Effects of simvastatin on relapse and remodeling of periodontal tissues after tooth movement in rats

Guanghong Han,a Yuanping Chen,b Jianhua Hou,c Chang Liu,d Chong Chen,e Jinliang Zhuang,f and Wei Mengg
Changchun, China

Introduction: Our objectives were to determine the effects of simvastatin on relapse and periodontal tissue remodeling after experimental tooth movement in rats and to explore the molecule mechanism. Methods: Thirty-two adult male Wistar rats were randomly divided into 2 groups. Bilateral mandibular first molars were moved mesially with nickel-titanium closed-coil springs in both groups. On the 21st day, the springs were removed, and dental casts were made. Animals in the experimental group began receiving simvastatin at a dose of 2.5 mg per kilogram per day for 4 weeks, and animals in the control group received 0.9% sodium chloride. The results were evaluated by model measuring and immunohistochemistry staining. Results: Relapse distances and relapse percentages were decreased in the simvastatin group compared with the controls. Osteoprotegerin expression increased, and RANKL decreased. Conclusions: These results indicate that simvastatin inhibits the bone-resorbing activity of osteoclasts while stimulating bone formation, probably by controlling the ratio of local osteoprotegerin to RANKL in the periodontal tissues. Therefore, it might be useful for retention. (Am J Orthod Dentofacial Orthop 2010;138:550.e1-550.e7)

Relapse is a major concern in orthodontics. It is a physiologic response of the supporting tissues to force application and can be attributed mainly to the occlusal stability and the increased mechanical tension exerted by the transseptal fiber system. It is believed that, during orthodontic tooth movement, stresses and strains are built up and stored in the periodontal and transseptal fiber systems. After removal of the orthodontic appliance, these stresses are released, and the teeth begin to relapse to their original positions. However, even though the transseptal fiber system is primarily responsible for the generation of forces on moved teeth, osteoclastic resorption and osteoblastic formation of surrounding alveolar bone are necessary for relapse. Because the relapse pressure persists until alveolar bone remodeling is completed, stimulation of alveolar bone production or inhibition of bone resorption after orthodontic tooth movement should effectively prevent the relapse of moved teeth.

The most common measure to overcome relapse is a retainer. Retention is a necessary procedure in orthodontics, and it is often prescribed for long periods in general, and even longer periods in adults and patients with periodontitis. Therefore, it is important to promote tooth stability at the new position, prevent relapse, and shorten the retention time for adults and patients with periodontitis. Recently, many studies have suggested that pharmacologic therapy might provide another mechanism to control tooth movement. Kim et al clarified that systemic administration of bisphosphonate in rats decreased the extent of relapse of moved molars via a mechanism involving impairment of the structure and resorptive functions of osteoclasts. Kanazaki et al reported that the osteoprotegerin (OPG) gene transfer to periodontal tissue inhibited the receptor activator of nuclear factor κB ligand (RANKL)-mediated osteoclastogenesis and significantly inhibited experimental tooth movement. However, in these studies, the control of tooth movement was considered to be due to inhibiting bone-resorbing activity of osteoclasts. In a recent study, relaxin, which is known to remodel soft tissues, was administered to dogs by gingival injection and had a significant preventive effect on relapse. Therefore, we
hypothesized that stimulating bone formation might be a method to makemovedtooth stable and reduce relapse.

Statins, 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA)-reductase inhibitor, are widely used for lowering serum cholesterol. Several statins, such as simvastatin and lovastatin, have anabolic effects on bone metabolism in vivo. Also, statins can stimulate osteoblastic bone formation in vitro and in vivo. Many researchers are using statins in oral research. A recent study reported, that at low concentrations, simvastatin had a positive effect on the proliferation and differentiation of human periodontal ligament (PDL) cells in vitro. Furthermore, an in-vivo study showed that simvastatin had a stimulatory effect on alveolar bone formation in rats with periodontitis. Statin and collagen grafts increased new bone formation in parietal defects in rabbits. Intraperitoneally injected simvastatin promoted osteogenesis around titanium implants measured as the bone contact ratio.

So far, no study concerning the effect of simvastatin on orthodontic tooth movement has been done. The purposes of this study were to investigate whether systemic administration of simvastatin in rats has an effect on relapse after orthodontic tooth movement and to explore the molecular mechanism of its role.

MATERIAL AND METHODS

All procedures involving animals were in strict accordance with the guidelines established by Jilin University for animal research and approved by the local ethics committee. Thirty-two male Wistar rats (age, 7-8 weeks; weight, 225 ± 25 g) were used in this study. All rats were bred in the Jilin University Laboratory Animal Center and housed in cages under controlled conditions of temperature (22°C), humidity (40%), and lighting (12 hour light and dark cycle) with free access to food and water.

