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RESEARCH ARTICLE

Scanning electron microscopy study of new bone formation following small and large defects preserved with xenografts supplemented with pamidronate—A pilot study in Fox-Hound dogs at 4 and 8 weeks

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ABSTRACT

The aim of the present study was to evaluate the feasibility of SEM and EDX microanalysis on evaluating the effect of porcine xenografts (MP3®) supplemented with pamidronate during socket healing. Mandibular second premolars (P2) and first molars (M1) were extracted from six Beagle dogs. P2 were categorized as small defects (SD) and M1 as large defects (LD). Four random groups were created: SC (small control defects with MP3®), ST (small test defects MP3® + pamidronate), LC (large control defects with MP3®), and LT (large test defects MP3® + pamidronate). At four and eight weeks of healing the samples were evaluated fisically through scanning electron microscopy (SEM), and chemical element mapping was carried out by Energy dispersive X-ray spectroscopy (EDX). After four weeks of healing, SEM and EDX analysis revealed more mineralized bone in ST and LT groups compared with control groups ($p < 0.05$). After eight weeks, Ca/P ratios were slightly higher for small defects (groups SC and ST); in SEM description, in both control and test groups, trabecular bone density was similar to the adjacent mineralized cortical bone. Within the limitations of this experimental study, SEM description and EDX elemental microanalysis have demonstrated to be useful techniques to assess bone remodelling of small and large defects. Both techniques show increased bone formation in test groups (MP3® modified with pamidronate) after four and eight weeks of healing.

1. Introduction

After dental extraction, the socket healing process involves a series of events that results in the loss of bone volume in both horizontal and vertical dimensions (Amler et al., 1960; Cardaropoli et al., 2003). Various techniques and methods have been proposed to overcome the natural resorptive process (Pietrokovski and Massler, 1967; Schropp et al., 2003; Araújo and Lindhe, 2005), and thus reduce the bone loss that accompanies natural healing (Heinemann et al., 2012; Vignoletti et al., 2012; Vittorini-Orgeas et al., 2013).

The scientific evidence does not provide clear guidelines regarding the type of biomaterial. The clinical success of bone substitute materials depends on their ability to support primary bone formation, regenerate mature bone and their chemical and physical properties (Pérez-Sánchez et al., 2010; Heinemann et al., 2015). In recent years, research has focused on improving bone substitutes and implant surfaces to achieve faster and better osseointegration by morphologic or biochemical modification. Biochemical modifications consist of the application of biologic mediators into the biomaterial, to improve bone quality and quantity (Gredes et al., 2015; Salomó-Coll et al., 2015).

One approach to minimizing bone loss could be to inhibit osteoclast action and consequently bone resorption. Bisphospho-

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nates are a group of drugs frequently used for the treatment of various bone diseases, including osteoporosis, malignant hypercalcemia, multiple myeloma, or Paget's disease (Benford et al., 2001; Montoya-Carralero et al., 2010). Experimental studies have reported that bisphosphonates directly inhibit bone resorption, promoting apoptosis of mature osteoclasts and suppressing the osteoclasts' multinucleated cells during the osteoclast differentiation process (Rogers et al., 1996; Rogers, 2003; Roelofs et al., 2006; Nagaoka et al., 2015). Traditionally, bisphosphonates have been administrated intravenously and orally. Reddy et al. (1995) observed that systemic administration of bisphosphonates prevented alveolar bone destruction associated with periodontal disease in Beagle dogs.

At the same time, it has been shown that the topical application of a bisphosphonate can minimize bone resorption following mucoperiosteal flap surgery (Yaffe et al., 1995, 1997, 2000, 2003); inhibit the progression of alveolar bone resorption in peri-implantitis (Shibutani et al., 2001); improve the osseointegration around dental implants (Ganguli et al., 2002; Yoshinari et al., 2002; Kajiwara et al., 2005; Aspenberg, 2014); improve the osteoconductive and regenerative capacity of a biomaterial (Houshmand et al., 2007); prevent the surface resorption of onlay bone grafts (Moller et al., 2014); or reduce post-extraction dimensional changes (Fischer et al., 2015).

Scanning electron microscopy, SEM, used to examine bone-to-biomaterial interfaces was first reported by Jasty et al. (1989). SEM imaging technique has been used to examine the implant-to-bone interface in descriptive studies, fracture healing or to evaluate implant surface roughness (Franch et al., 1998; Ottani et al., 2002; Sul et al., 2005; Botzenhart et al., 2015). SEM observation has sufficient resolution to allow exploration of the different biological processes of tissue healing involved in socket preservation techniques. SEM technique also offers the interesting possibility of describe and identify morphological changes on the cellular components of newly formed bone (Wierzchos et al., 2008). EDX analysis provides information on the chemical elements present in the biomaterial and surrounding tissues; included qualitative and quantitative microanalysis (Lindgren et al., 2010; Ramírez-Fernández et al., 2012). This method allows to calculate Ca/P ratio —indicative of the degree of bone mineralization—. The socket healing process starts with the formation of a coagulum, that is progressively replaced by a provisional matrix, which, thanks to calcium and phosphate deposits, transforms into woven bone and finally, with tissue maturation, becomes lamellar bone and bone marrow. Depending on bone maturation different amounts of the elements will be found (Amler et al., 1960; Cardaropoli et al., 2003).

