Atorvastatin-induced osteoclast inhibition reduces orthodontic relapse

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Introduction: The statin class of drugs enhances osteogenesis and suppresses bone resorption, which could be a plausible biologic mechanism for mitigation of orthodontic relapse. We aimed to determine whether atorvastatin (ATV) might affect orthodontic relapse and osteoclastogenesis by modulating expression of RANKL and osteoprotegerin (OPG), crucial molecules involved in bone turnover. Furthermore, we analyzed the adverse effects of ATV on femur turnover and endochondral ossification. **Methods:** Wistar rats were subjected to orthodontic tooth movement for 21 days, followed by removal of the appliance and ATV or saline solution administration. Up to 7, 14, and 21 days of ATV administration, tooth relapse was measured, and maxilla and femur sections were obtained and prepared for hematoxylin and eosin, TRAP, and immunohistochemical (RANKL and OPG) staining. **Results:** ATV decreased tooth relapse ($P = 0.03$) and osteoclast counts ($P = 0.04$), which were positively correlated ($P = 0.006$). Statin administration increased periodontal expression of OPG ($P = 0.008$), but not of RANKL protein. ATV administration also enhanced growth plate cartilage thickness. **Conclusions:** Statin-induced OPG overexpression reduces relapse after orthodontic tooth movement, in a phenomenon correlated with decreased osteoclast counts. This phenomenon sheds light on OPG as a molecular target that modulates maxillary bone metabolism and orthodontic relapse. (Am J Orthod Dentofacial Orthop 2017;151:528-38)

Despite the clinical relevance of orthodontic relapse, the cellular and molecular mechanisms involved in this event are not fully understood.[1-3] Yoshida et al[2] proposed that remodeling of the periodontal ligament fibers and alveolar bone are the main causes of relapse. In addition, Franzen et al[1] found that orthodontic relapse and orthodontic tooth movement (OTM) are associated with similar cellular adaptations, such as increased osteoclast differentiation in compression areas. Given this background, one could argue that endogenous or pharmacologic bone modulation to inhibit osteoclast resorption and promote osteoblast neoformation may have clinically relevant effects on the regulation of OTM and relapse.[3-8]

Statins are inhibitors of 3-hydroxy-3-methylglutaryl CoA reductase, the rate-limiting enzyme within the mevalonate pathway of cholesterol biosynthesis.[9,10] In addition to their cholesterol-lowering properties, statins have a series of pleiotropic and anti-inflammatory effects.[11] Studies have suggested that statins can influence bone turnover, enhancing osteogenesis and suppressing bone resorption.[3,12-19] These effects involve modulation of the receptor activator of nuclear kappa B (RANK), receptor activator of nuclear kappa B ligand (RANKL), and osteoprotegerin (OPG), ultimately promoting suppression of osteoclastogenesis.[3,15,17] In the bone system, RANKL is expressed on the osteoblasts, and as it binds to the RANK receptor expressed on hematopoietic osteoclast precursors, it induces rapid differentiation of these cells to mature osteoclasts. OPG is a decoy receptor produced by fibroblasts, osteoblasts, and even osteoclasts. This molecule will compete with RANK for RANKL binding, thus inhibiting differentiation of osteoclasts and inducing their apoptosis.

Apparently, the effects of statins on orthodontic relapse have been less explored. Han et al[3] observed that the ability of simvastatin to minimize tooth displacement was associated with decreased RANKL and increased OPG expression. They suggested an effective drug stimulation of bone neoformation, thus accelerating tooth stability and assisting the retention phase. Furthermore, Jin et al[20] found that simvastatin increases bone volume in rats affected by periodontal disease, with decreased RANKL expression apparently involved.

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Although statins seems to be well tolerated in adult and young patients,[21-23] long-term undesirable effects must be considered in clinical practice.[24] For instance, in-vitro and in-vivo preclinical studies have suggested that statins increase chondrocyte proliferation and longitudinal bone growth, which may preclude their use as an orthodontic pharmacologic strategy in children.[25,26]

In this study, we hypothesized that short-term atorvastatin (ATV) treatment in rats might reduce orthodontic relapse and osteoclastogenesis through modulation of RANKL and OPG expression. We also analyzed the adverse effects of ATV on long-bone turnover and endochondral ossification.

