

The Course of Freund's Adjuvant Induced Chronic Arthritis under the Influence of Calcium Channel Blockers

Khakimov Z. Z.¹, Bekova N. B.², Rakhmanov A. Kh.^{1,*}, Zaitseva O. A.¹

¹Tashkent Medical Academy, Tashkent, Uzbekistan

²Urgench Branch of the Tashkent Medical Academy, Urgench, Uzbekistan

Abstract The therapeutic and preventative effects of calcium channel blockers on the course of adjuvant-induced chronic arthritis and hematological blood parameters were studied in sexually mature male rats weighing 140-160 g. The aim of this work was to study the effects of amlodipine, diltiazem and cinnarizine on the course of adjuvant-induced arthritis (AIA) in comparison with diclofenac sodium. The AIA model in experiments is reproduced by injecting 0.1 ml of complete Freund's Adjuvant (CFA), which contains dead mycobacteria suspended in oil, into the hind right paw. Animals were intragastrically administered amlodipine, diltiazem, cinnarizine and diclofenac sodium at doses of 20 mg/kg, 20 mg/kg, 50 mg/kg and 10 mg/kg, respectively, once a day for 14 days. Similar studies were carried out in the second series of experiments for assessment of the therapeutic effects of the medicines. Treatment with the above medicines was carried out in similar doses from 15 to 28 days of experiment. It was found that calcium channel blockers have an inhibitory effect on the development of adjuvant-induced arthritis, especially diltiazem and cinnarizine, which effect was almost identical to diclofenac sodium. A more expressed correction of disturbances of hematological parameters was observed in animals treated with diltiazem in a therapeutic and preventative regimen.

Keywords Adjuvant-induced arthritis, Calcium channel blockers, Hematology

1. Introduction

Rheumatoid arthritis (RA) is one of the most common immune-inflammatory diseases, which is characterized not only by chronic erosive arthritis, but also by systemic damage of internal organs. It usually leads to disability and, as a consequence, a deterioration of the quality of life and a decrease in the life expectancy of patients [1]. In the treatment of this pathology, a significant breakthrough is associated with the introduction of glucocorticoid and cytostatic therapy. However, it is proved that treatment with these pharmacological drugs is insufficiently effective and accompanied by the development of a wide range of side effects. Due to the deciphering of the leading mechanisms of RA immunopathogenesis, a wide range of innovative drugs have been developed, such as genetically engineered biological drugs, monoclonal antibodies, recombinant proteins, and others [2]. Lack of effectiveness and high cost of these drugs were the most common reasons for discontinuing of pharmacotherapy [3]. In view of the wide

range of pharmacological effects, NSAIDs are one of the most commonly used group of drugs in medical practice, including in the treatment of RA. However, this group of drugs is characterized by the development of a line of specific side effects from the cardiovascular system, organs of the gastrointestinal tract, liver and kidneys [4,5,6,7]. The foregoing confronts tasks for medical science and pharmacology, in particular, the development of effective drugs for the treatment of this pathology. As the literature data show that RA is quite often diagnosed in elderly patients, in whom, along with the main diseases, pathology of the cardiovascular system is accompanied, in particular, hypertension, arrhythmias, angina pectoris, etc., and calcium channel blockers are often used for treatment them [8,9]. However, the effect of calcium channel blockers (CCBs) on the course of the chronic inflammatory process of the joints has been insufficiently studied.

Therefore, an assessment of the effect of calcium channel blockers on the course of RA would open up new possibilities for increasing the effectiveness of pharmacotherapy of this pathology.

The aim of this work was to study the effect of amlodipine, diltiazem and cinnarizine on the course of adjuvant-induced arthritis (AIA) in comparison with diclofenac sodium. We chose the last drug due to the fact that NSAIDs have not lost

* Corresponding author:

dr.ali.fl@mail.ru (Rakhmanov A. Kh.)

Received: Jan. 20, 2022; Accepted: Feb. 19, 2022; Published: Feb. 24, 2022

Published online at <http://journal.sapub.org/ajmms>

their positions in the treatment of arthritis [10].

