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Simulation of experimental chronic osteomyelitis

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Abstract

Introduction. In the existing models of osteomyelitis, there is no unified scheme for creating a pathological focus. To obtain reliable comparative data from Introduction There is no unified scheme for creating a pathological site in the existing osteomyelitis models. The location, the size of the defect, the dosage of the infecting agent and the carrier material are to be standardized to facilitate reliable comparative data from different authors and reproducibility of the experimental model. **The objective** was to demonstrate experimental results of simulated chronic osteomyelitis using a unified scheme for creating a pathological site. **Material and methods** An identical defect was simulated in the proximal tibia metaepiphysis of 15 rabbits using a four-sided cone-shaped drill with a diameter of 0.5 cm and a limiter to a depth of 0.5 cm. An allobone fragment impregnated with *Staphylococcus aureus* suspension with a microbial cell concentration of 1.0×10^8 CFU/mL was placed into the defect site. A part of sutures was removed from the middle third of the wound and the edges were diluted to initiate a fistula course at 3 postoperative days. An experimental model of chronic osteomyelitis was developed using unified parameters of location, defect size, dosage of the infecting agent and carrier material. The method was technically simple, required no additional infection and provided a chronic osteomyelitic process. Observation period was 21 days. The control of the model formation was produced through clinical observation, inflammatory changes in the peripheral blood, bacteriological, radiological and pathomorphological examinations. **Results** Postoperatively, the animals demonstrated a decreased physical activity, increased body temperature, impaired function of the operated limb, a non-healing fistula with an abundant purulent discharge of curd consistency formed at the site of the postoperative wound. Computed tomography showed a cavity with irregular sclerotic edges filled with multiple bone sequesters, edema of adjacent soft tissues and fistula at 21 postoperative days. Leukocytosis was observed in the peripheral blood. Bacteriological examination of the wound discharge showed growth of *Staphylococcus aureus*. Pathomorphological investigation indicated chronic osteomyelitis with bone defects in the proximal metaepiphysis of the tibia and necrotic areas, pronounced leukocyte infiltration, fragments of dissolving bone tissue, growth of connective tissue surrounding foci of chronic purulent inflammation. An experimental model of chronic osteomyelitis was developed using unified location parameters, defect size, dosing of the infecting agent and carrier material. The method was technically simple, required no additional infection and facilitated formation of a chronic osteomyelitic process for 21 days. **Discussion** We used allobone in our model to cause infection by impregnation of microbial suspension without additional removal of the carrier. The amount of infecting suspension to initiate osteomyelitic process to be absorbed by the allobone and avoid the death of the animal from septic complications was determined in the course of the study. For passive drainage of the wound, a fistula course was provided and its functioning maintained, with the osteomyelitic focus localized and survival of animals ensured throughout the experiment. **Conclusions** An experimental model of chronic osteomyelitis was demonstrated using a unified scheme for a pathological focus. The model allowed us to avoid generalization of the osteomyelitic process, ensure the survival of animals throughout the experiment and simulate the process being consistent with pathomorphological changes characteristic of human chronic osteomyelitis.

Keywords: chronic osteomyelitis, osteomyelitic defects, experimental model

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INTRODUCTION

The incidence of chronic osteomyelitis is reported to be as high as 10 % to 25 % among musculoskeletal diseases [1–4]. Poor results of treatment and recurrences can reach 40 % [5–7] with the incidence of disability in the patients exceeding 50 % [8, 9]. Improving surgical treatment of osteomyelitis requires preclinical experimental studies. The known models are labor-intensive and involve two stages, additional infection and repeated surgery which increases the risk of septic complications and death of the animal [10, 11]. The amount of the infectious agent varies over a wide range; exposure time [12, 13] and the method of introducing the infectious mixture onto the carrier are not considered [14, 15]. The simulated defects have different shapes and sizes that can interfere with the results of the experiment. The materials used as an infectious carrier include agar-agar solution [14], lavsan tissue [16], sterile quartz sand [17], physiological saline solution [18, 19], metal pin [20] and bone cement [21]. The location, the size of the defect, the dosage of the infecting agent and the carrier material are to be standardized to facilitate reliable comparative data from different authors and reproducibility of the experimental model.

