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MICROBIOLOGICAL CHARACTERISTICS OF EXPERIMENTAL MODELS OF OSTEOMIELITIS

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✓ ***Resume***

The aim of the study was to develop a methodology for the selection of various strains of microorganisms to create models of experimental acute and chronic osteomyelitis, to determine the properties of the formation of experimental osteomyelitis. It has been established that for the creation of acute and chronic experimental osteomyelitis, it is advisable to use collection strains as an infectious agent; to create a model of acute experimental osteomyelitis, it is recommended to use Staphylococcus aureus strains with high pathogenicity, and chronic experimental osteomyelitis pathogenic strains of Staphylococcus in -inflammatory process on the surface of the femur of animals is proven by clinical, microbiological and morphological signs.

Key words: model of expert osteomyelitis, white outbred rats, infecting microorganisms, collection strains, identification of microorganisms.

ЭКСПЕРИМЕНТАЛ ОСТЕОМИЕЛИТЛАР МОДЕЛЛАРИНИ ЯРАТИШНИНГ МИКРОБИОЛОГИК ЖИҲАТЛАРИ

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✓ ***Резюме***

Тадқиқот мақсади тажрибавий ўткир ва сурункали остеомиелитлар моделларини яратиш мақсадида турли микроорганизмлар штаммларини танлаш усулини яратиш, тажрибавий остеомиелитлар чақира олиш хусусиятини аниқлаш бўлди. Аниқланишича, ўткир ва сурункали тажрибавий остеомиелитлар моделларини яратиш учун инфицирловчи микроорганизмлар сифатида коллекцион штаммлар танланиши мақсадга мувофиқ, тажрибавий ўткир остеомиелит моделини яратиш учун Staphylococcus aureus нинг патогенлиги юқори штаммлари, тажрибавий сурункали остеомиелит моделини яратишда ассоциация кўринишида Staphylococcus aureus ва Pseudomonas aeruginosa танланган, ҳайвонлар сон суяги суяк устки қаватида йирингли-яллиғланиш жараёни кузатилгани микробиологик, клиник ва морфологик белгилар асосида исботланган.

Калит сўзлар: экспериментал остеомиелит модели, оқ зотсиз сичқонлар, инфицирловчи микроорганизмлар, коллекцион штаммлар, микроорганизмлар идентификацияси.

МИКРОБИОЛОГИЧЕСКИЕ ОСОБЕННОСТИ СОЗДАНИЯ ЭКСПЕРИМЕНТАЛЬНЫХ МОДЕЛЕЙ ОСТЕОМИЕЛИТОВ

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✓ ***Резюме***

Целью исследования было разработка методики выбора различных штаммов микроорганизмов для создания моделей экспериментальных острых и хронических остеомиелитов, определение свойств формирования экспериментальных остеомиелитов. Установлено, что для создания острых и хронических экспериментальных остеомиелитов в качестве инфицирующего агента целесообразно использование коллекционных штаммов, для создания модели острого экспериментального остеоомиелита рекомендуется использовать штаммы Staphylococcus aureus с высокой патогенности, а хронического экспериментального остеомиелита патогенные штаммы Staphylococcus aureus и Pseudomonas aeruginosa в ассоциации, развитие гнойно-воспалительного процесса на поверхности бедренной кости животных доказаны клиническими, микробиологическими и морфологическими признаками.

Ключевые слова: модель экспертментального остеомиелита, белые беспородные крысы, инфицирующие микроорганизмы, коллекционные штаммы, идентификация микроорганизмов.

Relevance

It has been established that the causative agents of acute and chronic osteomyelitis are various microorganisms, such as the genus Staphylococcus, Pseudomonas, Candida, members of the Enterobacteriaceae family, non-clostridial anaerobes, and others [1, 8].