2. Material and Methods

2.1. Experiments

The experiments were carried out on sexually mature white rats - males with an initial weight of 140-160 g, kept under standard vivarium conditions, quarantined for at least two weeks (with a natural light regime, at a temperature of 22-24°C and a relative humidity of 40-50%) using a standard diet. Experimental groups of animals were formed by 6 animals in each, taking into account the body weight. These experiments were carried out in accordance with the rules of good laboratory practice (GLP) for preclinical studies, as well as the rules and the International Recommendations of the European Convention for the Protection of Vertebrate Animals used in Experimental Research (1986). The study was approved by the Ethics Committee of the Ministry of Health of the Republic of Uzbekistan Protocol No. 6 dated September 23, 2021.

Adjuvant arthritis is one of the first experimental model used to test pharmaceuticals specialized for the treatment of RA. The experiment is implemented on white outbred white rats and the duration of the study is approximately 30 days. The similarity of AIA with human RA lies in the presence of edema of the extremities, degradation of cartilage, lymphocytic infiltration of the inflamed joint tissue, loss of their function, resorption of bone and periosteum [11]. The AIA model in experiments is reproduced by injection 0.1 ml of complete Freund's Adjuvant (CFA), which contains dead mycobacteria suspended in oil, into the hind right paw [12,13,14]. The AIA model was reproduced by intradermal injection of 0.1 ml of CFA (Chondrex, Inc., USA) into the dorsum of the hind paw, which contains killed mycobacterium H37RA at a concentration of 2 mg/ml suspended in oil, designed to reproduce AIA in rats [13]. AIA allows to induce an acute inflammatory reaction at the point of injection and an immunological reaction that develops after 9 days in the contralateral paw and other organs. The edema of the hind paw was observed from the very first to 15 days or more, depending on the duration of the experiment.

Due to study the preventative effect of medicines the animals were divided into several groups after the injection of CFA. Animals were intragastrically administered amlodipine, diltiazem, cinnarizine and diclofenac sodium at doses of 20 mg/kg, 20 mg/kg, 50 mg/kg and 10 mg/kg, respectively, once a day for 14 days. The similar studies were carried out in the second series of experiments for assessment the therapeutic effect of medicines. Treatment with the above medicines was carried out in similar doses from 15 to 28 days of experiment. After 3, 7, 10 and 14 days (preventative treatment), as well as after 21 and 28 days (treatment) of experiment, oncometric measurements of the affected paws were made by using a plethysmometer in all groups of animals [15]. Blood samples were taken from the

tail vein and the formula of blood indexes was determined on a hematological analyzer BC-3000 (Mindray, China). Then the animals were decapitated under general anesthesia and blood was collected for biochemical studies.

2.2. Statistical Analysis

The data obtained were processed by the method of variation statistics using the paired Student's test and one-way analysis of variance using the standard software package BIOSTAT 2009 with an assessment of the significance of indicators (Mean±Std error). Differences in the compared groups were considered significant at a significance level of 95% $p < 0.05$.

3. Results and Discussion

In the first series of experiments, we investigated the effect of the preventive action of CCBs on the course of chronic arthritis induced by CFA. The results of the experimental studies showed that the injection of CFA provoked a pronounced inflammatory process in rats. Already on the 3rd day after immunization, the rats became lethargic, aggressive, inactive, the coat became dull, tousled, and food consumption decreased. At the same time, after 3, 7, 10 and 14 days from the beginning of the experiment, there was a significant increase in the volume of the rat paws in comparison with the initial volumes by 256.4%; 269.0%; 283.6% and 290.9%, respectively. It was characteristic that there was some increase in the volume of other joints of the legs. All of these testified the development of chronic progressive, generalized, immune-dependent inflammation of the joints. In contrast, there was a less expressed increase in the volume of the paw in the group of rats preemptively treated with CCBs. So, under the influence of amlodipine, the increase in the volume of the paws was respectively 200.0%; 205.2%; 208.8% and 210.5%, compared with the initial value of paws in the indicated periods of observation. The index of inhibition of inflammation was 19.1%; 20.9%; 23.7% and 25.0% under the influence of medicine. A more pronounced pharmacotherapeutic effect was observed in the group of rats treated with diltiazem and cinnarizine. From the data in Figure 1, it is clear that the index of inhibition of inflammation by these medicines was 28.4 and 26.2% on the third day of the experiment, and after seven days, it was 29.7% and 27.7%, respectively. Continued administration of diltiazem and cinnarizine led to an increase in the noted effect, and on the 14th day of observation, the index of inhibition of inflammation was 33.1 and 30%, respectively. Similar in direction, but somewhat expressed action was stated in the group of animals receiving diclofenac sodium preventatively. In the indicated periods of observation, the index of inhibition of inflammation was 31.9%; 33.8%; 35.2% and 37.5%, respectively. As can be seen from the above data, calcium channel blockers have an inhibitory effect on the development of RA, which especially diltiazem and cinnarizine have a preventive effect almost identical

to diclofenac sodium.