The appliance design and placement were as previously described by Leiker et al. Briefly, the animals were anesthetized with ketamine hydrochloride, and a nickel-titanium closed-coil spring exerting an orthodontic force of 50 cN was ligated bilaterally to the maxillary first molars and incisors. The force was measured with a gauge. The rationale for the applied 50 cN force was that previous studies had demonstrated that a 40 to 60 cN force stimulated substantial molar tooth movement in rats. Experimental tooth movement was conducted for 21 days. Stone models of the rat maxillae in both groups were made every week. At the end of the experimental period, the spring appliances were removed, and precise impressions of all rat maxillae were taken by using silicone material with individual resin trays with the rats under anesthesia with light ether; stone models of the maxillae were also made. The rats were then randomly divided into 2 groups: a control group and a test group receiving simvastatin injections. Rats in the test group were given intraperitoneal injections of simvastatin, 2.5 mg per kilogram per day (JingXin Pharmaceutical, ZheJiang, China), for 4 weeks from the last day of tooth movement. Rats in control group received daily injections of 0.9% sodium chloride during the same period.

To quantify tooth movement, the distance between the distal grooves of the first and second molars was measured on the stone model of each animal. The models were trimmed so that they were parallel to the occlusion plane of the molars; the distance between the bottom and the occlusal plane was about 1 cm. The prepared models were scanned with Scan Maker (version 5.99, Samsung, Beijing, China) with a 1:1 proportion, and the distance was measured bilaterally with Photoshop (version 7.0, Adobe Photoshop, San Jose, Calif). All measurements were repeated 3 times by an examiner, and the mean of these measurements was used as the representative value of each distance. The following parameters were calculated on the basis of the measurements: (1) tooth movement (D0), the total tooth movement during the 21 days of orthodontic treatment; (2) relapse distance (Ra) of the various weeks, estimated by subtracting the distances of the various weeks from D0; and (3) percentage of relapse of the various weeks (PRn), the amount of Rn times 100 divided by D0.

At the end of drug treatment, the rats in both groups were perfused transcardially with 4% paraformaldehyde in 0.1 mol/L of phosphate buffer (pH 7.4) under deep anesthesia. The maxillae, including molars, were dissected, fixed overnight in 4% paraformaldehyde buffer, and then decalcified in 10% EDTA solution (pH 7.4) at 4°C for 4 weeks. The decalcified maxillae were dehydrated in a graded series of ethanol and embedded in paraffin. Eleven consecutive cross-sections were cut at 5 µm, and the sections numbered 1, 4, 7, and 11 were selected for staining with hematoxylin and eosin and strept-avidin-biotin-horseradish peroxidase complex immunohistochemistry. The detection of OPG and RANKL immunoreactivity was performed with polyclonal rabbit anti-OPG (1:250; Santa Cruz; sc-11383) and polyclonal goat anti-RANKL (1:250; Santa Cruz, sc-7628). The immunoreactivity of OPG and RANKL proteins was scored by using the Computer Image Analyzing System (HPIAS-1000, version 6.0, Media Cybernetics, Silver Spring, Md), and then the scores were converted to gray-scale values. Periodontal
tissues of the mesial roots of the first molars were examined. The expressions were measured on the pressure and tension sides in half of the mesial root area by a quantitative method, divided by the mesiodistal axis of the root.

Statistical analysis

The data were processed with SPSS software (version 11.5, SPSS, Chicago, Ill). The results were expressed as the means ± standard deviations. The data of Rn, PRn, OPG, and RANKL between groups were subjected to 1-way analysis of variance (ANOVA). The data of OPG and RANKL on the pressure and tension sides were subjected to paired t tests. The significance level was set at \( P < 0.05 \).

RESULTS

As shown in Table I, after simvastatin administration for 1 week and 4 weeks, the relapse distances in the test group were less than those in the control group (\( P < 0.01 \)). PRn in the test group was significantly lower compared with the control group (\( P < 0.001 \)). R1 and R4 of test group were 37.4% and 54.3% of the control group.

In the histologic examination, after appliance removal, the first molar relapsed toward the distal side. Therefore, the PDL on the distal side of the mesial root was considered to be the compressed side, and the mesial side of the PDL of the mesial root was the stretched side. Because tipping movement of the molars had occurred by force applications from placement and removal, we examined the PDL and the adjacent alveolar bones at the longitudinal midportion of the molar roots. In the immunohistochemistry examination, OPG was mainly distributed in fusiform mesenchymal cells around blood vessels and osteoblasts in the bone marrow, on the surface of the alveolar bone, and in fibroblasts in the periodontal tissue, odontoblasts, and dental pulp cells during normal tooth movement in the rats. RANKL was well expressed mainly in fusiform mesenchymal cells and extension cell processes around blood vessels, in addition to osteoblasts along the alveolar bone surface and a few weakly stained periodontal fibroblasts.

