Journal of Cranio-Maxillo-Facial Surgery 44 (2016) 1327–1332

Contents lists available at ScienceDirect

Journal of Cranio-Maxillo-Facial Surgery

journal homepage: www.jcmfs.com

The influence of oral administration of rosuvastatin on calvarial bone healing in rats

Akif Türer [a, *], Çiğdem Coşkun Türer [b], M. Cenk Durmuşlar [a], Umut Balli [b], M. Emin Önger [c]

[a] Department of Oral and Maxillofacial Surgery, Bülent Ecevit University, Zonguldak, Turkey
[b] Department of Periodontology, Bülent Ecevit University, Zonguldak, Turkey
[c] Department of Histology and Embryology, Ondokuz Mayıs University, Samsun, Turkey

ARTICLE INFO

Article history:
Paper received 28 November 2015
Accepted 14 April 2016
Available online 22 April 2016

Keywords:
Rosuvastatin
Bone healing
Stereological
Micro CT

ABSTRACT

The purpose of this study is to investigate the potential of the systemic administration of different doses of rosuvastatin (RSV) on autogenous grafted critical-sized cortical bone defects. Twenty-four rats were divided into three groups: Group C (control), Group RSV-2 and Group RSV-5. A 5-mm diameter critical-size defect was created in the calvarium of each animal. In Group C, the defect was filled by autogenous graft only and rats were given saline solution with oral gavage for 28 days. In Group RSV-2 defects were filled with autogenous graft and rats were given 2 mg/kg rosuvastatin with oral gavage for 28 days. In Group RSV-5 defects were filled with autogenous graft and rats were given 5 mg/kg rosuvastatin with oral gavage for 28 days. All animals were euthanized at 28 days postoperative. Stereologic and micro-CT analyses were performed. New bone area (NBA) and connective tissue volumes were measured. Stereologic analysis showed that Group RSV-5 and RSV-2 had significantly more new bone at 4 weeks compared with group C. Connective tissue volumes were also significantly higher in RSV applicated groups. New bone and connective tissue volumes' difference were not statistically significant between RSV groups. Micro-CT results were similar with stereologic analyses. Orally administered RSV enhances bone regeneration in critical size calvarial rat defects filled with autogenous graft furthermore possible inflammatory effect should be investigated.

1. Introduction

Segmental or large bone defects may occur due to trauma, resection or pathology and present important clinical difficulty for oral and maxillofacial surgeons (Kneser et al., 2006). The treatment of extensive bone defects may require use of grafting procedure for optimum bone formation (Young et al., 2009). Bone substitutes have been used by clinicians for the reconstruction of osseous defects and autogenous bone is accepted as the gold standard (Becker et al., 1996). However, because of poor blood supply, scarred dura or insufficient soft-tissue coverage large craniofacial defects may be needed extra modalities to help increase the chance of successful treatment (Hopper et al., 2001).

Statins, 3-hydroxy 3-methyl glutaril coenzyme A (HMG-CoA) reductase inhibitors, were first developed to control and treat patients with hyperlipidemia and hypercholesterolemia and Mundy et al. (1999) reported beneficial effects of statins on osteoporotic patients. In recent years, it has been shown that statins also have positive role on bone formation by modulate inflammation, enhance osteogenesis and angiogenesis (Maeda et al., 2003; Hernández et al., 2014; Tan et al., 2015).

Statins increase the expression of important osteoanabolic and angiogenetic factors such as bone morphogenetic protein (BMP)-2 and vascular endothelial growth factor (VEGF) (Mundy et al., 1999; Maeda et al., 2003). BMPs are active bone-inducing factors that act on immature mesenchymal cells, including osteoblasts, resulting in osteogenesis and BMP-2 is the most potent of the osteoinductive factors (Wozeny et al., 1988). VEGF is an angiogenic cytokine and may induce proliferation and differentiation of osteoblasts by stimulating endothelial cells to produce osteoanabolic factors (Wozney, 1995; Wong and Rabie, 2005).

Rosuvastatin (RSV) is a lipid lowering drug used to prevent cardiovascular disorders. RSV has long terminal-life and powerful effect according to simvastatin and atorvastatin (Karlson et al., 2016). This drug also has pleiotropic effects, including bone

* Corresponding author. Bülent Ecevit Üniversitesi, Diş Hekimliği Fakültesi Ağız, Diş ve Çene Cerrahisi AD, 67600 Zonguldak, Turkey. Tel.: +90 372 2613547.
E-mail address: akifturer@gmail.com (A. Türer).

http://dx.doi.org/10.1016/j.jcms.2016.04.021
1010-5182/

stimulation, promotion of vasculogenesis, and antiinflammatory effects (Monjo et al., 2010). Local effect of RSV on bone defects has been evaluated clinically and based on clinical parameters. (Pradeep et al., 2015a, b). Statins have extensive first metabolism in liver so they have low systemic bioavailability (Schachter, 2004). To investigate systemic effect of RSV on bone formation, higher dose of RSV should be used and tried on animal models to avoid side effects on human and find out right doses. Animal bone studies can be analyzed by histological methods but in clinical studies it may be an ethical problem to get a specimen from a patient for histological analyze. To the best of the authors' knowledge there is no study about systemic effect of RSV on bone defects.

