

Effect of flosteron on the content of acute-phase proteins and procalcitonin in infectious arthritis in rats

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Infectious arthritis was modeled on white male Wistar rats by injecting a suspension of Staphylococcus aureus microorganisms into the knee joint of animals, and the effects of the synthetic hormone flosteron were also studied. At the same time, the content of acute-phase proteins (ceruloplasmin, haptoglobin, C-reactive protein (CRP)) and procalcitonin (PCT) as markers of the development of the inflammatory process and bacterial infection was studied in the blood serum of rats. Determination of the content of haptoglobin, ceruloplasmin and CRP was carried out on a Cobas 311 biochemical analyzer; the PCT concentration on a Cobas 411 analyzer using Roche Diagnostics test systems. Analysis of the results showed that when flosteron was administered, the concentration of CRP increased by 1.7 times already on the 3rd day, and by more than 4 times on the 14th day. The content of ceruloplasmin and haptoglobin also increased. The PCT concentration was at the level of the control values. Under the conditions of modeling infectious arthritis, changes in the content of acute-phase proteins were observed. The greatest deviations from the reference values were found on the 14th day in animals that were injected with the hormone and S. aureus: the concentration of both CRP, the most objective biochemical marker of the inflammatory process, and PCT exceeded the physiological norm 12 times and more than 7 times, respectively. This indicates that the hormonal drug enhances the inflammatory process, which is confirmed by data that reflect changes in the content of acute-phase proteins. It can be assumed that the use of hormonal drugs contributes to the development of postoperative complications.

Key words: infectious arthritis; Staphylococcus aureus; flosteron; rats.

INTRODUCTION

Postoperative complications arising in patients with pathology of the musculoskeletal system, who used hormonal drugs, are being actively investigated. Currently, much attention is paid to the study of enzymes and biologically active proteins involved in the pathogenesis of the development of the inflammatory process, as well as biochemical markers that determine the activity of this process, including acute phase proteins (ceruloplasmin, haptoglobin, C-reactive protein (CRP)) [1, 2]. For example, there is a direct relationship between changes in the level, severity, and dynamics of clinical manifestations of inflammation: the higher the concentration of CRP, the greater the severity of the inflammatory process, and vice versa. That

is why this indicator is one of the most specific and sensitive clinical and laboratory indicators of inflammation. An important biochemical marker and instrument of bacterial infection in clinical practice is procalcitonin (PCT) [3-5]. PCT synthesis is induced by endotoxins (bacterial toxic substances) that are structural components of certain bacteria and are released only during lysis, i.e., during the disintegration of a bacterial cell [6-8].

Biochemical markers not only make it possible to carry out early diagnosis, but also provide information on the prognosis and course of pathology. In case of any violations of organs and tissues, including the occurrence of foci of inflammation, the body responds with a general adaptive response in the interests of the whole

organism. In inflammatory processes, including infections, many biological indicators change in connective tissues, in particular, the content of proteins in the blood and the appearance of acute-phase proteins, as well as procalcitonin.

Previously, we identified a certain pattern when comparing data, both in biological fluids and in tissues of patients with endoprosthetics of large joints and osteosynthesis with a complicated course of the disease. The more active the inflammatory process that develops after endoprosthetic and osteosynthesis, the more pronounced metabolic deviations from the norm in the metabolism of both the main protein of bone tissue collagen and glycosaminoglycans, which is accompanied by an increase in the activity of collagenase, alkaline phosphatase, as well as a change in the concentration of acute-phase proteins [6, 9].

The study of disorders in the metabolism of acute phase proteins and procalcitonin when using hormonal drugs in the experiment will make it possible to predict postoperative complications. It is important to understand and identify biological patterns, how the use of hormonal drugs affects postoperative complications.

The aim of our work was to determine the changes in the concentration of acute phase proteins and procalcitonin depending on the duration of the use of hormonal drugs and the prediction of postoperative complications in the experiment.

