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# Radiological Assessment of an Experimental Model of Human Osteomyelitis in Rabbits

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## Abstract

The purpose of this paper is to present a radiological assessment of the treatment performed on a model of human osteomyelitis induced in rabbits. In order to induce osteomyelitis in rabbits, a human strain of Staphylococcus aureus was injected in bone defects created in the rabbit tibia, a quantity of 0.2 ml being administered in each defect. We have created five groups of animals (positive control, negative control and treatment - 3 groups), the treatment groups receiving treatment in different phases of the disease (acute and chronic). The treatment consisted of copper and silver sub-millimeter-particles introduced in the same place with the Staphylococcus solution. Evaluation of installation and evolution of the disease was made by clinical, hematological, microbiological, radiological and histological monitoring. A separate study of radiological data is presented here. Radiological monitoring was done dynamically using a computerized radiology unit. Analyses were performed and scored individually and independently by three radiologists and specialists by evaluating the following parameters: periosteal relieves, bone architecture deformation, widening of bone shaft, neo-formation tissue and soft tissue deformation. The results of radiological evaluation show a mitigation of osteomyelitis as a result of the treatment and confirm that radiological analysis is a powerful tool in experimental models of this disease. The study was conducted in compliance with the welfare standards for animals used for scientific purposes.

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Peer-review under responsibility of the University of Agronomic Sciences and Veterinary Medicine Bucharest *Keywords:* osteomyelitis; radiological evaluation; Staphylococcus aureus

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# 1. Introduction

Despite numerous attempts with antibiotic treatment of osteomyelitis and other methods, osteomyelitis continues to raise serious clinical problems, being one of the most important posttraumatic and postoperative complications (Lazzarini et al., 2005). There are also many attempts to prevent the onset of osteomyelitis especially in the case of surgical procedures (Stallmannet et al., 2004; Mangram et al., 1999). Using a model of osteomyelitis in the tibia of rabbits (Coman et al., 2013), a new type of treatment based on copper (Cu) and silver (Ag) particles used together has been evaluated. For this purpose we have created several experimental groups, three of them using the treatment the others being used as positive and negative control groups.

Installation and evaluation of the disease and treatment monitoring was made by clinical, microbiological, radiological and histological assessment.

The current article was concentrated on a detailed assessment of radiological data. Radiological analysis both in experimental models and in clinical evaluations is an important tool for diagnosing osteomyelitis (Odekerken et al., 2013; Pineda et al., 2009). Radiological analyzes were performed in dynamics in well-defined time interval. Interpretation of the results was performed by three radiologists specialists (2 veterinarians and a human) independents one of other, using an independent assessment scheme literature (Smeltzer et al., 1997).

#### 2. Materials and Methods

# 2.1. Animals

The study was done in compliance with national and international standards for the protection of animals used for scientific purposes, and the project was approved by the ethics committee of the Cantacuzino Institute, Bucharest. The experiment was conducted in Baneasa Animal Facility, Cantacuzino Institute, Bucharest.

The animals that were used was New Zealand White rabbits (provided by Cantacuzino Institute, Bucharest), male and female. The rabbit mean age at the beginning of the experiment was 7 months and average individual weight was 2300 g. Rabbits were maintained in a temperature-controlled accommodation (16-210C) and controlled relative humidity (45-65%). Space has environmental conditions provided by a computerized system of air conditioning; installation ensures 15-20 air changes / hour. The food (provided by Cantacuzino Institute, Bucharest) and water were provided ad libitum throughout the experiment period. Accommodation was done in specific cages, 2-3 rabbits in cage.

## 2.2. Inoculum preparation

The microbial strain used in our study was *Staphylococcus aureus* ATCC 6538. In order to revitalize the bacterial culture preserved in our collection, after rehydration of the strain in nutritive broth (Biokar Diagnosis, Noack Romania SRL), three passages were made in the same media at 18-24 hours interval. The bacterial culture was then incubated at  $35-37^{\circ}$ C and a concentration of  $5x10^{6}$  Colony Forming Units (CFU)/ml was obtained from the last passage.

