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Selection of Influenced Agents in Formulating Experimental Osteomyelitis Modes

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Abstract Acute and chronic osteomyelitis is currently one of the most important medical problems that need to be addressed due to the incidence, complications, mortality and economic damage It is well known that osteomyelitis pathogens are microorganisms of various species, including Staphylococcus, Pseudomonas, Candida progenitors, some representatives of the Enterobacteriaceae family (Salmonella spp et al.), Noclostridial and other anaerobes. Influence of treatment on microorganisms and macro-organisms on special and non-specific protective factors does not allow to study dynamics of immuno-microbiological patterns of osteomyelitis, development and occurrence of osteomyelitis.

Keywords Experimental acute osteomyelitis, Experimental chronic osteomyelitis, White, Non-breeding mice, Infective agents

1. Introduction

To the present day, the immuno-microbiological aspects and pathogenetic mechanisms of osteomyelitis have not been properly assessed, as there is a need for immediate treatment with the detection of disease.

Therefore, the creation of experimental models of acute and chronic osteomyelitis will allow to study the microbiological and immunological aspects of these diseases.

To this day, many experimental models have been performed in a variety of laboratory animals (dogs, rabbits, rats, marine pigs) [4,9]. However, these studies did not reveal a comparative study of the main taxonomic features of the selection of infective agents and the relationship between selected microorganisms and antimicrobial factors.

Therefore, the aim of this research was to select the strains of infective microorganisms to form an experimental model of acute and chronic osteomyelitis and to determine their ability to call for experimental osteomyelitis.

2. Materials and Methods

120 white mice, weighing 2–3 months and weighing 18–22 grams, were used to form a model for experimental acute and chronic osteomyelitis.

The area where the experimental animals were kept was warm, light and dry, and poly cemented to prevent wild rodents from entering. The stored cages (which contained up to 20 mice per cage) were placed 30–70 cm above the floor. All animals were kept under control (quarantine) for a specified period of time (10 days) after being brought in for scientific work, and experiments were carried out to make sure they were not contagious or otherwise. The cages with experimental animals were cleaned and handled every morning according to generally accepted rules. Traditional ration was followed for timely feeding of mice. Mice that died during the experiment were disposed of according to these rules.

Before starting the experiments, the animals were grouped, transferred into separate cages, and re-assured their health. The cages were marked by groups. Infection and decontamination of microorganisms as experimental animals were carried out in accordance with traditional official rules and methods.

Given the differences in the incidence, duration, occurrence of pathogens in the form of monoculture and microorganisms associations, it is necessary to create separate models of acute and chronic osteomyelitis.

We used literature data and bacteriological data from the bioavailability (pus) obtained from 453 patients with acute and chronic osteomyelitis in our study when selecting infectious agents.

The following strains of Staphylococcus aureus were used to create a model for experimental acute osteomyelitis (TBO):

- Staphylococcus aureus 003994 / Wood-46 (gram-positive coccus, C-shaped, with golden pigment, typical enzyme-derived source - hemoculture);
- Staphylococcus aureus 003846/11 (gram-positive

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coccus, C-shaped, with a golden pigment; typical, isolated source of enzymatic properties - pus);
- Staphylococcus aureus 003851/2 (gram-positive coccus, C-shaped, with a golden pigment, typical enzyme-extracted source - pus);
- Staphylococcus aureus 003926 / M-4 (gram-negative coccus, C-shaped, with a golden pigment, typical enzyme-specific source - mucous throat);
- Staphylococcus aureus 004174 / MZ-85 (gram-negative coccus, C-shaped, with golden pigment, typical enzyme properties, isolated source - mucous in the nose).

Apparently, the selected staphylococci differed mainly by their isolated source. This was chosen in view of their increased pathogenicity.

Experiments on the formation of the experimental chronic osteomyelitis (ECO) model and the results of individual bacteriological studies were taken into account in the development of the experimental acute osteomyelitis (EAO) model.

Based on these microorganisms Association (2 strains) we should use:

- Staphylococcus aureus 003926 / M-4 (gram-negative coccus, C-shaped, with a golden pigment, typical enzyme-specific source - mucous throat);
- Pseudomonas aeruginosa 003480/237 (Gram-negative preparations, C-shaped, green pigment, typical enzyme-extracted source - pus).

We would like to thank the staff of the National Collection of Human Infections Research Institute for Epidemiology, Microbiology and Infectious Diseases of the Ministry of Health of the Republic of Uzbekistan for permission to use the collection strains for scientific work. All collection strains were stored in a freezer (40C) in semi-liquid environments.

All strains used were local strains isolated from patients with purulent inflammatory diseases in the country. The survey was completed in 2010-2014.

Statistical processing of the results was carried out with the use of a special program “Excel” for medical and biological tests on the Pentium-4 processor PC.

3. Results and Discussion

Prior to the development of planned experimental models, the collection strains were re-seeded and harvested in nutrient environments appropriate to their toxiconomical groups. Subsequently, all selected strains were identified based on morphological, tinktorial, cultural, enzymatic, toxicity and antigenicity properties study [2,3,5].

Colonies of Staphylococcus aureus were characterized by a typical, nutritious environment, with a smooth surface, a moist surface, and after 24 hours the color of the pigment. Additional microbiological tests examined urease, phosphatase, hyaluronidase, fibrinolytic activity. Staphylococcus aureus was characterized by high hemolytic activity, with the formation of α- and β-hemolysis in the food environment.