The results of animals have shown that there was a expressed changes in hematological parameters after injection of CFA (table 1). Thus, the number of blood leukocytes, lymphocytes and granulocytes increased by 172.9%; 135.8% and 64.35%, respectively, which caused an increase in the absolute content of the mixture of monocytes, basophils and eosinophils by 164.9%. Against

this background, in experimental rats the number of platelets increased by 51.7%, and thrombocyte rose by 49.2%.

Consequently, the expressed leukocytosis and lymphocytosis develop in animals under the influence of CFA, which indicates the development of chronic inflammation combined with circulatory disorders due to an increase in the number of platelets. These changes were most pronounced by the end of the 14th day of the experiment.

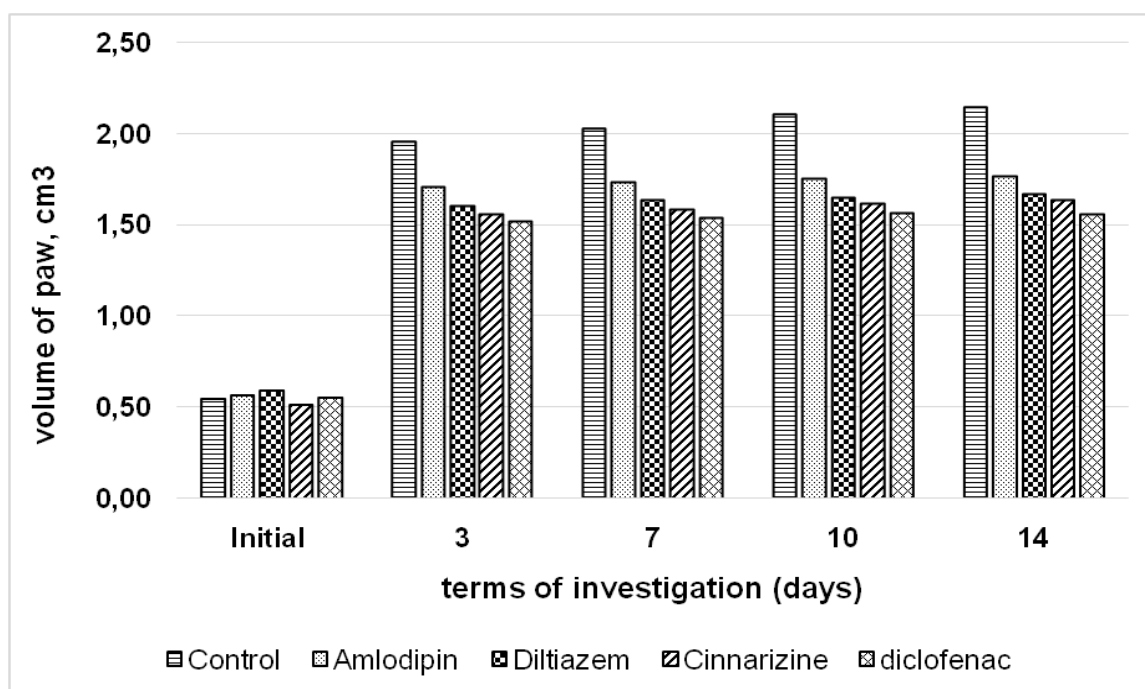


Figure 1. Influence of CCB and diclofenac sodium on the course of adjuvant-induced arthritis in preventive administration

Table 1. To study the effect of the preventative action of calcium channel blockers and sodium diclofenac on hematological parameters in adjuvant arthritis (M \pm std error, n = 6)