To create a model of experimental osteomyelitis, various microorganisms were used as an infectious agent. Katz S.A. (1954) and Nechaevskaya M.R. (1955) used anaerobic microorganisms, Matusis Z.E. (1959) used the genus Proteus. For this purpose, many researchers used the reference culture Staphylococcus aureus [4, 10], Le C.T. (1982) and Belzunegui J., Lopez L. (1997) used Salmonella spp. Tiller F.N., Tietze V. (1979) used Candida albicans to create acute hematogenous osteomyelitis, and Nondan C.N., Shinners E. (1979) and Matkurbanov A.Sh. [4] chose Pseudomonas aeruginosa for this purpose. Matkurbanov A.Sh. [5], Nuraliev N.A. et al. [6] used a museum strain of Staphylococcus aureus as an infectious agent. There is evidence of an infection experiment using a reference culture of Staphylococcus aurus and Pseudomonas aeruginosa to create a model of chronic osteomyelitis.

To create an experimental model of osteomyelitis, Gahukamble A.D. et al. [12] infected rabbits with the bacteria Propionibacterium acnes and Staphylococcus lugdunensis, and thus created a model of chronic osteomyelitis. After surgery, ways to optimize treatment were proposed based on the clinical aspects of this model.

Analysis of the published literature on the problem under study showed that using models of experimental osteomyelitis, it is possible to determine the formation, development and regularities of the course of acute and chronic osteomyelitis. Even if models of experimental osteomyelitis have been created, the problem of infectious agents has not been fully studied, the choice of an infectious microorganism that is suitable for all parameters has not been brought to its logical conclusion.

Since it is necessary to start treatment measures immediately after the diagnosis of the disease, today the immuno-microbiological aspects and pathogenetic mechanisms of various osteomyelitis are not fully assessed, the influence of various methods of treatment on the microorganism, specific and nonspecific factors of protection of the macroorganism, has not been fully studied.

Purpose of the study: To create a way to select different strains of gram-positive and gram-negative microorganisms in order to create experimental models of acute and chronic osteomyelitis, to assess the ability to cause experimental osteomyelitis.

Material and methods

In order to create experimental models of acute and chronic osteomyelitis, 150 white outbred mice of 2-3 months of age, weighing 18-22 grams of both sexes, were used.

The experimental animal room was warm, light and dry, and the floor was cemented to prevent the entry of wild rodents. Animal storage cages (there were 20 mice in one cage) were placed at a height of 30-70 cm.All animals brought for scientific work were kept in quarantine for 10 days, and after confirming the absence of infectious, parasitic and other diseases in the animals, the experiments were carried out. When feeding the mice, they adhered to the traditional diet [6]. At the end of the experiment, the euthanized mice were disposed of according to the same rules. When working with laboratory animals, we adhered to the safety rules and ethical principles of working with animals, accepted at the international level [2, 6, 11].

Taking into account the differences in the course, duration of the disease, the occurrence of pathogens as a monoculture or as an association of microorganisms, as well as the principles of treatment, it was decided to create separate experimental models of acute and chronic osteomyelitis. When choosing an infectious agent, we used materials on the study of biological material (pus) of 448 patients (380 adults and 68 children) with acute and chronic osteomyelitis; data of the microbial landscape were obtained as a result of bacteriological research. Collection strains were used to create an experimental model of acute and chronic osteomyelitis. They were kindly presented from the "National Collection of Microorganisms of Human Infections" of the Scientific Research Institute of Epidemiology, Microbiology and Infectious Diseases of the Ministry of Health of the Republic of Uzbekistan (now renamed the Republican Scientific and Practical Specialized Center for Epidemiology,

Microbiology, Infectious and Parasitic Diseases). The authors express their sincere gratitude to the staff of this collection. All used collection strains were stored in a refrigerator (40C) in a semi-liquid nutrient medium. All used strains were taken from patients with pyoinflammatory diseases living in the territory of Uzbekistan. The studies were carried out for the period 2010-2018.

In the course of statistical data processing, traditional statistical variational methods and the "Exsel" program on a Pentium-IV computer were used. When organizing and conducting research, the principles of evidence-based medicine were adhered to.

