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Dynamics of endothelial and inducible synthase nitric oxide in experimental osteoarthritis and its correction

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1st group is intact (n = 20). 2nd group is rats, which were modeled osteoarthritis without further correction and were withdrawn from the experiment in the first stage (7th day) (n=40). 3rd group is rats, which were modeled osteoarthritis without further correction and removed from the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group is rats, in which experimental osteoarthritis was corrected with NSAIDs (Diclofenac**)** and aminoguanidine and withdrawn from the experiment in the second stage (21st day) (n=20). 6th group is rats, where experimental osteoarthritis was corrected using NSAIDs and a 7% L-arginine solution and withdrawn from the experiment in the first stage (7th day) (n=20)

7th group is rats, in which experimental osteoarthritis was corrected with NSAIDs and 7% L-arginine solution and withdrawn from the experiment in the second stage (21st day) (n=20)

Animals were withdrawn from the experiment for the 7th day and the 21st day after the simulation of the pathological condition.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

During the experiment was found nitric oxide hyperproduction by increasing the activity of inducible NO synthase. It was found decreased endothelial NO synthase activity against the background of experimental osteoarthritis development and the induced inducible NO synthase activation. It has been proven aminoguadine correction effectiveness (inducible NO-synthase inhibitor) of endothelial dysfunction in osteoarthritis. It has been established the feasibility of using L-arginine as a corrective agent for endothelial dysfunction in experimental osteoarthritis. Correction agents comparative characteristics showed that the use of nitric oxide donor is more effective compared to inducible NO synthase inhibition.

Key words: osteoarthritis; experimental model; endothelial dysfunction; endothelial NO synthase; inducible NO synthas; aminoguanidine; L-arginine.

Introduction. OA incidence rate is 30-55% among all diseases of the bone and articular system [1, 2]. According to the WHO, in the next 10-15 years, osteoarthritis (OA) will be the fourth cause of disability in women, and the eighth - in men [3]. At this stage, osteoarthritis is considered as a complex disease, in which in process involves all joint structural components [4, 5]. 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 [6, 7]. Recently, attention has been paid to endothelial pathology development in patients with OA [8]. Significant role in this aspect is given to chronic inflammation, as endothelial dysfunction trigger mechanism.

Oxidative stress [9, 10], 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 [10, 11] 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 and inducible synthase activity was performed by spectrophotometric method [12, 13].

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 [14].

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 [15].

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

Dynamics of inducible nitric oxide synthase in rat blood during experimental osteoarthritis and its correction

Against the background of experimental osteoarthritis development, the activity of inducible NO synthase increased more than fourfold, indicating the development of the inflammatory process and serves as another confirmation of the progression of endothelial dysfunction (Table 1). Dynamics of inducible nitric oxide synthase activity in the first stage of the experiment is presented in Fig. 1. Decrease in activity of this enzyme in the second stage of the experiment in the group without correction is statistically insignificant. In the study of the impact of aminoguadine on the pathological process proved its effectiveness as an inhibitor of inducible NO synthase - revealed differences in comparison with the data of group 2 at the level of significance $p < 0{,}001$.

It was found that positive effect is more pronounced with prolonged administration of the drug: on the second stage was detected decrease of iNOS activity by 35.4 compared to the results of animals that received the same correction and were withdrawn from the experiment earlier (in the first stage). In our study, the most effective was the involvement of nitric oxide donor in the complex correction: at the first stage, the activity of the investigated indicator decreased by 73.4% (compared with the results of the group in which OA was simulated without further correction).

Table 1 - Inducible NO synthase activity dynamics 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
iNOS	3,66±0,09	17,32±0,31 p_{21}<0,001	15,83±0,33 p_{31}<0,001 p_{32}=0,002	14,00±0,42 p_{41}<0,001 p_{42}<0,001	9,05±0,47 p_{51}<0,001 p_{54}<0,001 p_{53}<0,001	4,61±0,27 p_{61}=0,003 p_{62}<0,001 p_{64}<0,001	3,90±0,27 p_{71}=0,42 p_{76}=0,038 p_{73}<0,001 p_{75}<0,001

All above mentioned confirms known literature data on the pronounced effect of L-arginine on the functional state of the endothelium and the reduction of oxidative stress on the background of inflammatory processes (Table 1).

Longer administration of arginine brought the level of iNOS activity closer to that of intact animals (no statistically significant differences between the data of the seventh and the first groups of experimental animals).

Endothelial nitric oxide synthase dynamic in the rats blood during experimental osteoarthritis and its correction

When analyzing this enzyme activity in the group of animals that were osteoarthrosis modeled without further correction, it was found to decrease by 37.5% in the first stage and by 36.3% in the second stage of the study compared with intact animals (Table 2). Obtained data together with the established increase in the activity of inducible NO synthase indicate physiological synthesis violation of nitric oxide.

It was also found that the correction of endothelial dysfunction with iNOS inhibitor only 6.5% restored endothelial nitric oxide synthase activity in the first stage (group 4) and the second (group 5) compared with the results of animals of group 2.

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

Table 2. – Endothelial NO synthase activity dynamic in rat 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
eNOS	0,736±0,081	0,460±0,015 p_{21}=0,003	0,469±0,007 p_{31}=0,004 p_{32}=0,57	0,490±0,011 p_{41}=0,007 p_{42}=0,12	0,524±0,004 p_{51}=0,009 p_{54}=0,018 p_{53}<0,001	0,615±0,010 p_{61}=0,16 p_{62}<0,001 p_{64}=0,001	0,765±0,093 p_{71}=0,73 p_{76}<0,001 p_{73}<0,001 p_{75}<0,001

That is, in the first stage, correction with a non-steroidal anti-inflammatory agent and nitric oxide donor restored the eNOS activity by 25.5% better, and in the second stage - by 46% better compared with the results of the group, which was corrected by a non-steroidal anti-inflammatory agent and an inducible 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 experimental OA: the effect in this group in the second stage was 24.4% better than in the first (Table 2).