Parameters Groups	leukocytes, $10^9/l$	Absolute content of lymphocytes, $10^9/l$	Absolute content of mix of monocytes, basophils and eosinophils, $10^9/l$	Quantity of granulocytes, $10^9/l$	Platelets in absolute number, $10^9/l$	thrombocyte, %
Intact	$8,72 \pm 0,38$	$7,66 \pm 0,54$	$1,34 \pm 0,12$	$6,11 \pm 0,47$	$351,20 \pm 32,52$	$0,368 \pm 0,027$
Control	$23,80 \pm 1,61$	$18,06 \pm 1,63$	$3,55 \pm 0,35$	$10,04 \pm 0,89$	$532,95 \pm 33,09$	$0,549 \pm 0,025$
P	< 0,001	< 0,002	< 0,002	< 0,02	< 0,02	< 0,01
Amlodipin	$12,77 \pm 1,41$	$12,88 \pm 1,22$	$2,66 \pm 0,30$	$7,87 \pm 0,53$	$482,05 \pm 30,81$	$0,466 \pm 0,017$
P	< 0,05	< 0,02	< 0,01	> 0,05	< 0,05	< 0,05
P₁	< 0,01	< 0,05	> 0,05	> 0,05	> 0,05	< 0,05
Diltiazem	$9,99 \pm 1,02$	$9,41 \pm 0,79$	$1,98 \pm 0,14$	$6,79 \pm 0,33$	$385,50 \pm 28,86$	$0,404 \pm 0,029$
P	> 0,05	> 0,05	> 0,05	> 0,05	< 0,02	> 0,05
P₁	< 0,001	< 0,01	< 0,01	> 0,05	< 0,02	< 0,002
Cinnarizine	$10,36 \pm 1,03$	$9,96 \pm 0,89$	$2,24 \pm 0,17$	$7,47 \pm 0,33$	$445,23 \pm 25,79$	$0,424 \pm 0,021$
P	> 0,05	< 0,05	< 0,05	> 0,05	> 0,05	> 0,05
P₁	< 0,01	< 0,01	< 0,02	< 0,02	> 0,05	< 0,02
Diclofenac	$9,12 \pm 0,79$	$8,87 \pm 0,64$	$1,78 \pm 0,15$	$6,47 \pm 0,33$	$409,07 \pm 21,68$	$0,390 \pm 0,035$
P	> 0,05	> 0,05	> 0,05	> 0,05	> 0,05	> 0,05
P₁	< 0,001	< 0,01	< 0,01	< 0,02	< 0,05	< 0,02

Note: P - statistically significant differences in comparison with intact group;

P₁ - statistically significant differences in comparison with control group.

Table 2. Study of the therapeutic effect of calcium channel blockers and sodium diclofenac on the course of adjuvant-induced arthritis ($M \pm m$, $n = 6$)

Groups	Volume of paws, cm^3 (days)			
	Исходный	14	21	28
Control	$0,60 \pm 0,02$	$\frac{2,20 \pm 0,13}{1,60 \pm 0,11}$	$\frac{2,22 \pm 0,11}{1,62 \pm 0,09}$	$\frac{2,16 \pm 0,06}{1,56 \pm 0,05}$
Amlodipin	$0,57 \pm 0,03$	$\frac{2,13 \pm 0,08}{1,56 \pm 0,08}$	$\frac{1,85 \pm 0,09}{1,28 \pm 0,08}$	$\frac{1,77 \pm 0,11}{1,20 \pm 0,10}$
P		$< 0,001$	$< 0,001$	$< 0,001$
P ₁		$> 0,05$	$> 0,05$	$< 0,05$
Diltiazem	$0,62 \pm 0,02$	$\frac{2,25 \pm 0,11}{1,63 \pm 0,10}$	$\frac{1,80 \pm 0,12}{1,18 \pm 0,11}$	$\frac{1,68 \pm 0,11}{1,06 \pm 0,10}$
P		$< 0,001$	$< 0,001$	$< 0,001$
P ₁		$> 0,05$	$< 0,05$	$< 0,02$
Cinnarizine	$0,52 \pm 0,02$	$\frac{2,09 \pm 0,10}{1,57 \pm 0,09}$	$\frac{1,72 \pm 0,08}{1,20 \pm 0,08}$	$\frac{1,60 \pm 0,11}{1,08 \pm 0,07}$
P		$< 0,001$	$< 0,001$	$< 0,001$
P ₁		$> 0,05$	$< 0,05$	$< 0,01$
Sodium diclofenac	$0,59 \pm 0,02$	$\frac{2,21 \pm 0,12}{1,62 \pm 0,11}$	$\frac{1,71 \pm 0,12}{1,12 \pm 0,11}$	$\frac{1,57 \pm 0,14}{0,98 \pm 0,14}$
P		$< 0,001$	$< 0,001$	$< 0,001$
P ₁		$> 0,05$	$< 0,05$	$< 0,02$

Note: in the numerator absolute indices of the paw volume, and in the denominator the difference of edema compared to the initial volume.