Result and discussion

In order to create an experimental model of acute and chronic osteomyelitis in selected animals under laboratory conditions, strains of Staphylococcus aureus were used. This choice was facilitated by the following main factors:

firstly, the obtained clinical and microbiological studies have shown that Staphylococcus aureus is the main causative agent of acute and chronic osteomyelitis in people of different ages living in different regions of the world; secondly, when using strains of Staphylococcus aureus as an infectious agent of acute and chronic osteomyelitis, the clinical signs of this disease are pronounced;

third, Staphylococcus aureus is easy to identify and differentiate by bacteriological methods;

fourthly, many researchers use this gram-positive microorganism as an infectious agent to create experimental models of acute and chronic osteomyelitis. The biological properties of Staphylococcus aureus used to create an experimental model of acute osteomyelitis in white outbred mice are given in table. one.

Identification of Staphylococcus aureus strains showed that all studied strains were identical in basic biological properties corresponding to their genus - morphological, tinctorial, cultural, enzymatic and antigenic characteristics [7]. , mucus from the throat or nose). When choosing the studied five strains, the fact that these microorganisms can be found in different biotopes of the body and can act as an etiological agent of the disease, and can also enhance their pathogenetic features, was taken into account.

Table 1

The main biological properties of Staphylococcus aureus strains for the creation of acute experimental osteomyelitis

Biological properties	Sequential number of collection strains				
	003994/ Wood-46	003846/ 11	003851/ 2	003926/ M-4	004174/ M3-85
Morphological	Coccus	Coccus	Coccus	Coccus	Coccus
Tinctorial	Gram "+"	Gram "+"	Gram "+"	Gram "+"	Gram "+"
Cultural	S shape, RFP	S shape, RFP	S shape, RFP	S shape, RFP	S shape, RFP
Enzymatic	Typical	Typical	Typical	Typical	Typical
A source	Hemoculture	Pus	Pus	Mucus from the throat	Mucus from the nose

Note: ZP - golden pigment.

Before carrying out experimental studies, the collection strains were sown in the media corresponding to their taxonomic groups. Then the selected strains were identified and differentiated based on the study of morphological, tinctorial, cultural, enzymatic, toxigenic and antigenic properties.

It was revealed that the colonies of Staphylococcus aureus slightly rose from the surface of the nutrient medium, the surface was smooth, moist, after 24 hours they were covered with a golden pigment. With the help of additional microbiological tests, the formation of urease, phosphatase and other taxonomic characteristics were studied, pathogenic factors - plasmacoagulase, hemolytic abilities, lecithinase and hyaluronidase activity were studied. It should be noted that Staphylococcus aureus strains are characterized by high hemolytic activity.

To create acute experimental osteomyelitis, the experimental method proposed by M.M. Soloviev was used. (1969) in our modification.

To form an experimental model of acute osteomyelitis, laboratory animals were infected with the selected five strains of Staphylococcus aureus twice.

At the first infection with Staphylococcus aureus, a mixture of the five aforementioned strains was used, before their use to enhance the infectious effect of microorganisms were cultivated separately in a liquid nutrient medium. After that, a fresh one-day culture was prepared for introduction into the animal's body.

According to the literature and our clinical observations, osteomyelitis mainly develops in the tubular bones. Taking this into account, the infection was carried out on the femur of the hind legs of a laboratory animal.

For this, the site of the operation was cleared of hairs 2-3 days before the operation. The animals were fixed with a traditional method, the upper part of the thigh of the hind legs was cut under local anesthesia, the bone was opened, the outer layer of the bone was damaged with a scalpel, and a one-day culture mixture of the selected strains of Staphylococcus aureus was injected in an amount of 0.1 ml at a concentration of 6x109 microbial bodies / ml (m t / ml). After that, the dissected part of the femur was sutured operatively. The animals were placed in cages separately from each other, then they were observed in a general vivarium.

The second infection was carried out on the 7th day of the beginning of the experiment in the same order, only, unlike other researchers, a mixture of a one-day culture of 5 strains was used (other authors used one strain for repeated infection). The rapid introduction of strains into the organism of laboratory animals is especially significant.

On visual observation, clinical signs of acute experimental osteomyelitis appeared on the 3rd day after secondary infection, that is, on the 10th day after the start of the experiment. The results of these observations were supplemented by a clinical visual study of the femur, where acute experimental osteomyelitis developed in laboratory conditions, and also by the study of the morphology of the tissues of the damaged part of the bone.

