Evaluation of the effects of topically applied simvastatin on titanium implant osseointegration

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ABSTRACT

Objective: The aim of this study was to evaluate the effect of topical simvastatin on the osseointegration of machined-surface titanium implants.

Material & methods: Sixteen female Sprague–Dawley rats were used in this study in a 4-wk experimental trial. The rats were divided into two groups: a test group ($n = 8$), in which local simvastatin was applied, and a control group ($n = 8$). In each of the 16 animals, a machined-surface titanium implant was surgically integrated into the tibial metaphysis. Prior to integration of the implants, 100 μ l of ethanol solution containing 5 mg of simvastatin was applied to the bone sockets. The implants were then immediately integrated into the bone sockets. No simvastatin treatment was applied in the control group. At the end of the 4-wk period, the rats were sacrificed, and the implants and surrounding bone tissue were removed. The bone–implant contact (BIC) and bone filling (BF) ratios of nondecalcified histological sections were then determined. The BIC ratio was defined as the ratio of the implant surface directly in contact with the bone to the total implant surface length. The BF ratio was determined by measuring the bone-filled area at a distance of 0.5 mm from implant.

Results: There were no statistically significant differences in the BIC and BF ratios of the test group and control group in terms of implant osseointegration ($p > 0.05$).

Conclusion: Within the limitations of this study, we conclude that topically applied simvastatin to implant surfaces does not affect osseointegration.

1. Introduction

Dental implant-supported prostheses are a scientifically accepted and widely used treatment option for the rehabilitation of partial and total edentulousness.¹ The success of a dental implant-supported prosthesis depends on successful osseointegration of the implant.^{2,3} Osseointegration is directly associated with the quality and quantity of the peri-implant bone tissues.^{3–5}

Dental implant-supported prosthetic treatment has increased, highlighting the need for methods to accelerate and improve the osseointegration process.⁶ Low bone quantity and quality are major problems in dental implant-supported prosthetic treatment. A number of previous studies demonstrated that osteoporosis impaired the osseointegration process.^{1,3,7} To increase osseointegration of dental implants, various biological agents, such as melatonin, vitamin D, growth hormone, and growth factors, have been applied to the implant surface, as well as of systemic substances, such as bisphosphonates.^{8–12}

Recent studies have demonstrated anabolic effects of statins, which are used to treat high blood cholesterol levels, on bone tissue.^{13,14} In

both in vivo and in vitro studies, statins reduced osteoclastic activity, increased osteoblastic differentiation and bone formation, and exerted beneficial effects on bone density.^{13–15} Statins also enhanced the release of biological mediators, such as bone morphogenetic protein-2 and vascular endothelial growth factor, thereby enhancing osteogenesis. As shown by in vitro studies, statin-coated titanium surfaces also promoted and stimulated osteoblastic differentiation.^{13–15}

This study aimed to investigate whether locally applied simvastatin to implant sockets was effective in improving osseointegration 4 wk after implant surgery using nondecalcified histological analysis methods.

2. Materials and methods

All experimental and surgical protocols were carried out in the experimental research center of Firat University, Elazig, Turkey. Ethical consent was provided by the animal experimental ethics committee of Firat University (Protocol Number: 2016/86). The rats used in this research were provided by the experimental research center of Firat

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Fig. 1. Machined surfaced titanium implant which installed in rat tibias.

University. All animal experiments were performed in accordance with Helsinki Declaration for the care and welfare of animals.

In this study, 16 female Sprague-Dawley rats weighing 300–320 g and aged 1–1.5 y from the same estrus period were used. The rats were housed in temperature-controlled cages in a 12-h dark, 12-h light environment until the end of the study. All animals were allowed free access to food and water during the experimental period.

