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Researc of pro- and anti-inflammatory cytokines dynamic on the background of endothelial dysfunction development induced by experimental osteoarthrosis

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Abstract

Study have been carried out on white Wistar line rats (age – 3 months, weight – 180-220 g). According to the tasks the animals were divided into 7 groups:

1st group is intact (n = 20). 2nd group is rats, which were modeled osteoarthritis without further correction and were withdrawn from the experiment in the first stage (7th day) (n=40). 3rd group is rats, which were modeled osteoarthritis without further correction and removed from the experiment in the second stage (21st day) (n=40). 4th group is rats, in which experimental osteoarthritis was corrected with nonsteroidal anti-inflammatory drugs (NSAIDs) (Diclofenac) and aminoguanidine and removed from the experiment in the first stage (7th day) (n=20). 5th group is rats, in which experimental osteoarthritis was corrected with NSAIDs (Diclofenac) and aminoguanidine and withdrawn from the experiment in the second stage (21st day) (n=20). 6th group is rats, where experimental osteoarthritis was corrected using NSAIDs and a 7% L-arginine solution and withdrawn from the experiment in

the first stage (7th day) (n=20). 7th group is rats, in which experimental osteoarthritis was corrected with NSAIDs and 7% L-arginine solution and withdrawn from the experiment in the second stage (21st day) (n=20). Animals were withdrawn from the experiment for the 7th day and the 21st day after the simulation of the pathological condition. NSAIDs (Diclofenac), aminoguanidine and L-arginine were administered from the beginning of the study.

It were found during the experiment, increased levels of Interleukin 1β and Interleukin 10 in the simulated osteoarthrosis pathogenesis. It has been established positive dynamics of these cytokines in the endothelial dysfunction correction at osteoarthritis with the aminoguadine correction. It was revealed more pronounced efficacy of using L-arginine as a corrective means of impaired endothelial function in experimental osteoarthritis. Comparative characteristics of correction agents has shown that the use of nitric oxide donor is more effective than incubation of inducible NO synthase. It was proved normalization of endothelial functional status indicators in the group of animals treated with L-arginine as a part of complex correction of osteoarthrosis was proved.

Key words: osteoarthritis; experimental model; endothelial dysfunction; Interleukin 1β; Interleukin 10; aminoguanidine; L-arginine.

Introduction

Today, the problem of the musculoskeletal system has become of great socio-economic importance. An essential component among patients are patients with osteoarthritis. OA prevalence in the US was 13.2 million in 2002, 14.5 million in Europe, 6.6 million in Japan [1-4]. In recent years, the number of patients with peripheral joints and spine injuries has been steadily increasing, and, unfortunately, there has been a tendency to develop the disease in young people [5-7]. According to many authors there is a certain pattern in the sexual development of osteoarthritis in different age groups. If men under 50 are more commonly diagnosed with OA, then in the older age, arthrosis develops more often in women. It has been observed that women mostly develop hemarthrosis and men suffer from hip OA [8]. According to statistics, the disease prevalence increases by 2-10 times in the age range from 30 to 65 years. If at the age of 29 years 8,4% of a person per 1000 population suffer from this disease, then at 33-39 years - 42,1, 40-49 years -191,9 and at 50-59 years - 297,2 per 1000 persons. In people older than 65 years, this pathology is diagnosed in 879.7 people per 1000 population [9-11]. Despite the generally favorable prognosis, the risk of disability in osteoarthritis can be compared with the risk associated with cardiovascular

disease. According to WHO, only OA of the knee joints ranks fourth among the causes of disability in women and eighth in men. This pathology among the disabilities ranks second after ischemic heart disease [12-14].

Materials and methods

Study have been carried out on white Wistar line rats (age – 3 months, weight – 180-220 g). According to the tasks the animals were divided into 7 groups:

1st group is intact (n = 20).

2nd group is rats, which were modeled osteoarthritis without further correction and were withdrawn from the experiment in the first stage (7th day) (n=40).

