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Comparative characteristics of inducible NO synthase inhibitor and nitric oxide donor in endothelial dysfunction correction caused by osteoarthrosis under experimental conditions

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1st group is intact (n = 20). 2nd group is rats, which were modeled osteoarthritis without further correction and were withdrawn from the experiment in the first stage (7th day) (n=40). 3rd group is rats, which were modeled osteoarthritis without further correction and removed from the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5th group is rats, in which experimental osteoarthritis was corrected with NSAIDs (Diclofenac) and aminoguanidine and withdrawn from the experiment in the second stage (21st day) (n=20). 6th group is rats, where experimental osteoarthritis was corrected

using NSAIDs and a 7% L-arginine solution and withdrawn from the experiment in the first stage (7th day) (n=20). 7th group is rats, in which experimental osteoarthritis was corrected with NSAIDs and 7% L-arginine solution and withdrawn from the experiment in the second stage (21st day) (n=20)

Animals were withdrawn from the experiment for the 7th day and the 21st day after the simulation of the pathological condition.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.

We have obtained the following results:

The increase in the content of von Willebrand factor (VWF) in the animals blood proves that endothelial dysfunction is an important part of experimental osteoarthritis pathogenesis. It's revealed the tendency which directed on normalization of the endothelial dysfunction investigated marker at correction by aminoguadine as a part of complex therapy. L-arginine involvement in the complex correction in experimental osteoarthritis more pronouncedly normalized the VWF level, which indicates the endothelial function normalization. The use of nitric oxide donor is more effective in comparison with the inhibition of inducible NO synthase also in the endothelial nitric oxide synthase activity analysis.

Key words: osteoarthritis; experimental model; endothelial dysfunction; Von Willebrand factor; endothelial NO synthase; aminoguanidine; L-arginine.

Introduction. Osteoarthritis (OA) is considered as a complex disease in which all structural components of the joint are involved in the process [1, 2]. Several authors define OA as a heterogeneous diseases group of different etiology with identical clinical, morphological and biological manifestations, which are based on cartilage, subchondral bone, synovial membrane, ligament, capsule and paraartiular muscle damage [3, 4]. Recently, attention has been paid to endothelial pathology development in patients with OA [5]. Significant role in this aspect is given to chronic inflammation, as endothelial dysfunction trigger mechanism.

Oxidative stress [6, 7], endothelins production and endoperoxides, which are vasoconstrictors, are defined as causes of violation the functioning and endothelial vessels layer structure violation in osteoarthritis. Inflammatory cytokines that violate nitric oxide production [7, 8] also play a role.

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:

Determination of endothelial synthase activity was performed by spectrophotometric method [9, 10].

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 [11].

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 (Vedensky B.P., Galchenko S.E., Kovalev G.O., 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 [12].

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

Based on obtained results, we can assert that endothelial dysfunction develops on the background of a modeled osteoarthritis: established increasing of von Willebrand factor (VWF), which is a commonly used endothelial dysfunction (ED) markers. (Table 1).

Table 1. Von Willebrand factor levels' dynamic 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
VWF	83,3±3,4	94,9±1,6 p_{21}=0,004	93,0±0,9 p_{31}<0,001 p_{32}=0,31 insignificant	91,2±0,8 p_{41}=0,032 p_{42}=0,047	90,8±0,4 p_{51}=0,047 p_{54}=0.19 insignificant p_{53}=0,38 insignificant	86,5±0,7 p_{61}=0,36 insignificant p_{62}<0,001 p_{64}=0,014	84,2±0,8 p_{71}=0,79 insignificant p_{76}=0,032 p73<0,001 p_{75}<0,001

At the first research stage, an increase indicator content in experimental animals blood of 2nd group was found to be 13.9% compared with the data of intact animals. At the second

stage, differences between first group and rats results that weren't correct for the modified osteoarthritis were 11.6%, which indicates that the adaptive properties of the organism are insignificant for the restoration of the functional state of the endothelium (differences between second and third groups data are statistically insignificant).