The animals were randomly divided into two groups, as follows:

Control group: Vehicle solution (100 μ l ethanol) was applied to the bone socket before the installation of the implants (4 mm long and 2.5 mm in diameter) (Fig. 1). In each animal, a machined-surface titanium implant (TiAl₆ Va₄) (Bioinfinity Dental Implant; Avrupa Dental Implant Corporation, Istanbul, Turkey) was then surgically integrated into the tibial metaphysis. No other treatment was applied during this 4-wk trial.^{15,16}

Test group: Before the installation of the titanium implants (4 mm long and 2.5 mm in diameter) (Fig. 1), simvastatin in ethanol solution (100 μ l of ethanol containing 5 mg of simvastatin) was applied to the bone sockets in accordance with the method described by Yang et al. who achieved successful results in terms of osseointegration.¹⁵ In each animal, a machined-surface TiAl₆ Va₄ implant (Bioinfinity Dental Implant; Avrupa Dental Implant Corporation) was then immediately surgically integrated into the tibial bone socket.^{15,16} Prior to implant placement, the implants were cleaned in an ultrasonic bath and sterilized by autocaving (125 °C for 15 min).

2.1. Surgical procedure

In all cases, the surgical procedures were undertaken with the rats under general anesthesia. General anesthesia was achieved by an intramuscular injection of 35 mg/kg of ketamine hydrochloride and 5 mg/kg of xylazine. All the surgical procedures were performed under sterile conditions. After general anesthesia was achieved, the tibial skin was washed and shaved. A linear incision 1.5–2 cm long was made on the skin in the tibial crest. After the skin incision, a periosteal elevator was used to reach the metaphyseal part of the tibial bone. The implant sockets were created using a round-point drill, 1.8 mm taper. For the preparation of the implant sockets, 2.3-mm tapered drills were used at 500 rpm under sterile saline perfusion to avoid bone tissue necrosis. Surgical integration of the implants was done with primary

stabilization. The implants were inserted using a force of 15 Ncm. Bone wax was used only to seal the hole and prevent leakage after the integration of the implants, not inserted into the implant sockets. Bone wax is an excellent hemostatic material and is commonly used during bony surgeries. Bone wax is composed of beeswax, paraffin, and isopropyl palmitate.¹⁷

After implant placement, the tibial skin, soft tissue, and muscles were located in the original positions and sutured using 4-0 polyglactin resorbable sutures. Upon completion of the surgical procedures, an antibiotic (40 mg/kg cephalosporin) and analgesic (0.1 mg/kg tramadol hydrochloride) were administered for 3 d to prevent infection and pain. The same operator performed all the surgical procedures.

2.2. Histological analysis

The rats were sacrificed 4 wk after the surgical placement of the titanium implants, and the implants and surrounding bone tissues were removed for histological analysis.¹ Based on the study of Bozoglan et al.,¹ a waiting period of 4 wk was considered sufficient for osseointegration. The specimens were fixed in a 10% formaldehyde solution for 7 d and then embedded in 2-hydroxyethylmethacrylate resin for non-decalcified histological analysis. The implants and surrounding bone tissues were cut in the middle of each block using an Exakt® (Germany) microtome. All the sections were ground using an Exakt grinder, and sections 50 μ m thick were obtained for light microscope analysis.³ Histological staining for BIC and bone filling (BF) analysis was performed using toluidine blue. Bone filling and BIC was analyzed using a light microscope and an image analyzer at the Department of Microbiology Laboratory, Faculty of Medicine, Firat University, Elazig, Turkey. Blinded and calibrated histomorphometry was performed using the stereological software system (Nikon, Tokyo, Japan) by an author. The bone implant contact ratio (%) was defined as the ratio of the implant surface directly in contact with the bone to the total implant surface length.^{3,5,12} The BF (%) ratio was determined by measuring the bone-filled areas at a distance of 0.5 mm from the titanium implant (mesial, distal, and apical portion of the implant).¹⁸

2.3. Statistical analysis

The SPSS software program was used for the statistical analysis. The mean and standard deviation of the data were calculated. For data analysis, the Student's *t*-test was used. A statistically significant difference was accepted at a *p* value of < 0.05.

