Atorvastatin inhibits osteoclastogenesis and arrests tooth movement

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Introduction: In addition to their cholesterol-lowering effects, the statin class of drugs appears to enhance osteogenesis and suppress bone resorption, which could be a clinical concern during orthodontic treatment. In this animal study, we aimed to determine whether atorvastatin (ATV) affects orthodontic tooth movement (OTM) through osteoclast inhibition. Furthermore, we analyzed the potential adverse effects of ATV on long-bone turnover and endochondral ossification. **Methods:** Rats were administered ATV (15 mg/kg) or saline solution via gavage (n = 12 animals/group), starting 2 weeks before initial OTM. Tooth displacement was measured after 7, 14, and 21 days. Histologic sections of the maxilla and femur were obtained after 14 and 21 days of OTM and stained (hematoxylin and eosin; TRAP assay) for histomorphometric analysis. **Results:** ATV was associated with significant ($P < 0.05$) reductions in OTM and osteoclast counts. Independently of drug administration, OTM increased the number of osteoclasts and reduced the bone-volume ratio compared with the control maxillae without OTM. Long-term statin administration did not appear to affect femoral endochondral ossification. **Conclusions:** This experimental study showed that the long-term use of ATV can significantly promote osteoclast inhibition and slow the OTM in the first week in rats. Under physiologic conditions, the drug did not affect bone turnover and endochondral ossification. (Am J Orthod Dentofacial Orthop 2018;153:872-82)

Epidemiologic studies have shown a significant increase in the prevalence of diseases such as obesity and hyperlipidemia in adults,[1,2] with the latter regarded as the main cause of coronary atherosclerosis.[3] According to Mercado et al,[2] 36.7% of adults, or 78.1 million persons aged 21 years or over, in the United States alone were on or eligible for lipid-lowering treatment; 55.5% of them were taking cholesterol-lowering medications. The drugs most widely used for this purpose are the statins, which lower cholesterol levels by inhibiting hydroxymethylglutaryl-coenzyme A reductase, the rate-controlling enzyme of the mevalonate pathway.[4,5] Studies suggest that, in addition to their cholesterol-lowering effects, statins may influence bone turnover, enhancing osteogenesis and suppressing bone resorption.[6-14] Attempts to ascertain the mechanism of statin-regulated bone anabolism have suggested 3 aspects: promotion of osteogenesis, inhibition of osteoblast apoptosis, and suppression of osteoclastogenesis.[15] Although the effects of statins on bone anabolism have been widely demonstrated in laboratory studies,[6-14] their clinical effects are not convincing.[16]

The biologic reaction of periodontal tissues during orthodontic tooth movement (OTM) is characterized by an aseptic acute inflammatory response in the early stages, followed by a transient, aseptic chronic inflammation. Chemokines, cytokines, and growth factors are the main molecules that orchestrate this inflammatory response, which is followed by osteoclastogenesis and bone resorption, and then by osteoblast and new bone formation.[17] Current biologic knowledge raises the possibility that pharmacologic modulation of these periodontal cellular and molecular responses may affect OTM, as shown in various experimental models.[11,18-22] Research has demonstrated that statins reduce OTM and relapse; however, the biologic mechanisms underlying these clinical effects are unknown.[11,12] Since statins are among the most commonly prescribed pharmaceutic agents for prevention of cardiovascular

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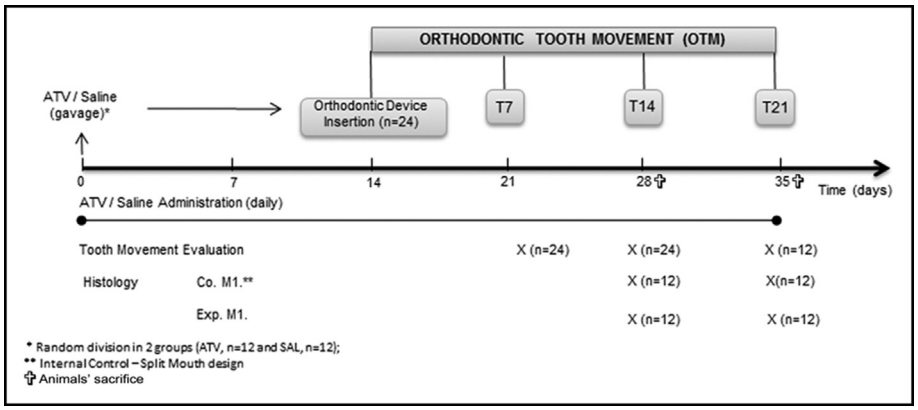