The objective was to demonstrate experimental results of simulated chronic osteomyelitis using a unified scheme for creating a pathological site.

MATERIAL AND METHODS

The experiment was produced in the vivarium of the Federal State Budgetary Educational Institution of Higher Education "PIMU" certified by the State Veterinary Service of the Nizhny Novgorod Region as specified in the order of the Ministry of Health and Social Development of the Russian Federation No. 708n dated August 23, 2010 "On approval of the rules of laboratory practice", in accordance with the "European Convention for the Protection of Vertebrate Animals Used for Experiments" or for other scientific purposes" (Strasbourg, 18.05.2014) and equipped under the "Specific pathogen free" (SPF) category to maintain conventional animals of purity class 2 (MD).

The model was simulated with a 1gram allograft bone placed in a solution of a microbial suspension with a density of 1 g/mL for 15 minutes and a concentration of microbial cells of 6.0×10^8 CFU/mL. Control weighing (electronic balance DX-200WP) showed the absorption of 0.01 gram of a microbial suspension solution with a microbial cell concentration of 1.5×10^8 CFU/ML by allograft bone confirmed by bacteriological cultures using the method of serial dilutions. The allograft bone with the impregnated microbial suspension was frozen at -70 ˚C prior to application. The content of microorganisms was maintained at the control measurement before experimental use.

Surgery was performed under combined anesthesia in the supine position of the animals. Access was made on the medial surface of the proximal metaepiphysis of the tibia. An identical bone defect was simulated with a 0.5 cm tetrahedral cone-shaped drill and a depth limiter of 0.5 cm. The allograft bone infected with a microbial suspension matching the size of the defect was placed in the defect zone and the wound was sutured layer-by-layer. The sutures were partially removed from the middle third of the wound with the edges diverged to initiate a fistulous tract after 3 days of surgery. The specimen were taken for bacteriological examination after 7 days. Animals were withdrawn from the experiment by air embolism after 21 days of the experiment and computed tomography was performed to assess the formation of an osteomyelitic focus. The specimen obtained were fixed in 10 % formalin and decalcified in a special decalcifying solution (Biodec) at a temperature of 37 ºC for 24 hours. Then the experimental material was subjected to dehydration in alcohols of increasing concentration starting from 70 % alcohol and up to absolute. The specimen were embedded in paraffin after being placed in xylene. Standard histological examination was produced using *Thermo Scientific Excelsior ES* Tissue Processor. Paraffin blocks were made using *Thermo Fisher Scientific HistoStar* Embedding Workstation. Serial sections 4–6 microns thick were obtained with *Thermo Scientific Microm HM* 325 Rotary Microtome. Sections were stained with hematoxylin and eosin. Leica DM 2500 microscope, ×5, ×10, ×20, ×40, ×100 objective, and ×10 eyepiece were used for morphological evaluation of the experimental specimen. Hemoglobin, erythrocyte and leukocyte levels were measured on the first and 21st days of the experiment to determine the systemic reaction of the body. The results of the experiment were recorded using MS Office Excel spreadsheets (2007) and processed using medstatistic.ru software. Paired Student's t-test was used for related populations at a significance level of $p < 0.05$.

RESULTS

The animals showed decreased physical activity, increased body temperature and a dysfunction of the operated limb after 3 postoperative days. A moderate hemorrhagic discharge was seen 3 days after removal of some sutures with divergence of the edges of the postoperative wound and a purulent discharge of a liquid or curdled consistency observed after 7–10 days. A non-healing fistulous tract formed after 14 days of the experiment. The fistulous tract continued to function after 21 days of the experiment, the supportability of the operated limb restored, appetite improved and body temperature returned to normal.

CT scans performed for the proximal metaepiphysis of the tibia of the animals after 21 days of surgery showed a cavity with uneven sclerotic edges filled with multiple bone sequesters, edema of the adjacent soft tissues and a fistulous tract (Fig. 1).

