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CHARACTERISTICS OF ORGANIC ORGANISMS ABLE TO CAUSE ACUTE AND CHRONIC EXPERIMENTAL OSTEOMYELITIS

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ABSTRACT

The goal was to select infectious strains of microorganisms for the formation of experimental acute and chronic osteomyelitis. For this purpose, Staphylococcus aureus and Pseudomonas aeruginosa were selected. They were introduced 2 times in the amount of 6x109 mt / ml in each laboratory animal. Microbiological, clinical, and morphological signs of experimental acute and chronic osteomyelitis were observed in the pathological focus.

Keywords: acute and chronic experimental osteomyelitis, laboratory animals, strains of microorganisms.

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ХАРАКТЕРИСТИКИ ОРГАНИЧЕСКИХ ОРГАНИЗМОВ, СПОСОБНЫХ ВЫЗЫВАТЬ ОСТРЫЙ И ХРОНИЧЕСКИЙ ЭКСПЕРИМЕНТАЛЬНЫЙ ОСТЕОМИЕЛИТ

АННОТАЦИЯ

Целью был отбор инфекционных штаммов микроорганизмов для формирования экспериментального острого и хронического остеомиелита. Для этого были отобраны Staphylococcus aureus и Pseudomonas aeruginosa. Их вводили 2 раза в количестве 6x109 мт / мл каждому лабораторному животному. В патологическом очаге наблюдались микробиологические, клинико-морфологические признаки экспериментального острого и хронического остеомиелита.

Ключевые слова: острый и хронический экспериментальный остеомиелит, лабораторные животные, штаммы микроорганизмов.

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ЎТКИР ВА СУРУНКАЛИ ЭКСПЕРИМЕНТАЛ ОСТЕОМИЕЛИТНИ КЕЛТИРИБ ЧИҚАРАДИГАН ОРГАНИК ОРГАНИЗМЛАРНИНГ ХУСУСИЯТЛАРИ

АННОТАЦИЯ

Мақсад экспериментал ўткир ва сурункали остеомиелитни шакллантириш учун микроорганизмларнинг юқумли штаммларини танлаш эди. Бунинг учун Staphylococcus aureus ва Pseudomonas aeruginosa танланган. Улар ҳар бир лаборатория ҳайвонига 6x109 мт / мл миқдорида 2 марта юборилган. Патологик ўчоқда экспериментал ўткир ва сурункали остеомиелитнинг микробиологик, клиник ва морфологик белгилари кузатилди.

Калит сўзлар: ўткир ва сурункали экспериментал остеомиелит, лаборатория ҳайвонлари, микроорганизмларнинг штаммлари.

Introduction. The causative agents of acute and chronic osteomyelitis are microorganisms belonging to different species, including the genera Staphylococcus, Pseudomonas, Candida, members of the Enterobacteriaceae family, non-clostridial anaerobes, and others [1, 2, 12].

To date, the immuno-microbiological aspects and pathogenetic mechanisms of osteomyelitis of various forms have not been clearly evaluated, but there is a need for an immediate diagnosis and treatment, the effect of treatment on the microorganism, as well as specific and non-specific protective factors of the macroorganism, which does not allow to study everything in dynamics.

Therefore, the creation of experimental models of acute and chronic osteomyelitis allows you to fully study the microbiological and immunological aspects of these diseases.

To date, experimental models have been carried out on laboratory animals such as dogs, rabbits, white rats and guinea pigs [7, 8, 11, 13]. However, in these studies, in a comparative study of the main taxonomic characteristics of their generation and species during the selection of pathogenic microorganisms, the relationship between the selected strains of pathogens and the antimicrobial factors of the body was not revealed.

The purpose of the study: The selection of strains of microorganisms belonging to different species for the induction of acute and chronic osteomyelitis in an experiment with the subsequent identification and assessment of their ability to cause experimental osteomyelitis.

Materials and methods. In order to induce experimental acute and chronic osteomyelitis, 120 white thoroughbred mice aged 2-3 months and weighing 18-22 g were used as laboratory animals.

The place where the experimental animals were kept was warm, bright and dry, and the floor was cemented to prevent the penetration of wild rodents. They were placed in cages (up to 20 mice were in one cage) at a height of 30-70 cm above the floor. All animals were quarantined 10 days after they were brought for a scientific experiment, and it was carried out after they were convinced that they did not have any infectious or other diseases. The cells that contained the experimental animals were cleaned and treated every morning in accordance with generally accepted rules. For timely feeding of mice, a traditional diet was used [8]. Mice that died during the experiment were disposed of in accordance with these rules.

