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Original research

Effect of rifampin in combination with allogeneic, alloplastic, and heterogenous bone grafts on bone regeneration in rat tibial bone defects

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ABSTRACT

Purpose: The aim of this study was to evaluate the efficacy of rifampin with allogeneic, alloplastic, and heterogeneous bone graft substitutes on osteogenesis of experimentally created bone defects in rat tibias.
Materials and methods: Twenty-eight male Wistar albino rats were used in this study. In each animal, two bone defects (10 mm length × 3 mm width × 2 mm depth) were created in the left and right tibias, respectively. The animals were divided into four groups. In Group 1, the right defects were irrigated with rifampin alone, and the left defects were irrigated with sterile saline alone. In Group 2, the right defects were filled with rifampin and allogeneic bone graft, and the left defects were filled with allogeneic bone graft alone. In Group 3, the right defects were treated with rifampin and alloplastic bone graft, and the left defects were filled with alloplastic bone graft alone. In Group 4, the right defects were filled with rifampin and heterogeneous bone graft, and the left defects were filled with heterogeneous bone graft alone.
Results: The animals were sacrified on the 21st postoperative day. Histopathological analysis of samples was performed to evaluate the process of bone regeneration and the presence of spongeous bone, cortex bone, and bone marrow. Bone union ($p = 0.023$) and spongeous bone ($p = 0.030$) values were higher in Group 1A (rifampin alone) than those in Group 1B (saline alone). Bone union ($p < 0.001$) and spongeous bone ($p < 0.001$) values in Group 2B (allograft + saline) were higher than those in Group 2A (allograft + rifampin). These differences were statistically significant.
Conclusions: Topical rifampin can accelerate the bone repair process, but the combination of rifampin and allogeneic bone grafts can also reduce new bone formation in osseous defects. Further studies involving long-term follow-up with a larger number of cases and different antibiotic agents should be conducted. These will provide additional data regarding new bone formation, especially in contaminated bone defects, resulting from use of antibiotic-supplemented bone grafts.

1. Introduction

Bone grafts are widely used for the repair, restoration and reconstruction of bone defects in oral and maxillofacial surgery. The following three processes were defined by Garg in 1999 to be associated with the fate of bone grafts: osteogenesis, osteoinduction, and osteoconduction. Osteogenesis is formation of bone, osteoinduction is the process by which osteogenesis is induced, and osteoconduction is a physiologic process whereby a conductor provides a physical matrix for deposition of new bone tissue [1]. Among the wide range of bone-graft materials, autogenous bone is widely accepted as the gold standard. Although autogenous bone is the best material for use in grafts, many substitutes for bone tissue have been proposed to reduce or replace the harvesting of autogenous bone, various types of allografts and alloplasts (natural or synthetic bone substitutes) have been developed. Bone grafting materials derived from human, bovine, and synthetic sources represent a viable alternative [2]. Recently, many additional substitutes (antibiotics, platelet-rich plasma, platelet-rich plasma, etc.) combined with graft materials for this purpose have been proposed to prevent infection of the surgical area and promote the effectiveness of the bone graft [3–6].

☆ AsianAOMS: Asian Association of Oral and Maxillofacial Surgeons; ASOMP: Asian Society of Oral and Maxillofacial Pathology; JSOP: Japanese Society of Oral Pathology; JSOMS: Japanese Society of Oral and Maxillofacial Surgeons; JSOM: Japanese Society of Oral Medicine; JAMI: Japanese Academy of Maxillofacial Implants.

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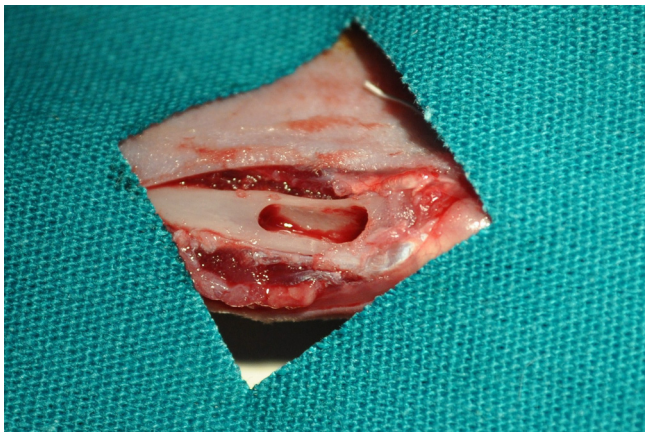

Fig. 1. Clinical view of the surgical prepared rat tibial bone defect.