Fig 1. Experimental study design. Saline solution or ATV (15 mg/kg) was administered daily via gavage from day 0 through day 35. At day 14, mesial displacement of the right first molars was begun. Total displacements were evaluated at 7, 14, and 21 days of OTM (*T7*, *T14*, and *T21*, respectively); histologic analyses (henatoxylin and eosin and TRAP) were performed at days 14 and 21. The left maxilla served as the internal control (no OTM).

diseases, their plausible effect of arresting tooth movement could be a concern in orthodontic practice.[4,5]

Clinical trials have shown that statins are well tolerated in adult and younger populations,[23-25] but long-term data regarding the impact of statin therapy on growth and development are limited.[25,26] In a study by Macpherson et al,[23] treatment of children and adolescents with statins over 2 years had no impact on height, weight, body mass index, and sexual maturation. On the other hand, preclinical studies have suggested that these drugs increase chondrocyte proliferation and longitudinal bone growth, which could have implications for their clinical use in the pediatric population.[27,28] These discrepancies between clinical trials, in-vitro experiments, and in-vivo studies are puzzling, and further research is necessary to investigate the potential adverse effects of statins on bone turnover and endochondral ossification.[23-28]

In an attempt to mimic the clinical perspective of orthodontic treatment in patients taking statins, we developed an experimental animal model in which high-dose statin administration was started before OTM, simulating a protocol used in humans. We hypothesized that atorvastatin (ATV) treatment in rats might reduce OTM by inhibiting osteoclastogenesis. Furthermore, we analyzed its potential adverse effects on long-bone turnover and endochondral ossification.

MATERIAL AND METHODS

Twenty-four male Wistar rats (age, 6 weeks; weight, approximately 330-340 g) was the sample size estimated by the resource equation method. They were housed four to a cage, under a 12-hour light and dark cycle, at a controlled ambient temperature of 23°C, and given food and water ad libitum. All animal care and use procedures were conducted in keeping with the internationally accepted guidelines in the National Institutes of Health Guide for the Care and Use of Laboratory Animals[29] and were approved by the relevant institutional ethics committee of the School of Dentistry of Federal University of Rio Grande do Sul (number 28401) in Brazil.

The animals were randomly divided into 2 groups: experimental (ATV, n = 12) and control (saline solution [SAL], n = 12). Those in the experimental group received atorvastatin (ATV; Medley Farmacêutica, Suzano, Brazil), 15 mg per kilogram daily via gavage, and those in the control group received 0.1 mL of phosphate-buffered saline solution via the same route. Saline solution or drug administration continued until the animals were killed (Fig 1).

After 14 days of saline solution or drug administration, the animals were anesthetized with ketamine and xylazine (80 mg/kg and 5 mg/kg, respectively) for orthodontic appliance placement. This procedure consisted of placing a superelastic nickel-titanium closed-coil spring between the maxillary right first molar and incisors, as described elsewhere.[12,30-33] Our protocol was based on previous studies demonstrating that 50 cN of force is sufficient to provide substantial OTM.[11,22,32,33] The device was kept in place for 21 days (Fig 1) to generate mesial displacement of the first molar. In a split-mouth design, the maxillary right side of each rat served as the experiment (OTM), and the maxillary left side, without OTM, served as internal control. Throughout the study, the animals were evaluated weekly for weight gain or loss, appliance breakage, and gingival or other soft tissue inflammation.