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Effects of local application of simvastatin on bone regeneration in femoral bone defects in rabbit

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ABSTRACT

Simvastatin (SIM), which is widely used in hyperlipidemia treatment, has also attracted attention due to its anabolic effects on bones. This study is designed to investigate the effectiveness of 2 mg SIM combined with 3 different carriers as delivery systems. Bone defects were surgically created in the femoral bones of 14 New Zealand white rabbits. The carriers used were the inorganic bovine bone graft (BOS), the hydroxyapatite combined with calcium sulfate (HACS), and the collagen sponge (COS). The bone defects were divided for each time period into 7 groups, as follows: passive control group (CONT), active control groups (BOS), (HACS) and (COS) (no simvastatin), and groups (BOS + SIM), (HACS + SIM) (carrier and simvastatin combination). Animal were sacrificed at 4 and 8 weeks postoperatively, and bone defects areas were prepared for histological examination and histomorphometric evaluation. Analysis of variance demonstrated statistically significant differences between groups depending on the carrier used. At 4 weeks, the BOS + SIM group presented higher rates of new bone formation, whereas at 8 weeks more new bone formation was noted for the HACS + SIM group. This study suggests that local application of simvastatin, combined with an appropriate carrier, can promote new bone formation.

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1. Introduction

The development of statins (HMG-coA) as therapeutic agents in hypercholesterolemia has been of great importance due to their safe and targeted action in the reduction of high levels of cholesterol in the blood (Marou et al., 2000). Recent research data has demonstrated that the effect of statins is not limited to their lipid-lowering properties, but they also present pleiotropic action. The effects of statins in the nervous, cardiovascular, and skeletal systems as well as their immunological response are well documented. Statins are also involved in several cellular functions associated with the improvement of endothelial dysfunction, namely, the antioxidant, anti-inflammatory, and anticoagulant effect, the stabilization of atherosclerotic plaques, the inhibitory effect on transplant rejection, the antitumor activity in laboratory animals, and the anabolic effect on bone tissue (Davignon and Laaksonen, 1999; Mundy et al., 1999; Bellosta et al., 2000).

Clinical *in vivo* and *in vitro* studies revealed that statins reduce osteoclast activity and activate osteoblast differentiation and bone formation (Mundy et al., 1999; Maeda et al., 2001; Grasser et al., 2003; Song et al., 2003; Staal et al., 2003; Maeda et al., 2004; Baek et al., 2005; Hughes et al., 2007; Ayukawa et al., 2008). In particular, they increase the expression of bone morphogenetic protein−2 (BMP-2) and vascular endothelial growth factor (VEGF) (Mundy et al., 1999; Sugiyama et al., 2000; Maeda et al., 2003). Because of these properties, it is considered that statins may be an important therapeutic option in the treatment of osteoporosis, fractures, and bone defects.

The systemic (oral) administration of statins presents a limited positive effect on bone healing because of their high hepatic action; statins do not accumulate on the bone as do bisphosphonates. Furthermore, high doses of oral administration may increase the risk for liver damage and kidney disease (Brown, 2008). The local application of statins directly to the bone defect area using

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appropriate grafted materials that are used as carriers and can achieve a controlled and gradual release of the substance increases their effectiveness on skeletal tissues.

Mundy et al. were the first who reported the *in vivo* activation of bone formation by the use of statins (Mundy et al., 1999). According to *in vitro* and *in vivo* studies, simvastatin is considered a statin suitable for bone growth activation (Garrett et al., 2001). Several studies have demonstrated that high concentrations of simvastatin increase bone formation and decrease bone resorption (Maritz et al., 2001). Simvastatin presents local and systemic anti-inflammatory activity, but may cause clinical symptoms (inflammation) when applied locally in high concentrations (Thylin et al., 2002). Several researchers have studied the effect of locally administered simvastatin in various concentrations (Thylin et al., 2002; Wong and Rabie, 2003, 2005; Stein et al., 2005; Nyan et al., 2007; Ma et al., 2008; Moriyama et al., 2008). Some of them are the ACS (absorbable collagen sponges) (Boyne et al., 1997; Howell et al., 1997), the DBM (decalcified bone matrix) (Toriumi et al., 1991; Sigurdsson et al., 1996), hyaluronate (hyalorunan) (Hunt et al., 2000), the inorganic bovine bone graft (deproteinized bovine bone matrix) (Jang et al., 2003; Wong and Rabie, 2003), hydroxyapatite (HA) (Koempel et al., 1998), calcium phosphate (CP) (Wikesjo et al., 2002), calcium sulfate (CS) (Nyan et al., 2007), PGA/PLGA (polylactic acid/polyglycolic acid copolymer) (Boyne and Shabahang, 2001), and others.

The purpose of this study was to investigate the local action of simvastatin in bone regeneration. More specifically, our study examined and evaluated histologically the effect of simvastatin local application on bone defects of laboratory animals (rabbits) at 4 and 8 weeks postoperatively, and evaluated the effectiveness of three grafted materials that were used as carriers. These materials were the bovine inorganic bone graft (BOS), the hydroxyapatite with calcium sulfate (HACS) and the collagen sponge (COS).

