

Effect of local administration of simvastatin on orthodontic tooth movement in rabbits

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Introduction: Maintaining tooth anchorage during orthodontic treatment has challenged orthodontists and threatening the success of some orthodontic therapy. The objective of this study was to evaluate the effect of local administration of simvastatin on orthodontic tooth movement. **Methods:** Nickel-titanium coil springs were used to induce orthodontic tooth movement in 10 white New Zealand rabbits for 21 days. A split-mouth design was implemented where one mandibular quadrant received local administration of simvastatin and the corresponding mandibular quadrant received control vehicle solution on a weekly basis. Magnitudes of tooth movement were measured on 3-dimensional models of the experimental teeth. Animals were killed at the end of the experimental period to allow histomorphometric analysis of alveolar bone modeling. **Results:** The total magnitude of tooth movement in the quadrant receiving simvastatin was significantly less than that in the quadrant receiving control vehicle solution. Local administration of simvastatin resulted in a significant percentage of inhibition of tooth movement of $39.8 \pm 22.6\%$. Histomorphometric analysis revealed a significant reduction in the numbers of osteoclasts and areas of active bone-resorptive lacunae hindering bone resorption processes in the quadrant receiving simvastatin. **Conclusions:** Local administration of simvastatin can reduce the rate and magnitude of orthodontic tooth movement. Moreover, local administration of simvastatin diminishes bone resorption processes associated with orthodontic tooth movement reducing the number of osteoclasts and the subsequent area of active bone resorption. (Am J Orthod Dentofacial Orthop 2019;156:75-86)

Orthodontic tooth movement is based on bone modeling that occurs after the application of mechanical forces.¹ At present, despite the efficacy of orthodontic techniques, there are a number of circumstances in which treatment efficiency might be improved by modulating the activity of osteoclasts and, consequently, bone turnover.

Maintaining tooth anchorage during orthodontic treatment has challenged orthodontists since the beginning of orthodontic treatment. Conventional methods for improving tooth anchorage aim at redirecting such

forces to skeletal structures or distributing them over a larger number of teeth.²

Poor patient cooperation has prompted orthodontists to develop alternate solutions that minimize requirements for patient compliance and improve the prediction of treatment outcome.³ However, little consideration has been directed to safe pharmacologic therapy aimed at regulating tooth movement. Various systemically and locally administered agents have been reported to impede tooth movement in animal models, including bisphosphonates,^{4,5} estrogen,^{6,7} and nonsteroidal antiinflammatory drugs.^{8,9} Although the mechanisms of action are different, tooth movement is ultimately decreased by modification of the modeling process of the dental supporting tissues. These findings prompted the possibility of pharmacotherapeutic strategies designed to manipulate alveolar bone modeling to minimize tooth movement.

Statins, such as simvastatin, lovastatin, and pravastatin, were primarily developed to inhibit cholesterol biosynthesis and reduce its plasma levels.¹⁰ Statins are considered to be first-line therapeutic agents for

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reducing myocardial infarction, coronary mortality, and the incidence of stroke or transient ischemic attack in patients with coronary heart disease and hypercholesterolemia.¹¹ The overall excellent long-term safety profile of statins is well documented.¹²

Inhibition of the 3-hydroxy-3-methyl-glutaryl-coenzyme-A reductase enzyme and the subsequent blockage of the mevalonate pathway is probably the most important mechanism of inhibition of bone resorption by simvastatin.¹³ Yazawa et al¹⁴ showed that relatively low concentrations of simvastatin promoted cell proliferation and osteoblastic differentiation. Yoshii et al¹⁵ demonstrated an increase in the volume and density of newly formed bone in critical-sized segmental femoral defects following the administration of statin in rats. Holwegner et al¹⁶ showed that intraarticular injection of statins resulted in overall increase in ramus height, condylar width, condylar bone surface density, and bone volume preserving normal condylar growth.

Statins, including simvastatin, have shown biologically significant antioxidant,¹⁷ antiinflammatory,^{18,19} and anabolic effects on osteoblastic bone formation which could prove to be beneficial in the field of orthodontics.¹⁴ Han et al²⁰ revealed that the systemic administration of simvastatin could minimize relapse through inhibiting the bone-resorbing activity of osteoclasts as well as stimulating bone formation. Mirhashemi et al²¹ showed that the reduced tooth movement following treatment with statins in Sprague-Dawley rats supported the osteogenic potential of this drug group along with its preventive effect on bone resorption.

