ORIGINAL ARTICLE

Local Application of Simvastatin to Rat Incisor Socket: Carrier-dependent Effect on Bone Augmentation

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SYNOPSIS
It has been reported that simvastatin, which is a cholesterol synthesis inhibitor and a therapeutic drug for hypercholesteremia, stimulates bone morphogenetic protein 2 expression in osteoblasts, suggesting potentiality of simvastatin in local bone augmentation. We prepared bovine atelo-collagen or calcium sulfate containing simvastatin and applied these materials to the rat lower incisor sockets. After 4 weeks, the bone of the extracted site was examined radiographically and histologically. Calcium sulfate containing simvastatin remarkably increased the thickness of the alveolar bone. This effect was not observed when simvastatin alone was applied or when atelo-collagen was used as a drug carrier. Although further studies are required, the present results indicate that simvastatin augments bone around tooth socket, which is carrier-dependent.

Key words: simvastatin, bone regeneration, tooth socket

INTRODUCTION
Prosthodontic treatment with dental implants is currently promising and the number of patients, who wish to be treated with dental implants, is increasing. However, decrease in height and width of alveolar ridge after tooth extraction causes difficulties in dental implant treatment. Thus, it is useful to develop a method to augment alveolar ridge after tooth extraction.

On the other hand, statins, which inhibit cholesterol synthesis, are clinically used as therapeutic drugs for hypercholesteremia. In 1999 Mundy and his collaborators have reported that simvastatin, one of the statins, enhances bone formation via stimulating bone morphogenetic protein 2 (BMP2) expression in osteoblasts and that local intermittent injection of simvastatin to the mouse head increases the bone thickness. Therefore, we speculated that a material, which contains simvastatin and releases it,
would augment bone. In the present study, we prepared materials containing simvastatin, applied them to rat incisor sockets and examined the bone of the extracted site radiographically and histologically.

MATERIALS AND METHODS

The Committee of the Animal Experiment of Tokyo Medical and Dental University approved this study. Thirty-six Wistar male rats (10 weeks old) were used. Simvastatin was provided by Merck & Co., Inc., Whitehouse Station, NJ, USA. Bovine type-I atelo-collagen (2% solution, KOKEN Co., Ltd. Tokyo, Japan) and calcium sulfate (grade for thin layer chromatography, Wako Pure Chemical Co. Ltd. Osaka, Japan) were used as drug carriers. Five hundred μl of collagen solution with or without 2 mg simvastatin was freeze-dried in 0.5 ml materials was done in aseptic condition.

Our group has recently established an experimental model of rat incisor socket healing. Right mandibular incisor of each rat was cut at the gingival margin level every three days three times under ether anesthesia. Three days after the third cut, the incisor was extracted. The animals were divided into 6 groups and the socket of each group was differently treated as shown in Table 1. Animals were sacrificed 4 weeks after the incisor extraction under excess chloroform anesthesia and the mandibles were removed for analysis. Whole mandible was photographed with soft X ray and bone mineral content (BMC) of the extracted site was measured with dual-energy X-ray absorptiometry for small animals (DXA, DCS-600, Aloka Co. Ltd.) (Fig. 1). Values of BMC of each group was presented as mean and standard deviation and statis-

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
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<tbody>
<tr>
<td>Control</td>
<td>Socket was not treated.</td>
</tr>
<tr>
<td>S</td>
<td>Simvastatin was directly applied to the socket after the extraction.</td>
</tr>
<tr>
<td>Col</td>
<td>Freeze-dried collagen was applied to the socket.</td>
</tr>
<tr>
<td>Col-S</td>
<td>Freeze-dried collagen containing simvastatin was applied to the socket.</td>
</tr>
<tr>
<td>P</td>
<td>Plaster of Paris (calcium sulfate) was applied to the socket.</td>
</tr>
<tr>
<td>P-S</td>
<td>Plaster of Paris containing simvastatin was applied to the socket.</td>
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Fig. 1 The area of BMC measurement. BMC in the area including the extracted site, the anterior region of the medial plane of the first molar, was measured.
P-S group the augmentation of the bone of the extracted site was prominent visually and the thick alveolar bone of the tooth socket of the P-S group was evident in the soft X-ray photograph (Fig. 2). This bone increase was not prominent in other groups including the S and Col-S groups. The results of DXA analyses were presented in Fig. 3. Corresponding with the soft X-ray photograph, BMC of the extracted site of P-S group was significantly high compared to the other groups. As
shown in Fig. 4, in P-S group, the thick alveolar bone was observed in the transverse section of the extracted site (Fig. 4).

**DISCUSSION**

It has been reported that intermittent injection of simvastatin to mouse head increases bone thickness. Thus, it is likely that local continuous release of simvastatin in the vicinity of the bone could augment bone. Thylin et al. have used methylcellulose as a drug carrier of simvastatin and applied this combination on the mouse calvaria. Wong and Rabie used collagen sponge to apply simvastatin to bone defect. In the present study, we used collagen and calcium sulfate as drug carriers for local application of simvastatin. Collagen is the most abundant matrix protein in the body and a favorable scaffold for regeneration. Collagen sponge or sheet has been clinically used as dressing material for wound healing including tooth socket healing. Calcium sulfate, which is also called “Plaster of Paris”, is an absorbable material in the body and it has been applied as a bone substitute to bone defect in some countries. These two carriers are biocompatible and they did not affect BMC of the extracted site.

Notably, in the present study the application of P-S augmented the bone of the extracted site whereas neither S group nor Col-S group showed such an augmentation effect. When simvastatin alone was locally applied to the socket, it would rapidly spread to the whole body. When simvastatin was mixed with collagen and applied to the socket, it would remain there for a longer time than simvastatin alone. Wong and Rabie applied collagen sponge containing 0.5 mg simvastatin to the bone defects of the parietal bone of rabbits and demonstrated that this material stimulated...
bone repair compared to the collagen application. The reason why we did not observe any bone increase in Col-S group is not clear. The difference of animals and experimental models might explain this. When simvastatin was mixed with calcium sulfate and applied to the socket, it would be gradually released together with the degradation of calcium sulfate. Calcium sulfate can probably keep simvastatin for longer period than collagen. Therefore, it is conceivable that the slow release of simvastatin would be a key to augment bone.

Ayukawa and his collaborators have demonstrated that systemic administration of simvastatin to rats increases osteogenesis around titanium implants, which strongly suggests the potentiality of this compound in dental implant treatment. In the present study we demonstrated that the local application of simvastatin with calcium sulfate as a carrier thickened the alveolar bone of the socket. Simvastatin is chemically synthesized and it is chemically stable at room temperature. Furthermore, it is inexpensive. Adverse and beneficial effects of this compound have been well examined and proved. Thus, local application of this compound to the tooth socket after tooth extraction would not exert serious side effect and it might clinically preserve alveolar ridge after tooth extraction.

ACKNOWLEDGEMENTS

This research was supported by the grant for Center of Excellence Program for Frontier Research on Molecular De-

struction and Reconstruction of Tooth and Bone in Tokyo Medical and Dental University. This research was also supported by a grant-in aid (15659475) from the Ministry of Education, Science and Culture, Japan.

REFERENCE


(Received: March 14 / Accepted: March 25)

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85