2. Materials and methods

This study was conducted in a total number of 14 adult male white rabbits (New Zealand white rabbits, *Oryctolacus cuniculus*), weighing 3.0–3.5 kg. Fifty-six bone defects were surgically created (4 bone defects per animal, 2 bone defects per femur). Half of the animals (7) were sacrificed in the 4th week and the rest in the 8th week. The groups were as follows: BOS + SIM, BOS, HACS + SIM, HACS, COS + SIM, COS, and CONT. The BOS, HACS, and COS groups were the active control groups, and CONT was the passive control group. The animals were sustained during the study period in an appropriately designed area of Experimental Surgery of the Second Surgical Clinic of the Medical School of the Aristotle University of Thessaloniki (Installation Experimentation EL 54 BIO 09) in accordance to the regulations and conditions set by the Veterinary Service of Thessaloniki Prefecture, which approved the conduct of this study (Protocol No. 13/4865, Thessaloniki 13/04/2009). The study was approved by the Ethics Committee of the Dental School of the Aristotle University of Thessaloniki, which ensures that all procedures described in this study were conducted in accordance with the principles of the Regulation Ethics Research Committee of the Aristotle University of Thessaloniki (Protocol No./Date: 185/17.4.10).

2.1. Surgical procedure

All animals were anaesthetized with xylazine (Rompun) and ketamine (Ketaset) injections (concentration 5 ml and 2 ml, respectively) (Rompun-Bayer AG, Leverkusen, Germany, Bayer Corporation, Agriculture Division, Animal Health, Kansas, USA, Ketaset-Fort Dodge Animal Health Division of Wyeth, Fort Dodge, IA). After disinfection of the surgical field with iodine solution (Betadine) and proper preparation of hair removal (shaving), an incision of 4–5 cm was made on the skin and the muscle layer, until the femur was adequately exposed.

Two bone defects 5 mm in diameter and 2 mm in depth were surgically prepared in each femur of each animal by a low-speed surgical motor (Kavo Intrasurg 500) with a carbide drill (2 mm), under constant saline irrigation. A titanium cylinder specifically prefabricated for this purpose with dimensions of 4 mm in diameter and 4 mm in height was placed in each bone defect, to be filled with the three different simvastatin carriers. In the passive control group, the titanium cylinders remained empty (CONT group). The purpose of their use was to achieve the same volume of carrier used in all cases (Figs. 1 and 2).

The carriers that were used are the following: 1) the collagen sponge (Bioteck, Biocollagen (TO); 2) the inorganic bovine bone graft (Bio-Oss, Geistlich Pharma AG, Wolhusen, Switzerland), and 3) the hydroxyapatite with calcium sulfate (Ostim PerOssal, Biomaterials GmbH et Co. KG, Dieburg, Germany). The simvastatin concentration used was 2 mg (Artemis Biotech, Themis Medicare Limited, Industrial Development Area, Jeedimetla, Hyderabad). All bone defects were covered by Gore-Tex nonabsorbable membrane. We used 4/0 absorbable and nonabsorbable sutures for the muscle layer and the skin, respectively.

Half of the animals were sacrificed in the 4th week and the rest in the 8th week. The femurs were removed, and the bone defect areas were histologically examined and analyzed by the Laboratory of Pathology of Medical School of Aristotle University of Thessaloniki.

2.2. Histological preparation and histomorphometry

The bone specimens were fixed in neutral formaldehyde (volume ratio material/formaldehyde 1/10) for 48 h at room temperature. For the decalcification procedure, the bone specimens were immersed in solution (Calci-Clear Rapid of National Diagnostics) and remained at constant temperature for 2–4 days. The process was strictly controlled and 2- to 3-mm incisions were performed after achieving the appropriate composition of the specimens. Subsequently, the specimens were kept in an automatic system for 24 h and, after their inclusion in paraffin blocks, histological sections of 8–10 μm were obtained with a conventional microtome. These histological sections were stained with hematoxylin–eosin. Where necessary, for technical reasons, the preparation was enriched with specific contractual stains such as Masson and Van Gieson trichromatic staining of connective tissue. The histological

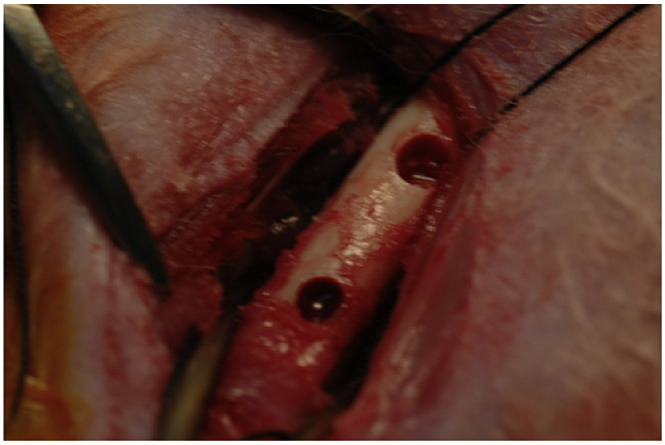

Fig. 1. Bone defects were surgically created.

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