The purpose of this study is to investigate the potential of the systemic administration of different doses of rosuvastatin (RSV) on autogenous grafted critical-sized cortical bone defects.

2. Materials and methods

2.1. Animals

Twenty-four 6-8 week-old Wistar rats were used. The animals were put in standard cages which were placed in rooms with a relative humidity rate of 40–60% and temperature of 22 ± 1 °C. The illumination system of the room was configured to automatically provide 12 h of day and 12 h of night and this study was approved by the Animal Experimentation Committee of Bülent Ecevit University, Zonguldak, Turkey.

2.2. Surgical procedure

All surgeries were performed under sterile conditions in an animal laboratory surgical suite. The rats were anesthetized by intramuscular injection of 3 mg/kg xylazine hydrochloride (Rompuns, Bayer, Leverkusen, Germany) and 35 mg/kg ketamine hydrochloride (10% Ketasol; Richter Pharma AG, Wels, Austria). After aseptic preparation, a semilunar incision was made and a full thickness flap was reflected exposing the parietal and frontal bones. A 5-mm diameter critical size calvarial defect was performed with a trephine used in a low-speed handpiece under continuous sterile saline irrigation. Care was taken during the surgery not to damage the dura mater. All defects were filled by autogenous grafts, the flap was sutured with resorbable 4/0 polyglactin 910 sutures (Vicryl; Ethicon, Somerville, NJ, USA). For postoperative infection control, 10 mg/kg cefazolin sodium (Sefazol; M Nevzat, Istanbul, Turkey) was injected to animals and metamizole sodium (Novalgin, Aventis, Turkey) as analgesic, for 5 days after the operation.

2.3. Experimental groups

Rats were divided into three groups of eight rats each:

Group C (Control): the defects were filled with autogenous graft and rats were given saline solution with oral gavage for 28 days.

Group RVS-2: the defects were filled with autogenous graft and rats were given 2 mg/kg rosuvastatin with oral gavage for 28 days.

Group RSV-5: the defects were filled with autogenous graft and rats were given 5 mg/kg rosuvastatin with oral gavage for 28 days.

RSV was pulverized to powder and dissolved in sterile distilled water.

Autogenous bone grafts were harvested from left tibia of rats. Medial surface of the left legs of the subjects were shaved and disinfected the area with povidone iodine solution. The legs were given the flexion position and longitudinal incisions of 20–25 mm were made periosteally in order to reach the medial surfaces of the tibia. The medial surfaces of the tibia were exposed with blunt dissection and soft tissues were excluded. Autogenous bone graft, covering the cortex and medulla layers of the bone was obtained by using round-tipped, stainless steel drill with a diameter of 3 mm under sterile saline solution.

Four weeks after surgery, the animals were euthanized with a lethal injection of anesthetics. The skin was dissected, the calvaria removed, and immediately immersed in a 10% tempered solution of formaldehyde. Micro CT analyses were performed before decalcification.

2.4. Micro-CT

The specimens were scanned using micro-CT (Skyscan 1174; Micro Photonics Inc., Allentown, PA, USA). Before beginning micro-CT scannings, flat-field setups are arranged. Then, scanning was performed with a spatial resolution of 15 μm using 2800 ms 50 kV and 800 μA at a 0.7° rotation steps with 3 frames for a total of 180°. All images were taken in three-dimensional reconstruction with the NRECON software, and then the collected data were evaluated with CTAn software. In that part of analysis, upper and lower borders of the defect are assigned and the region is selected without any healthy tissue contribution in the defect as region of interest between these upper and lower limits. Settings of dark and low gray regions are done for each tissue. After all of these settings 3D analysis performed with only volume of mineralised new bone formation without graft materials was calculated.

2.5. Stereology analysis

The samples were decalcified using formic acid (%5) for 21 days. After the decalcification process, the samples were fixed in 10% formaldehyde, dehydrated in a graded alcohol series, and cleared in xylol for light microscopic examination. After dehydration, specimens were embedded in fresh paraffin. Sections were cut using a microtome (Leica RM 2135; Leica Instruments, Nussloch, Germany). Each paraffin block was serially cut into 7-μm thickness. For volumetric estimation procedure, every 20th section was selected through a set of consecutive paraffin sections from each sample. Choosing the first section was done randomly. All of the sections were sampled from each sample in a systematic random manner. Selected sections were stained with hematoxylin–eosin (H-E) and photographed on the stereology analysis system (Stereo-investigator 9.0, Microbrightfield, Williston, VT, USA) using a light microscope (Leica M 4000 B, Germany) with a digital color camera attachment (Microbrightfield, Williston, VT, USA).

Unbiased Cavalieri method was applied to the light microscopic images for the stereological estimation of volume of new bone area (Vn). Point counting test grids were used for the estimation of these parameters. These grids were used to estimate volume of new bone area (Vn). The point density of the point counting grids was designed to obtain an appropriate coefficient of error (CE) for interesting area in images of the serial sections (Odaci et al., 2003). CE and coefficient of variation (CV) were estimated according to Gundersen and Jensen' formula (Gundersen and Jensen, 1987). The test grid with systematic array of points was randomly placed on the screen of PC. The volume of each interesting area in all sections was estimated with following formula:

$$\text{Volume} = t \times a/p \times \sum p$$

('t', section thickness; 'a/p', representing area of each point on the point counting grid; '$\sum$p', total number of the points hitting the interesting area).