P – statistically significant differences in comparison with the initial volume;

P₁ – statistically significant differences in comparison with control group at the corresponding study period.

Table 3. To study the influence of the therapeutic effect of calcium channel blockers and sodium diclofenac on hematological parameters in adjuvant arthritis ($M \pm m$, $n = 6$)

Parameters Groups	leukocytes, $10^9/l$	Absolute content of lymphocytes, $10^9/l$	Absolute content of mix of monocytes, basophils and eosinophils, $10^9/l$	Quantity of granulocytes, $10^9/l$	Platelets in absolute number, $10^9/l$	thrombocyte, %
Intact	$7,81 \pm 0,74$	$7,21 \pm 0,57$	$1,48 \pm 0,13$	$6,49 \pm 0,44$	$280,03 \pm 7,86$	$0,345 \pm 0,032$
Control	$20,85 \pm 1,66$	$16,54 \pm 1,23$	$3,89 \pm 0,41$	$9,17 \pm 0,76$	$530,20 \pm 28,24$	$0,607 \pm 0,016$
P	$< 0,001$	$< 0,001$	$< 0,01$	$< 0,05$	$< 0,001$	$< 0,001$
Amlodipin	$13,25 \pm 1,38$	$11,36 \pm 1,37$	$2,78 \pm 0,28$	$7,77 \pm 0,39$	$448,67 \pm 40,44$	$0,488 \pm 0,024$
P	$< 0,02$	$< 0,05$	$< 0,01$	$> 0,05$	$< 0,01$	$< 0,02$
P ₁	$< 0,02$	$< 0,05$	$> 0,05$	$> 0,05$	$> 0,05$	$< 0,01$
Diltiazem	$9,24 \pm 0,56$	$8,77 \pm 1,26$	$2,06 \pm 0,27$	$7,26 \pm 0,24$	$375,53 \pm 25,32$	$0,384 \pm 0,025$
P	$> 0,05$	$> 0,05$	$> 0,05$	$> 0,05$	$< 0,02$	$> 0,05$
P ₁	$< 0,002$	$< 0,01$	$< 0,02$	$> 0,05$	$< 0,02$	$< 0,002$
Cinnarizine	$10,01 \pm 1,01$	$9,46 \pm 0,81$	$2,11 \pm 0,19$	$7,33 \pm 0,33$	$449,23 \pm 25,79$	$0,412 \pm 0,044$
P	$> 0,05$	$< 0,05$	$< 0,05$	$> 0,05$	$< 0,02$	$> 0,05$
P ₁	$< 0,01$	$< 0,01$	$< 0,02$	$> 0,05$	$> 0,05$	$< 0,02$
Diclofenac	$8,79 \pm 0,95$	$8,28 \pm 0,68$	$1,73 \pm 0,15$	$6,79 \pm 0,57$	$330,21 \pm 29,62$	$0,351 \pm 0,032$
P	$> 0,05$	$> 0,05$	$> 0,05$	$> 0,05$	$> 0,05$	$> 0,05$
P ₁	$< 0,002$	$< 0,002$	$< 0,01$	$< 0,05$	$< 0,01$	$< 0,001$

Note: P - statistically significant differences in comparison with intact group;

P₁ - statistically significant differences in comparison with control group.