The aim of the present study was to investigate the effects of local administration of simvastatin on the magnitude of orthodontic tooth movement and related alveolar bone modeling. The null hypothesis was that the local administration of simvastatin has no effect on either orthodontic tooth movement or associated alveolar bone modeling.

MATERIAL AND METHODS

This study was conducted in accordance with ARRIVE guidelines²² for conducting animal studies. The study was conducted after approval by the Ethics Committee, Faculty of Dentistry, Alexandria University, Egypt.

Calculation of the required sample size was performed with the use of the formula for studies comparing paired continuous data.²³ Based on the results of a previous study yielding a standard deviation of 0.548 mm,²⁴ it was determined that a conservative sample size of 9 rabbits would be sufficient to detect an effect size of 0.7 mm difference in a split-mouth

design at a study power of 90% and a significance level of 0.05. Sample size was corrected to a total of 10 rabbits to allow for 10% expected sample attrition.²⁵

Ten 16-week-old healthy male white New Zealand (*Oryctolagus cuniculus*) rabbits were included in the study. The included rabbits had body weights of 2.5–3.5 kg and normal development of dentition. All experimental procedures were performed under general anesthesia to maximize accessibility during operation. Ketamine (Ketamine Alfasan 10%; Alfasan, Woerden, The Netherlands) was injected into the paravertebral muscles at a dose of 35 mg/kg. Xylazine (Xylaject Injectable Solution; ADWA, 10th of Ramadan City, Egypt), a muscle relaxant, was administered in the same manner at a dose of 5 mg/kg. Adequate depth of anesthesia was determined by visual inspection of tongue reflex when a dental mirror was inserted in the oral cavity.

The rabbits were kept under standardized conditions of light and dark schedule at the animal house. Animals had free access to a soft standard nutritional diet to minimize the incidence of appliance breakage.

A prospective randomized split-mouth experimental trial was implemented in each rabbit. The appliance used to promote tooth movement was similar to the model described by Pithon and Ruellas.²⁶ All rabbits received an orthodontic appliance consisting of a 13-mm nickel-titanium closed coil spring (Nickel Titanium Closed Coil Springs, 13 mm, Light; Jiscop, Gunpo-si, Gyeonggi-do, Korea) stretched between the mandibular first premolars and mandibular incisors bilaterally. The spring was stretched in situ until providing a force of 100 cN as measured with the use of a force gauge (Correx Gauge; Haag-Streit, Koeniz, Switzerland). A ligature wire was used to encircle the mandibular second premolar, first molar, and second molar and tied in an attempt 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).

Each mandibular quadrant was randomly assigned to one of the experimental groups: (A) the control group, where local injection of control vehicle solution was performed on days 0, 7, and 14; and (B) the simvastatin group, where local injection of simvastatin solution was performed on days 0, 7, and 14. The assignment of each mandibular quadrant to one of the experimental groups was performed with the use of a computer generated list of random numbers. The individual generating the randomization list was blinded to the treatment groups.

Simvastatin powder (Simvastatin PHR1438; Sigma-Aldrich, Saint Louis, Mo) was prepared at a concentration of 0.5 mg/480 μ L solution. Pluronic F127

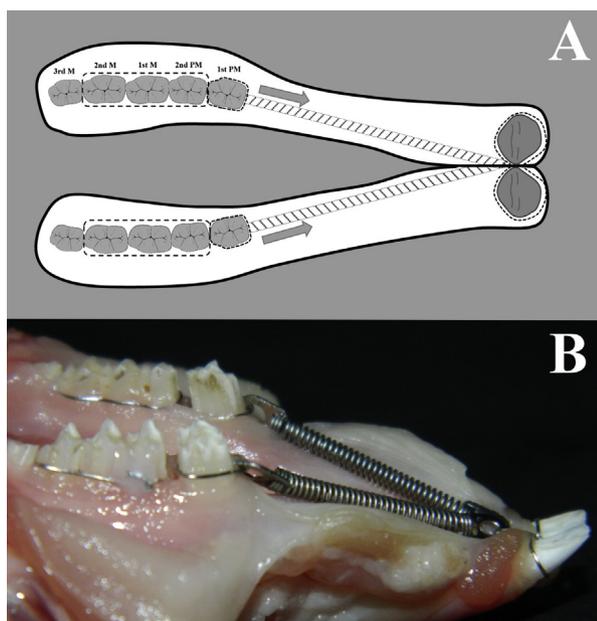


Fig 1. A, Schematic diagram showing an occlusal view of the orthodontic appliance used in the study. Arrows indicate the direction of experimental tooth movement. *PM*, premolar; *M*, molar. **B**, Lateral view of experimental orthodontic appliance in situ.