As shown in Table II, in the comparison of OPG and RANKL expression in the experimental and control groups, the differences in RANKL were statistically significant (\( P < 0.001 \)).

After the paired t tests (Table III), the scores of OPG observed on the tension side were slightly higher than those on the compressed side of the PDL, whereas the alterations were not statistically significant (\( P > 0.05 \)).

**Table I.** Relapse distances and percentages 1 week and 4 weeks after administration of simvastatin (mean ± SD)

<table>
<thead>
<tr>
<th>Group</th>
<th>Relapse distance (mm)</th>
<th>Relapse percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 week</td>
<td>4 weeks</td>
</tr>
<tr>
<td>Control</td>
<td>0.265 ± 0.034</td>
<td>0.359 ± 0.051</td>
</tr>
<tr>
<td>Simvastatin</td>
<td>0.099 ± 0.02*</td>
<td>0.195 ± 0.032*</td>
</tr>
</tbody>
</table>

*\( P < 0.01 \) compared with the control group; †\( P < 0.001 \) compared with the control group.

**Fig 1.** In the control rats on the compressed side, resorption lacunae were observed on the alveolar bone surface with bone formation (a). Occasionally, a few osteoclasts were observed (b). OPG was expressed weakly. AB, alveolar bone; D, dentin (immunohistochemistry, times 200).

In the control group on the compressed side, the surfaces of alveolar bone were rough, along which there were a few monolayer ancipital osteoblasts. A few osteoclasts formed Howship’s resorption lacunae with new bone deposition. The PDLs were still irregularly arranged. OPG in the PDL was weakly and positively expressed (Fig 1), but RANKL in the PDL was strongly and positively expressed (Fig 2).

In the test group, on the compression side, bundles of dense fibers and fibroblasts were regularly distributed, and the Sharpey’s fibers were stretched into the osteoid layers. The surfaces of bone and tooth were smooth. Rows of osteoblasts were arranged on the surface of the alveolar bone, which showed a round profile and plenty of cytoplasm. The osteoid layers were thick with many deeply stained hematoxylin lines, even forming finger protrusions. There were plenty of blood vessels. OPG in the PDL was strongly positively expressed (Fig 3), but RANKL in the PDL was weakly expressed (Fig 4). Most of the osteoclasts were clearly localized around blood vessels in the vascular canals of the alveolar bones (Fig 5) but were apparently sparse in the PDL proper on the compressed side.
The score of RANKL observed on the tension side was slightly lower than that on compressed side of the PDL, whereas the alteration was not statistically significant \( (P > 0.05) \). The ratio of OPG and RANKL in the PDL was less than 1 in the control group, but the ratio of the experimental group was more than 1 (Table IV).

**DISCUSSION**

In a previous study, it was demonstrated that, in rat molars, the number of osteoclasts steadily increased on the compressed side of the PDL from 1 day to 7 days after experimental force application but decreased markedly in all periodontal tissues by day 14.\(^{16}\) Therefore, at 21 days after experimental force application, the moved rat molars were considered to have been stable for at least a week. In our study, we found that, in the control group, the relapse after 1 week was the fastest, almost half of the total distance; the speed of relapse slowed in the next 2 weeks; after 4 weeks, the relapse was almost complete, by comparing \( R_n \) and \( PR_n \) at 1 week with those at 4 weeks. Possibly, relapse energy stored in the collagenous periodontal and transseptal
fiber systems was gradually released after spring removal, resulting in faster and greater relapse in 1 week. As the energy dissipated, the speed and extent of relapse stepped down until the remodeling process of the PDL was stable. Van Leeuwen et al.\textsuperscript{17} found that relapse started immediately, not only without retention, but also after retention.

Relapse in the control rats, with the more active tooth movement, was both greater and faster. However, the trend was not obvious in the test group, because simvastatin administration speeded the remodeling of the PDL. At 3 weeks in the relapse process, we found that the first molars in the test groups did not loosen, but those in the control group loosened by $1/14$. This study confirmed that simvastatin might be a potentially important agent in controlling relapse. Most studies reported that resorption of the alveolar bone was associated with OPG/RANKL in the PDL. In the in-vitro study of Wada et al.\textsuperscript{22} the results suggested that human PDL fibroblastic cells produce and secrete OPG, and the authors speculated that 1 biological role of OPG in the PDL might be the protection of the tooth from attack by osteoclasts.