3. Results

Due to improper preparation (e.g., separation of the implant from tissue or separation of the bone from the histological prepare), one specimen was removed from the test group, and one was removed from the control group. The histological BIC analysis results in the control and test groups are shown in Table 1. The mean BIC ratio in the control group was 48.63% $\pm$ 15.02%, whereas it was 45.79 $\pm$ 9.86% in the test group (Fig. 2a and b). There was no statistically significant difference in the BIC ratios of the two groups. The BF ratios of the groups are shown in Table 2. The mean BF ratio in the control group was 31.43 $\pm$ 11.80%, whereas it was 34.29 $\pm$ 7.9% in the test group

Table 1

Bone implant contact ratio (%) of the test group-simvastatin and controls. Statistically significant differences was not detected between the groups-Student T test- (*P* > 0.05).

Groups	N	Mean (%) $\pm$ Sd	<i>P</i> *
Test-Simvastatin	7	45.79 $\pm$ 9.86	> 0.05
Control	7	48.63 $\pm$ 15.02

Fig. 2. Non-decalcified histologic images of the A: Test and B: Control Groups, (40 times magnification, Methylene blue). Yellow Line: Implant surface not contacting bone (α), Red Line: Implant surface in contacting with bone (β), Total implant surface: ϵ , Bone Implant Contact Ratio (%): $\epsilon \cdot \alpha(\beta) / \epsilon$. Bone filling detected by measuring the bone filled areas distance 0.5 mm from implant (mesial, distal and apical portion of implants). Bone filling areas (δ : Blue Line), non-bone areas (ϵ : Brown Line), total area ($\tilde{A}$). Bone filling Ratios (%): $\tilde{A} \cdot \delta / \tilde{A}$ ($\delta / \tilde{A}$). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 2

Bone filling ratio (%) of the test group-simvastatin and controls. Statistically significant differences was not detected between the groups- $\ast$ Student T test- $(P > 0.05)$.

Groups	Mean (%) $\pm$ Sd	P*
Test-Simvastatin (n = 7)	31.43 $\pm$ 11.8	> 0.05
Control (n = 7)	34.29 $\pm$ 7.87	

(Fig. 2a and b). There was no statistically significant difference in the BF ratio of the two groups.

4. Discussion

The use of biocompatible materials for the treatment of peri-implant bone tissue has been extensively investigated in the literature.^{3,8–10,12,19,20} To improve the regeneration capacity of peri-implant bone tissue, the materials investigated include growth hormone, melatonin hormone, bone morphogenetic proteins, hydroxyapatite-calcium deposits, fluoride coatings, and magnesium.^{3,8–10,12,19,20}

A number of studies have investigated the relationship between statins and bone metabolism.^{21–23} Simvastatin enhanced bone formation by stimulating osteoblast proliferation and differentiation.^{21–23} Systemic administration, including oral and intraperitoneal administration, of statins improved the osseointegration of pure titanium implants in healthy and osteoporotic rats.^{21–23} Various experimental studies have confirmed the beneficial effects of statins on bone tissue healing, with different local administration methods. Recent studies have focused on local administration of drugs that have the potential to improve osseointegration.¹⁵ Statins increased bone defect regeneration when administered locally in cranial/mandibular bone defect models.¹⁵ These studies used local applications of drug-releasing collagen and gelatin sponges, both of which tend to dissolve in local application sites, or local injection techniques.^{21–23} Piskin et al.²⁴ showed that simvastatin-loaded electrospun nanofibers improved bone tissue mineralization according to a histological and microcomputed tomography analysis. Nyan et al.²⁵ applied simvastatin to porous implant surfaces by homogenous wetting and demonstrated clear effects on implant osseointegration. Although the method of local administration and animal models were different, the results were similar. Based on histological findings in an ovariectomized animal model, Wen Fang et al.²¹ reported

