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EFFICIENCY OF DETERMINATION OF ACUTE PHASE PROTEINS AND PROCALCITONIN UNDER THE CONDITIONS OF EXPERIMENTAL INFECTIOUS ARTHRITIS IN MICE

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Acute phase proteins, i.e. ceruloplasmin, haptoglobin, C-reactive protein (CRP) and procalcitonin are the markers that characterize the inflammatory process. C-reactive protein is one of the central components of the acute phase (AF), and is a generally accepted indicator of inflammatory processes.

Aim. To establish the level and the efficiency of determination of acute-phase proteins (CRP, haptoglobin, ceruloplasmin), as well as procalcitonin under the conditions of modeled infectious arthritis.

Materials and methods. Experimental studies were conducted on 52 white male Wistar rats. A model of infectious arthritis was created for seven days by daily injection of 0.02 ml of *S. aureus* 108 No. 209 into the knee joint of a rat. The animals were divided into groups and vivarium control (group I). The following dosage scheme was used for the experimental groups: a single daily injection of 0.02 ml of flosteron into the knee joint for seven days (group II); daily single administration for seven days of 0.02 ml of S. aureus 108 No. 209 (III group); daily one-time intermittent (every other day) administration for seven days of 0.02 ml of flosteron and 0.02 ml of *S. aureus* 108 No. 209 into the knee joint (group IV). The effectiveness of the drugs was observed 3 and 14 days after administration.

Results. It was established that the concentration of haptoglobin was significantly increased in the blood serum of rats after 3 and 14 days in all studied groups of animals compared to the control. The greatest increase compared to control values was noted 3 days after the seven-time injection of *S. aureus* 108 #209 into the knee joint. However, after 14 days it was already not so evident, and was significantly lower (by 85.33%) compared to the measurement after three days. Only the rats receiving intermittent (every other day) injections of 0.02 ml of flosteron and 0.02 ml of *S. aureus* 108 No. 209 into the knee joint demonstrated a statistically significant increase in the level of haptoglobin (by 775.08%, $P < 0.05$) compared to the control, and 77.78% reduced compared to the measurement after three days. The concentration of ceruloplasmin in blood serum increased in all experimental rats during the entire observation period and differed little between 3 and 14 days. The content of C-reactive protein in blood serum increased in all studied groups of rats without exception, which proves its high specificity for detecting inflammatory processes of various severity. The concentration of procalcitonin was most significantly increased by 235.0% 3 days after alternating (every other day) administration of 0.02 ml of flosterone and 0.02 ml of *S. aureus* 108 No. 209. It was slightly lower (by 120.0%) under the same conditions experiment after 14 days. This indicator significantly increased (by 65%) 14 days after the 7-time introduction of *S. aureus* 108 #209. In the experimental animals of other groups, the PCT concentration did not change.

Conclusions. The determination of haptoglobin reflects the initial activation of the inflammatory process, which was enhanced by the hormonal drug flosteron. However, its determination over a longer period of time can be efficient as well, because numerous factors lead to a bacterial infection, reinforcing each other. At the same time, the synthesis of ceruloplasmin is being activated exactly during the first three days of the infectious process, which makes it an informative marker for detecting early infectious complications. The dynamics of changes in the level of C-reactive protein in blood serum showed the highest correlation with the activity of the infectious process, which proves its high efficiency for detecting inflammatory processes of various severities, choosing adequate treatment and predicting the course of the disease.

Key words: haptoglobin, ceruloplasmin, C-reactive protein, procalcitonin, infectious arthritis.

An important role in the pathogenesis of inflammatory diseases of the joints is attributable to the changes in biochemical indicators, namely, the increase of acute-phase proteins content in blood. There is a direct relationship between the change in their level, the severity and dynamics of clinical manifestations of inflammation. In this way, they have advantage over the determination of the number of leukocytes, platelets, leukocyte formula, leukocyte intoxication index, ESR. Although the effectiveness of modern microbiological tests is highly specific, the time required to obtain results may not be acceptable. However early diagnosis of inflammatory joint diseases is vital to prevent devastating complications.