Before the start of the experiments, the animals were divided into groups, transferred to separate cells, their health was restored, and the cells were noted in groups. Experimental animals were infected with selected strains of microorganisms in accordance with traditional methods. When working with laboratory animals, ethical principles of working with them were observed, as well as generally recognized rules for biological safety [3, 14].

Given the course and duration of the disease, the emergence of pathogens in the form of monocultures, associations of microorganisms, as well as differences in the principles of treatment, it was necessary to create separate models of experimental acute and chronic osteomyelitis.

In the selection of infectious agents, the microbial landscape data obtained as a result of bacteriological studies of biological material (pus) in 448 patients (380 adults and 68 children) with acute and chronic osteomyelitis we studied was used.

Collectible strains were used to induce acute and chronic experimental osteomyelitis in laboratory animals. They were taken from the National Collection of Human Infections Microorganisms of the Scientific Research Institute of Epidemiology, Microbiology and Infectious Diseases of the Ministry of Health of the Republic of Uzbekistan. The authors thank the collection staff for this. All strains used were stored in a refrigerator (40C) in a semi-liquid nutrient medium.

All strains used were local strains isolated from patients with purulent-inflammatory diseases in our country. Studies were conducted in 2010-2018.

Statistical processing of the results was carried out using traditional methods of variation statistics, using the Excel program on a personal computer. When organizing and conducting research, the principles of evidence-based medicine are strictly observed.

The results obtained and discussion. Staphylococcus aureus strains were used to induce experimental acute osteomyelitis in selected laboratory animals. For this, we had the following reasons:

- firstly, the results showed that they are the main causative agents of acute and chronic osteomyelitis in humans;
- secondly, clear clinical manifestations have been identified when this pathogen causes acute and chronic osteomyelitis;
- thirdly, the identification of these gram-positive cocci is well established in bacteriological laboratories;
- fourthly, the ability of this pathogen to cause acute and chronic osteomyelitis in people, regardless of place of residence;
- fifthly, most researchers have used this pathogen as an infectious agent that causes experimental acute and chronic osteomyelitis in laboratory animals.

Data on these microorganisms used to induce acute experimental osteomyelitis are shown in Table 1.

Table 1

The main biological parameters of the strains of Staphylococcus aureus used to induce acute experimental osteomyelitis

Biological properties	Sequence number of strains				
	003994/ Wood-46	003846/ 11	003851/ 2	003926/ M-4	004174/ M3-85
Morphological properties	Coccus	Coccus	Coccus	Coccus	Coccus
Tinctorial properties	Gram "+"	Gram "+"	Gram "+"	Gram "+"	Gram "+"
Cultural properties	S shape, golden pigment	S shape, golden pigment	S shape, golden pigment	S shape, golden pigment	S shape, golden pigment
Enzymatic properties	Typical	Typical	Typical	Typical	Typical
Dedicated source	Hemoculture	Pus	Pus	Throat mucus	Nasal mucus

It was found that the Staphylococcus aureus strains selected for laboratory animal infections exhibited typical characteristics for all biological properties (morphological, tinctorial, cultural,

enzymatic and other characteristics) during bacteriological identification, differing mainly in the source of excretion (blood culture, pus, mucus from the throat or nose).

When choosing five strains of these microorganisms at once for the induction of experimental acute osteomyelitis, it was taken into account that these microorganisms are found in various biotopes of the body and can be the etiological agent of osteomyelitis, regardless of the age of the person, in addition, these strains enhance mutual pathogenic properties.

Before the planned experimental studies, the collection strains were re-seeded with nutrient media corresponding to their taxonomic groups. After that, the selected strains were identified to the genus and species based on the study of morphological, tinctorial, cultural, enzymatic, toxigenic and antigenic properties [4, 5, 9].

The colonies of Staphylococcus aureus were typical, convex, the surface was smooth, moist and after 24 hours had the ability to highlight golden pigment. Using additional microbiological tests, taxonomic signs, such as the formation of urease, phosphatase and others, as well as pathogenicity factors - plasmocoagulase and hemolytic ability, lecithinase and hyaluronidase activity. It should be noted that Staphylococcus aureus was characterized by high hemolytic activity.