3rd group is rats, which were modeled osteoarthritis without further correction and removed from the experiment in the second stage (21st day) (n=40).

4th group is rats, in which experimental osteoarthritis was corrected with nonsteroidal anti-inflammatory drugs (NSAIDs) (Diclofenac) and aminoguanidine and removed from the experiment in the first stage (7th day) (n=20)

5th group is rats, in which experimental osteoarthritis was corrected with NSAIDs (Diclofenac**)** and aminoguanidine and withdrawn from the experiment in the second stage (21st day) (n=20)

6th group is rats, where experimental osteoarthritis was corrected using NSAIDs and a 7% L-arginine solution and withdrawn from the experiment in the first stage (7th day) (n=20)

7th group is rats, in which experimental osteoarthritis was corrected with NSAIDs and 7% L-arginine solution and withdrawn from the experiment in the second stage (21st day) (n=20)

Animals were withdrawn from the experiment for the 7th day and the 21st day after the simulation of the pathological condition. NSAIDs (Diclofenac), aminoguanidine and L-arginine were administered from the beginning of the study.

Blood samples were taken for the biochemical study of the following parameters:

Interleukin 1β and Interleukin 10 levels dynamic were determined by enzyme immunoassay (ELISA/EIA).

Aminoguadine is a selective inhibitor of inducible NO synthase (iNO-synthase), given to experimental animals at a dose of 15 mg/kg/day in the form of a solution in the free drink mode [15].

Nitric oxide donor administration a solution of L-arginine (SIMESTA, made in China, quality standard USP32) was carried out by intragastric injection of L-arginine solution in

0.9% sodium chloride solution at a dose of 500 mg / kg (Pokrovsky M.V., Pokrovskaya T.G., Korchakov V.I., etc., 2008) through a syringe with a feeding tube.

Both drugs were administered throughout the experiment.

Research was conducted in accordance with the "Rules for carrying out works using experimental animals", approved by the Order of the Ministry of Health of Ukraine No. 249 of 01.03.2012 and the Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruel Treatment" (as amended on December 15, 2009, and 10/16/2012)

Destructive-dystrophic process of cartilage tissue was modeled by knee joint criodamadge**.** One-time intraarticular injection was performed with solution of cooled ethanol (Vvedensky BP, Galchenko SE, Kovalev GO, 2011).

Choice of this modeling method is justified by the fact that it does not require surgery, allows to standardize the experimental reproduction of the pathological process, reduces the risk of complications and does not lead to paraarticular tissues damage. This model provides a high frequency of consequences of local and general changes in the body in response to a modeled pathological process [16].

Before using parametric, normality-based statistical distribution methods, it were used to test the series of quantitative data for normality using the Shapiro–Wilk test. Due to the normal distribution of digital data in the samples, was used Student's parametric criterion.

Research results

Interleukin 1 β dynamics in rats blood.

Obtained from the research data show inflammatory process development in animals, which simulated the pathological process. In 2nd group, in which animals didn't undergo correction of the simulated OA, was found this indicator value increasing more than one and a half times as compared with the data of intact rats. (Table 1). In 3rd group animals, it was found that proinflammatory cytokine level decreased by 2.6% in comparison with previous group. Foregoing indicates that after 30 days, in the group without are involved correction compensatory restorative processes, but not in a significant way. Proinflammatory cytokine dynamic at the first stage of the experiment is presented in Fig. 1

Next step is to compare groups that received correction using standard therapy and aminoguanidine with data from intact animals and rats without correction. Thus, comparing data of 1st and 4th groups, are found differences at significance level $p < 0.001$. That is, at this stage, the fourth group results aren't close to intact animals values yet. But it should be noted that Interleukin 1β level is 52.3% lower in the fourth group than in the second, which indicates the correction efficiency.