(Sigma-Aldrich) acted as the carrier for the simvastatin in group B. Pluronic control vehicle solution without simvastatin was administered in group A.

Two routes of local administration were implemented in each quadrant (Fig 2). Intraligamentous injection was performed with the use of an intraligamentous injector (Saniject; Saniswiss, Geneva, Switzerland) into the mesial periodontal space of mandibular first premolar, delivering 180 μ L of solution. Submucosal injection was performed with the use of a 0.5-mL insulin syringe with a 31-gauge ultrafine needle (Insumed 31G Insulin Syringe 31G \times 8 mm; Picosolution, Artsana, Grandate, Italy) in close proximity to the mesial surface of the mandibular first premolar, delivering 300 μ L of solution. The solutions were injected by an operator blinded to the contents of the syringes.

Impressions of experimental teeth were performed on the 7th, 14th, and 21st days with the use of injection type silicone vinyl polysiloxane impression material (3M ESPE Express Vinyl Polysiloxane Impression Material—Fast Set; 3M ESPE Dental Products, Saint Paul, Minn) loaded into previously fabricated custom trays. The impressions were then poured with the use of an improved die stone (Elite Rock Dental Stone, Zhermack, Badia Polesine, Rovigo, Italy).

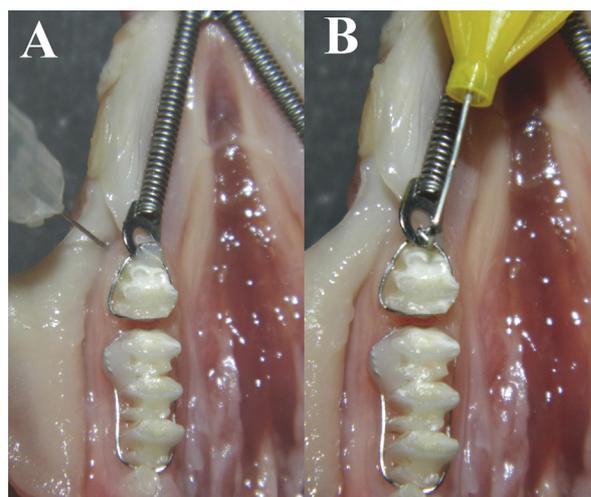


Fig 2. A, Submucosal and **B**, intraligamentous injections of working solutions at the experimental sites. Submucosal injection was performed with the use of an insulin syringe with a 31-gauge ultrafine needle in close proximity to the mesial surface of the premolar. Intraligamentous injection was performed with the use of a Saniject intraligamentous injector into the mesial periodontal space of mandibular first premolar.

Casts of the experimental teeth were scanned with the use of a 3D scanner (InEos X5 Scanner; Sirona Dental Systems, Bensheim, Germany) to create 3-dimensional (3D) models in STL file format. With the use of Viewbox software (Viewbox for Windows, Version 4.0.1.7; Dhal Software, Kifissia, Greece), the models were oriented according to the mandibular occlusal plane determined by the cusp tips of the mandibular molars and second premolar. Two planes were drawn perpendicular to the mandibular occlusal plane. The first plane was drawn touching the most distal contact area of the distal surface of the mandibular first premolar. A second plane was drawn touching the most mesial contact area of the mesial surface of mandibular second premolar. The amount of tooth movement was determined by measuring the linear distance (parallel to the mandibular occlusal reference plane) between the 2 constructed planes (Fig 3). Measurements were performed with the use of the ruler tool of the Viewbox software. The aforementioned method, similar to that proposed by Vieira et al²⁷ with modifications, was performed to minimize the measurement errors from the tipping and extrusion movements that occurred on the mandibular first premolar. Magnitudes and weekly incremental rates of tooth movement were calculated in both experimental groups.