Acute-phase proteins, i.e. ceruloplasmin, haptoglobin, C-reactive protein (CRP) are markers that characterize the inflammatory process and show a high correlation with disease activity. C-reactive protein is one of the central components of the acute phase (AF) and is a generally recognized indicator of inflammatory processes. An increase in the concentration of CRP precedes the increase in ESR and the increase in the number of peripheral blood neutrophils in bacterial infections. Its content increases rapidly in the first 6–8 hours (by 20–100 times, sometimes by 1000 times) when inflammation increases and also decreases quickly with amelioration of the inflammation. That is why this indicator is one of the most specific and sensitive clinical and laboratory indicators of inflammation, and it is widely used to monitor and control the effectiveness of therapy for bacterial and viral infections, chronic inflammatory diseases.

Ceruloplasmin is intensively synthesized during the first two days from the beginning of the infectious process. Its level nearly doubles in response to inflammation, injury, or infection. Haptoglobin is an acute phase protein that binds free hemoglobin and has anti-inflammatory properties but can have a pro-inflammatory effect on the joint. It plays a role in the inflammatory process of bone destruction through bradykinin and thrombin, stimulating the formation of prostaglandin E2, which leads to bone resorption [1, 2].

Procalcitonin protein (PCT) is an important biochemical marker for the diagnosis of inflammatory processes in the joints. This indicator makes it possible to assess the degree of development of the inflammatory process and sepsis and distinguish bacterial infection from non-bacterial infection [3–5]. In the presence of viral infections, the concentration of PCT demonstrate only minor increase, while in the presence of a significant bacterial infection, it increases. In patients without infectious complications, the PCT level quickly returns to normal values. Moreover, a high concentration that does not decrease, or a secondary increase in PCT are considered to be the predictors of sepsis [6]. The synthesis of PCT is induced by endotoxins – bacterial toxic substances, which are structural components of typical bacteria and are released only during lysis, that is, during the breakdown of a bacterial cell [7–9].

There is no doubt that acute-phase proteins and PKT take a direct part in the development of the inflammatory process [1, 2, 6, 8]. An increase in their level in the blood serum of patients, in most cases, indicates the destruction of the cartilage and bone tissue of the joints [10, 11]. However, the inconsistency of biochemical data in certain inflammatory joint diseases and their treatment protocols, as well as the lack of comparability of results complicate both the diagnosis of the disease and the effectiveness of further treatment. There is currently no consensus as to which biochemical markers are most reliable for tracking the level of inflammation and/or infection. Therefore, studies on monitoring the level of acute-phase proteins ceruloplasmin, haptoglobin, C-reactive protein and procalcitonin in order to determine the most effecient marker of them are relevant for the detection of early infectious complications and strategies for its elimination.

The aim of the study was to evaluate the level and efficiency of determination of acute-phase proteins (CRP, haptoglobin, ceruloplasmin), as well as procalcitonin under the conditions of modeling infectious arthritis.

Materials and Methods

The experiment was conducted on 52 white male Wistar rats. An infectious arthritis model was created for seven days by daily injection (once a day) of 0.02 ml of *S. aureus* 108 No. 209 into the knee joint of a rat. The animals were divided into experimental groups — and vivarium control. The following drug administration model was used for the experimental groups: daily administration (once a day) for seven days of 0.02 ml of flosterone into the knee joint (II group); daily administration (once a day) for seven days of 0.02 ml of *S. aureus* 108 No. 209 (III group); daily administration (once a day) alternately (every other day) for seven days of 0.02 ml

of flosterone and 0.02 ml of *S. aureus* 108 No. 209 into the knee joint (IV group). Animals were decapitated under ether anesthesia after 3 and 14 days of the experiment after drug administration. All animals were under the supervision of a veterinarian in the standard conditions of the accredited vivarium of the Bohomolets Institute of Physiology of the National Academy of Sciences of Ukraine, in compliance with the general principles of bioethics in accordance with the international principles of the European Convention on the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes (Strasbourg, 1986) under the natural light/dark cycle. Animals had free access to water.