Table 1 - Dynamics of Interleukin 1 β in the blood of rats during experimental osteoarthritis and its correction

Group	Intact	OA without correction I stage	OA without correction II stage	OA with NSAIDs correction and aminoguanidine I stage	OA with NSAIDs correction and aminoguanidine II stage	OA with NSAIDs correction and L-arginine I stage	OA with NSAIDs correction and L-arginine II stage
№ п/п	1	2	3	4	5	6	7
IL-1β	35,54±0,86	92,46±1,53 p_{21}<0,001	90,08±1,09 p_{31}<0,001 p_{32}=0,077	44,11±0,85 p_{41}<0,001 p_{42}<0,001	42,84±1,32 p_{51}<0,001 p_{54}=0,42 slightly p_{53}<0,001	43,38±1,14 p_{61}<0,001 p_{62}<0,001 p_{64}=0,61	40,15±0,98 p_{71}=0,002 p_{76}=0,038 p_{73}<0,001 p_{75}=0,11

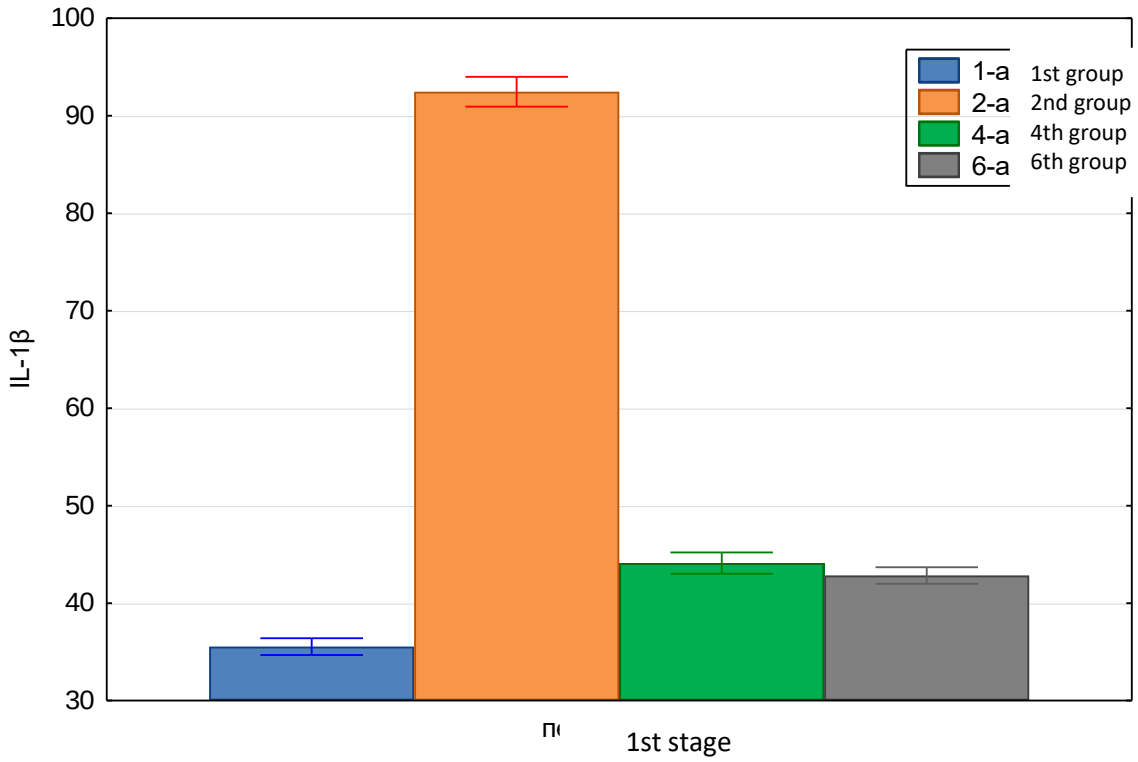

Figure 1 - Dynamics of Interleukin 1β level at the first research stage

Fifth group received the same corrective remedies combination as fourth, but longer time. Animals were withdrawn from the experiment at the second research stage. Proinflammatory cytokine level in this group is slightly more than the fourth approximates to intact animals values (difference between 1st and 4th groups data was 24, 1%, and the 1st and

5th - already 20.5%, differences when comparing 4th and 5th groups results are statistically insignificant).