OPG expressions in both sides of the PDL in the simvastatin group were higher than those in the control group, and OPG was strongly and positively expressed. All results showed that OPG/RANKL signaling is negatively regulated by the soluble receptor antagonist protein OPG, which inhibits signaling through RANK and induces osteoclast apoptosis.\textsuperscript{21} RANKL signaling is essential for osteoclast differentiation, activation, and survival. Any drug that regulates OPG and RANKL might have an effect on osteoclast differentiation, activation, and apoptosis indirectly. Many researchers have reported that resorption of the alveolar bone was associated with OPG/RANKL in the PDL. In the in-vitro study of Wada et al.\textsuperscript{22} the results suggested that human PDL fibroblastic cells produce and secrete OPG, and the authors speculated that 1 biological role of OPG in the PDL might be the protection of the tooth from attack by osteoclasts.

Table II. Comparison of OPG gray-scale values of the PDL around the alveolar bone surface (mean ± SD)

<table>
<thead>
<tr>
<th>Group</th>
<th>Compression side</th>
<th>Tension side</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>127.28 ± 2.15</td>
<td>133.90 ± 4.55</td>
</tr>
<tr>
<td>Simvastatin</td>
<td>152.78 ± 2.42*</td>
<td>154.61 ± 1.64*</td>
</tr>
</tbody>
</table>

* $P < 0.001$ compared with the control group.

Table III. Comparison of RANKL gray-scale values of the PDL around the alveolar bone surface (mean ± SD)

<table>
<thead>
<tr>
<th>Group</th>
<th>Compression side</th>
<th>Tension side</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>143.12 ± 1.69</td>
<td>141.30 ± 0.71</td>
</tr>
<tr>
<td>Simvastatin</td>
<td>121.75 ± 2.05*</td>
<td>121.46 ± 1.17*</td>
</tr>
</tbody>
</table>

* $P < 0.001$ compared with the control group.

Table IV. Ratio of OPG and RANKL in the PDL (mean)

<table>
<thead>
<tr>
<th>Group</th>
<th>Compression side</th>
<th>Tension side</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.889</td>
<td>0.948</td>
</tr>
<tr>
<td>Simvastatin</td>
<td>1.255</td>
<td>1.273</td>
</tr>
</tbody>
</table>

Fig 6. The experimental group. On the stretched side, the PDLs were regularly arranged, with thick osteoid layers. The osteoblasts were distributed in rows, and the nuclei were round. OPG was expressed strongly and positively. The staining extent of OPG was more than that on the compressed side. AB, alveolar bone; D, dentin (immunohistochemistry, times 200).
that simvastatin might regulate the level of OPG and RANKL indirectly to affect bone remodeling, inhibit osteoclastic resorption, and promote bone anabolism, by controlling BMP-2 expression to increase OPG and reduce RANKL.

In this study, RANKL expression on the compressed (distal) side was slightly higher than that on the tension (mesial) side in both groups. This suggests that the relapse direction is distal, opposite to the original tooth movement. OPG expression on the tension side was slightly higher than on the compressed side (Figs 3 and 6). The result means that the elastic relapse force of the PDL is less than the orthodontic force during the relapse process. This method, which increases bone anabolism and accelerates PDL remodeling, should raise the probability of tooth stability in the new place and reduce the retention period. Moreover, it suggests that the interactive network of bone-resorbing and anti-resorptive cytokines and hormones converges at the OPG/RANKL system. When the ratio of OPG/RANKL is less than 1, it implies that OPG has a relative excess, and then bone formation is promoted. When the ratio is more than 1, it implies that RANKL has a relative excess, and then osteoclast activation is promoted. As shown in Figure 4, the ratio was less than 1 in the control group. It suggested that the osteoclast trend was stronger than the bone anabolism, and moved tooth would return to their original positions under the relapse force of the PDL, until reaching the initial position.

It is interesting that the distribution of osteoclasts in the experimental group was different from that in the control group. Most osteoclasts in the former were clearly localized around blood vessels in the vascular canals of the alveolar bones but were apparently sparse in the PDL proper on the compressed side. The result is consistent with that of Kim et al3 in a bisphosphonate study. It suggests that simvastatin increases the speed of alveolar bone remodeling. Cementum block was observed on the cement surface on the stretched side. This was possibly because cementum block was overproliferated and secreted a matrix to form a block under simvastatin stimulation, after cement resorption on the stretched side (initially the compressed side). This observation confirms that simvastatin promotes bone formation in the PDL after orthodontic tooth movement.

**CONCLUSIONS**

Simvastatin inhibited the relapse of experimentally moved rat molars by stimulating PDL remodeling and increasing alveolar bone formation. In addition, simvastatin decreased the extent of relapse via regulating OPG and RANKL expression to control osteoclastic resorption activity and then stimulating bone formation. Further investigations are needed to confirm the effect of simvastatin on helping orthodontic patients with periodontitis.

This research was supported by JiLin Provincial Science and Technology Department of China (No.200705348).

**REFERENCES**