MATERIAL AND METHODS

Thirty-six male Wistar rats, age 6 weeks, weighing approximately 330 to 340 g, were used in the experiments. The animals were housed 4 to a cage, under a 12-hour light and dark cycle, at a constant temperature of 23°C, and received food and water ad libitum. All animal handling and care procedures were conducted in keeping with internationally accepted guidelines (Guide for the Care and Use of Laboratory Animals)[27] and were approved by the ethics committee of the School of Dentistry of Federal University of Rio Grande do Sul in Brazil (CEUA 23145).

After induction of anesthesia with ketamine (80 mg/kg) and xilazyne (5 mg/kg), a superelastic closed nickel-titanium coil spring exerting a force of 50 cN was inserted unilaterally, between the maxillary right first molar and incisors, as described in the split-mouth design.[1,17,28-30] Our protocol was based on previous demonstrations that 50cN provides substantial tooth movement.[1,3,4,8,30] The device was kept in place for 21 days to generate mesial displacement of the first molar. Whereas the maxillary right molar was used for experimental tooth movement, the maxillary left molar served as the internal control with no orthodontic tooth movement. Throughout the study, the animals were evaluated weekly for weight gain or loss, appliance breakage, and signals of gingival or other soft tissue inflammation. After 20 days of OTM, the animals were randomly divided into 2 groups: control (saline solution [SAL], n = 18) and experiment (ATV, n = 18). ATV, 15 mg per kilogram, was given daily (Medley Farmacêutica, Campinas, São Paulo, Brazil), via gavage, for 7, 14, or 21 days (Fig 1, *A*). Rats in the control group received 0.1 mL of phosphate-buffered saline, daily, via gavage. On day 21, the appliance was removed, marking the start of the relapse phase (Fig 1, *A*). The experimental time points were set at 7, 14, and 21 days after appliance removal (Re7, Re14, and Re21, respectively).

Using dental stone (Durone; Dentsply, York, Pa), precise plaster models of the maxilla were obtained from impressions made with silicone material (Perfil; Vigodent, Rio de Janeiro, São Paulo, Brazil). Impressions were obtained every 7 days under anesthesia (Fig 1, *A*) in both groups. The occlusal surfaces were photographed (DSC#H10; Sony, Tokyo, Japan) at 300 dpi and magnified (4 times) using Image J software (version 1.44; National Institutes of Health, Bethesda, Md; 2011). A 100-mm ruler was placed next to the casts to calibrate measurements. The mean distance between the distal surface of the first molar and the mesial surface of the second molar, measured at 3 distinct points on each photo, was considered for analyses (Fig 1, *B*). Total tooth movement during the 21 days of orthodontic treatment was recorded (baseline). At 7, 14, and 21 days after appliance removal, the distances between the first and second molars were measured and recorded. Based on the baseline and the final measurements, the percentage of relapse in each animal was calculated, and the values were averaged for each group, as described in previous studies.[3]

At each experimental time point, 12 animals (6 per group) were killed with an overdose of ketamine and xylazine. The maxillae and distal left femurs were immediately dissected and fixed by submerging for 24 hours in 10% buffered formalin. The specimens were demineralized in 10% EDTA (pH 7) for 30 to 60 days. The samples were then dehydrated through an ethanol series and embedded in paraffin. Fifteen semisuccessive cross-sections were cut at 5 μm, and the sections numbered 1, 5, 10, and 15 were selected for staining with hematoxylin and eosin, tartrate-resistant acid phosphatase (TRAP), and immunohistochemicals (RANKL and OPG). The cut plane was considered satisfactory when the overall length of the maxillary mesial root was observed (cervical to apical region). Furthermore, the distal root of the first molar also was observed.

Briefly, for TRAP staining, histologic sections were selected and incubated in acetate buffer (pH 5.0) containing naphthol AS-MX phosphate (Sigma-Aldrich, St Louis, Mo), Fast Red Violet LB Salt (Sigma-Aldrich), and 50 mmol per liter of sodium tartrate. The sections were counterstained with hematoxylin.

Histomorphometric analyses of the maxilla specimens were performed considering the following subgroups: experimental hemimaxillae from the ATV animals, control hemimaxillae from the ATV animals, experimental hemimaxillae from the SAL animals, and control hemimaxillae from the SAL animals. Comparisons across subgroups were performed at each time point (7, 14, and 21 days) or by analysis of overall means. For evaluation of the femur specimens, the sample was

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