 450±35 mg/kg (Belous 2005).

Objectives

In connection with this, the objective of this experiment was to study the indices characterizing the red and white hematoipoiesis in a clinical blood examination in animals with a simulated inflammation process against a background of pharmacocorrection with original thiadiazine derivatives.

Materials and methods

The present pharmacological experiments were carried out on 48 white pedigree mature rats of both sexes weighing 170–210 g in the autumn in a certified pharmacological laboratory. The animals throughout the experiment period were kept in vivarium conditions on a standard diet, with no more than six individuals in a cage in accordance with the rules for working with laboratory animals. The conditions of keeping animals and the manipulations carried out with them were in compliance with the requirements from the the guidelines for the ethical review of biomedical research (Habriev 2005). Before the beginning of the experiment, all the animals were carefully examined, considering their weight, age, motor activity and condition of hair.

In the experiment, there were used the minimum number of animals (6 in the group) acceptable for statistical processing and getting reliable results, and the minimum number of experimental groups, to achieve the goal and to solvr the research problems, i.e the total number of animals.

As an experimental model of inflammation, a pathological process that develops in animals after a single injection of a formalin solution (1%-0.2 ml) into the region of the right retromandibular fossa – the projection of the parotid salivary gland – was used. For the experiments, the tetrahydropyrido [2,1-b] [1,3,5] thiadiazine derivatives II, III and V were selected, since they showed the strongest anti-inflammatory, analgesic and antipyretic properties in the previous experiments.

After external examination and rejection, the rats were divided into eight groups: intact (rats without pathology), control (inflammation), referent 1 (inflammation+diclofenac sodium 0.5 mg/kg), referent 2 (inflammation+analgin 5 mg/kg), referent 3 (inflammation+indomethacin 5 mg/kg), experiment 1 (inflammation+TD0330 5 mg/kg), experiment 2 (inflammation+TD0470 5 mg/kg), experiment 3 (inflammation+TD0472 5 mg/kg). The administration of the drugs was carried out for 14 days at the above doses. After the end of the experiment, blood was taken from the femoral vein. The standard methods were used to determine the number of erythrocytes, hemoglobin, color index, ESR, leukocytes and neutrophils.

The statistical processing of the data was carried out using the software package Statistica. In connection with the use of a small number of experimental specimens in the experiment, an analysis of the experimental data in respect of normality of distribution indicated an insufficient sample size for obtaining significant differences in the processing of data by classical parametric methods. With further analysis of the numerical results of the experiment, valid ranges of values for most of the indicators were determined, which allowed using nonparametric statistical methods, including the Wilcoxon signed-rank test (Kobzar' 2006).

Results and discussion

As we can see from Fig. 2 and Table 1 of the data, the number of erythrocytes in the blood of the animals in the

control group with subacute parotitis significantly decreased ($p < 0.03$) by 16.8% compared to the values recorded in the intact group. In reference groups 1, 2 and 3, no significant differences were found.

The use of tetrahydropyrido [2,1-b] [1,3,5] thiadiazine derivatives II, III, and V in animals with experimental mumps was accompanied by a significant increase in the number of erythrocytes in comparison with the control group. Thus, the administration of compound V resulted in the restoration of and administration of compound III promoted an increase in the number of erythrocytes in rats with experimentally modeled parotitis to the level of the intact animals (Fig. 2). The data of the conducted experimental studies concerning the hemoglobin level in the blood of the rats of the control group showed a sharp significant decrease (by 16.3%) in comparison with the indicator registered in the intact group (Fig. 3, Table 1).

The referents used do not have the same effect on the red blood-germ cells. From Fig. 2 we can see, that a 14-day application of indomethacin in the animals with experimental parotitis resulted in a significant decrease in the amount of hemoglobin in the blood by 21.4% compared to the indicator in the intact group of animals.

The condensed derivatives of thiadiazine III and V used in the experimental groups were able to restore the hemoglobin index against the background of the simulated inflammatory process of the parotid salivary gland to the normal values (Table 1).

The values of the color index shown in Fig. 4 and in Table 1, show a significant ($p < 0.03$) decrease in the value of this parameter in the control group of rats in comparison with the intact group similar to that found in the rats in reference groups 2 and 3, which received analgin and indamethacin as a treatment for subacute parotitis. In the animals of experimental groups receiving samples III and V, significant differences in the colour index were found from the parameters in the control group.