that more bone tissue formed around implant surfaces and more BIC was detected in a group treated with simvastatin as compared with that in a control group. Wen Fang et al.²¹ also reported an increase in BIC and new bone formation around simvastatin- and hydroxyapatite-coated implants. In the same study they reported that simvastatin increased implant osseointegration in osteoporotic rats via an electrochemical loading process. In an experimental study, Yang et al.²⁶ reported that simvastatin-loaded porous implant surfaces appeared to improve implant osseointegration in an ovariectomized rat model. In the simvastatin-treated group, they observed significantly greater new bone formation around the surfaces of the simvastatin-applied implants, in addition to increased BIC as compared with a control group. In another experimental study, Yang et al.²⁷ also reported that simvastatin-loaded porous titanium implant surfaces encouraged osteogenic differentiation of preosteoblasts and enhanced osseointegration. Based on in vitro and in vivo research In an ovariectomized rat model, Zhao et al.²⁸ showed that alkaline phosphatase and osteocalcin activities increased 4 and 7 d after surgery with titanium implants loaded with simvastatin and a calcium phosphate coating. They reported that the bone area and BIC significantly increased in the simvastatin-loaded implant group as compared with the controls 4 and 7 d after surgery. They concluded that topical application of simvastatin and implants coated with calcium phosphate increased osteoblast function and BIC in ovariectomized rats.²⁸ In the present study, we did not detect a statistically significant difference (BIC and BF) in the test (simvastatin treated) group versus the control group. We attributed this finding to the use of machined-surface titanium implants. In similar study, Naito et al.¹² compared osseointegration after the application of three doses of topical vitamin D to machined-surface implants. After 6 wk, they detected no statistically significant difference between the test and control groups for BIC. They attributed this result to the machined-surface implants not sustaining a sufficient vitamin D concentration or damage to proteins during the coating procedure. They suggested that rough-surfaced implants could improve osseointegration and alter the potential effects of topical vitamin D applications. Yang et al.²⁶ investigated the effect of locally applied simvastatin to roughened implant surfaces on implant osseointegration. The data in our study can be evaluated in this respect.

Thus far, most studies about osseointegration have focused on the systemic administration of simvastatin. However, when statins are topically applied or continuously released from an implant, they are 50–80 times more effective in inducing bone formation than when administered via the peroral route or injected subcutaneously. Previous research demonstrated that simvastatin was released immediately from implant surfaces and that the drug's half-life was short. Due to the short half-life of simvastatin, the expected effect may not have been detected in this study.²¹ However, in an experimental study by Pauly et al.,²⁹ locally applied simvastatin impaired osseointegration of an intramedullary titanium implant in rat femurs as compared with controls over an 8-wk period. Pauly et al.²⁹ reported biomechanical results showing a significant decrease in the fixation force for high-dose simvastatin-coated implants when compared with that in two control groups (uncoated and polymer [poly(D,L-lactide)] coated). In the same study, histomorphometry revealed a significant reduction in total and direct BIC with the high-dosage simvastatin implants as compared with the controls. Total contact was reduced with the low-dosage simvastatin implants when compared with that of the uncoated controls. Furthermore, there was a significant reduction in new bone formation around the high-dosage simvastatin-coated implants as compared with that in both the control groups, (uncoated and polymer-coated implants). In another experimental study, Nyan et al.²⁵ detected new bone formation on the implant surface in the bone marrow cavity in simvastatin-loaded implant groups 2 wk post-treatment. They reported that the peri-implant bone volume and mineral apposition rate of the simvastatin-loaded groups were significantly higher than those of control and micro-arc oxidized groups 2 wk post-treatment. However, the bone

volume showed no further increase by 4 wk, with the peri-implant bone volume in all the groups appearing similar, especially in the marrow space areas. Yang et al.²⁶ investigated bone responses of simvastatin-loaded porous implant surfaces in ovariectomized rat tibias. They used ethanol containing simvastatin similar to Nyan et al.²⁵ In the simvastatin-loaded groups, the implants showed a significantly greater bone area and higher BIC ratio as compared with the controls at all the follow-up times (1, 2, 4, and 12 wk). Give the prolonged release of simvastatin and the results of Nyan et al.²⁵ and Yang et al.,²⁶ we applied an ethanol solution containing simvastatin to the bone sockets in the present study.

5. Conclusion

Within this study's limitations, local application of simvastatin did not affect the osseointegration of machined-surface titanium implants. Further studies using different implant surfacing methods are needed to clarify the effects of local simvastatin applications on osseointegration.

Declaration of competing interest

The authors declare there is no conflict of interest.

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