A statistically insignificant decrease in hemoglobin and erythrocytes levels was noted in animals that demonstrated the stable condition. The level of leukocytes increased from $7.673 \pm 1.095 \times 10^9$/L to $9.667 \pm 1.793 \times 10^9$/L ($p = 0.003$) which was interpreted as an indicator of a local inflammatory process (Fig. 2).

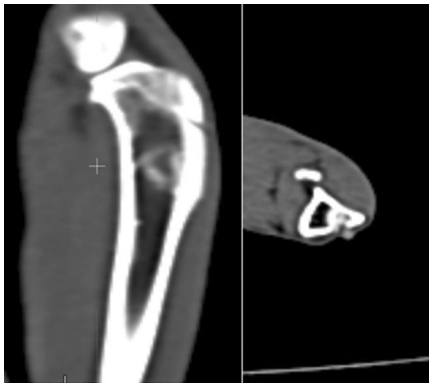

Fig. 1 Computed tomography of the osteomyelitic focus in the proximal metaepiphysis of the right tibia produced after 21 days of surgery

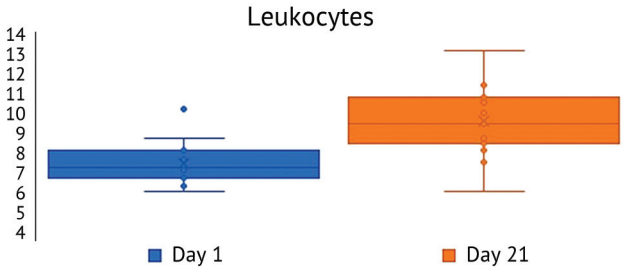

Fig. 2 Serum leukocyte level on the first (10^9/l) and 21st days of the experiment

Bacteriological examination indicated the growth of Staphylococcus aureus detected in all rabbits. Macroscopic study of the experimental specimen demonstrated the samples of the proximal metaepiphysis of the tibia represented by bone-soft tissue fragments measuring 2 × 2 cm with rounded defects 0.5 × 0.5 cm filled with purulent discharge and foci of soft bone in the center. Brown areas presumably hemorrhages were visualized along the periphery of the bone defect (Fig. 3).

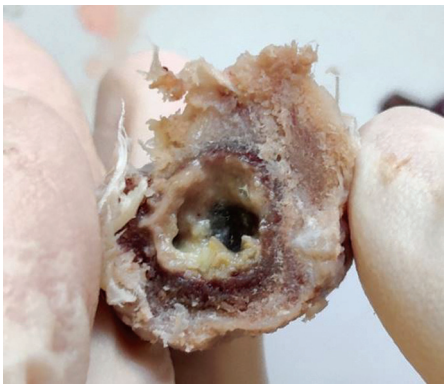

Fig. 3 Osteomyelitic focus of the tibial metaepiphysis

Microscopic examination revealed bone defects of the proximal metaepiphysis of the tibia with areas of necrosis, severe leukocyte infiltration, fragments of melting bone tissue, proliferation of connective tissue surrounding the foci of chronic purulent inflammation which indicated chronic osteomyelitis (Fig. 4).