As can be seen from Fig. 5, the erythrocyte sedimentation rate increases 4-fold in the group of laboratory ani-

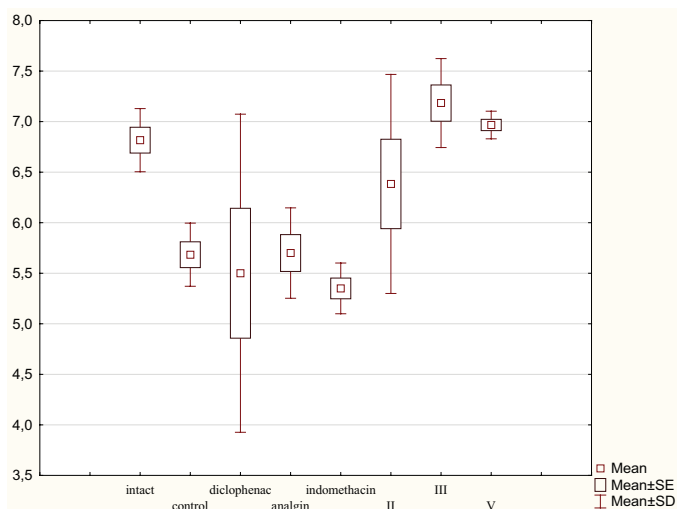
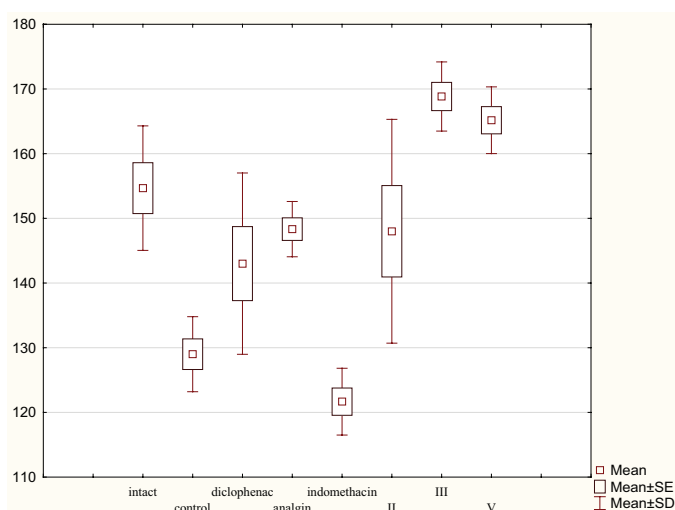


Figure 2. The effect of diclofenac 0.5 mg/kg, analgin 5 mg/kg and indomethacin 5 mg/kg per the number of erythrocytes in the blood of the rats studied groups after treatment for subacute parotitis

Table 1. The effect of diclofenac 0.5 mg/kg, analgin 5 mg/kg and indomethacin 5 mg/kg on the index of the clinical analysis of blood of the rats in the groups under study

Group of animals	Number of erythrocytes	Hemoglobin	Colour index	Erythrocyte sedimentation rate	Number of leukocytes
Intact	6.82±0.31 p<0.05	154.67±9.63 p<0.05	0.74±0.03 p<0.05	1.67±0.82 p<0.05	6.92±1.72 p<0.1
Control	5.68±0.31	129.00±5.80	0.58±0.03	6.67±1.63	5.08±0.64
Diclophenac	5.50±1.57 p>0.5	143.3±14.01 p<0.05	0.59±0.03 p<0.05	1.83±1.33 p<0.05	9.12±0.45 p<0.05
Analgin	5.70±0.45 p>0.5	148.33±4.27 p<0.05	0.57±0.03 p<0.6	2.00±1.10 p<0.05	4.03±0.14 p<0.05
Indomethacin	5.35±0.25 p<0.1	121.67±5.16 p<0.1	0.66±0.03 p<0.6	1.83±0.75 p<0.05	3.32±0.31 p<0.05
II	6.38±1.08 p<0.5	148.00±17.3 p<0.6	0.71±0.03 p<0.5	2.17±1.17 p<0.05	5.65±2.19 p<0.6
III	7.18±0.44 p<0.05	168.83±5.34 p<0.05	0.77±0.11 p<0.05	1.67±1.21 p<0.05	7.55±1.45 p<0.05
V	6.97±0.14 p<0.0277	165.17±5.15 p<0.05	0.66±0.13 p<0.05	1.33±0.52 p<0.05	4.88±0.17 p<0.5

p – is calculated in relation to the control.