Measurements were performed in a blinded fashion by a single investigator. The intraexaminer errors for

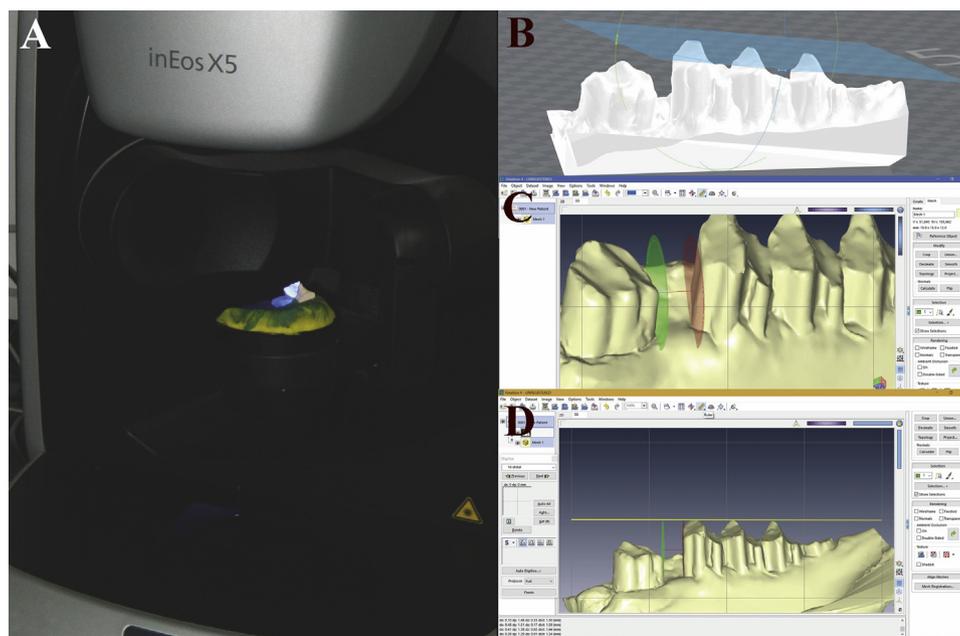


Fig 3. Measurement of magnitude of tooth movement. **A**, InEos X5 Sirona Scanner used to scan dental casts. **B**, 3D models were first oriented according to the mandibular occlusal plane. **C**, Two planes were constructed touching the most distal and most mesial contact areas of the mandibular first and second premolars, respectively. **D**, The magnitude of tooth movement was determined by measuring the linear distance (parallel to the mandibular occlusal reference plane) between the 2 constructed planes. Measurements were performed with the use of the ruler tool of the Viewbox software.

tooth movement measurements were assessed by repeating the measurements of 30 randomly selected 3D models, 2 weeks apart by the same investigator. Intra-class correlation coefficient (ICC) was calculated to assess intrarater reliability.

At the end of the experimental period, 3 animals were randomly selected for histologic analyses. After they were killed, their mandibles were dissected, cut into halves, fixed, and decalcified. Parasagittal serial sections of 6 μm thickness were obtained, and 5 randomly selected sections per specimen were processed. The sections were stained with hematoxylin and eosin. Sections along the mesial aspect of the root of the mandibular first premolar in each of the experimental groups A and B were evaluated under a light microscope (Zeiss Primo Star Light Microscope; Carl Zeiss, Oberkochen, Germany) equipped with a 5-megapixel digital camera, and images of representative areas were captured and described. Histologic sections including the coronal and middle third of the root of the mandibular first premolar and the adjacent alveolar bone were examined.

After image scale calibration with the use of Image J Software (Image J Software for Windows, version 1.50i; U.S. National Institutes of Health, Bethesda, Md), a square grid overlay with a side length of 1 mm was

superimposed over the histologic sections, with the 2 opposite sides of the square perpendicular to the root surface of the mandibular first premolar. Histomorphometric parameters, including number of osteoclasts, area of active bone-resorptive lacunae, and number of capillaries, were evaluated within the designated square area according to the method previously described by Igarashi et al.²⁸ Number of osteoclasts, as an indicator of bone resorption, was performed on the pressure side along the alveolar bone surface adjacent to the mesial aspect of the root of the mandibular first premolar. Cells were considered to be osteoclasts if they were multinucleated eosinophilic cells, containing round nuclei and located on the alveolar bone surface or residing in bone-resorptive lacunae. Number of osteoclasts was expressed as number of cells per square mm of bone area. In a similar manner, the sizes of active bone-resorptive lacunae were measured in both experimental groups. Active bone-resorptive lacunae were defined as resorptive cavities in which osteoclasts could be seen. The size of a lacuna was measured with the use of a virtual line joining the edges of the lacuna. Resorptive lacunae with depths $<3 \mu\text{m}$ were not included. The sum of the sizes of the lacunae in each section was considered to represent the active bone-resorptive area.