Given the potential benefits of bisphosphonates and the scientific evidence of pamidronate reducing alveolar bone resorption; the aim of the present study was to evaluate the feasibility of SEM and EDX microanalysis on evaluating the bone healing of small and large defects filled with a bisphosphonate (pamidronate) combined with collagenized porcine material, at four and eight weeks.

2. Material and methods

The study used six male Fox-Hound dogs of 1.5 ± 0.5 years of age and weighing 12–13 kg each. The study protocol was designed following Spanish and European guidelines (2007/526/CE) for animal experiments. The experiment was approved by the Ethics Committee for Animal Research of the University of Murcia (Spain). Following the European Union Council Directive of February 1st 2013, Royal Decree 53/2013 (BOE no. 34, s. I, p. 11370).

2.1. Surgical procedure

The animals were pre-anesthetized with acepromazine (0.12%–0.25 mg/kg), buprenorphine (0.01 mg/kg) and medetomidine (35 μg/kg). The mixture was injected intramuscularly in the femoral quadriceps. Then an intravenous catheter was inserted (diameter 22 or 20 gauge) into the cephalic vein, and propofol was infused at the rate of 0.4 mg/kg/min at a slow constant infusion rate. Conventional dental infiltration anaesthesia (articaine 40 mg, 1% epinephrine) was administered at the surgical sites. These procedures were carried out under the supervision of a veterinary surgeon.

2.2. Tooth extraction and grafting procedures

In both quadrants of the lower jaws, second premolars (P2) and first molars (M1) were used as experimental sites. The alveoli corresponding to P2 were qualified as small defects (SD) and M1 as large defects (LD).

Teeth were sectioned with a carbide tungsten drill; the roots were removed with forceps, without damaging the remaining bony walls. Sulcular marginal incisions were made along the vestibular and lingual areas adjoining the alveoli, separating tissues in order to make the crestal hard tissue walls visible (Fig. 1). Prior to graft placement, the external dimensions of the post extraction sockets were measured using a calliper and recorded. The extraction sockets mean bucco-lingual alveolar ridge measurements were as follows: 3.8 ± 0.21 for P2 and 5.6 ± 0.07 mm for M1.

Using a split-mouth design, the alveoli (SD and LD) corresponding to the right hemi-mandible were used as controls (C) and were filled with MP3® (OsteoBiol, Tecnoss Dental, Turin, Italy) porcine collagenated bone, after rehydration with sterile saline. Left hemi-mandible defects (SD and LD) were filled with MP3® prehydrated with pamidronate (Novartis Pharma, Basel, Switzerland) as test sites (T). Pamidronate solution was prepared by dissolving 90 mg pamidronic acid in 10 ml saline (9 mg/ml) and mixed with 2.0 cc (approximately 4 g) of 600–1000 μm particles of porcine bone.

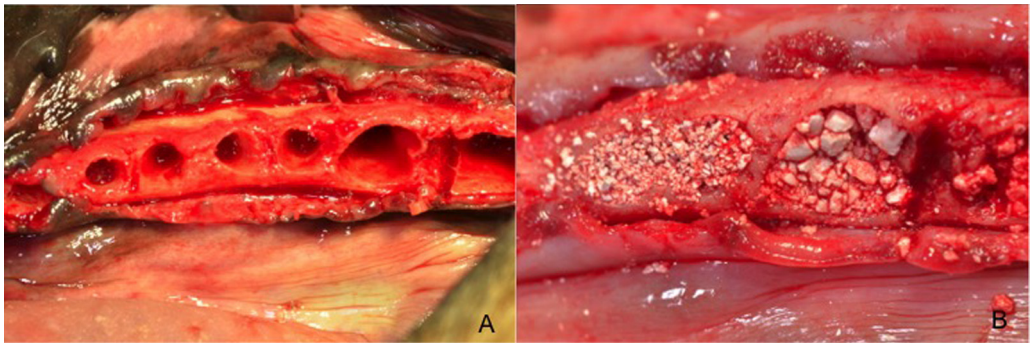

Fig. 1. Overview of a dogís mandible: (a) small and large defects after tooth extraction, (b) small and large defects filled with MP3® with and without pamidronate.