**Figure 3.** The effect of diclofenac 0.5 mg/kg, analgin 5 mg/kg, indomethacin 5 mg/kg and the test substances on the amount of hemoglobin in the blood of the rats of the groups under study after treatment for subacute parotitis

mals with experimental parotitis (control). In all reference groups and in all experimental groups, there are significant differences in comparison with the control.

Comparing the values of leukocytes in the blood of the rats with a simulated inflammatory process with those of the intact animals (Table 1), a decrease of 26.6% can be seen. The reference medications, which were used, showed heterogeneity of their action with towards white blood cell lineage of the rats with subacute parotitis. Thus, the administration of diclofenac sodium leads to a significant increase in the number of leukocytes under the experimental conditions, whereas the use of analgin in the animals with inflammation of the parotid salivary gland can reduce the leukocyte count by

41.85% in comparison with the intact group. An even more pronounced depression of white blood cell lineage growth was found in reference group 3 (indomethacin). There was a decrease of 52% there in comparison with the intact group and of 34.5% in comparison with the control group.

Comparing the total number of leukocytes in the blood of the rats receiving substance V with the analogous values of the rats of the control group, no significant differences were revealed (Fig 6, Table 1). In the experimental group of the rats receiving substance III, the total number of leukocytes exceeded the control by 48.6% and had a comparable value with that of the animals of the intact group.

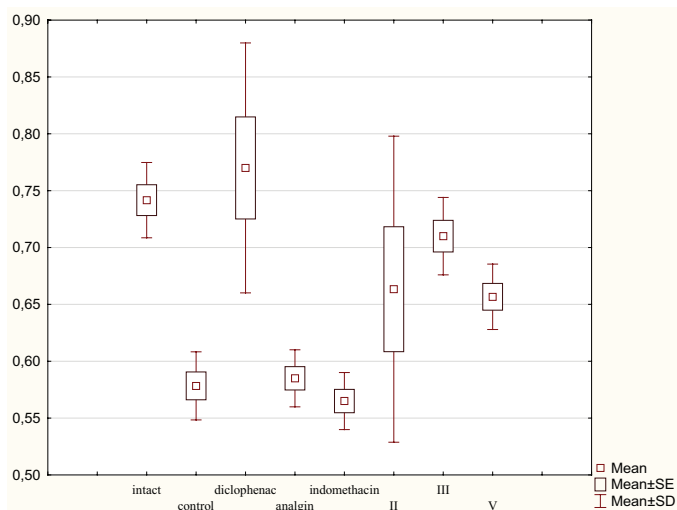


Figure 4. The effect of diclofenac 0.5 mg/kg, analgin 5 mg/kg, indomethacin 5 mg/kg and test substances on colour index of the blood of the rats of the study groups, after treatment for subacute parotitis

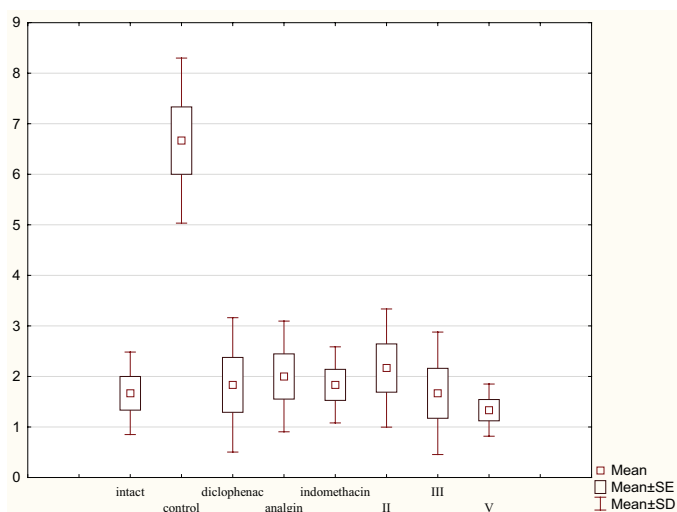


Figure 5. The effect of diclofenac 0.5 mg/kg, analgin 5 mg/kg, indomethacin 5 mg/kg and test substances on the sedimentation rate of erythrocytes in the blood of the rats of the study groups, after treatment for subacute parotitis