From a clinical point of view, edema was observed in the projection of the infected femur, such clinical manifestations as increased body temperature, careful stepping on the injured paw, and dragging the paw with difficulty. Also, they noted the bulging of the hairline, inactivity, lack of appetite, the slow manifestation of various conditioned and unconditioned reflexes.

Bacteriological studies showed that mainly Staphylococcus aureus was identified from the pus of the pathological focus in 96.7% of cases (in other cases, no growth was noted), the average concentration of microorganisms was noted in the amount of 8x1011 colony-forming units / ml (CFU / ml).

Other gram-positive and gram-negative microorganisms were not identified from the pathological focus. Apparently, the following factors contributed to this:

- in the pathological focus, other microorganisms, except for Staphylococcus aureus, could not multiply in sufficient quantities during the short period of the experiment;
- the selected strains of Staphylococcus aureus were introduced into the pathological focus for the formation of experimental osteomyelitis in sufficiently large quantities, in addition, other pathogens were prevented from entering the pathological focus during the operation;
- the absence of various infectious and pyoinflammatory diseases in laboratory animals (white outbred mice) before the start of the experiment, which would have entered the focus of the hematogenous route;
- a comparatively increased antagonistic ability of the effect of Staphylococcus aureus on other gram-positive and gram-negative microorganisms, therefore, other microorganisms were not sown from the pathological focus.

In addition, morphological studies made it possible to detect purulent-inflammatory signs on the damaged surface.

Thus, observing the clinical, microbiological and morphological signs, it was possible to induce acute experimental osteomyelitis in 96.7% of laboratory animals involved in the experiment. This shows that the choice of the infectious agent for forming the model was made correctly.

In the formation of chronic experimental osteomyelitis, the results of experience on the creation of an experimental model of acute

osteomyelitis, the results of microbiological studies of patients were used. As a result, it became known that in chronic osteomyelitis, a high sowing rate of Staphylococcus aureus is detected, and also often in the form of an association of microorganisms with other microorganisms, including the main representative of nosocomial infections Pseudomonas aeruginosa.

Based on the above, for the formation of an experimental model of chronic osteomyelitis, it was decided to use associations of microorganisms of 2 species – Staphylococcus aureus and Pseudomonas aeruginosa. Of the 5 collection strains (serial numbers 003994 / Wood-46, 003846/11, 003851/2, 003926 / M-4, 004174 / M3-85) used to create an experimental model of acute osteomyelitis, we selected the most pathogenic strain 003926 / M -4 for the formation of a model of chronic osteomyelitis.

Both strains of microorganisms were kindly submitted by the National Collection of Microorganisms for Human Infections. The biological properties of the strains are presented in table. 2.

Table 2

Basic biological parameters of microorganism strains used to form an experimental model of chronic osteomyelitis

Biological properties	***Staphylococcus aureus***	***Pseudomonas aeruginosa***
Serial number	003926/M-4	003480/237
Morphological	Coccus	Bacterium
Tinctorial	Gram "+"	Gram "-"
Cultural	S shape,	S shape,
Enzymatic	golden pigment	green pigment
Source of receipt	Typical for the species	Typical for the species

When identified by a bacteriological method, the selected Staphylococcus aureus strain (serial number 003926 / M-4) showed all the properties typical of the genus and type of microorganism.

After repeated sowing of Pseudomonas aeruginosa into the nutrient medium, all the typical features of the bacteria in their taxonomic group were revealed: the colonies are typical, there was a green pigment, a peculiar smell, lysis zones and the phenomenon of "rainbow sheen". Detection of the phenomenon of "rainbow sheen" is a characteristic sign of the pathogenicity of Pseudomonas aeruginosa [3]. This means that the collection strain we have chosen has a high virulence. I would like to emphasize that this microorganism is widespread as a causative agent of chronic pyoinflammatory processes, a hospital strain, it is characterized as a "problem" microorganism with resistance to antibiotics.

When forming a model of chronic experimental osteomyelitis, the method [5] proposed by MM Soloviev was used. (1969) in our modification.

