Effect of local administration of simvastatin on postorthodontic relapse in a rabbit model

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Introduction: Posttreatment relapse is a major challenging clinical issue. The objective of this study was to evaluate the effect of local administration of simvastatin on posttreatment relapse. **Methods:** Orthodontic tooth movement was induced in 10 white New Zealand rabbits. After 21 days of active tooth movement, the orthodontic appliances were removed, and the experimental teeth were allowed to relapse for 21 days. During the relapse phase, 1 mandibular quadrant received local simvastatin administration, and the other received the control vehicle solution on a weekly basis. Three-dimensional models of the experimental teeth were created to allow the measurement of experimental tooth movement and posttreatment relapse. The animals were killed at the end of the relapse phase for histomorphometric analysis of alveolar bone remodeling. **Results:** The mean relapse percentages were 75.83% in the quadrant receiving the control vehicle solution and 62.01% in the quadrant receiving simvastatin. Neither the relapse magnitude nor the relapse percentage showed a significant difference between the 2 quadrants. Histomorphometric analyses showed that local simvastatin administration yielded a significant reduction in the area of active bone-resorptive lacunae and a significant increase in newly formed bone area. **Conclusions:** Although local administration of simvastatin aids in bone remodeling associated with posttreatment relapse by reducing the area of active bone resorption and upregulating bone formation, it did not significantly minimize posttreatment relapse. (Am J Orthod Dentofacial Orthop 2018;153:861-71)

Relapse is a physiologic response of the supporting tissues to force application and has been a major challenging clinical issue with respect to the goals of successful orthodontic treatment.[1,2] The classic approaches to orthodontic retention primarily involve the use of fixed or removable retainers whose caveats include reliance on patient compliance and a long duration of retention to ensure stability.[3]

Various systemically and locally administered agents have been reported to reduce the amount of relapse in animal models, including bisphosphonate,[4] osteoprotegerin,[5] relaxin,[6] and bone morphogenetic proteins.[7] Although the mechanisms of action are different, relapse is decreased by modification of the remodeling process of the dental supporting tissues. These findings prompted the possibility of safe pharmacotherapeutic strategies designed to manipulate alveolar bone remodeling to minimize postorthodontic relapse.

A hypolipidemic agent, simvastatin, has recently been expected to serve as a therapeutic agent for osteoporosis. These drugs, known as statins, represent a new approach for treating osteoporosis; one that emphasizes building new bone to replace bone that has deteriorated.[8] Of 30,000 natural compounds, Mundy et al[9] identified statins as the only natural product that specifically increases bone morphogenetic protein-2 (BMP-2) gene expression in osteoblasts and increases levels of bone formation proteins in these cells. There was also a striking increase in osteoblast cell numbers after statin application, since BMP-2 is one of the most potent growth factors targeting bone formation in vivo, which stimulates osteoblastic differentiation and proliferation.[10]

Inhibition of the 3-hydroxy-3-methyl-glutaryl-coenzyme-A reductase enzyme and the subsequent blockage of the mevalonate pathway is probably the most

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All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest, and none were reported.
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Submitted, July 2017; revised and accepted, October 2017.
0889-5406/$36.00

https://doi.org/10.1016/j.ajodo.2017.10.024

important mechanism of inhibition of bone resorption by simvastatin.[11] Yazawa et al[12] showed that relatively low concentrations of simvastatin promote cell proliferation and osteoblastic differentiation. Lee et al[13] observed increased bone formation and higher maximum force to fracture after local application of statins in rat mandibles. Ozec et al[14] showed that local application of simvastatin significantly increased the density of bony defects in rat mandibles.