METHODS

Experimental studies were carried out on 31 white Wistar male rats weighing 250-300 g. An experimental model of infectious arthritis was created by daily injection of 0.02 ml of *S. aureus* 10⁸ No. 209 into the knee joint of rats for three days. Experimental animals were divided into groups. In I group (control) rats were on the standard diet of the vivarium. For experimental groups, the following model of drug administration was used: daily administration of flosteron at a dose of 0.45 mg/kg body weight in the knee joint for three days (group II); daily three injections of 0.02 ml of *S. aureus* 10⁸ No. 209

(group III); three alternating (every other day) injection of 0.02 ml of flosteron and 0.02 ml of *S. aureus* 10⁸ No. 209 in the knee joint (group IV). The effectiveness of the drugs was observed 3 and 14 days after administration. All animals were under the supervision of a veterinarian in standard conditions of an accredited vivarium of Bogomoletz Institute of Physiology, National Academy of Sciences of Ukraine, Kyiv, in compliance with the general principles of bioethics in accordance with the international principles of the "European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes" (Strasbourg, 1986) in the natural light/dark cycle. The animals had free access to water.

Determination of haptoglobin, ceruloplasmin and CRP was carried out in blood serum on a Cobas 311 biochemical analyzer, the concentration of procalcitonin (PCT) was determined on a Cobas 411 analyzer using the Roche Diagnostics test systems. The results were statistically processed using the OriginPro 8.5 software package. The average values of the obtained indicators (\bar{x}) with standard deviations (SD) were determined. The likelihood of a difference between the control and experimental samples was assessed by the Student's *t* test. At $P < 0.05$, the changes were considered significant.

RESULTS AND DISCUSSION

Analysis of the results obtained in the study of blood serum of experimental animals on the 3rd day after 3-fold administration of the hormonal preparation flosteron showed that the content of haptoglobin was within the physiological norm characteristic of intact animals. Indicators reflecting the content of ceruloplasmin revealed a tendency for this protein to increase. The content of CRP increased by 72% relative to the norm. The PCT concentration remains at the level of normal values without significant changes (Table 1).

Biochemical indicators obtained on the 14th day of observation in this group of animals indicate an increase in the content of

Table 1. Changes in the content of acute-phase proteins and procalcitonin in blood serum in experimental animals after 3-fold local administration of the hormonal preparation flosteron and *S. aureus*

Indicators	Control (n = 10)	Flosteron		S. aureus 10 ⁸ No. 209	
		3 days (n = 7)	14 days (n = 7)	3 days (n = 7)	14 days (n = 7)
Haptoglobin, g/l	0.317 ± 0.004	0.326 ± 0.01	0.423 ± 0.008**	0.330 ± 0.008	0.560 ± 0.010**
Ceruloplasmin, g/l	0.022 ± 0.001	0.033 ± 0.001**	0.037 ± 0.001**	0.190 ± 0.001	0.023 ± 0.001
C-reactive protein, ng/ml	0.011 ± 0.003	0.019 ± 0.005	0.046 ± 0.009**	0.061 ± 0.042	0.022 ± 0.002*
Procalcitonin, ng/ml	0.020 ± 0.000	0.019 ± 0.003	0.026 ± 0.001**	0.018 ± 0.001	0.018 ± 0.001

*P < 0.05, **P < 0.001 relative to values in control rats

haptoglobin by 33% from the norm. The content of ceruloplasmin increased by 68% relative to the control. The concentration of C-reactive protein increased significantly in relation to the norm, namely, more than 4 times. Indicators of procalcitonin were within the normal range with a tendency to increase (Table 1). The data obtained indicate an intensification of the pathological process, especially the indicators of CRP, one of the key acute phase proteins, reflecting the degree of the inflammatory process.

On the 3rd day after the 3-fold administration of *S. aureus*, the content of haptoglobin was within the physiological norm with a tendency to increase. More pronounced changes were observed in the content of ceruloplasmin. So, the content of this protein in absolute indicators was 0.190 ± 0.001 g/l, or 4.6 times higher than the norm. The most significant changes are found in the content of CRP, where its concentration increased more than 5 times in relation to the norm. The PCT content remains within the physiological norm (Table 1).

The results obtained during the examination of experimental animals, which were 3 times injected into the knee joint with a suspension with *S. aureus* microorganisms on the 14th day of observation, show that the content of haptoglobin increased by 77% relative to the control group of animals. The content of ceruloplasmin was within the physiological norm. The concentration of CRP has increased twice in relation to the norm. Indicators of procalcitonin were within the normal range (Table 1).

The results obtained in the study of the blood serum of experimental animals, which were injected with the hormone and a suspension with the microorganism *S. aureus*, on the 3rd day of observation indicate an increase in the concentration of haptoglobin by 38% of the norm. At the same time, indicators reflecting the content of ceruloplasmin also increased, namely by 86%. The concentration of the most sensitive acute-phase protein CRP exceeded the control values by more than 6 times. The PCT content was within the normal range with a slight decrease (Table 2).