To induce experimental acute osteomyelitis, we used the traditional method of MM Soloviev, which he proposed in 1969 [7] in our modification.

Laboratory animals were infected with Staphylococcus aureus strains twice to induce acute experimental osteomyelitis. In both cases, a one-day culture of a mixture of the five Staphylococcus aureus strains mentioned above was used. To enhance the infectious effect before use, they were seeded into fresh nutrient media and one-day cultures were obtained.

From literature [1, 6, 7, 10] and our clinical and bacteriological studies, it was known that osteomyelitis mainly develops in tubular bones. In this regard, the femur artificially injured by us was infected.

For this, the operating area was completely cleared of wool 2-3 days before the experiment. After the mice were fixed in the traditional way, the upper part of the femur was opened under local anesthesia, the bone was opened and the upper layer was injured using a surgical scalpel, then this injured surface was injected with a one-day culture of selected Staphylococcus aureus strains in an amount of 0.1 ml at a concentration of 6 × 109 microbial bodies ml (bw / ml). The femur was then sutured surgically, laboratory animals were separated from each other, and they continued to be kept under the general conditions of vivarium.

The second infection was carried out in the same way on the 7th day after the start of the experiment, except that, unlike other researchers [7], a one-day culture of a mixture of five strains was used (other authors used one, the most pathogenic strain for re-infection) . The introduction of the strains to laboratory animals was deemed appropriate immediately.

Clinical signs of acute experimental osteomyelitis in laboratory animals were observed 3 days after infection with microorganisms. The results of these observations were supplemented by a visual examination of the surface of the pathogenic focus, developed in acute experimental osteomyelitis, bacterioscopic and bacteriological examination of pus, and morphological examination of the affected area of the bone.

In the projection of a clinically infected hip, such signs as edema, general and local temperature increase, numbness of the affected leg and difficulties with its movement were visualized. Tousled hair was also observed, low mobility, lack of appetite, slow manifestation of various conditioned and unconditioned reflexes.

Staphylococcus aureus was mainly identified from pus with bacteriological pathological damage (96.7%) - with an average concentration of 8x1011 colony forming units / ml (CFU / ml), other microorganisms were not identified.

Morphologically in the tissues of the pathological focus were found signs of purulent-inflammatory process.

Thus, based on the established microbiological, clinical and morphological features, acute experimental osteomyelitis was induced in 96.7% of laboratory animals involved in the

experimental study. This showed that the microorganism strains for infection were correctly selected to form a model of acute experimental osteomyelitis.

In the induction of chronic experimental osteomyelitis, the results of experiments on the formation of acute experimental osteomyelitis were taken into account, taking into account the high inoculation of Staphylococcus aureus from patients with chronic osteomyelitis, their frequent identification as an association of microorganisms, and the frequent inoculation of Pseudomonas aeruginosa (primary) biological material of a patient with chronic osteomyelitis.

Based on the foregoing, a decision was made to infect laboratory animals to induce chronic experimental osteomyelitis using an association of microorganisms from 2 different strains - Staphylococcus aureus and Pseudomonas aeruginosa.

Both strains, as in the previous case, were kindly provided by the National Collection of Human Infections Microorganisms. Their biological properties are shown in table 2.

Table 2

Basic biological parameters of microorganism strains used to induce chronic experimental osteomyelitis

Biological properties	Staphylococcus aureus	Pseudomonas aeruginosa
Serial number	003926/M-4	003480/237
Morphological properties	Coccus	Bacterium (rod-shaped)
Tinctorial properties	Gram "+"	Gram "-"
Cultural properties	S shape, golden pigment	S form, green pigment, specific odor
Enzymatic properties	Typical	Typical
Dedicated source	Throat mucus	Pus

With bacteriological identification of Staphylococcus aureus, genus- and species-specific biological properties were obtained.

After plating Pseudomonas aeruginosa in nutrient media, it acquired all the typical signs corresponding to its taxonomic group: the colonies were typical, formed a green pigment, felt a characteristic odor, lysis zones and the “iris corolla” phenomenon. The definition of the phenomenon of the “iris” is a characteristic feature of the pathogenicity of Pseudomonas aeruginosa [4].

Therefore, the collection strain we selected was highly pathogenic. It is known that this microorganism is a “problem” microorganism that causes chronic purulent-inflammatory processes, which is widespread in hospitals as a hospital strain, characterized by high resistance to antibiotics.