Certainly, positive point is that Interleukin 1β level in the fifth group is 52.4% lower than in the group 3, in which animals were also excluded from the experiment in the second stage, but didn't receive corrective medications.

Next, we compared group results of animals that received standard correction in combination with L-arginine solution, which is a nitrogen oxide donor with data from other rats groups.

At the first stage, pro-inflammatory interleukin level in this group is closer to intact animals values than group 4 (difference in intact animals and rats comparison in group number 6 is 22.1%).

Also, this group result is 53.1% better than animals without correction. It is noteworthy that nitric oxide donator inclusion before corrective therapy is more effective than inducible NO synthase inhibitor induction in Interleukin 1β analysis change: sixth group result was better at 35.6% ($p < 0.001$).

Proinflammatory cytokine dynamic at the second stage of the research is presented in Fig. 2.

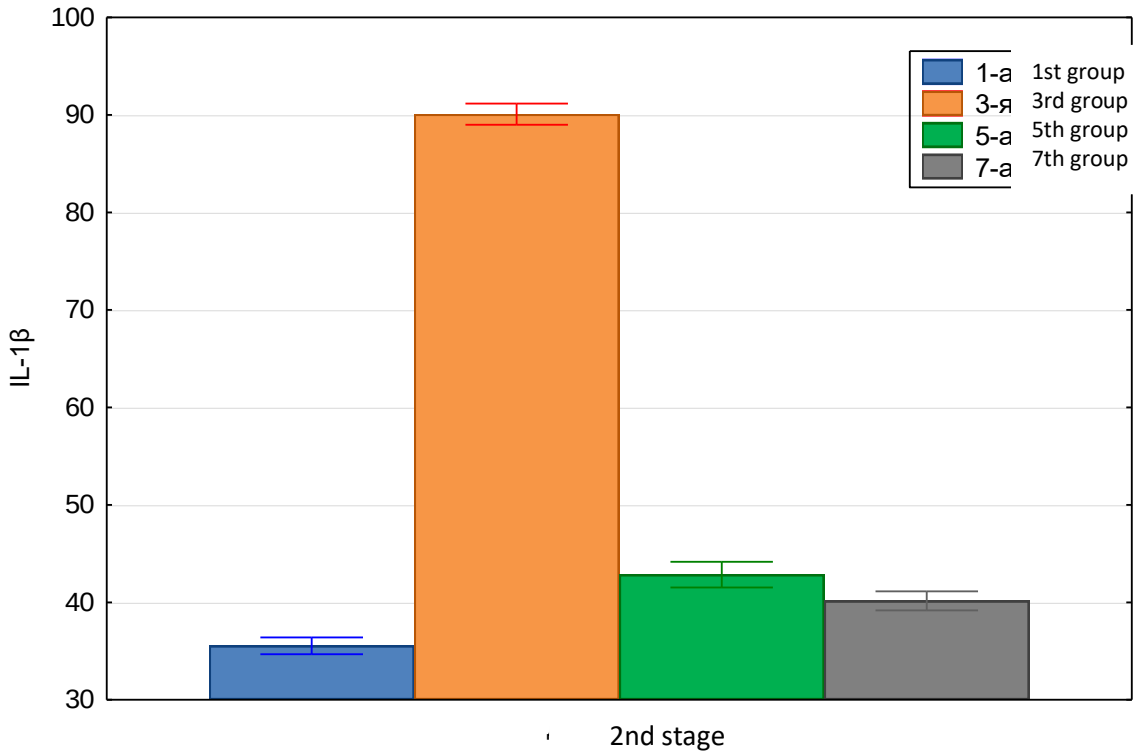

Figure 2 – Dynamics of Interleukin 1β level at the second research stage

Interleukin 10 dynamics in rats blood.

