The Effects of Simvastatin on Osseo-Integration Around Titanium Implants in Posterior Maxilla of Osteoporotic Rats

N. Doan, Z. Du, J. Xiao, P. Reher, W. Xia, R. Crawford, P. Reher, S. Ivanovski, F. Yang, Q.T. Duong, J. Jiang, and Y. Xiao

Abstract

Objective This study aims to evaluate the relationship between implant placement, poor quality bone, simvastatin, and osseo-integration of surface-treated implants in the posterior maxilla of osteoporotic rats. *Materials and methods* Sixty-four female Sprague-Dawley rats, aged 3 months old were used in this study, divided into three groups: Sham-operated (SHAM; n = 20), ovariectomized (OVX; n = 20) and ovariectomized treated with simvastatin (OVX + SIM; n = 20). Two rats from the SHAM and two from the OVX groups were used to verify osteoporosis. Eighty-four days following ovariectomy, screw-shaped titanium implants were immediately placed into mesial root sockets of the posterior maxilla. Simvastatin was administered orally at 5 mg/kg each day after the implant placement in the OVX + SIM group. The animals were sacrificed at either 28 or 56 days from the date of implant insert and the undecalcified tissue sections were processed for histological analysis. The osseo-integration indices used were: bone formation rate (BFR), bone to implant contact (BIC), and bone density (BD). *Results* The osseo-integration indices (BFR, BIC and BD) in the three groups demonstrated significant differences among the SHAM > OVX + SIM > OVX group, which implied that simvastatin could promote bone mineralization in OVX rats. *Conclusion* This study shows for the first time that simvastatin can positively affect the osseo-integration indices, and successfully promoted osseo-integration in the posterior maxilla in OP rats.

Keywords

Simvastatin • Dental implants • Osteoporosis • Posterior maxilla • Rats

1 Introduction

With The phenomenal pace of dental implant development in the last two decades has led to widespread studies in both humans [1] and animals [2]. As the implant success rate improves, dental implantologists have to deal with much more complex issues encountered in those patients with medical conditions such as osteoporosis [3]. Osseo-integration or the process of incorporation of a dental implant into the beneficiary bone, consists of a series of incidents that can be affected by several issues such as site

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selection, surgical techniques, systemic and local conditions, and medication used [2–4]. There is sufficient evidence that success rates of implant clinical procedures markedly reduce with age and certain systemic conditions, such as osteoporosis [4–6]. Poor bone quality and quantity, such as those found in osteoporosis, may produce an unfavourable effect on osseo-integration [2, 3]. In spite of the proposition of the undesirable consequence of dwindling bone quantity or osteoporosis on the success rate of implant therapy, animal research has been able to demonstrate the enhanced properties of statins on osseo-integration in poor quality bone osteoporotic rats [7].

There have been many published studies [8–18, 20–39] looking at the effects of simvastatin on osseo-integration of dental implants in osteoporotic subjects; however, not one of them has concentrated on STIs in the posterior maxilla of osteoporotic rats. This study attempts to be the first of its kind to fill this knowledge gap. The working hypotheses are: (1) Osteporotic (OVX) rats have lower osseo-integration indices than normal (SHAM) rats; (2) Simvastatin in conjunction with surface-treated implants (STIs) can enhance osseo-integration in osteoporotic rats. Thus, the aim of this study is to evaluate these hypotheses by assessing the association between bone formation during osseo-integration of surface-treated implants in the posterior maxilla of osteoporotic (OVX) rats treated with simvastatin.

2 Materials and Methods

2.1 Experimental Design

This study was conducted following a protocol approved by the Animal Care and Use Committee of Fujian Medical University, and a similar research approach employed previously by Du et al. [2, 6]. Sixty-four female Sprague-Dawley rats, aged 3 months old were used in this study, divided into three groups: Sham-operated (SHAM; n = 20), ovariectomized (OVX; n = 20) and ovariectomized treated with simvastatin (OVX + SIM; n = 20). Two rats from the SHAM and two from the OVX groups were used to verify osteoporosis. Eighty-four days following ovariectomy, screw-shaped titanium implants were immediately placed into mesial root sockets of the posterior maxilla. Simvastatin was administered orally at 5 mg/kg each day after the implant placement in the OVX + SIM group. The animals were sacrificed at either 28 or 56 days from the date of implant insert and the undecalcified tissue sections were processed for histological analysis. The osseo-integration indices used were: bone formation rate (BFR), bone to implant contact (BIC), and bone density (BD). Statistical methods-Variations in bone quantity among the three groups were measured by one-way analysis of variance (ANOVA) followed by Fisher's LSD post hoc test (a = 0.05).