Chronic experimental osteomyelitis was induced by us on the basis of the method of MM Soloviev, which he proposed in 1969 [7].

For this, the operating area was completely cleared of wool 2-3 days before the experiments. After the mice were fixed in the traditional way, the upper part of the femur was cut under local anesthesia, the bone was opened and 3% acetic acid was applied to the surface in an amount of 0.1 ml. This acid was selected as a toxic, stressful and damaging factor before infection with microorganisms. It was carefully and carefully administered only on the surface of the bone. Acetic acid was applied only after the transfusion needle touched the hard part of the bone. Attempts have been made to prevent this acid from entering the bloodstream, since it causes hemolysis of red blood cells in the blood and the death of laboratory animals.

4 days after acetic acid was applied to the bone, Staphylococcus aureus 003926 / M-4 was injected into the same affected area at a concentration of 6x109 mt / ml, which caused acute experimental osteomyelitis. On the 7th day of infection, the strain Pseudomonas aeruginosa 003480/237 was introduced into this primary focus at a concentration of 6x109 mt / ml.

To increase infection, both strains were seeded separately in nutrient broth and standardized. When introducing cultures of microorganisms into white, outbred mice, the need for immediate administration of an infected agent was taken into account.

The reason for using various strains of microorganisms for the induction of chronic experimental osteomyelitis was that they have a wide range of antigenic stimuli, which leads to high microbial sensitization of the body, providing a diverse spectrum of pathogenic factors. In-depth bacteriological studies showed that there were practically no antagonistic effects between the used strains of microorganisms.

Infection of laboratory animals with different types of microorganisms with different antigenic and pathogenic properties led to a longer duration of the purulent process in the femur (30 days or more). Clinical, bacteriological and morphological signs identified in the acute manifestation of the pathogenic process also manifested themselves in the chronic form. All studies have shown the development of chronic experimental osteomyelitis in laboratory animals.

The results showed that when choosing infectious microorganisms to cause acute and chronic experimental osteomyelitis, the following features should be considered:

- you need to make sure that the selected strains were identified by genus and species, and were also as a monoculture;
- you need to make sure that the pathogenicity of infectious agents was high, in addition, the culture of microorganisms was one-day;
- the need to use a monoculture of microorganisms in the induction of acute experimental osteomyelitis should be taken into account, and in the formation of chronic experimental osteomyelitis, an association of microorganisms (2 strains) must be used, since the chronic pathological process takes a long time and there is a high probability of getting other types of microorganisms in the outbreak;
- for infection of laboratory animals, the concentration of cultures of microorganisms should be at least 6x109 m.t. / ml;
- pay attention to the fact that the effectiveness of infection depends on the type, level of pathogenicity, concentration of the microorganism and the time of administration of the infecting agent;
- the formation of acute and chronic experimental osteomyelitis should be proved not only by microbiological parameters, but also by clinical and morphological signs.

Conclusions.

1. Collective strains were selected as infectious microorganisms for the induction of acute and chronic experimental osteomyelitis, since they are identified to the species and almost all biological properties are known.

2. To induce acute experimental osteomyelitis, 5 different strains of Staphylococcus aureus were introduced into the pathological focus (femur) of the organism of white outbred mice at a concentration of 6x109 mt / ml 2 times at 7-day intervals. In the pathological focus nab

References

1. Abujazar U.M .. Alkhodjaev S.S., Zhankin B.A., Dzhaksibaev M.N., Zubi Yu.Kh., Kilybaev A.K., Asymgevakov R.A., Eshmetova M.K. Chronic osteomyelitis and its clinical and immunological characteristics // Bulletin of Kazan NMU. - Kazan, 2014. - No. 1. - S.246-248.
2. Babushkina I.V., Mamonova I.A. Gladkova E.V. The etiological role of pathogens of chronic osteomyelitis and the effect of copper nanoparticles on the clinical strains of Staphilococcusaurens // Bulletin of Perm University. - Perm, 2014. - Issue 2. - S.52-56.
3. Zharmukhamedova T.Yu., Semushina S. G., Pakhomova I. A., Pimenov M. S., Murashev A. N. International rules for working with laboratory animals during preclinical trials // Toxicological Bulletin. - Moscow, 2011. - No. 4 (109). - S. 2-9.

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