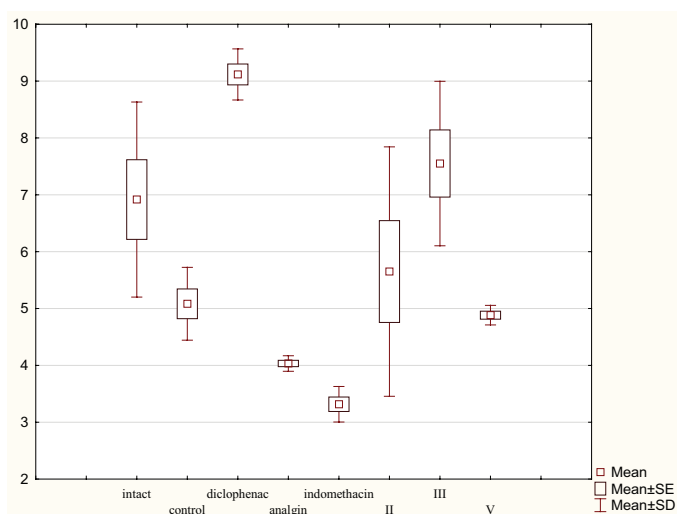


Figure 6. The effect of diclofenac 0.5 mg/kg, analgin 5 mg/kg, indomethacin 5 mg/kg and test substances on the total number of leukocytes in the blood of the rats of the study groups, after treatment for subacute parotitis

Conclusion

Thus, the results obtained confirm the data about a high hematotoxicity of indamethacin and analgin. The studies of three tetrahydropyrido [2,1-b] [1,3,5] thiadiazine derivatives with high anti-inflammatory activity identified compounds **III** (3-(3-chloro-4-methylphenyl)-8-(2-methoxyphenyl)-6-oxo-3,4,7,8-tetrahydro-2H, 6H-pyr-

ido [2,1-b] [1,3,5]-thiadiazine-9-carbonitrile) and **V** (3-(4-chlorophenyl)-8-(4-ethoxy-3-methoxyphenyl)-6-oxo-3,4,7,8-tetrahydro-2H, 6H-pyrido [2,1-b] [1,3,5] thiadiazine-9-carbonitrile) as not showing hematotoxicity.

The question of the mechanisms of action of these drugs remains open. In the future, a promising direction of research is looking for points of application of these tetrahydropyrido [2,1-b] [1,3,5] thiadiazine derivatives.