3 Results

3.1 Day 28

In the OVX group (Fig. 1b), there was less freshly developed bone near the implant in contrast to the SHAM (Fig. 1a) and the OVX + SIM (Fig. 1 c) groups at day 28 after implant placement. There was a smaller number of osteoblasts in the recently established bone bed near the implant, and the bone matrix around the implant was slim and sporadic (Fig. 1b). The presence of osteoclastic activity was seen in the freshly established bone in the OVX group (Fig. 1b). Additionally, the cancellous bone further from the implant exterior appears to have fewer mineralized trabeculae in the OVX group than in the SHAM and OVX + SIM groups (Fig. 1a–c). At day 28, the morphology of the newly produced bone near the implants in both the SHAM and OVX + SIM groups displayed similar features (Fig. 1a, c). In contrast to the OVX group, both the OVX + SIM and SHAM groups displayed more bone surrounding the implants in terms of the matrix width and the continuous link of mineralized mass surrounding the implant surface (Fig. 1a–c). In the OVX + SIM group, the majority of the newly formed bone matrix surrounding the implant seemed to be not as mature as in the SHAM group (Fig. 1a, c).

3.2 Day 56

At 56 days (Fig. 1d–f) after implant placement, the histological data disclosed more newly created bone concealing the implant surface than at 28 days in all 3 groups (Fig. 1a–c vs. Fig. 1d–f). The differences in both the SHAM and OVX + SIM groups were minimal, as the recently created bone on the implant surface turned out to be denser with time (Fig. 1a, c vs. Fig. 1d, f). In the OVX group (Fig. 1e), the quantity of new bony tissue surrounding the implant surface was less compared with the new bone surrounding the implant shown in the OVX + SIM and SHAM groups (Fig. 1d, f). In contrast to the SHAM and OVX + SIM groups, the OVX group shows more signs of both osteoblastic and osteoclastic action in the bone base adjacent to the implants (Fig. 1b, e). In addition, at the cancellous bone further from the implant surface, it was noticeable that fewer mineralized trabeculae were found in the OVX group (Fig. 1e) in contrast to the SHAM (Fig. 1d) and OVX + SIM groups (Fig. 1f). In contrast, at the cortical zone, the implant surfaces were concealed with established lamellar bone, and no major changes were observed among the three groups.

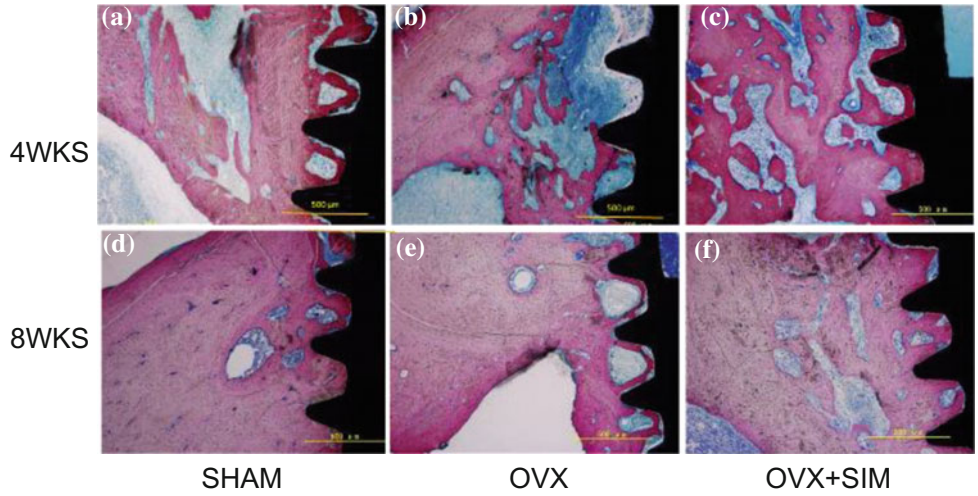

Fig. 1 Histological evaluation of bone to implant contact (*BIC*) and bone density (*BD*) at 4 weeks (**a**, **b**, and **c**) and 8 weeks (**d**, **e**, and **f**) (4× magnification using methylene blue-basic fuchsin staining)

