DEVELOPMENT OF AN EXPERIMENTAL MODEL OF INFECTED BONE VOID IN THE ULNA OF RABBITS

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ABSTRACT

Objective: Develop a model that allowed the study of bone regeneration in infection conditions. Method: A 15 mm defect was surgically created in the rabbit ulna and inoculated with $5x10^8$ colony-forming units (CFU) of S. aureus. Surgical debridement was performed two weeks after and systemic gentamicin was administered for four weeks. Animals were followed up to 12 weeks to evaluate infection control and bone regeneration. Result: Bone regeneration was inferior to 25% of the defect in radiological and histological analysis. Conclusion: Infected bone defect of 15 mm in the rabbit ulna was unable to achieve full regeneration without further treatment. ***Level of Evidence V, Experimental Study.***

Keywords: Bone diseases. Infectious. Bone regeneration. Rabbits.

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INTRODUCTION

The loss of large bone segments remains one of the most challenging problems in orthopedic surgery,[1,2] and is frequently associated with infection.[2-5] Experimental models are useful for assessing the efficacy of new treatments, and should be reproducible, well controlled and afford the application of standardized methods of analysis.[6]

Evans, Nelson and Harrison[7] described a method for creating experimental infection in rabbit forearms, which has become one of the most important for studying osteomyelitis. However, in this model infection produces alterations in the bone architecture such as osteolysis, sequestration, sclerosis and diaphyseal enlargement,[7,8] which render it unsuitable for analyzing regeneration. The aim of this study was to develop a model of infected bone gap/defect that would allow the adequate evaluation of bone regeneration.

MATERIAL AND METHODS

Staphylococcus aureus inoculum

Samples of the selected strain were seeded in blood agar and left in the incubator at a temperature of 35ºC for 24 hours. After this period the material was diluted in sterile saline until it reached the turbidity corresponding to 10^{10} CFU/ml. A sample of the solution was then diluted to 10^4 and read in the Neubauer chamber (Herka Intercolor), confirming the concentration, and seeded in CLED agar to test the viability of the inoculum.

Experimental model

The study was approved by the ethics committee in animal experimentation of Faculdade de Medicina de Ribeirão Preto da Universidade de São Paulo, and twelve female rabbits weighing between 2.5 and 3.5kg were used. The animals were anesthetized using ketamine hydrochloride (40mg/Kg) and xylazine (8mg/Kg) applied via intramuscular route, followed by continuous venous infusion of 1% ketamine. The right ulna was exposed surgically and a 15mm bone segment from the distal third was resected without the periosteum using a bone saw. After this the segment was placed back on the defect and 0.05 cc of the inoculum ($5x10^8$ CFU) was introduced into the medullary canal using a 0.3 cc syringe. The periosteum and skin were sutured closed and analgesia with ketoprofen (2/mg/Kg IM) was performed for three days after each surgical procedure. Two weeks later the wound was debrided, the bone fragment collected for culturing, all the necrotic tissues removed and the wound cleaned with 40ml of 0.9% sterile saline solution. Two animals were euthanized soon after the debridement, and the others received 6mg/Kg/day IM gentamicin for four weeks.

All the authors declare that there is no potential conflict of interest referring to this article.

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The bone fragment collected in debridement was cultivated on blood agar and tested for antimicrobial sensitivity (Walkaway-96, Baxter Diagnostics Inc.).
Administration of fluorochromes was performed in two animals, alizarin (10mg/Kg) four weeks after debridement, calcein (10mg/Kg) at eight weeks and oxytetracycline (10mg/Kg) 48 hours before euthanasia.
Radiographs were taken after debridement and at 4 and 8 weeks. The animals were euthanized after 12 weeks with sodium thiopental (50mg/Kg IV) and the piece was submitted to radiography with high resolution film (Kodak Insight®).

Radiographic and histological analysis

The infection was evaluated radiographically according to the degree of destruction of the bone architecture and of the periosteum[9] and regeneration based on the size of the bone defect and on the void filling percentage.
For histology the region of the bone void was decalcified with 7.5% nitric acid, soaked in paraffin and sectioned lengthwise. Hematoxylin-eosin and Gram were used for staining.[10,11] The samples with fluorochromes were submitted to dehydration in increasing gradations of alcohol and then were embedded in acrylic resin. The cross-sections were stained with a mixture of methylene blue, azure II and pararosaniline or left unstained for fluorescence.[12] The histological analysis used the scale described by Ambrose et al.[11]

Statistics

SPSS 15.0 software was used for data analysis with significance level of $p = 0.05$.

RESULTS

Eleven wounds (92%) had clinical signs of infection at the time of debridement. The average size of the bone defect measured in the intraoperative period, increased from 15mm before the inoculum to 16 mm after debridement ($p = 0.01$). Two animals died during the follow-up of causes not related to the study. After 12 weeks all the wounds were healed and without signs of infection. *S. aureus* was the only agent isolated in the samples analyzed. Periosteal reaction was observed on the borders of the bone void in 58% of the animals in the initial radiographs, considered secondary to surgical handling and not to infection. No radiographic alterations related to infection were observed at the end of study. The average length of the bone defect decreased from 16mm after debridement to 11mm at the end of the follow-up. Union (defined as bone regeneration above 25% of the defect) occurred in only one fourth of the bone voids. The mean percentage of gap filling rose from 3% in the fourth week after debridement to 6% after 12 weeks. (Figure 1)
The presence of bacteria was not observed in the histological control, yet signs of intraosseus inflammation were identified according to Table 1. After 12 weeks the percentage of void filling was below 25%, with most of the regenerate material originating from the periosteum of the intraosseus membrane. The fluorescence showed that bone deposition occurred predominantly during the first four weeks, and was minimal at 12 weeks. (Figure 2)