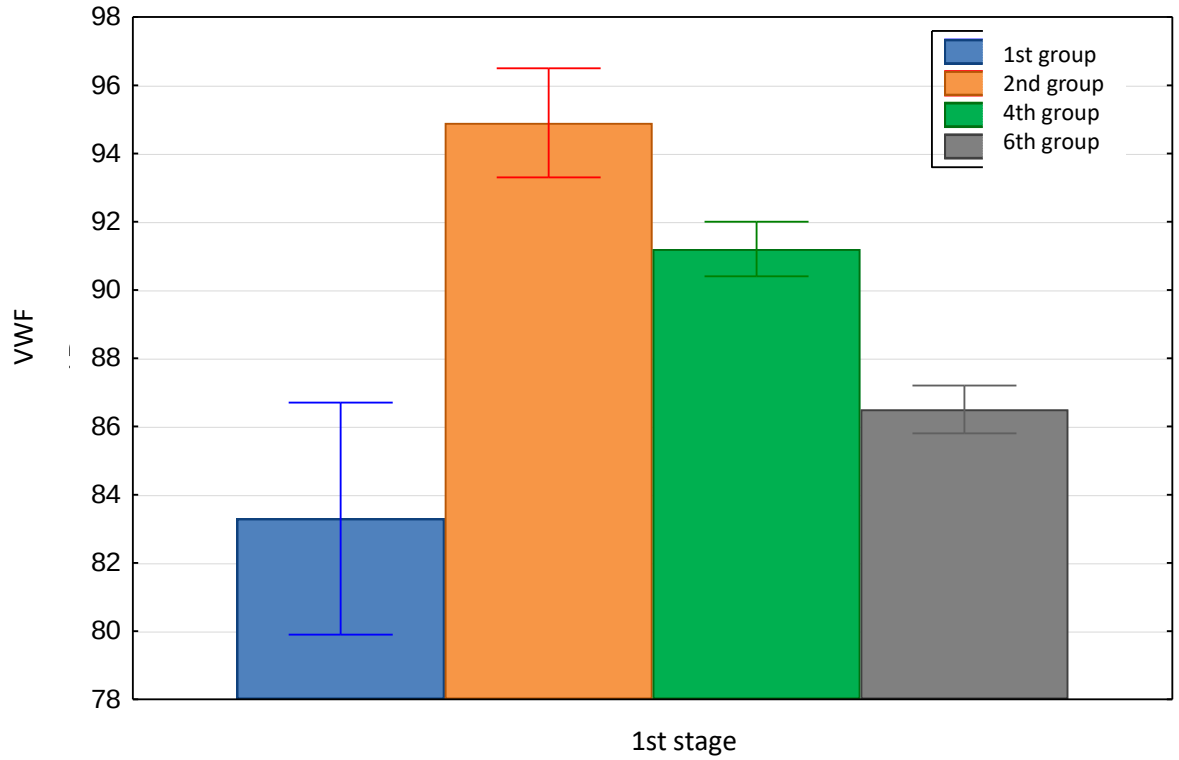

Figure 1 – Dynamics of VWF in the first stage of the experiment

The level' dynamics ED studied marker at the first stage of the experiment is presented in Fig. 1.

Aminoguanidine effectiveness review' s in combination with NSAIDs, it was found that in the first stage, its level decreased with respect to animals groups data without correction by 3.9%, and in the second - by 2.4%, that is, there no pronounced positive inducible NO- syntase inhibitor effect for endothelium functional state marker normalization. Results of the study groups that modeled OA corrected using NSAIDs and L-arginine, was found Von Willebrand factor improvement to be 8.9% in the first stage and 9.5% in the second (differences in both cases is with statistic significant at $p < 0.001$) (Table 1). Foregoing indicates that use of L-arginine is more appropriate for endothelial dysfunction correction in experimental osteoarthritis.

Dynamics of VWF in the second stage of the experiment is presented in Fig. 2.