On the 14th day after the start of the experiment in this group of animals, the studied parameters indicate a deepening of the inflammatory process: the concentration of haptoglobin increased by 66%, and the content of ceruloplasmin increased more than 6 times relative to the control. The concentration of CRP, the most objective biochemical marker of the inflammatory process, exceeded the physiological norm by 12 times. PCT indicators during these periods of the study also increase more than 7 times (Table 2).

Analysis of biochemical parameters reflecting the metabolism of acute-phase proteins in a group of experimental animals that were injected with the hormonal drug flosteron, *S. aureus*, as well as their combined administration, indicate a change in the concentration of these proteins in comparison with the reference values (control group of animals). The most significant changes are observed in the content of CRP. So, in the group of animals that were injected with flosteron, already on the 3rd day after the start of

Table 2. Changes in the content of acute-phase proteins and procalcitonin in the blood serum of experimental animals after 3-fold local administration of the hormonal preparation flosteron and *S. aureus*

Indicators	Control (n = 10)	Flosteron + <i>S.aureus</i> 10 ⁸ No. 209	
		3 days (n = 7)	14 days (n = 7)
Haptoglobin, g/l	0.317 ± 0.004	0.436 ± 0.008**	0.526 ± 0.003
Ceruloplasmin, g/l	0.022 ± 0.001	0.041 ± 0.003**	0.137 ± 0.01
C-reactive protein, ng/ml	0.011 ± 0.003	0.07 ± 0.004	0.131 ± 0.01
Procalcitonin, ng/ml	0.020 ± 0.001	0.017 ± 0.002	0.145 ± 0.02

*P < 0.05, **P < 0.001 relative to values in control rats

the experiment, the concentration of this protein increases 1.7 times in relation to the reference indicators, on the 14th day it increases more than 4 times. The same trend persists in the group of animals that were injected with *S. aureus*. The largest deviations from the control values are found in the group of animals that were injected with both the hormone and infected with *S. aureus*. This indicates that the hormonal drug enhances the inflammatory process, which is confirmed by data that reflect the metabolism of acute-phase proteins. It can be assumed that the use of hormonal drugs contributes to the development of postoperative complications. In case of high deviations in the metabolism of acute-phase proteins in comparison with the reference values, in order to prevent the development of postoperative complications, it is necessary to take into account the indicators of these proteins and conduct a therapy that helps to stabilize these disorders.

From all the above it can be concluded that changes in the content of acute-phase proteins in the serum of rats after administration of flosteron and *S. aureus*, as well as their combined action, were unidirectional, but had their own characteristics. This was manifested in a more significant increase in the concentration of haptoglobin, ceruloplasmin, and C-reactive protein 14 days after drug administration. The largest deviations were observed in rats, which were injected three times alternately (every other day) with flosteron and *S. aureus* 10⁸ No. 209 in the knee joint. Such changes suggest

that the hormonal drug flosteron contributed to the intensification of the inflammatory process. Our data are consistent with the results obtained by Jevsevar, et al. and Bedard, et al., who did not provide convincing evidence to support the use of glucocorticoids for the treatment of osteoarthritis and did not find a clear balance between the benefits and potential harms of this treatment [10, 11]. According to the literature, plasma procalcitonin increases rapidly during bacterial infections and can therefore be used to detect infection in patients with osteoarthritis, reflecting the severity of the infection [12]. However, the concentration of procalcitonin in our experiment did not change significantly. We believe that the range of effectiveness of intra-articular therapy depends on the type and concentration of the drug used and can have significant clinical significance in the treatment of arthritis if it is carried out according to the correct indications and using the right technique.