Determination of haptoglobin, ceruloplasmin and CRP was carried out on a Cobas 311 biochemical analyzer using Roche Diagnostics test systems. The concentration of procalcitonin (PCT) was determined on a Cobas 411 analyzer using Roche Diagnostics test systems. Statistical processing of the obtained results was carried out using the Origin Pro 8.5 program package. The average values of the obtained indicators (x) with standard deviations (SD) were determined. The probability of difference between the control and experimental samples was estimated by the Student's t test. At $P < 0.05$, changes were considered significant.

Results and Discussion

The analysis of the results of changes in the concentration of haptoglobin in the blood serum of rats showed its statistically significant increase in relation to the control values after three doses in all experimental groups by 226.18% (II group), 801.85% (III group) and 172.24% (IV group), respectively. The concentration was the highest after three days under the conditions of 7-time injection of 0.02 ml of *S. aureus* 108 No. 209 into the knee joint. Compared with rats of II and IV groups, the increase was 177.46% and 231.28%, respectively (Table 1).

Under the same experimental conditions, after 14 days only in rats after alternating (every other day) injection of 0.02 ml of flosterone and 0.02 ml of *S. aureus* 108 No. 209 into the knee joint (group IV), demonstrated the greatest increase in the level of haptoglobin by 775, 08% ($P < 0.05$) compared to the control. Whereas in rats of the II group, its content remained almost at the same level as after the measurement three days later. The concentration of haptoglobin in blood serum of animals of the III group was higher than the control values by 117.67%. However, it was significantly lower (by 85.33%) compared to the measurement the third day of administration (Table 2).

The concentration of ceruloplasmin in the blood serum of rats of the II group compared to the control increased almost equally by 77.27% and 81.82%, respectively ($P < 0.001$) 3 and 14 days after the last administration of flosteron. It increased by 286.36% and 122.73% after 3 and 14 days after 7-time injection of 0.02 ml of *S. aureus* 108 No. 209 into the knee joint. Whereas three days after alternating (every other day) administration of 0.02 ml of flosterone and 0.02 ml of *S. aureus* 108 No. 209 increased by 613.64% and even more after 14 days – by 640.91% after the last administration 0.02 ml of *S. aureus* 108 No. 209 (Table 1, 2).

The highest concentration of C-reactive protein was 3 and 14 days after the introduction of 0.02 ml of *S.aureus* 108 No. 209 in rats of the III group. After 3 days, it differed from the control values by 2363.04% and 1890.91% after 14 days . These values were somewhat lower in animals of the II group 3 and 14 days after administration of 0.02 ml of flosteron 1118.18% and 1163.04%, respectively compared to the control. After 3 and 14 days of alternating (every other day) administration of 0.02 ml of flosterone and 0.02 ml of *S.aureus* 108 No. 209, the level of C-reactive protein was 1754.55% and 1727.27%, respectively, of the control values (Table. 1, 2).

The largest increase (by 235.0%) in the PCT level occurred 3 days after alternating (every other day) administration of 0.02 ml of flosteron and 0.02 ml of *S.aureus* 108 No. 209. It was slightly lower (by 120.0%) after 14 days for the same conditions of the experiment. This indicator probably increased by 65% 14 days after the 7-time administration of *S.aureus* 108 No. 209 (Tables 1, 2). In other experimental groups, the PCT concentration in animals did not change.

It follows from the above data that the concentration of haptoglobin probably significantly increased in the blood serum of rats both after 3 and 14 days in all studied groups of animals compared to the control. The greatest increase relative to the control values was noted 3 days after the seventh injection of *S. aureus* 108 No. 209 into the knee joint. However, after 14 days it was already not so significant and significantly lower (by 85.33%) compared to the measurement after three days. Only in

Table 1. **The content of acute-phase proteins and procalcitonin in the blood serum of experimental animals with 7-fold local administration of the hormonal drug flosteron and *S. aureus*108 N 209**