For this, the operation site was completely cleared of hairline 2-3 days before the operation. The mice were fixed in the traditional way, the upper part of the thigh of the hind legs was cut under anesthesia, the bone was opened, and before infection the upper part of the femur was treated with 0.1 ml of 3% acetic acid as a stress and damage factor. The treatment was carried out carefully, only on the surface of the bone. Acetic acid was injected after the transfusion needle reached the hard part of the bone. The acid was injected carefully so that it did not enter the blood vessel, since if it enters the bloodstream, hemolysis of erythrocytes may occur and the animal may die.

4 days after exposure to acetic acid, the pathological focus where inflammation was caused was infected with strains of Staphylococcus aureus, at a concentration of 6x109 bw / ml, which caused chronic experimental osteomyelitis. On the 7th day of infection, Pseudomonas aeruginosa strains (serial number 003480/237) were introduced into this focus at a concentration of 6x109 bw / ml.

In order to increase the efficiency of infection, both strains were cultivated in liquid nutrient medium separately and standardized. Of particular importance was the rapid introduction of one-day fresh cultures of microorganisms into the body of white outbred mice.

The reason for the use of various microorganisms in order to form an experimental model of chronic osteomyelitis is that they stimulate a broad antigenic stimulus, lead to a strong microbial sensitization of the body, and provide a variety of pathogenic factors.

In-depth bacteriological studies have shown the practical aspect of the absence of antagonistic interaction of both strains of microorganisms with each other.

Infection with microorganisms with various antigenic and pathogenic properties led to a prolonged purulent-inflammatory process in the bone tissue (up to 60 days or more). In the chronic type of the disease, clinical, bacteriological and morphological signs were also manifested as in acute osteomyelitis. All the results obtained proved the formation of experimental chronic osteomyelitis in laboratory animals.

At the end of the research, the results obtained showed that it is necessary to pay attention to the following features when choosing infectious microorganisms in order to form an experimental model of acute and chronic osteomyelitis:

- after identification of the selected strains for infection to the genus and species, you need to make sure that this is a monoculture;
- to prove the high pathogenic properties of strains of microorganisms before introduction into laboratory animals, as well as the need for infection with a one-day fresh culture;
- take into account the need to use a microorganism of one type in acute experimental osteomyelitis, as well as the use of an association of microorganisms (two or more different strains) in the formation of a model of experimental chronic osteomyelitis, since the chronic form lasts a long time and there is a likelihood of other pathogens entering the pathological focus;
- to ensure the concentration of cultures of microorganisms used during infection, not less than 6x109 MT / ml;
- pay attention to the dependence of effectiveness on the variety of microorganisms that caused acute and chronic experimental osteomyelitis, on the degree of pathogenicity, time of administration and concentration in the pathological focus.

The medical effectiveness of the proposed methods lies in the fact that it became necessary to form experimental models of acute and chronic osteomyelitis, taking into account the fact that when assessing the effectiveness of any treatment, it is necessary to take into account possible difficulties in assessing the microbiological and immunological aspects of this pathology.

The creation of these experimental models will allow testing new methods of treatment, new modern medicines. This, in turn, will increase the medical efficiency of patient care.

The social effectiveness of the proposed method is that the created experimental models of acute and chronic osteomyelitis will allow the implementation of the proposed various methods of treatment into healthcare practice without prejudice to the health of patients, and clinically evaluate the dosage of new drugs.

This, in turn, will improve the quality of life of patients through adequate treatment. An increase in the effectiveness of treatment will lead to a rapid recovery of the patients' ability to work, a decrease in disability from this ailment.

The economic efficiency of the proposed method lies in the fact that the introduction of a method for selecting an infectious microorganism in order to create experimental models of acute and chronic osteomyelitis, will save money on each series of experimental studies, and also increase the efficiency of experimental studies by 15%.

Findings

1. The choice of collection strains as infectious microorganisms for the creation of experimental models of acute and chronic osteomyelitis was expedient, since they have been identified and all their biological properties are known. The use of hospital strains is not advisable.
2. To create a model of experimental acute osteomyelitis, 5 pathogenic strains of Staphylococcus aureus were injected twice into the injury site (femur) of experimental animals (white outbred mice) at a concentration of 6x109 MT / ml with an

interval of 7 days. During the observation period, all clinical, microbiological and morphological signs of experimental acute osteomyelitis appeared in the pathological focus.