In anti-inflammatory cytokine research, it was established its increasing in all groups in which animals were modeled osteoarthritis (Table 2). Most pronounced - in rats, which didn't undergo pathological condition correction. Thus, in the second group, Interleukin 10 level increased by more than 3.5 times ($p < 0.001$). At the second stage, was found this indicator reduction in the group without correction (group number 3) to 4.8%, which indicates the presence of compensatory restorative processes in experimental animals organism, but its influence is unexpressed.

In the rats group, which receiving NSAIDs in combination with the NO-syntase inhibitor was found significant positive dynamics: anti-inflammatory cytokine research results were 55.3% better than those without correction ($p < 0.001$). However, this correction didn't lead to complete investigated index normalization: Interleukin 10 level in the 4th group was 59.5% higher than in intact animals.

Anti-inflammatory cytokine level dynamic is presented at Fig. 3. and Fig. 4

Table 2 - Dynamics of Interleukin 10 in the rats blood during experimental osteoarthritis and its correction

Group	Intact	OA without correction I stage	OA without correction II stage	OA with NSAIDs correction and aminoguanidine I stage	OA with NSAIDs correction and aminoguanidine II stage	OA with NSAIDs correction and L-arginine I stage	OA with NSAIDs correction and L-arginine II stage
№ п/п	1	2	3	4	5	6	7
IL-10	17,12± 0,69	61,05±1,08 $p_{21}<0,001$	58,14± 1,30 $p_{31}<0,001$ $p_{32}=0,017$	27,31± 0,72 $p_{41}<0,001$ $p_{42}<0,001$	25,12± 0,85 $p_{51}<0,001$ $p_{54}=0,056$ $p_{53}<0,001$	25,54±0,83 $p_{61}<0,001$ $p_{62}<0,001$ $p_{64}=0,12$	23,44±0,95 $p_{71}<0,001$ $p_{76}=0,11$ $p_{73}<0,001$ $p_{75}=0,19$

In the second stage, animals results which receiving the same therapy were as follows (Table 2): difference in comparison with intact animals data is lower than in the previous stage, and already makes up 46.7%. It was found an anti-inflammatory cytokine reduction to be 8% compared to group 4 (whose rats received the same correction but were withdrawn from the experiment in the first stage). The improvement was found to be 56.8% ($p < 0.001$) compared with the group data, which didn't correct the pathological process during 30 days.