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ВПЛИВ ФЛОСТЕРОНУ НА ВМІСТ ГОСТРОФАЗОВИХ БІЛКІВ ТА ПРОКАЛЬЦИТОНІНУ ПРИ ІНФЕКЦІЙ- НОМУ АРТРИТІ У ЩУРІВ

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На білих щурах-самцях моделювали інфекційний артрит введенням у колінний суглоб суспензії мікроорганізмів

Staphylococcus aureus, а також вивчали ефекти синтетичного гормону флостерону. При цьому в сироватці крові щурів досліджували вміст гострофазних білків (церулоплазміну, гаптоглобіну, С-реактивного білка – СРБ) і прокальцитоніну (ПКТ) як маркерів розвитку запального процесу і бактеріальної інфекції. Вміст гаптоглобіну, церулоплазміну і СРБ визначали на біохімічному аналізаторі Cobas 311, концентрацію ПКТ – на аналізаторі Cobas 411 з використанням тест-систем Roche Diagnostics. Аналіз результатів показав, що при веденні флостерону вже на 3-тю добу збільшується концентрація СРБ в 1,7 раз, на 14-ту добу – більш ніж у 4 рази. Також збільшувався вміст церулоплазміну і гаптоглобіну. Концентрація ПКТ була на рівні контрольних значень. В умовах моделювання інфекційного артриту спостерігали зміни в вмісті гострофазних білків. Істотне відхилення від референтних значень виявили на 14-ту добу у тварин, яким вводили гормон і *S. aureus*: концентрація СРБ, найбільш об'єктивного біохімічного маркера запального процесу, перевищила фізіологічну норму в 12 разів, ПКТ – більш ніж в 7 разів. Це свідчить про те, що гормональний препарат сприяє посиленню запального процесу. Можна припустити, що застосування гормональних препаратів спричиняє розвиток післяопераційних ускладнень.

Ключові слова: інфекційний артрит; *Staphylococcus aureus*; флостерон; щури.

REFERENCES

1. Velkov VV. Procalcitonin and C-reactive protein in modern laboratory diagnostics. Clin Lab Consult. Part 1. 2008;25(6):46-52. [Russian].
2. Magomedov AM, Gerasimenko SI, Boychuk BP, Krinitskaya OF, Kravchenko EN, Arshulik MA. Metabolism of acute-phase proteins in patients with trauma of the lower extremities long bones. Bull Ortop Traumatol Prosthet. 2014;1:24-8. [Russian].
3. Bukhanova DV, Belov BS, Tarasova GM, Dilbaryan AG. Procalcitonin test in rheumatology. Clinician. 2017;11(2):16-23. [Russian].
4. Lapin SV, Maslyansky AL, Lazareva NM, Vasilyeva EYu, Totolyan AA. The value of the quantitative determination of procalcitonin for the diagnosis of septic complications in patients with autoimmune rheumatic diseases. Clin Lab Diagnost. 2013;1:28-33. [Russian].
5. Shen CJ, Wu MS, Lin KH, et al. The use of procalcitonin in the diagnosis of bone and joint infection: a systemic review and meta-analysis. Eur J Clin Microbiol Infect Dis. 2013;32(6):807-14.
6. Magomedov S, Kravchenko OM, Kolov GB, Shevchuk AV. Procalcitonin as a biochemical marker in the diagnosis of inflammatory processes (literature review). Bull Ortoped Traumat Prosthet. 2018;1:63-7. [Ukrainian].
7. Fuchs T, Stange R, Schmidmaier G, Raschke MJ. The use of gentamicin-coated nails in the tibia: preliminary results of a prospective study. Arch Orthoped Trauma Surg. 2011;131(10):1419-25.
8. Wallbach M, Vasko R, Hoffmann S, et al. Elevated procalcitonin levels in a severe lupus flare without infection. Lupus. 2016;25(14):1625-6.
9. Magomedov AM, Polishchuk LV, Kuzub TA, Kolov GB, Gordiy AS. Metabolism of connective tissue in patients with purulent complications after osteosynthesis of fragments of long bones. Trauma. 2016;17(1):111-15. [Russian].
10. Jevsevar DS, Brown GA, Jones DL, Matzkin EG, Manner PA, et al. The American Academy of Orthopedic Surgeons evidence-based guideline on: treatment of osteoarthritis of the knee, 2nd edition. J Bone Joint Surg Am. 2013 Oct 16;95(20):1885-6.
11. Bedard NA, DeMik DE, Glass NA, Burnett RA, Bozic KJ, Callaghan JJ. Impact of clinical practice guidelines on use of intra-articular hyaluronic acid and corticosteroid injections for knee osteoarthritis. J Bone Joint Surg Am. 2018 May 16;100(10):827-34.
12. Tsujimoto K, Hata A, Fujita M, Hatachi S, Yagita M. Presepsin and procalcitonin as biomarkers of systemic bacterial infection in patients with rheumatoid arthritis. Int J Rheumat Dis. 2018 Jul;21(7):1406-13.

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