Indicators	Control $n = 10$	Hormone (flosterone)		Infection (*S. aureus*108 N 209)	
		3 days, $n = 7$	14 days, $n = 7$	3 days, $n = 7$	14 days, $n = 7$
Haptoglobin, g/l	0.317±0.004	1.034±0.011	1.016±0.006**	2.859±0.047	0.690±0.021**
Ceruloplasmin, g/l	0.022±0.001	0.039±0.003**	0.040±0.001**	0.085±0.021	0.043±0.005
C-reactive protein, ng/ml	0.011±0,003	0.134±0.013	0.139±0.011**	0.271±0.018	0.329±0.042*
Procalcitonin, ng/ml	0.020±0.001	0.023±0.,001	0.020±0.001**	0.019±0.002	0.033±0.005

*$P < 0,05$: **$P < 0.001$.

Table 2. **The content of acute-phase proteins and procalcitonin in the blood serum of experimental animals after the 7th local administration of the hormonal drug flosteron+ *S. aureus*108 N 209**

Indicators	Control $n = 10$	Flosteron + *S. aureus* 108 N 209	
		3 days, $n = 7$	14 days, $n = 7$
Haptoglobin, g/l	0.317±0.004	0.863±0.028**	2.774±0.052
Ceruloplasmin, g/l	0.022±0.001	0.157±0.013**	0.163±0.015
C-reactive protein, ng/ml	0.011±0,003	0.104±0.014	0.201±0.023
Procalcitonin, ng/ml	0.020±0.001	0.067±0.002	0.044±0.004

*$P < 0,05$: **$P < 0.001$.

rats after a 14-day alternating (every other day) administration of 0.02 ml of flosterone and 0.02 ml of *S. aureus* 108 No. 209 into the knee joint, a probable increase in the level of haptoglobin by 775.08% ($P < 0.05$) was observed compared to the control and decreased by 77.78% compared to the measurement after three days. We believe that this indicator reflects, first of all, the primary activation of the inflammatory process, which was enhanced by the hormonal drug flosteron. Therefore, its determination can be effective over a longer period of time, as bacterial infections lead to several processes, reinforcing each other.

The concentration of ceruloplasmin in blood serum increased in all experimental rats during the entire observation period with minor differences between 3 and 14 days. Probably, the synthesis of ceruloplasmin increases precisely during the first three days of the infectious process, which leads to an increase in its content. The abruption of further increase of this indicator is most likely due to the depletion of the enzyme antioxidant reserve of the serum, of which ceruloplasmin is a component, due to its ability to bind free oxygen radicals and inactivate them.

Among all acute-phase proteins, the concentration of C-reactive protein in the blood demonstrated the highest increase in all studied groups of rats without exception, which proves its high efficiency in detecting inflammatory processes of various severity.

The concentration of procalcitonin in the conditions of our experiment significantly changed in the blood serum of rats 14 days after the introduction of *S.aureus* 108 #209 and after 3 and 14 days under the combined influence of flosterone and *S.aureus* 108 #209.

Therefore, with increasing bacterial infection, this indicator is more specific.

Thus, according to our data, the determination of haptoglobin reflects, first of all, the primary activation of the inflammatory process, which was enhanced by the hormonal drug flosteron. Therefore, its definition can be effective over a longer period of time, as typical, multiple factors lead to bacterial infection, reinforcing each other. At the same time, the synthesis of ceruloplasmin increases exactly during the first three days of the infectious process, which turns it into an effective marker for detecting early infectious complications. The dynamics of changes in the level of C-reactive protein in blood serum showed the highest correlation with the activity of the infectious process, which proves its high efficiency for detecting inflammatory processes of various severity, choosing adequate treatment and predicting the course of the disease.

Prospects

Further study of longer use of a hormonal drug (flosterone) is in an experiment.

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Conflict of interest

The authors declare no conflict of interest during the preparation of the article.