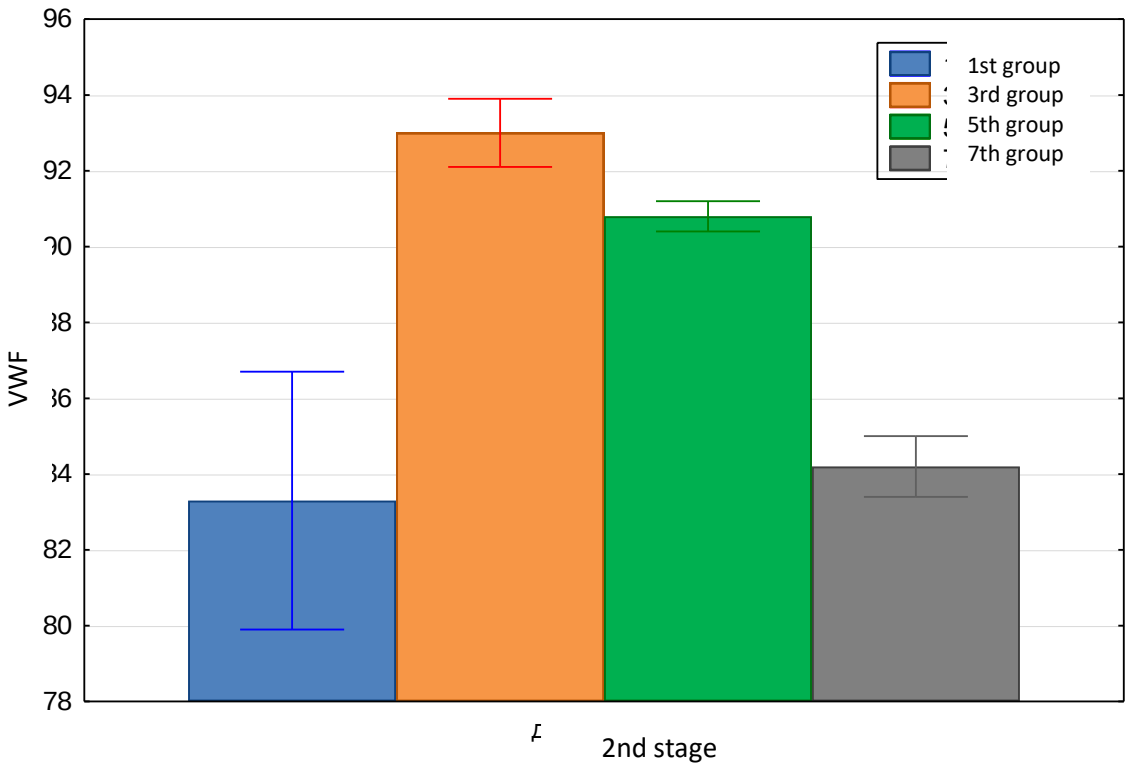

Figure 2 – Dynamics of VWF in the second stage of the experiment

According to the literature, it is known that the link between increased VWF concentration in the blood with the vascular endothelial degree exposure has been repeatedly proven in a number of model experiments in rats with endotoxemia and mechanical endothelial damage [13-15], as well as in a number of clinical observations. [16].

Von Willebrand factor is synthesized by endothelial cells and circulates in blood plasma at a concentration of about 10 pg/ml [16]. The most significant role of VWF is the mediator function in the vascular-platelet interaction at the adhesion stages, platelet aggregation [17, 18]. In these reactions, VWF acts as a bridge between the subendothelial structures of the damaged vascular wall and platelets, as well as between individual platelets [19].

It is also known that VWF is synthesized with a certain "stock", VWF molecules that do not participate in the implementation of physiological functions accumulate in the endothelial cells' intracellular organelles - Weibel-Palade bodies, where they are subject to multimerisation and post-translational modification, and from where they can be quickly mobilized [16].

Nitric oxide endothelial synthase dynamics in rats blood at experimental osteoarthritis and its correction.

It was found that the endothelial functional status violation correction by inhibitor of iNOS only restored the endothelial nitric oxide synthase activity in the first stage (group 4) and by 11.7% in the second group (group 5) by 6.5% compared to the results animals of group №2.

Correction of the pathological state by means of the involvement of L-arginine in the complex therapy gave a more pronounced effect (Figure 3): in the first stage, the activity of the eNOS increased by 33.7% compared with the data of group number 2, and in the second stage - by 63.1% (also in comparison with a group of rats, to which the pathological state was modeled without further correction).