Statins, including simvastatin, showed biologically significant antioxidant,[15] anti-inflammatory,[16] and anabolic effects on osteoblastic bone formation that could prove beneficial in orthodontics. Han et al[17] showed that the systemic administration of simvastatin could minimize postorthodontic relapse by inhibiting the bone-resorbing activity of osteoclasts and stimulating bone formation. Moreover, the impressive long-term safety profile of simvastatin renders it a suitable agent for use in orthodontic therapy.[18]

In this study, we used an experimental rabbit model to explore the effect of local administration of simvastatin on postorthodontic relapse. It was hypothesized that the osteoinductive effects of simvastatin on the dental supporting tissues as well as the inhibition of alveolar bone resorption might minimize postorthodontic relapse. The null hypothesis was that the local administration of simvastatin has no effect on either postorthodontic relapse or associated alveolar bone remodeling.

MATERIAL AND METHODS

This study was conducted in accordance with the ARRIVE guidelines[19] for animal studies and after approval from the ethics committee, Faculty of Dentistry, Alexandria University in Egypt.

Calculation of the required sample size was performed using the formula for studies comparing paired continuous data.[20] Based on the results of a previous study yielding a standard deviation of 0.548 mm,[21] we determined that a conservative sample of 9 rabbits would be sufficient to detect an effect size of a 0.7-mm difference in a split-mouth study with a power of 90% and a significance level of 0.05. Since we expected that some animals might not tolerate the experimental procedures and survive until the end of the experimental period, the sample size was increased to a total of 10 rabbits to allow for a 10% expected attrition.[22]

Ten 16-week-old healthy male white New Zealand rabbits (*Oryctolagus cuniculus*) were included in the study. They had a body weight between 2.5 and 3.5 kg with normal development of dentition and mandibular incisors, premolars, and molars. All experimental procedures were performed under general anesthesia by intramuscular injection of ketamine (ketamine alfasan 10%; Alfasan Group of Companies, Woerden, The Netherlands) and xylazine (xyla-ject injectable solution; Adwia Pharmaceuticals, 10th of Ramadan City, Egypt).

The rabbits were kept under standardized light and dark cycles at the animal house of Alexandria Medical Research Institute. They had free access to a soft standard diet to minimize the incidence of appliance breakage.

A prospective randomized split-mouth experimental trial was implemented in each rabbit. The appliance used to produce tooth movement was similar to the model described by Pithon and Ruellas.[23] All rabbits received an orthodontic appliance consisting of a 13-mm nickel-titanium closed-coil spring (Jiscop, Gyeonggi-do, Korea) stretched between the mandibular first premolars and incisors bilaterally. The activation force of the spring was measured with a force gauge (Correx; Haag-Streit, Koniz, Switzerland) so that the spring provided a force of 100 cN. A ligature wire was used to encircle the mandibular second premolar, first molar, and second molar and tied to stabilize them in position and minimize the influence of interseptal fibers. The appliance was left in place for 21 days to achieve appreciable tooth movement (Fig 1).

At the end of active tooth movement, the orthodontic appliances were removed, and the experimental teeth were allowed to relapse for 21 days. Each mandibular quadrant was randomly assigned to 1 of 2 experimental groups. In the control group (A), the teeth were allowed to relapse with a local injection of control vehicle solution on days 21, 28, and 35, and in the simvastatin group (B), the teeth were allowed to relapse with a local injection of simvastatin on days 21, 28, and 35. The assignment of each mandibular quadrant to an experimental group was performed using a computer-generated list of random numbers. The investigator (M.M.) generating the list was blinded to the treatment groups.

Simvastatin powder (PHR1438; Sigma-Aldrich, St Louis, Mo) was prepared at a concentration of 0.5 mg per 480 μl of solution. Pluronic F-127 (Sigma-Aldrich) acted as the carrier for the simvastatin in group B. The pluronic control vehicle solution without simvastatin was administrated in group A.

Two routes of local administration were implemented in each quadrant (Fig 2). Intraligamentous injection was performed using an intraligamentous injector (Sanijject; Saniswiss, Geneva, Switzerland) into the distal periodontal space of the mandibular first premolar delivering 180 μl of solution. Submucosal injection was performed using a 0.5-ml insulin syringe with a 31-gauge ultrafine needle (Insumed 31G × 8 mm; Pic Solution, Artsana, Grandate, Italy) close to the distal