Research result discussion

It is well known from the literature that the increase in activity of one of the nitric oxide synthases occurs against the background and at the expense of the other. Therefore, one of the objectives of our study was to compare the activity of inducible and endothelial nitric oxide synthase at each of the steps.

It should be noted that NO exerts a cytoprotective effect and enhances blood circulation in microvessels only under conditions of its weak generation. When excess nitrogen oxide synthesized by inducible NO synthase, it exhibits its cytotoxic properties. For a time not exceeding a few seconds, in biosystems, NO self-oxidizes into inactive metabolites: nitrite and nitrate (NO_2 and NO_3) [16-18]. Nitric oxide is produced by cells from L-arginine, mediated by the action of nitric oxide synthase, which catalyzes the formation of NO and citrulline using NADPH electron donors. There are three isoforms of the enzyme: inducible and NO synthase, endothelial NO synthase, and neuronal NO synthase [19].

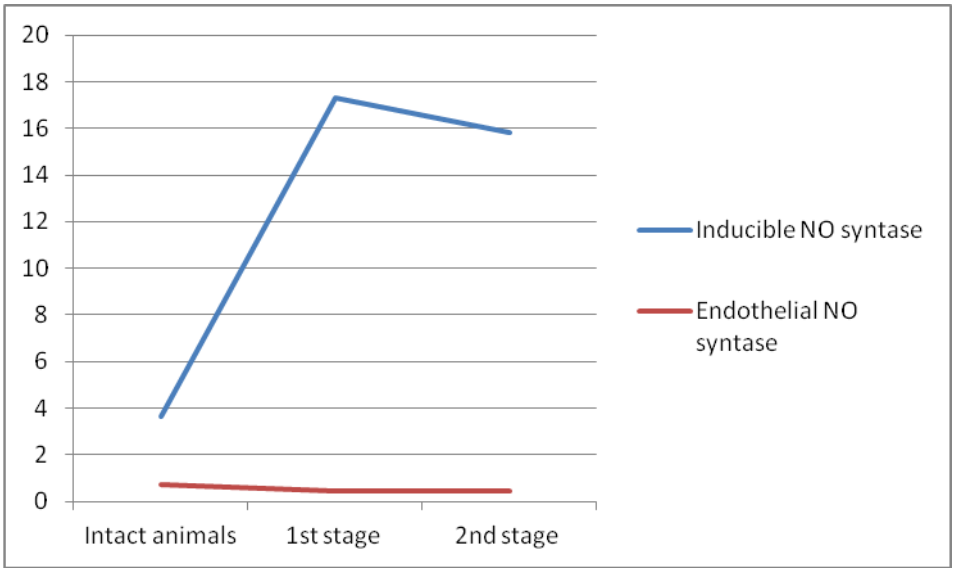

Figure 1. - Endothelial and inducible nitric oxide synthase activity dynamic against the background of experimental osteoarthrosis

Although all isoforms form NO, each is specific, both in its mechanisms of action and localization, and in its biological effects. Therefore, they are introduced into the inducible and constitutive nitric oxide synthases [20, 21]. All three isoforms of the active forms of the enzyme are represented by homodimers. In each monomer, a calmodulin binding domain, a reductase domain, an oxygenase domain, and a terminal sequence that is specific to each of these isoforms are distinguished. The principal catalytic differences of isoforms are that calcium is required for activation of endothelial and neuronal NO synthases, whereas calmodulin is so strongly bound to inducible NO synthase that it does not require calcium. NO synthases are unique in their structure and have a large number of cofactors: heme and calcium-modulin, FAD, FMN and three dissimilar substrates - oxygen, arginine and NADPH [22, 23]. It should be noted that dimers of endothelial and neuronal NO synthase are more stable than dimers of inducible NO synthase. The inducible form is a soluble protein, while the other two isoforms are membrane-binding [24].

Constitutive NO synthase, which has two isoforms - NOS-1 and NOS-3, which is constantly in the cytoplasm, depending on the concentration of calmodulin and calcium. It also promotes the release of a small amount of nitric oxide in response to physical and receptor stimulation. Under the influence of acetylcholine, glutamate or histamine, calcium enters the cells, where it binds in the cytosol to a single complex with calmodulin. This complex, as a co-factor, activates the NOS. Under the influence of constitutive NOS, a small

amount of NO is formed, which implements local regulation. NO activates the cellular enzyme guanylate cyclase, resulting in the formation of cyclic guanosine monophosphate, which mediates all the effects of NO [20].

It should also be noted that decrease in endothelial nitric oxide synthase activity is one of the evidence of endothelial dysfunction development. Detection of endothelial functional disorders caused by osteoarthrosis (in particular the influence of the inflammation process) will help to develop a more comprehensive and effective approach to the development of new methods of correction of the specified pathological process**.**

Conclusion:

1. During the experiment, was established nitric oxide overproduction by increasing the activity of inducible NO synthase.

2. Ir has been proved reduced activity of endothelial NO synthase against the background of experimental osteoarthritis and the activation of inducible NO synthase.

3. It has been proved effectiveness of the aminoguadine correction (inducible NO synthase inhibitor) of endothelial dysfunction in osteoarthritis.

4. It is established that the use of L-arginine as a corrective means of impaired endothelial function in experimental osteoarthritis.

5. Comparative characterization of the correction agents has shown that the use of nitric oxide donor is more effective than the inducible NO synthase inhibition.

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