Table I. Magnitudes and weekly incremental rates of tooth movement in experimental groups A and B

	Group	Day 7	Day 14	Day 21	Total % of inhibition*
Magnitude of tooth movement (mm)	A	0.81 ± 0.14	1.29 ± 0.11	1.77 ± 0.15	39.8 ± 22.6%
	B	0.41 ± 0.08	0.71 ± 0.20	1.04 ± 0.38	
	Group	Week 1	Week 2	Week 3	Average weekly incremental rate (mm)
Weekly incremental rate of tooth movement (mm)	A	0.81 ± 0.14	0.48 ± 0.07	0.47 ± 0.15	0.59 ± 0.05
	B	0.41 ± 0.08	0.31 ± 0.21	0.33 ± 0.19	

*Percentage of paired alternative group.

Table II. Post hoc tests showing pairwise comparisons between weekly incremental rates of tooth movement in group A

Weekly incremental rates in group A	Mean difference (mm)	SE	Significance*	95% CI for difference*	
				Lower	Upper
Week 1–week 2	0.334 [†]	0.066	0.003	0.136	0.533
Week 2–week 3	0.009	0.058	1.000	−0.167	0.185

*Bonferroni adjustment for multiple comparisons; [†]The mean difference is significant ($P < 0.05$).

Histologic analysis was performed in a blinded manner by a single investigator. Histomorphometric parameters were remeasured by the same investigator 2 weeks apart on 15 randomly selected slides. ICC was calculated to assess intrarater reliability.

Statistical analysis

Statistical Package for Social Sciences Software (IBM SPSS Statistics for Windows, version 23; IBM, Armonk, NY) was used for conducting statistical analysis.

Regarding intragroup comparisons, Mauchly test was used to assess the sphericity between each pair of scores in the same experimental group. In case the assumption of sphericity was not verified, Greenhouse-Geisser correction was applied. One-way repeated-measures analysis of variance (ANOVA) was conducted to test the presence of significant differences between each group’s means, followed by post hoc tests to investigate the differences between each group’s means.

For comparisons between different groups, Kolmogorov-Smirnov and Shapiro-Wilk tests were conducted to assess the normality assumption. Once verified, paired-samples *t* test was conducted to compare mean values between different experimental groups. Otherwise, Wilcoxon signed ranks test was conducted. Differences with *P* values less than the 0.05 level of significance were considered to be significant.

RESULTS

One experimental rabbit failed to survive and was excluded from the study. Because the percentage of

the attrition was similar to the estimated percentage during the sample size calculation, the animal was not substituted. The rest of the animals tolerated the experimental procedures well, with no discernible effects on overall health.

Mean values and standard deviations were calculated for the magnitudes of experimental tooth movement on days 7, 14, and 21 as well as the weekly incremental rates of tooth movement during the 1st, 2nd, and 3rd weeks and their corresponding average values in groups A and B. A summary of the calculated data is presented in Table I.

The estimated ICC for the repeated measurements of tooth movement was 0.994, revealing strong evidence for the reliability of the performed measurements.

For group A, the results of the Mauchly test of sphericity for weekly incremental rates of tooth movement revealed a value of 0.663, confirming the assumption of sphericity. The results of the repeated-measures ANOVA test revealed a significance value ($P < 0.001$) denoting the presence of a significant difference between means of weekly incremental rates of tooth movement in group A. Table II presents pairwise comparisons with a Bonferroni correction for weekly incremental rates of tooth movement in group A. The only significant difference was reported between the weekly incremental rates of tooth movement during the 1st and 2nd week ($P = 0.003$).