After pseudomonas aeruginosa cultivation in food environments, it acquired all the typical features of its taxonomic group: colonies formed a typical, green pigment, specific odor, lysis zones, and the phenomenon of the rainbow. The phenomenon of “rainbow shine” is a characteristic sign of the pathogenicity of Pseudomonas aeruginosa [2]. This means that the strains we select have high virulence.

Modeling of the AEO Solovyov M.M. [8] was conducted using the method proposed by him, based on the supplements we propose. Experimental animals were infected twice with selected microorganisms to form EAO model.

In the first infusion, a mixture of 5 strains of Staphylococcus aureus was used. In order to enhance the anti-inflammatory effect, they were grown separately in juice before use. Infection was performed in the thigh bone, taking into account the development of osteomyelitis in the bone marrow. To do this, the surgery site was completely cleaned from the wool 2-3 days before. After mice fixation was performed under local anesthesia in the projection of the upper jaw, the bones were opened and the upper layer was injured by surgical scalpel, with a daily culture of selected strains of 6x109 micrograms / ml (m.t. / ml) at the concentration.

The second infection was done on the 7th day, using only one strain - Staphylococcus aureus 003926 / M-4, which outperformed the other strains of Staphylococcus aureus due to its strong pathogenicity.

After both infusions, laboratory animals were surgically sutured to the skull.

Signs of EAO were visible in 3-4 days after infection with microorganisms. The results of these observations were complemented by a visual examination of the advanced bone marrow, bacterioscopic pus, morphological examination of the affected bone.

In this infected area there are visual and traumatic pus, inflammatory symptoms such as swelling, swelling of the affected foot, difficulty moving, temperature rise, clinical stiffness, low mobility, no appetite, slow manifestation of various conditioned and unconditional reflexes was characterized by morphological signs.

The symptoms revealed that the ECO had developed. Therefore, infective agents (microorganisms) have been selected correctly in the formation of the ECO model.

The method of Solovyov M.M. [8] was based on the supplements we proposed.

Prior to infection with microorganisms to trigger ECO, the upper portion of the thigh was treated with 0.1 ml of 3% acetic acid as a toxic, stressful and damaging factor. It was carefully inserted only on the bone. Acetic acid was administered only after the transfusion needle hit the hard part of the bone. After that, the infective material was sent to the infected area. We have tried to prevent this acid from getting into the blood vessels because it causes hemolysis of the red blood cells and the laboratory animal is killed.

We observed, for the same reason, the lethal outcome of

10% (n = 5) laboratory animals during the experiment.

After 4 days of acetic acid exposure, the anti-inflammatory factor was infected at a concentration of 6x109 m.t / mL with the Staphylococcus aureus 003926 / M-4 strain caused by EAO. On the 7th day of the infection, the Pseudomonas aeruginosa 003480/237 strain was placed in the primary oven at a concentration of 6x109 m./mL. This is a "problematic" microorganism that is characterized by natural antibiotic resistance, which is common in hospitals as a hospital strain, which causes chronic purulent inflammatory processes.

Both strains were extracted and standardized separately in nutritious juice to enhance the effectiveness of infestation. It was also taken into account the feasibility of urgent action to introduce the mass of microorganisms into white, non-breeding mice.

The reason for the use of various microorganisms for ECO formation is that they cause extensive antigen stimulation, leading to strong microbial sensitization in the body and providing a wide range of pathogenic factors. Advanced bacteriological studies have shown that the antagonistic effects of both strains on each other are insignificant.

Infection with microorganisms with different antigenicity and pathogenicity has resulted in prolonged prolonged bone maturation (15–30 days or more). All studies showed that ECO were formed in laboratory animals.

Our results showed that the following characteristics should be taken into account when selecting infective agents (microorganisms) for the formation of ECO and EAO models:

- making sure that the selected strains are bacteriologically identified and are in monoculture;
- Identification of pathogenicity and virulence characteristics in the identification of infective agents (microorganisms) before infestation of laboratory animals;
- The use of microorganisms strains in the EAO and the use of the microorganisms association (2 strains) in ECO process is chronic and the other pathogens are more likely to get into the furnace;
- ensuring that the concentration of microorganisms (infective agents) is not less than 6x109 mL / ml;
- attention should be paid to the type of microorganisms caused by an acute or chronic process, the degree of their virulence, time and concentration in the furnace.

4. Conclusions

1. Different, high-virulence strains of Staphylococcus aureus were injected into the affected area (thigh bone) at a rate of 6x109 m.t / ml for 7 days to form an experimental acute osteomyelitis model in experimental animals (white, non-breeding mice). All signs of purulent inflammation (experimental acute osteomyelitis) were manifested in the pathological source.

2. 2 different strains (Staphylococcus aureus and Pseudomonas aeruginosa) were selected to form the experimental model of chronic osteomyelitis. Since the 15th day of infusion, purulent inflammation has been observed in the bone marrow of the skull.

3. The strains of microorganisms (Staphylococcus aureus and Pseudomonas aeruginosa) used to induce experimental acute and chronic osteomyelitis have been fully identified and have high virulence.

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