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ЕФЕКТИВНІСТЬ ВИЗНАЧЕННЯ ГОСТРОФАЗНИХ ПРОТЕЇНІВ І ПРОКАЛЬЦИТОНІНА ЗА УМОВ МОДЕЛЮВАННЯ ІНФЕКЦІЙНОГО АРТРИТУ У МИШЕЙ

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Гострофазні протеїни церулоплазмін, гаптоглобін, С-реактивний протеїн (СРП) і прокальцитонінє маркерами, які характеризують запальний процес. С-реактивний протеїн — один із центральних компонентів гострої фази (ГФ) і є загальновизнаним показником запальних процесів.

Мета. Визначити рівень і ефективність визначення гострофазних протеїнів (СРП, гаптоглобіну, церулоплазміну), а також прокальцитоніну за умов моделювання інфекційного артриту.

Матеріали і методи. Експериментальні дослідження було проведено на 52 білих щурах-самцях лінії Вістар. Модель інфекційного артриту створювали протягом семи діб, щоденним уведенням 0,02 мл *S. aureus* 108 № 209 у колінний суглоб щура. Тварин було розподілено на групи — I віварний контроль. Для експериментальних груп було застосовано таку модель уведення препарату: щоденне одноразове введення протягом семи діб по 0,02 мл флостерону в колінний суглоб (II група); щоденне одноразове введення протягом семи діб по 0,02 мл *S. aureus* 108 № 209 (III група); щоденне одноразове поперемінне (через день) уведення протягом семи діб по 0,02 мл флостерону і 0,02 мл *S. aureus* 108 № 209 у колінний суглоб (IV група). Ефективність дії препаратів спостерігали через 3 і 14 діб після введення.

Результати. Встановлено, що концентрація гаптоглобіну вірогідно зросла у сироватці крові щурів як через 3, так і 14 діб у всіх досліджуваних групах тварин порівняно з контролем. Найбільше підвищення відносно контрольних значень спостерігалось через 3 доби після семиразового введення *S aureus* 108 №209 у колінний суглоб. Однак через 14 діб воно вже було не таким суттєвим і значно нижчим (на 85,33%) порівняно з вимірюванням через три доби. Лише у щурів після 14-добового поперемінного (через день) уведення по 0,02 мл флостерону та 0,02 мл *S. aureus* 108 № 209 у колінний суглоб спостерігали вірогідне зростання рівня гаптоглобіну на 775,08% ($P < 0,05$) порівняно з контролем і на 77,78% зниженим порівняно з вимірюванням через три доби. Концентрація церулоплазміну в сироватці крові зростала у всіх експериментальних щурів за весь період спостереження і мало відрізнялася між 3 і 14 добами. Вміст С-реактивного протеїну в сироватці крові зростав у всіх без винятку досліджуваних групах щурів, що доводить його високу специфічність для виявлення запальних процесів різної важкості. Концентрація прокальцитоніну найбільше — на 235,0% вірогідно підвищувалася через 3 доби після поперемінного (через день) уведення по 0,02 мл флостерону та 0,02 мл *S. aureus* 108 № 209. Дещо нижче на 120,0% за тих самих умов експерименту через 14 діб. На 65% цей показник вірогідно зріс через 14 діб після 7-разового введення *S. aureus* 108 №209. У решти експериментальних тварин концентрація ПКТ не змінювалася.

Висновки. Визначення гаптоглобіну відображає, перш за все, первинну активацію запального процесу, посиленню якої сприяв гормональний препарат флостерон. Проте його визначення може бути ефективним через більш тривалий термін, оскільки до бактеріальної інфекції призводять декілька чинників, посилюючи один одного. В одночас синтез церулоплазміну посилюється саме впродовж перших трьох діб інфекційного процесу, що перетворює його у результативний маркер для виявлення раннього інфекційного ускладнення. Динаміка змін рівня С-реактивного протеїну в сироватці крові продемонструвала найвищу кореляцію з активністю інфекційного процесу, що доводить його високу ефективність для виявлення запальних процесів різної важкості, вибору адекватного лікування та прогнозу перебігу захворювання.

Ключові слова: гаптоглобін, церулоплазмін, С-реактивній протеїн, прокальцитонін, інфекційний артрит.