# 2.3. Treatment

The two different treatments consisted of two silver and four copper particles per one bone defect used together (treatment particles), in one treatment and physiological saline solution containing silver and copper ions (treatment solution), in the other. The particle diameters were in the 400-500 microns range and the shape was spherical.

# 2.4. The animals groups

In this study we used 74 rabbits divided into five groups as follows:

- Group 1 3 rabbits only created bone defect;
- Group 2 12 rabbits created bone defect and induction of osteomyelitis;

- Group 3 12 rabbits created bone defect, osteomyelitis induction and treatment in same time with induction of disease;
- Group 4 41 rabbits bone defect created, osteomyelitis induced; treatment given at 30 days after bacterial inoculation (Group 4.1. treatment particles and Group 4.2 treatment solution). Group 4.3 received treatment at 60 days after bacterial inoculation, particle treatment;
- Group 5 6 rabbits created bone defect and placed the particles used as treatment.

## 2.5. The surgical procedure

The surgical procedure was undertaken as follows: at all rabbits under total anesthesia (35 mg/kg ketamine + 7 mg/kg xylazine, IM) and aseptic conditions, inoculation the left tibia was approached for inoculation and right tibia remains the control. Contention supine, the proximal third of the tibia of the left leg was prepared for surgical intervention with local cutting and betadine disinfection. At all groups the surgical procedure was identical: the skin on the antero-medial shaft of the tibia was incised directly down to the bone, exposing 3-4 cm. Two bone defects were then drilled perpendicularly at 5-10 mm distance one from the other, starting at 5 mm distance from the femoral-tibial-patellar joint to the medullar cavity. For drilling was used a medical drill (Makita Inc.) using a 1.1 mm drilling pin. Induction of osteomyelitis was obtained by inoculation of Staphylococcus aureus solution (0.2 ml/ hole) using a 27G needle, followed by introduction of 1 mm diameter cotton mesh balls (previously held in the solution of Staphylococcus aureus). The cotton meshes were made using two 10 cm cotton threads (detached from standard medicinal cotton mesh) rolled together into a small ball. Cotton meshes were introduced with pointed tweezers. The treatment was inserted into each hole using a trocar (the particles) together with cotton mesh (Group 3) or after removing the cotton mesh (Groups 4). In Group 4 rabbits, the treatment was introduced as follows: the rabbits were totally anesthetized, the wound was opened, the cotton mesh was removed and the treatment was placed. In all rabbits and all interventions skin defect was sutured with the tissue adhesive (Surgibond) and over suture was applied a protective layer of aluminum (Aluspray).

After each surgery, analgesics (Ketofen - 3 mg / kg SC) were given for 3 days.

## 2.6. Radiological analysis

Animal monitoring was undertaken in dynamics, using standard clinical (clinical condition, weight, temperature, hematology) microbiological (identification strain of *Staphylococcus aureus*), histological and radiological assessments. The study lasted 90 days, and in each group, a fixed number of animals were euthanized following a pre-set protocol.

Euthanasia was done by an overdose of anesthetic.

Radiological monitoring was done by examination of both tibia of the rabbits using a computerized radiology unit consisting of a high frequency generator and radiogenic tube Toshiba giving maximum quality and radiation dose to very low shutter speeds, the parameters being kept constant at all examinations. The soft X-ray apparatus is integrated with 605 preset programs depending on the species, size, organs examined, dedicated veterinary practice and a radiological system CR (Computed Radiography) for digitizing the acquired images.

The investigated tibias were coordinated by a specific processing algorithm: Frequency Tuning Multi-Algorithm (4VET clinic - Bucharest).

Monitoring was done at different time intervals as follows: Group 1 and Group 5: at 30 days' time interval; Group 2, 3 and 4 at 15 days' time interval.

The radiological data were interpreted by three doctors, radiology specialists, independently of each other.