DISCUSSION

Rabbit ulna segmental bone defect is a well-established model in the study of bone regeneration.[13-19] Inocula ranging between 10^5-10^8 in a segment of devitalized bone produce infection rates

Table 1. *Results of the histological analysis.*

Histological grading scale		
Categories	Debridement	12 weeks
Presence of intraosseous bacteria		
Yes	100%	0
No	0	100%
Intraosseous inflammation		
Severe	100%	50%
Moderate		25%
Mild		25%
None		0
Bone Neoformation		
Minimal < 25%	100%	38%
Mild 25-50%		38%
Moderate 50-75%		24%
Total 75-100%		0

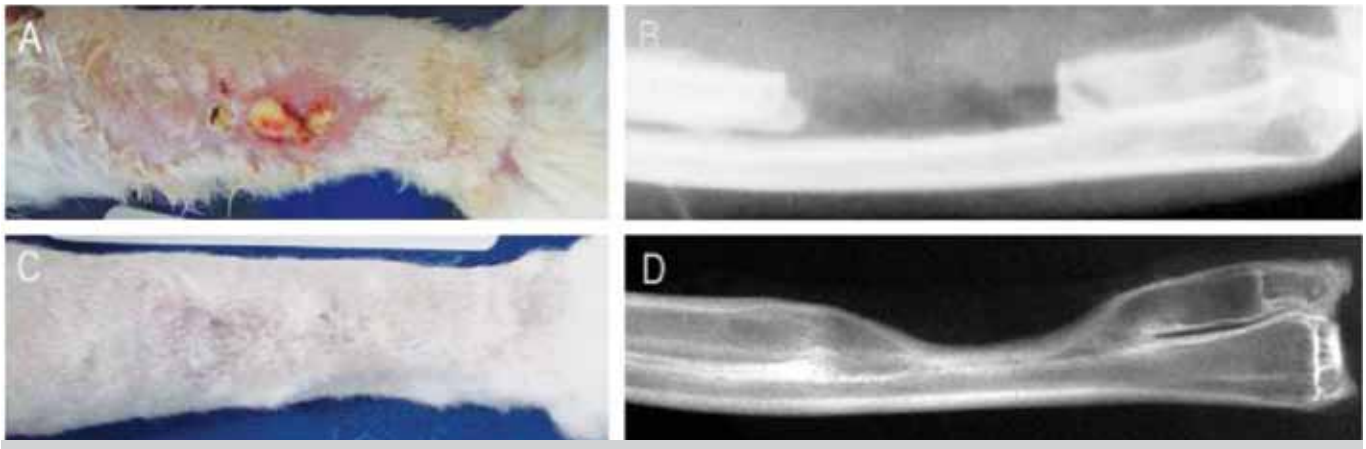

Figure 1. *(A) – Wound before the debridement. (B) – Bone void after debridement. (C) – Wound at 12 weeks (D) – Incomplete regeneration of the bone void at 12 weeks.*

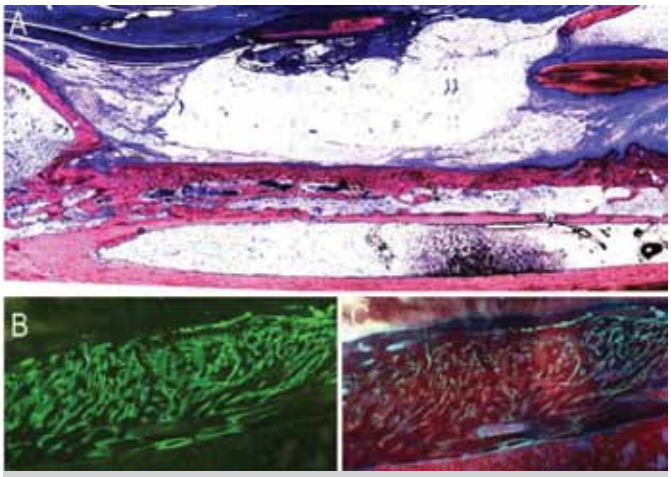

Figure 2. *(A) – Longitudinal section showing unfilled bone void and predominant deposition along the radial border (methylene blue, azure II, pararosaniline) bone 2.5x. (B) – Bone regeneration in the 4th week (alizarin) 2.5 x. (C) – Bone regeneration in the 12th (oxytetracycline) 2.5 x.*

above 90%, with the time to debridement ranging from two to four weeks.[7,8,11] Longer periods are associated with intense destruction of the bone architecture and disorganized periosteal reaction, with the infection extending through the bone,[7,8,11,20,21] hindering the radiographic and histological evaluation of regeneration.

In this study infection was obtained in 100% of the animals without important destruction of the bone architecture after two weeks. With debridement there was an increase in the bone void and four weeks of systemic antibiotics were sufficient to control the infection, with no signs of recurrence during follow-up. After 12 weeks the bone regeneration of the void was below 25% both in the radiologic and histological evaluation.

CONCLUSION

An infected bone void of 15 millimeters in rabbit ulna can be considered a "critical void" since spontaneous regeneration is not achieved. This model proved to be predictable and well controlled, and as such, appropriate for studying bone regeneration under infection conditions.

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