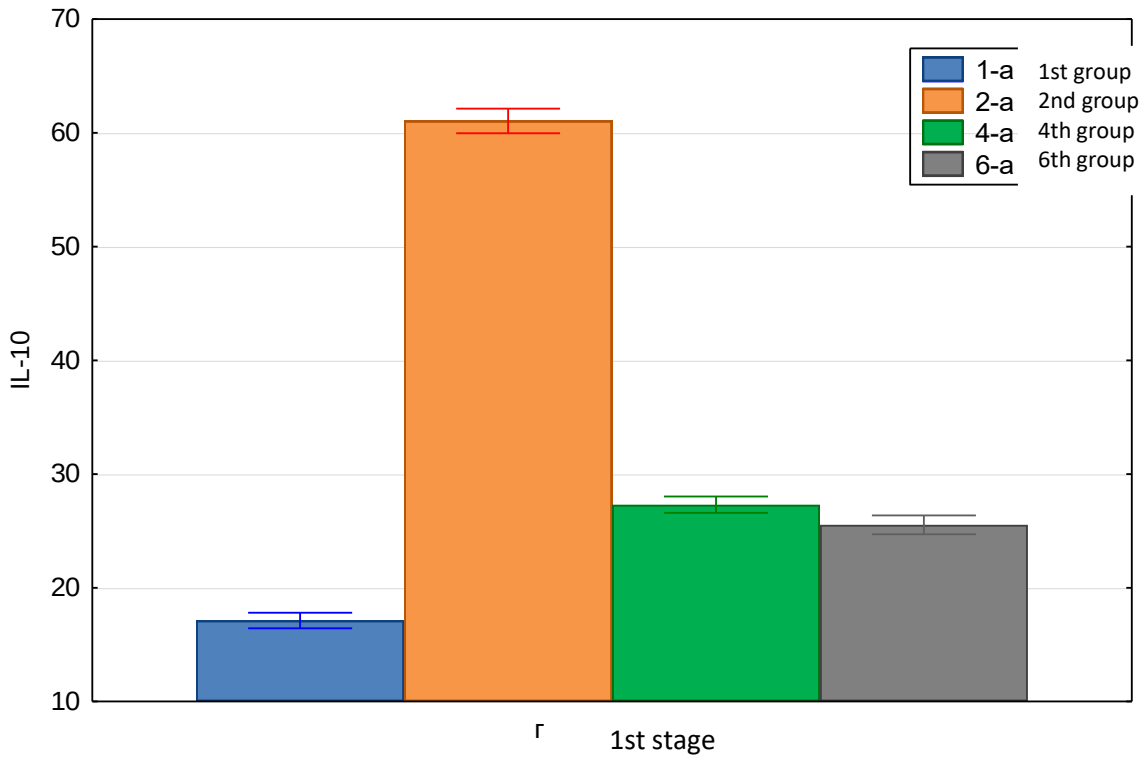

Figure 3 – Dynamics of Interleukin 10 level at the first research stage

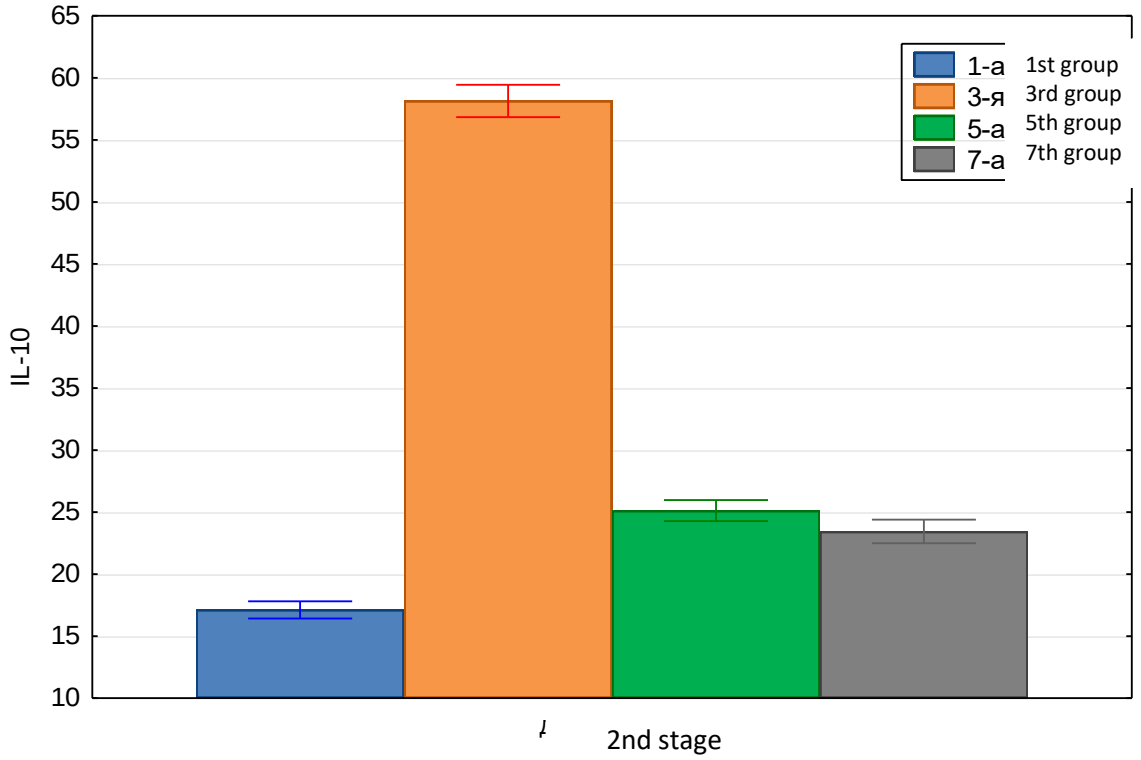

Figure 4 –Dynamics of Interleukin 10 level at the second research stage

Next, consider the group results, in which pathological condition was corrected by combining standardized therapy and L-arginine solution. On first stage, it was found that Interleukin 10 levels exceeded its value in intact animals by 49.2%, which is the best result compared to the group receiving NSAIDs in combination with aminoguanidine at the same stage (level in 6th group is less than 4th group results by 6.5%). It was also found that the result of this group is 58.2% better than in the group, which has not been corrected by experimental osteoarthritis.

At the second stage, it's confirmed the more pronounced efficiency of attracting a nitric oxide donor to the complex correction. So in the group with its use it was found that in the second stage anti-inflammatory cytokine activity is 59, 7% less than in the group without correction at the same stage. Difference from intact animals data is already smaller in comparison with another group data with correction and makes up 36,9%.

Research result discussion

In our research has been demonstrated the important roleof proinflammatory cytokines activation in the pathogenesis of experimental osteoarthritis.

Interleukin 1β is secretory cytokine that performs more than 50 different biological functions, both locally and systemically. It is produced as an inactive precursor, but under the action of caspase 1 is converted into an active cytokine. Realizing its effects, interleukin 1β enhances MMPs synthesis and activates interleukin 1 dependent intracellular responses, thereby blocking the activity of the key enzyme responsible for glycosamine synthesis and enhancing cartilage destruction. In chondrocytes, it is able to enhance the synthesis of minor collagens of the first and third types and inhibit the synthesis of the second and ninth collagens types [17-19].