3. In the formation of experimental chronic osteomyelitis, associations of microorganisms - Staphylococcus aureus and Pseudomonas aeruginosa were selected. The strains were injected into the femur of animals at a concentration of 6x109 bw / ml twice with an interval of 2 days. On the 15th day of infection, a purulent-inflammatory process on the upper part of the femur was confirmed in terms of clinical, microbiological and morphological properties. The pathological process lasted 60 days or more, which confirms the chronic nature of the process.
4. After complete identification and determination of the pathogenic properties of the strains Staphylococcus aureus and Pseudomonas aeruginosa are recommended for use as an infectious agent for the formation of experimental models of acute and chronic osteomyelitis.

LIST OF REFERENCES:

1. Babushkina I.V., Mamonova I.A. Gladkova E.V. The etiological role of causative agents of chronic osteomyelitis and the effect of copper nanoparticles on clinical strains of Staphylococcus aureus // Bulletin of Perm University. - Perm, 2014. - Issue 2. - P.52-56.
2. Zharmukhamedova T.Yu., Semushina S.G., Pakhomova I.A., Pimenov M.S., Murashev A.N. International rules for working with laboratory animals during preclinical tests // Toxicological Bulletin. - Moscow, 2011. - No. 4 (109). - P.2-9.
3. Iskhakova Kh.I., Vakhidova Kh.M., Shadmanova N.A. Classification, ecology and differentiation of Pseudomonas aeruginosa and other non-fermenting gram-negative bacteria (NGOB) // Teaching aid. - Tashkent, 2010 .-- 36 p.
4. Matkurbanov A.Sh., Ismailov E.A., Rakhmanova S.S., Batyrbekov A.A. Assessment of the state of immunological parameters in experimental acute osteomyelitis // Methodical recommendations. - Urgench, 2011 .-- 11 p.
5. Matkurbanov A.Sh. Experimental ўtkir va surunkali osteomyelitlarda immun tizim olati va uning kўrsatkichlari dynamics: td. ilmiy darazha olish uchun diss ... - Toshkent, 2012 .-- 124 b.
6. Nuraliev N.A., Bektimirov A.M.-T., Alimova M.T., Suvonov K.Zh. Rules and methods of working with laboratory animals in experimental microbiological and immunological research // Methodical manual. - Tashkent, 2016 .-- 34 p.
7. Keys to bacteria Bergy. Edited by Hoult J, Krieg N, Snit R, Staley J., Williams S. - Moscow: Mir Publishing House, 1997. - Volume 1-2. - 389 p.
8. Saginova D.A., Teleubaev B.E., Koshanova A.A. Microbial landscape in patients with chronic osteomyelitis and bacteriological efficiency of local antibiotic transport to S. aureus strains // Medicine. - Almaty, 2018. - No. 1 (187). - P.44-48.
9. Tsybin A.A., Badrintsev V.S., Mashkov A.E., Slesarev V.V., Sultonov Sh.R. Osteomyelitis in children: Tula, publishing house "Aquarius", 2016. - 384 p.
10. Reizner W., Hunter J.G., O'Malley N.T., Southgate R.D., Schwarz E.M., Kates S.L. A systematic review of animal models for *Staphylococcus aureus* osteomyelitis // Eur Cell Mater. - 2014. - Vol 27. - N25. - P.196-212.
11. Tyurin E.A., Checkan L.V., Marinin L.I., Dyatlov I.A. Professional risks faced by microbiological laboratory workers and their mitigation measures // Health Risk Analysis – 2014. - N3. – P.6-12.
12. Gahukamble A.D., McDowell A., Post V., Salavarrieta Varela J., Rochford E.T. *Propionibacterium acnes* and *Staphylococcus lugdunensis* cause pyogenic osteomyelitis in an intramedullary nail model in rabbits // J Clin Microbiol. - 2014. - Vol 52(5). - P.1595-1606.

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