The following parameters were evaluated: periosteal relieves, bone architecture deformation, widening of bone shaft, neo-formation tissue and soft tissue deformation.

### 3. Results and Discussions

A summary of the opinions of the three specialists is presented in the following:

No pathological change was observed in Group 1 and Group 5 at any investigation time;

- In group 2 (inoculated, not treated), installation of osteomyelitis specific changes were observed since day 15, the lesions being present up to the end of experiment (90 days); (Table 1 and Figure 1);
- Group 3 (inoculated and treated in the same time) installation of osteomyelitis specific changes were observed since day 15, but at a lower intensity than in group 2, and the lesions diminished as time passed.

In addition, the percentage of animals presenting bone tissue neo-formation was high (Table 1 and Figure 2).

Lesions	Group/day	15	30	45	60	75	90
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Periosteal relieves	2	60	60	60	75	75	66
	3	65	54	45	6	0	0
Bone architecture deformation	2	20	40	40	50	47	47
	3	23	23	24	0	0	0
Widening of bone shaft	2	0	0	20	20	33	50
	3	0	17	14	0	0	0
Neo-formation tissue	2	0	0	0	0	16	16
	3	0	13	43	50	50	50
Soft tissue deformation	2	75	54	50	50	30	16
	3	66	44	38	17	33	33

Table 1. Average lesions found in the analysis of groups 2 and 3 (in %)



Figure 1. Radiological images in the dynamic from a rabbit of group 2



Figure 2. Radiological images in the dynamic from a rabbit of group 3

Group 4 rabbits presented osteomyelitis characteristic lesions until application of treatment. Mitigation of lesions and an increase of the percentage of animals presenting neo-formation tissue were observed in time (Table 2 and Figures 3, 4, 5).

Lesions	Group/day	15	30	37	45	60	75	90
Periosteal relieves	4.1.	54	45	15	8	11	-	-
	4.2.	45	45	4	0	6	-	-
	4.3.	54	51	-	45	45	0	0
Bone architecture deformation	4.1.	22	14	16	22	11	-	-
	4.2.	19	18	8	13	0	-	-
	4.3.	14	22	-	19	19	11	11
Widening of bone shaft	4.1.	0	4	16	16	0	-	-
	4.2.	0	4	4	0	0	-	-
	4.3.	0	4	-	7	7	11	12
Neo-formation tissue	4.1.	0	0	4	54	56	-	-
	4.2.	0	0	0	43	50	-	-
	4.3.	0	0	-	0	4	44	75
Soft tissue deformation	4.1.	45	52	47	42	0	-	-
	4.2.	54	54	34	38	17	-	-
	4.3.	62	57	-	15	24	22	17

Table 2. Average lesions found in the analysis of groups 4 (in %)



Figure 3. Radiological images in the dynamic from a rabbit of group 4.1.



Figure 4. Radiological images in the dynamic from a rabbit of group 4.2.



Figure 5. Radiological images in the dynamic from a rabbit of group 4.3

Radiological investigation is an effective tool in evaluation of the treatment of osteomyelitis (Odekerken et al., 2014). In our study, radiological analysis revealed that our treatment did not cause toxicity or bone damage (Group 5). In the animal group where the disease was induced but no treatment applied, the lesions sharply increased in severity. In contrast, it was clearly observed that in the treated groups, regardless of time of treatment application (with or after disease induction) the lesions declined. Also, the radiological investigations evidenced the growth of neo-formation tissue in the treated rabbits. The radiological results were confirmed by other analysis including histopathology (which will be published).

# 4. Conclusions

Osteomyelitis is still an important health problem despite numerous attempts of finding a cure. Therefore the analysis of a possible new treatment using animal models is welcome.

Both induction and treatment of osteomyelitis were clearly evidenced by radiological investigations.

The radiological analysis showed efficacy of our proposed treatment in all treatment stages.

The study confirms that radiology is a powerful instrument for the evaluation of osteomyelitis at different evolutional stages.

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