Thus, on the seventh day of this pathological process development in all experimental groups, the level of Interleukin 1β was significantly increased (at the level of statistical significance $p < 0.001$). It should be noted that already at this stage there was a positive effect of correcting the means: in the group in which experimental osteoarthrosis was adjusted by NSAIDs and aminoguadine the level of proinflammatory cytokine was lower than in the group without correction ($p < 0.001$), in the group in which as the NSAIDs and the nitric oxide donor differences in group 2 were found to have the same level of significance in the correction means. It is also noteworthy that the involvement of L-arginine slightly reduced the level of IL 1β to a greater extent (although the difference is not statistically significant).

What about the data obtained from the analysis of Interleukin-10, which is an anti-inflammatory marker, in our study, we established the following. In all study groups, it was

found to increase compared to intact rats (p <0.001), indicating an active response of the body to the development of the inflammatory process. Maximum increase at this stage was set in the group in which the modeled pathology was not corrected. In the groups in which the modeled pathological process was corrected, was detected less pronounced increase in this marker (p <0,001) (compared with the results of the group without correction). Comparing two experimental osteoarthritis correction methods, it should also be noted that the results of the group receiving NSAIDs and nitric oxide donors are slightly better (6.5%) than the correction of NSAIDs and aminoguanidine, which indicates a more pronounced positive effect of L-arginine under these conditions.