We found a different picture in the group of animals that received CCB. Thus, the number of leukocytes, lymphocytes and granulocytes compared with the control decreased by 46.3%; 28.7% and 21.6% in animals receiving amlodipine, by 58.0%; 47.9% and 32.4% - in animals receiving diltiazem, as well as by 54.6%; 42.8% and 27.6% - in animals receiving cinnarizine. It is noteworthy that against this background, there was a decrease in the absolute content of the mixture of monocytes, basophils and eosinophils by 25.1%; 44.2% and

33.8%, respectively, in the animals of the indicated groups. It should be noted that these changes in hematological parameters were accompanied by a decrease in the number of platelets and thrombocrit by 9.5% and 15.1%, respectively, in the group receiving amlodipine, by 16.4% and 22.7% in the group receiving diltiazem, 13.1% and 15.5% in the group receiving cinnarizine preventively.

As can be seen from the presented material, the most significant effects in the correction of disturbances of

hematological parameters were observed in animals preventively receiving diltiazem. These changes under the influence of the latter did not differ significantly from the indexes of the groups of animals that were preventively treated with diclofenac sodium.

Thus, injection of CFA in animals leads to pronounced changes in peripheral blood parameters, indicating the development of chronic immune inflammation, and drugs of the CCB group clearly eliminate them, especially diltiazem, which does not differ in its pharmacological activity from a classic NSAID drug - diclofenac sodium.

Summarizing the results of the first series of experiments, we can conclude that preventive administration of CCBs prevent the development of chronic immune-inflammatory process clearly showing a decrease in the development of arthritis and elimination of hematological parameters.

Further, we studied the effect of CCBs on the course of chronic inflammation induced by CFA in the therapeutic application, since the overwhelming majority of patients seek medical help after a clinical manifestation of pathology, where pharmacological agents are used in therapeutic intention. Considering this circumstance, it seemed important to study the effect of CCBs on the course of AIA during their therapeutic administration. As can be seen the results shown in table 2, a high level of paw volume is stably maintained in animals with the AIA model in comparison with initial volume of paws and it was more than 270.0-266.0% in the next two weeks of the experiment. The paw volume gradually decreases under the influence of calcium channel blockers in rats. So, in amlodipine treated group, the volume of the paws was respectively 224.5 and 210.5% compared to the initial volume after one and two weeks from the beginning of treatment. We determined a more expressed effect in the group of animals treated with diltiazem, in which the paw volume at the indicated observation periods was 190.3% and 170.9%, and in cinnarizine treated group was 237.7% and 207.7%, respectively. AIV of amlodipine, diltiazem and cinnarizine were respectively 21.0%; 27.2% and 25.9% after seven days of treatment. After two weeks of treatment, these values were respectively 23.1%; 32.0% and 30.7%. It is noteworthy that diltiazem was not statistically significantly inferior to diclofenac sodium in terms of its anti-inflammatory efficacy, in which AIV was 30.9% and 37.2% in the studied periods.

Consequently, calcium channel blockers clearly inhibit the development of RA in experimental animals both in the case of preventative and therapeutic administration, and in terms of their effectiveness, they are equal to the effects of the classical representative of NSAIDs the gold standard-diclofenac sodium [16].

It is known that from a clinical point of view, it is important to establish the effectiveness of pharmacological agents in their therapeutic use by objective laboratory indicators, including hematological analysis. To establish the effectiveness of CCB for these purposes, a special series of experiments was carried out, in which pharmacological agents were administered after the reproducing a diffuse

clinical picture of AIA model in experimental animals. In these experiments, treatment with drugs of the CCB group was carried out for two weeks at the same doses as in the previous series of experiments. Analysis of the results of the experimental studies showed that after the end of the experimental therapy, there were significant positive changes in the parameters of peripheral blood. So, the number of leukocytes, lymphocytes and granulocytes increased respectively by 167.0%; 129.4% and 41.3% in AIA animals' group, so in amlodipine treated group these parameters decreased by 36.4%; 31.3% and 15.3%, respectively. We also noted a decrease in these indicators in the diltiazem treated group by 55.7%; 47.0% and 21.0%, and in cinnarizine treated group's animals the indicated values were 52.0%; 42.8% and 20.0%. Against this background, there were a decrease in the absolute content of a mixture of monocytes, basophils and eosinophils respectively by 31.3% 47.0% and 42.8 in amlodipine, diltiazem and cinnarizine treated animals. We found similar changes in the group of animals treated with sodium diclofenac, in which the number of leukocytes, lymphocytes, the absolute content of the mixture of monocytes, basophils, eosinophils and the number of granulocytes decreased by 57.8%; 50.0%; 55.5% and 25.9%, respectively. From the data in table 3, the therapeutic effect of the studied drugs of the CCB group and diclofenac sodium is accompanied by a statistically significant decrease in the platelet count and thrombocrit value, especially when diltiazem was used. At the same time, it was not significantly inferior to diclofenac sodium by its activity.