Rifamycins are semisynthetic macrocyclic antibiotics derived from natural rifamycin B. They exhibit broad-spectrum bactericidal activity against gram-positive and gram-negative microorganisms and are thus frequently preferred for treatment of infected surgical or traumatic cutaneous wounds [7]. It is a cornerstone of the treatment of staphylococcal osteoarticular infections, particularly those of implanted material. Rifampin is a bactericidal antibiotic that diffuses very well within bone tissue and bacterial biofilms. The mechanism of action is inhibition of bacterial DNA transcription to mRNA independently from bacterial division, which results in activity against even dormant *Staphylococcus specific pair of primers* (spp.) organisms [8]. There are 2 important observations worth noting. First, rifampin use does not seem antagonistic to other antibiotics in human studies. Second, although rifampin seems to be well tolerated in most patients with *Staphylococcus aureus* infections, some degree of intolerance occurs. Rifampin therapy may be reasonable in infections in which cure rates are not high, assuming patients are at low risk for toxic effects from rifampin or significant drug–drug interactions (eg, with anticoagulants and immunosuppressive medications [9]. This informations might facilitate determination of the appropriateness of rifampin for infections the etiologic agents of which are known, and might also be useful for determination of optimum dosage. The aim of this study was to evaluate the effect of this rifamycin antibiotic with different types of bone graft on the healing of experimentally created bone defects.

2. Materials and methods

This research was conducted with the approval of the Dicle University Animal Care and Use Ethical Committee (permit no: 2011-18), obtained from the Dicle University Center of Experimental Medical Research (DÜSAM). Twenty-eight male Wistar albino rats weighting 250–400 g were used. All animals were housed in metallic cages in a temperature – ($23 \pm 10\,^{\circ}C$) and humidity – (60–80%) controlled room under regular light and dark conditions. The animals were anesthetized using an intraperitoneal injection of 5 mg/kg ketamine (Ketalar®; Eczacıbaşı, Turkey) and xylazine HCl (Rompun®; Bayer, Turkey) into the gluteal region. The skin was shaved and scrubbed with an antiseptic solution (1% iodine). A 2-cm longitudinal incision was made along the frontal aspect of both tibias, and flaps were raised to expose the bone tissue. Standard bone defects (10 mm long, 3 mm deep, and 2 mm wide) involving the cortical and cancellous bone layers were created with a round dental bur at a low rpm under irrigation with sterile saline and suction (Fig. 1). The animals were divided into four experimental groups.

Fig. 2. β-Tricalcium phosphate hydrated with rifamycin.

Group 1: The right bone defects were irrigated with rifampin (Rif 1.5 mL/125 mg; Koçak Farma, Turkey) alone, and the left defects were irrigated with sterile saline alone.

Group 2: The right surgical defects were filled with rifampin and allogeneic bone graft (Raptos; Citagenix, Canada), and the left defects were filled with allogenic bone graft alone.

Group 3: The right defects were treated with rifampin and synthetic bone graft (β-tricalcium phosphate) (β-TCP) (4Bone; MIS, France) (Figs. 2 and 3a), and the left defects were filled with β-TCP alone (Fig. 3b).

Group 4: The right bone defects were filled with rifampin and heterogeneous bone graft (Bio-Oss^{R}; Geistlich Farma AG, Switzerland), and the left defects were filled with heterogeneous bone graft alone.

The periosteum was closed using 5-0 vicryl sutures (Ethicon®; Edinburgh, UK), while the skin flaps were closed using 5-0 nylon sutures (Ethicon®; Edinburgh, UK). Antibiotics (gentamicin, 0.05 mL/kg) were administered intramuscularly once daily for 5 days postoperatively. The rats were sacrified by an overdose of sodium thiopental (Pentothal®) to assess the bone healing response on the 21st postoperative day. A skin incision with a periosteal flap was used to expose the tibial bone, and the previously grafted site was widely excised. The samples were fixed in 10% buffered neutral formalin for 24 h and then decalcified in a formic acid–hydrochloride acid combination for 24 h. After rinsing with tap water, the samples were dehydrated in increasing concentrations of ethanol and embedded in paraffin. Tissue sections of 5- to 7-μm thickness were prepared in the transverse plane and stained using hematoxylin-eosin, Mallory-Azan, and Masson's trichrome staining methods [10]. The tissue sections were examined and imaged by means of a Nikon Eclipse E800 light microscope and Nikon Coolpix 4500 digital camera. The histopathological scoring system first reported by Heiple et al. and then modified by Lane and Sandhu was used for evaluation of bone union and determination of the spongeous, cortex, and bone marrow values of the experimentally created bone defect areas [11,12] (Table 1).

3. Results

The histological characteristics of each surgical bone cavity within each group are described. The bone regeneration process and presence of spongeous bone, cortex bone, and bone marrow were evaluated (Tables 2 and 3).