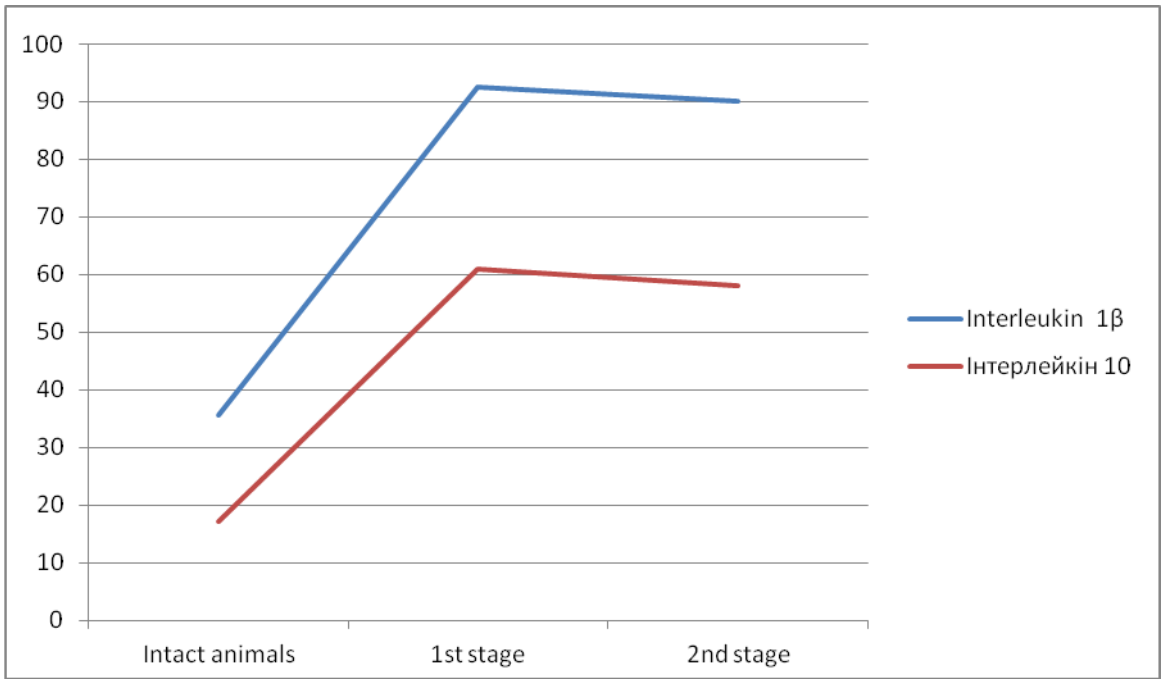

Figure 1 - Inflammatory markers dynamic against the background of experimental osteoarthritis

At the second stage in the group, where were simulated the pathological process without correction revealed that the level of proinflammatory cytokine remains significantly elevated (p <0.001 compared with intact animals), the compensatory capacity of the experimental animals showed only a decrease in IL 1β by only 2.6 %. In both groups, in which the pathological process was corrected, normalization of the investigated index was found at the level of significance p <0.001 compared with the data of the group without correction. In the group receiving non-steroidal anti-inflammatory agent in combination with

nitric oxide donor revealed the most pronounced normalization of the marker of the inflammatory process - no statistical differences when compared with intact animals, indicating a positive effect of this method of correction on the developed inflammatory process osteoarthritis.

At the second stage of the anti-inflammatory cytokine research, the following data were obtained. In the group, which did not correct the modeled osteoarthrosis, the level of anti-inflammatory cytokine remains high ($p < 0.001$), the compensatory capacity of the animal organism aimed at normalization of the indicator was not expressed (no statistical significance).

Conclusion

1. It was proved expediency of using the selected experimental model of osteoarthrosis.
2. It were found during the experiment, increased levels of Interleukin 1β and Interleukin 10 in the simulated osteoarthrosis pathogenesis.
3. It has been established positive dynamics of these cytokines in the endothelial dysfunction correction at osteoarthritis with the correction of aminoguadine.
4. It was revealed more pronounced efficacy of using L-arginine as a corrective means of impaired endothelial function in experimental osteoarthritis.
5. Comparative characteristics of correction agents has shown that the use of nitric oxide donor is more effective than incubation of inducible NO synthase.
6. It was proved normalization of endothelial functional status indicators in the group of animals treated with L-arginine as a part of complex correction of osteoarthrosis was proved.

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