Summarizing the above data, it can be stated that CCBs exhibits a distinct therapeutic effect in animals with developed autoimmune arthritis, which is manifested in the elimination of disturbances of hematological parameters in laboratory animals.

4. Conclusions

1. Calcium channel blockers clearly suppress the development of chronic autoimmune inflammation in experimental animals with adjuvant-induced arthritis.
2. The pharmacological effects of calcium channel blockers are convincingly confirmed in the elimination of disturbances of hematological parameters.
3. The results of these experimental studies can be the basis for introducing changes in the pharmacotherapy of patients with rheumatoid arthritis who are simultaneously taking calcium channel blockers.

REFERENCES

- [1] Nasonov E.L., Leela A.M., 2019, Rheumatoid Arthritis: Achievements and Unresolved Issues. Therapeutic Archives, 91, 5, 4-7.

- [2] Nasonov E.M., 2019, New directions of pharmacotherapy of immunoinflammatory rheumatic diseases. Therapeutic Archives, 91, 8, 98-107.
- [3] Aronova E.S., Lukina G.V., Glukhova S.I., 2020, Survival with genetically engineered biological therapy in bionic patients with rheumatoid arthritis: data from a retrospective 12-month follow-up. Therapeutic archive, 92, 5, 39-45.
- [4] Asfandiyarova N.S., Filippov E.V., 2020, The use of non-steroidal anti-inflammatory drugs for polymorbid pathology. Therapeutic Archives, 92, 1, 82-88.
- [5] Kalagova A.V., Aylarova N.R., Panagov Z.G., 2019, NSAIDs - gastropathy in patients with rheumatoid arthritis. Bulletin of Science and Education, 1, 55, 97-100.
- [6] Klimov L.Ya., Aksyonov A.G., Popova E.V., 2018, Fulminant hepatic failure while taking acetaminophen. Medical Bulletin, 11, 79-83.
- [7] Ivashkin V.T., Baranovsky A.Yu., Raikhelson K.L., 2019, Medicinal lesions of the liver (clinical guidelines for physicians). Russian journal of gastroenterology, hepatology, caloproctology, 29, 1, 101-131.
- [8] Kukes V.G., 2021, Clinical pharmacology: textbook: edited by V.G. Kukes, D.A. Sychev, 6th ed., Rev. and additional - M.: GEOTAR-Media, 1024.
- [9] Kharkevich D.A., 2017, Pharmacology. 12th edition M.: "GEOTAR-Media". 760.
- [10] Zavodovsky B.V., Sivordova L.E., Polyakova Yu.V., 2020, Evaluation of the safety, tolerability and efficacy of the first domestic generic drug of aceclofenac in patients with undifferentiated arthritis. Therapeutic Archives, 92, 5, 61-68.
- [11] Bendele A., 1999, Sustained blood levels of interleukin-1 receptor antagonist in animal models of arthritis. Arthritis Rheum., 42, 498-506.
- [12] Allison A., Byars N., 1986, An adjuvant formulation that selectively elicits the formation of antibodies of protective isotypes and of cell-mediated immunity, J. Immunol. Methods, 95, 157-168.
- [13] Voleeva I.Kh., Titarenko A.F., Khaziakhmetova V.N. Ziganshina L.E., 2016, Antioxidant activity of xymedon on a model of chronic autoimmune inflammation, Experimental and Clinical Pharmacology. 79, 1, 33-37.
- [14] Ivanova E.A. Matyushkin A.I., Voronina T.A., 2019, Influence of hemantanum in a dosage form for external use on the inflammatory process in rats caused by complete Freud's adjuvant. Experimental and Clinical Pharmacology. 82, 4, 23-27.
- [15] Kopyeva T.N., 1980, Pathology of rheumatoid arthritis. M.: Medicine, 208.
- [16] Ivanova E.A., Voronina T.A., 2018, The effect of diclofenac sodium on the level of histamine and serotonin in acute exudative inflammation in rats. Pharmacokinetics and Pharmacodynamics, 2, 12-15.