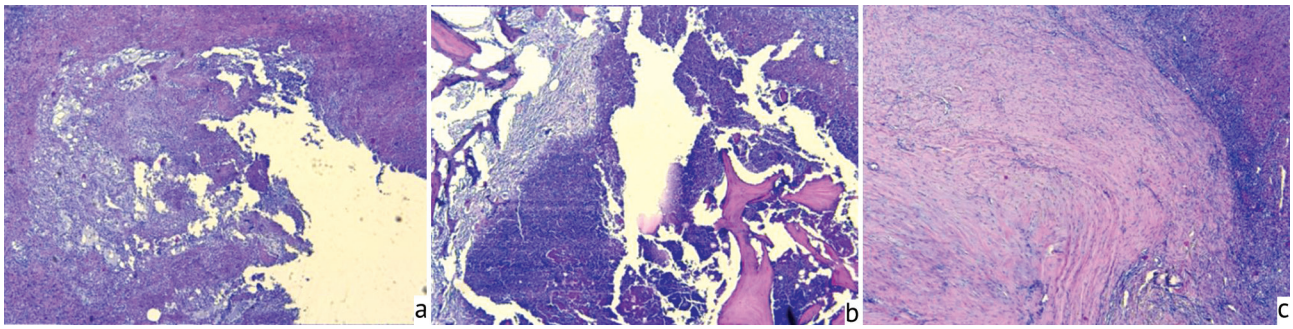

Fig. 4 (a) leukocyte infiltration of the bone defect and necrotic areas; (b) fragments of melting bone tissue; (c) proliferation of connective tissue surrounding the focus of chronic purulent inflammation. Stained with hematoxylin and eosin, ×20

DISCUSSION

A unified scheme for creating a pathological focus that required no additional infection, repeated surgical interventions, removal of the carrier to initiate the osteomyelitic process in all animals was used for developing a model of chronic osteomyelitis. We do not share the point of view of the authors of the patent [10] who suggested that preliminary sensibilization was needed for the development of the osteomyelitic process in all animals. We share the opinion of other authors [14] and believe that sensibilization increases the risk of generalization of the osteomyelitic process and can lead to the death of the animal.

The method of introducing the infecting agent and the material of the carrier are important for the development of an osteomyelitic model. The use of physiological saline solution as an infectious carrier and injection of the suspension results in extensive abscesses, fistulas of soft tissues, sepsis and death of animals [11]. Additional filling is used to localize the infectious suspension at the injection site with greater risk of generalization of the osteomyelitis in the absence of wound drainage [14].

An injection version of infection to develop an osteomyelitic focus in the distal metaepiphysis of the femur was offered by the authors of another patent [13]. The method we used led to death of 3 out of 5 rabbits due to septic complications. We rejected the model and changed the localization of the focus to the anterior medial surface of the proximal tibial metaepiphysis that allowed us to avoid complications. The use of viscous substances, gelatin, agar-agar as a carrier could help restrict the spread of the infectious suspension into the surrounding tissues [10, 14]. The use of such carrier materials that require additional intervention for removal (sterile quartz sand, bone cement, lavsan thread) can be inappropriate. We used allograft bone for our model as the most physiological material to induce local infection by impregnating a microbial suspension without additional removal of the host.

We simulated a fistulous tract for passive drainage of the wound 3 days after surgery and maintained its functioning during 21 days to provide localization of the osteomyelitic focus. This was practical to avoid local,

systemic complications and ensured the survival of animals throughout the entire period of the experiment. We determined the amount of infectious suspension absorbed by the allograft bone which was necessary for the development of the osteomyelitic process and to avoid the death of the animal from septic complications. This allowed for avoiding antibiotic therapy for the rabbits which is a common cause of death resulting from dysbacteriosis and dysfunction of the gastrointestinal tract. Chronic osteomyelitis simulated in earlier models occurred at 8 weeks [12], 6 weeks [11, 13, 18, 19], 30 and 31 days [14, 15]. The model offered allowed for a focus of chronic osteomyelitis develop and was confirmed by histological examination within 21 days reducing material costs and reducing the duration of the experiment.

CONCLUSION

An experimental model of chronic osteomyelitis developed with a unified scheme for creating a pathological focus was demonstrated [22]. A standard cone-shaped defect 0.5 cm in diameter and 0.5 cm deep was simulated in the proximal metaepiphysis of the tibia of the animals and filled with allograft impregnated with a suspension of *Staphylococcus aureus* with a concentration of microbial cells of 1.0×10^8 CFU/mL.

The method offered allowed us to avoid local and systemic complications of the osteomyelitic process, ensure the survival of animals throughout the experiment, reduce the model formation time to 21 days and simulate the process corresponding to pathomorphological changes characteristic of humans. The method was technically simple, easily reproducible, reducing material costs and the timing of experiments aimed at improving methods of treating osteomyelitis. Therefore, we could achieve our goal by demonstrating the results of an experimental model of chronic osteomyelitis simulated using unified parameters of localization, defect size, dosing of the infecting agent and carrier material to facilitate reliable and comparable results.

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