References

- Belous YB (2005) Eticheskaya ekspertiza biomeditsinskih issledovaniy [Ethical review of biomedical research]. Moskva. <http://perviydoc.ru/v42046> [in Russian]
- Brune K, Patrignani P (2015) New insights into the use of currently available non-steroidal anti-inflammatory drugs. *Journal of Pain Research* 20(8): 105–118. <https://doi.org/10.2147/JPR.S75160> [PubMed]
- Bybik EY, Frolov KA, Yaroshevskaya OG, et al. (2016) The search for new analgesics in the series of tetrahydropyrido[2,1-b][1,3,5] thiadiazine derivatives. “Scientific bulletins of Belgorod State University. Series: Medicine. Pharmacia [Nauchnie vedomosti Belgorodskogo gosudarstvennogo Universiteta. Seriya Medicina. Farmaciya] 5(226): 135–140. [in Russian]
- Clauw DJ (2015) Diagnosing and treating chronic musculoskeletal pain based on the underlying mechanism(s). *Best Practice & Research: Clinical Rheumatology* 29(1): 6–19. <https://doi.org/10.1016/j.berh.2015.04.024> [PubMed]
- Collantes E, Curtis S, Lee K, et al (2002) A multinational randomized, controlled, clinical trial of etoricoxib in the treatment of rheumatoid arthritis. *BMC Family Practice* 3: 10. <https://doi.org/10.1186/1471-2296-3-10> [PubMed] [PMC]
- Dotsenko VV, Frolov KA, Kryvokolisko SG, Pekhtereva TM, et al (2014) Design and synthesis of pyrido[2,1-b][1,3,5]thiadiazine library via uncatalyzed Mannich-type reaction. *ACS Combinatorial Science* 16: 543–550. <https://doi.org/10.1021/co5000807> [PubMed]
- Dotsenko VV, Frolov KA, Kryvokolisko SG et al. (2015) Amino-methylation of morpholinium and N-methylmorpholinium 3,5-dicyano-4,4-dimethyl-6-oxo-1,4,5,6-tetrahydropyridine-2-thiolates. *Chemistry of Heterocyclic Compounds* 52(2): 116–127. <https://doi.org/10.1007/s10593-016-1843-5>
- Eisenach JC, Curry R, Rauck R, et al. (2010) Role of spinal cyclooxygenase in human postoperative and chronic pain. *Anesthesiology* 112(5): 1225–1233. <https://doi.org/10.1097/ALN.0b013e3181d94dc0> [PubMed] [PMC]
- Habriev RU (2005) Manual on experimental (preclinical) study of new pharmacological substances [Rukovodstvo po eksperimentu tal'nomu doklinicheskomu izucheniy novykh farmakologicheskikh veshstev], Medicina, Moscow, 832 p. [In Russian]
- Karateev AE, Karateev DE, Davydov OS (2017) Pain and inflammation. Part the analgesic potential of anti-inflammatory drugs. *Rheumatology Science and Practice* 55(1): 58–67. <https://doi.org/10.14412/1995-4484-2017-58-67> [In Russian]
- Karateev AE, Nasonov EL, Yakhno NN, et al. (2015) Clinical guidelines “Rational use of nonsteroidal anti-inflammatory drugs (NSAIDs) in clinical practice”. *Sovremennaya Revmatologiya [Modern Rheumatology Journal]* 9(1): 4–23. <https://doi.org/10.14412/1996-7012-2015-1-4-23> [In Russian]
- Kobzar' AI (2006) Applied Mathematical Statistics [Prikladnaya matematicheskaya statistika]. Fizmatlit, Moscow, 816 pp. [In Russian]
- Kroon FP, van der Burg LR, Ramiro S, et al. (2015) Non-steroidal antiinflammatory drugs (NSAIDs) for axial spondyloarthritis (ankylosing spondylitis and non-radiographic axial spondyloarthritis). *The Cochrane Database of Systematic Reviews* 17(7): CD010952. <https://doi.org/10.1002/14651858.CD010952.pub2> [PubMed]
- Nasonov EL (2014) Progress in rheumatology in the early 21st century. *Sovremennaya Revmatologiya [Modern Rheumatology Journal]* 8(3): 4–8. <https://doi.org/10.14412/1996-7012-2014-3-4-8> [In Russian]
- Nasonov EL (2015) Methotrexate in rheumatoid arthritis – 2015: New facts and ideas. *Nauchno-Prakticheskaya Revmatologiya [Rheumatology Science and Practice]* 53(4): 421–433. <https://doi.org/10.14412/1995-4484-2015-421-433> [In Russian]
- Osolodkin DI, Kozlovskaya LI, Dueva EV et al. (2013) Inhibitors of tick-borne flavivirus reproduction from structure-based virtual screening. *ACS Medicinal Chemistry Letters* 4 (9): 869–874. <https://doi.org/10.1021/ml400226s> [PubMed] [PMC]
- Rainsford K (2007) Anti-inflammatory drugs in the 21st century. *Subcellular Biochemistry* 42: 3–27. https://doi.org/10.1007/1-4020-5688-5_1 [PubMed]
- Smolen JS, Aletaha D, McInnes IB (2016) Rheumatoid arthritis. *Lancet* 388(10055): 2023–2038. [https://doi.org/10.1016/S0140-6736\(16\)30173-8](https://doi.org/10.1016/S0140-6736(16)30173-8) [PubMed]

Author contributions

- **Elena Y. Bybik**, Doctor of Medical Sciences, Professor, Head of the Department of Fundamental and Clinical Pharmacology; e-mail: Helen_bibik@mail.ru; the author set goals and objectives of the experiments and developed a model of pathology.
- **Oksana G. Yaroshevskaya**, Assistant, Department of Fundamental and Clinical Pharmacology, e-mail: chog@rambler.ru; the author conducted analysis and interpretation of the results and worked on the analysis of literature review.
- **Andrey V. Demenko**, Candidate of Medical Sciences, Associate Professor of the Department of Pharmaceutical Chemistry and Pharmacognosy; e-mail: pathecology@mail.ru; the author carried out mathematical-statistical processing.
- **Konstantin A. Frolov**, Candidate of Chemical Sciences, Assistant of the Department of Pharmaceutical Chemistry and Pharmacognosy; e-mail: ka.frolov@inbox.ru; the author designed the biochemical part of the experiment.
- **Viktor V. Dotsenko**, Doctor of Chemical Sciences, Head of the Department of Organic Chemistry and Technology; e-mail: victor_dotsenko@bigmir.net; the author designed the biochemical part of the experiment.
- **Sergey G. Kryvokolysko**, Doctor of Chemical Sciences, Professor, Head of the Department of Pharmaceutical Chemistry and Pharmacognosy; e-mail: ksg-group-lugansk@mail.ru; the author designed the biochemical part of the experiment.