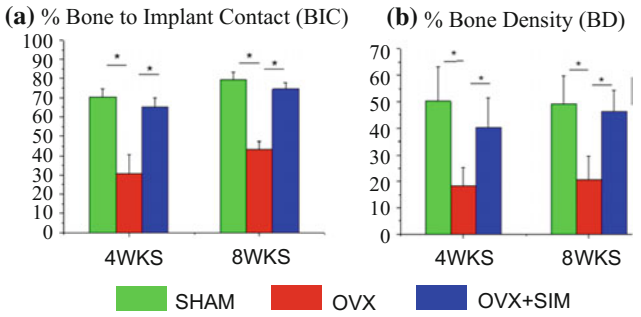

Fig. 2 Graphs of bone to implant contact (*BIC*) and bone density (*BD*). BIC (**a**) and BD (**b**) illustrate the inferiority and statistically significant differences of the OVX groups as compared with the SHAM ($P < 0.05$) and OVX + SIM groups ($P < 0.05$). Compared with the 4-week groups, the 8-week groups show BIC increased by the three groups and BD appears to be denser in the OVX and OVX + SIM groups but not in the SHAM group

4 Disscussion

In the present study, it was observed that after 28 days, the OVX group had lower BIC and BD in contrast to the SHAM and OVX + SIM groups. This phenomenon suggests that the OVX group may have developed the characteristic alterations of bone turnover seen in osteoporosis (increased bone resorption and reduced bone formation). In the OVX + SIM group, the amount of BIC and BD was not different from the SHAM group. This implies that simvastatin may partially reverse the different turnover attribute of osteoporosis via improvement of osteoblast activity and differentiation, and diminished osteoclastic activity.

At 28 and 56 days (Fig. 2), the BFR, BIC and BD of surface-treated implants in both the OVX + SIM and SHAM groups were considerably greater in contrast to the OVX group except for the BFR which was lower at 56 days than at 28 days, indicating that simvastatin stimulates bone growth around titanium implants throughout the initial phases of osseo-integration, and as more bone is in contact with the implant, saturation may have approached its end point. Consequently, this may have resulted in the slowing down of BFR.

At 56 days, BIC and BD in the OVX + SIM group were better than in the OVX group but less than in the SHAM group. These effects signify that simvastatin continued to stimulate osteoblastic activity as the recently grown bone near the implant aged.

With the addition of simvastatin not only more mineralized bone was formed but it emerged to become closer to the implant surface as demonstrated with calcein and alizarin staining. This implies that simvastatin had somehow up-regulated the expression of BMB-2 mRNA in osteoblasts to produce more bone [19] and also through the VGF pathway [40]. The order of staining intensity of mineralized bone by the three groups was SHAM > OVX + SIM > OVX and the BFR order was similar, namely: SHAM > OVX + SIM > OVX. However, even in the presence of simvastatin, the BFR of the OVX + SIM was still inferior that of the SHAM group. Therefore, it can be assumed that osteoporotic model used in this study works well in the presence of simvastatin and may have helped to lessen the effect of osteoporosis. The BFR of the OVX + SIM group was better than that of the OVX group, but it was slightly

inferior to the SHAM group. This implies that further research is needed on the direct application of simvastatin to implant surface and medicaments needed to improve osseo-integration in osteoporotic subjects.

5 Conclusion

In conclusion, the present study is the first of its kind that has shown the enhancing effect of simvastatin on osseo-integration of dental implants in the posterior maxilla of osteoporotic rats. It has also demonstrated that new bone formation and mineralization activity are positively correlated with osseo-integration of surface-treated implants in osteoporotic rats treated with simvastatin.

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Conflict of Interest The authors declare that they have no conflict of interest.

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