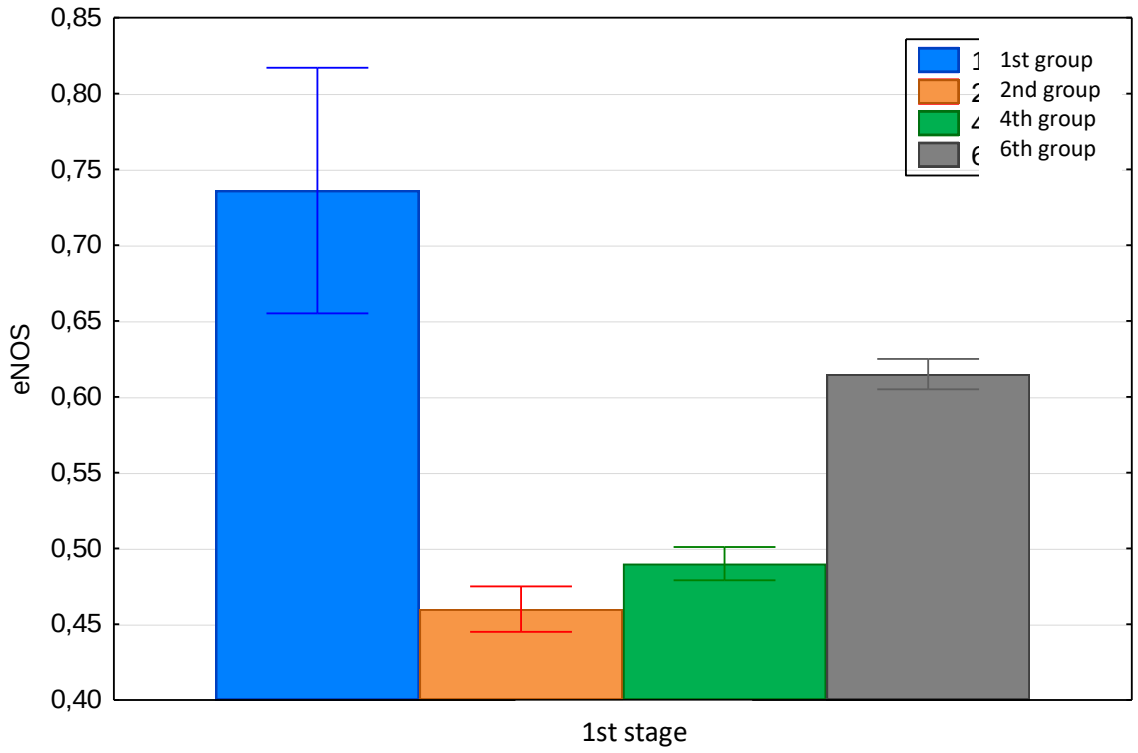

Figure 3 – Dynamics of endothelial NO syntase in the first stage of the experiment

That is, in the first stage, the correction with a NSAIDs and a nitric oxide donor restored the activity of eNOS to 25.5% better, and in the second stage - by 46% better than the group's results, which was corrected by NAIDs and an inhibitor of NO synthase inhibitor. It should be noted that the effectiveness of the effect of L-arginine on the course of the pathological process increases with the duration of its use in the experimental OA: the effect in this group in the second stage was 24.4% better than the first.

Dynamics of endothelial NO syntase is presented in Fig.4.

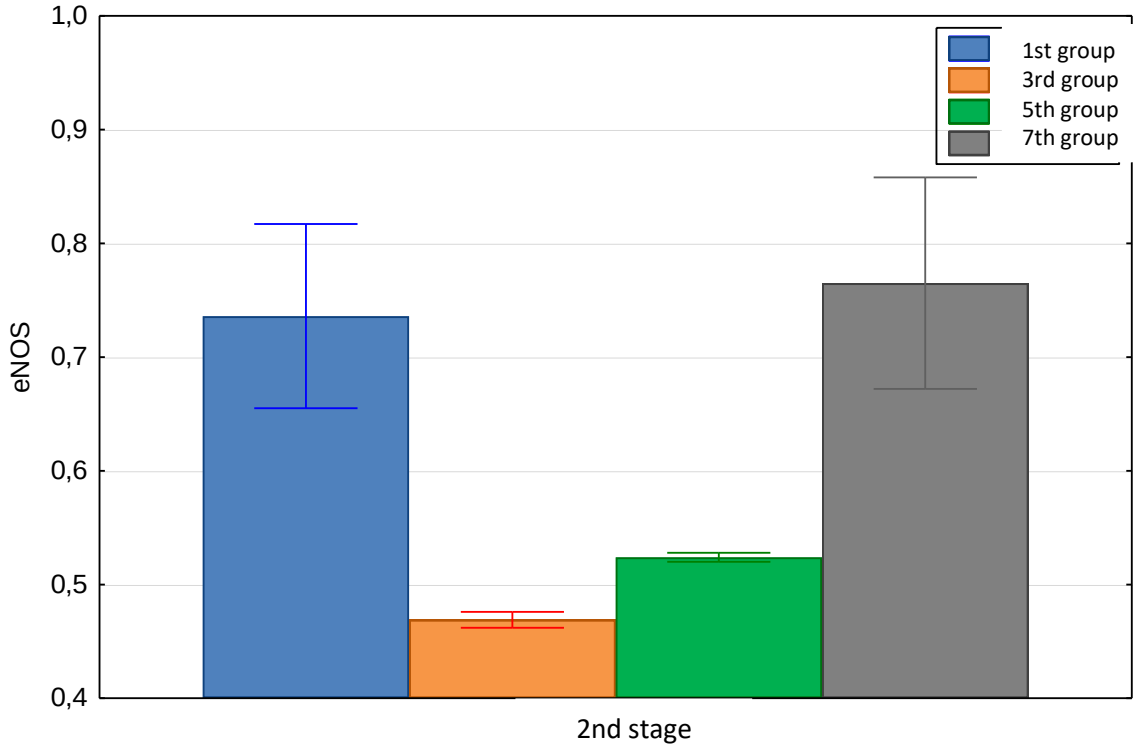

Figure 4 – Dynamics of endothelial NO syntase in the second stage of the experiment

Conclusions

1. The increase in the content of von Willebrand factor (VWF) in the animals blood proves that endothelial dysfunction is an important part of experimental osteoarthritis pathogenesis.

2. It's revealed the tendency which directed on normalization of the endothelial dysfunction investigated marker at correction by aminoguadine as a part of complex therapy.

3. L-arginine involvement in the complex correction in experimental osteoarthritis more pronouncedly normalized the VWF level, which indicates the endothelial function normalization.

4. The use of nitric oxide donor is more effective in comparison with the inhibition of inducible NO synthase also in the endothelial nitric oxide synthase activity analysis.

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