For group B, the results of the Mauchly test of sphericity for weekly incremental rates of tooth movement revealed a significant value ($P = 0.031$), indicating

Table III. Paired-samples *t* test comparing total cumulative magnitudes and weekly incremental rates of tooth movement in groups A and B

Comparison	Paired differences						Significance (2-tailed)
	Mean (mm)	SD	SEM	95% CI of difference			
				Lower	Upper		
Pair 1 Total cumulative movement magnitudes (group A–group B)	0.721*	0.455	0.152	0.372	1.070	0.001	
Pair 2 Week 1 movement (group A–group B)	0.407*	0.200	0.067	0.253	0.561	0.000	
Pair 3 Week 2 movement (group A–group B)	0.174	0.255	0.085	−0.022	0.371	0.075	
Pair 4 Week 3 movement (group A–group B)	0.140	0.316	0.105	−0.103	0.383	0.220	
Pair 5 Average weekly movement (group A–group B)	0.242*	0.151	0.050	0.126	0.358	0.001	

*The mean difference is significant ($P < 0.05$).

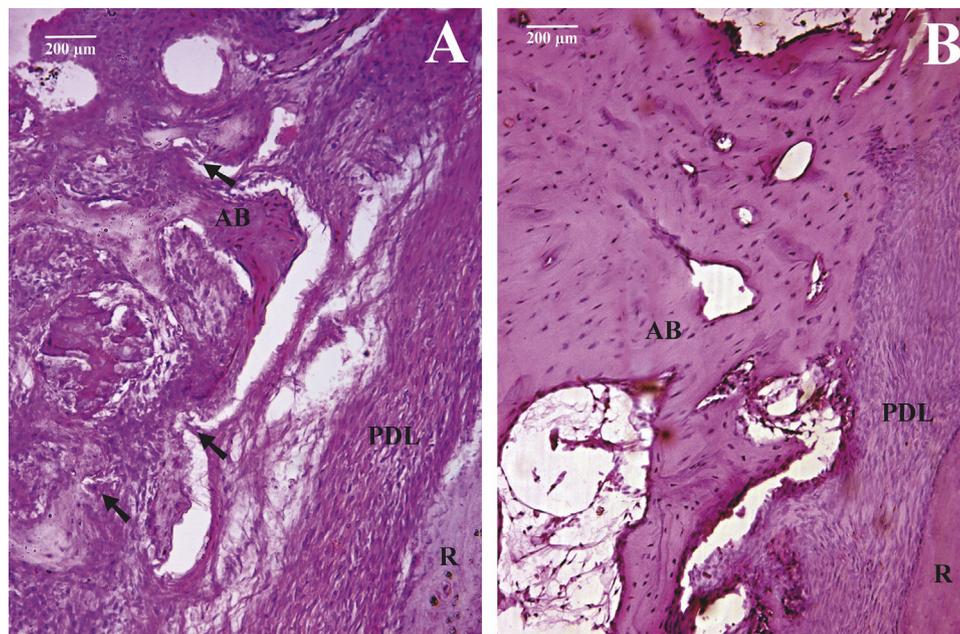


Fig 4. Light microscopic images of the mesial aspect of the mandibular first premolar in **A**, the control group and **B**, the simvastatin group. In the control group (**A**), extensive areas of bone resorption were observed with widespread intensive resorption foci (arrows). In the simvastatin group (**B**), relative reduction in bone-resorptive activity was noted. Alveolar bone exhibited a smoother surface, being uniform at times and cut at others. *R*, root of mandibular first premolar; *PDL*, periodontal ligament; *AB*, alveolar bone. Hematoxylin and eosin staining.

violation of the assumption of sphericity. The results of the repeated-measures ANOVA test with a Greenhouse-Geisser correction ($F(1.228, 9.826) = 1.421$; $P = 0.271$) excluded the presence of a significant difference between weekly incremental rates of tooth movement in group B.

Results of Kolmogorov-Smirnov and Shapiro-Wilk normality tests for total cumulative magnitudes of tooth movement and weekly incremental rates of tooth movement confirmed the assumption of normality.

As illustrated in [Table III](#), results of paired-samples *t* tests revealed the presence of a significant difference ($P = 0.001$) between total cumulative magnitudes of tooth movement in groups A and B showing significant reduction in group B. Moreover, the results revealed a significant difference in the weekly incremental rates of tooth movement between groups A and B during the 1st week only ($P < 0.001$). However, no significant difference in the weekly incremental rate of tooth movement was reported during the 2nd ($P = 0.075$)

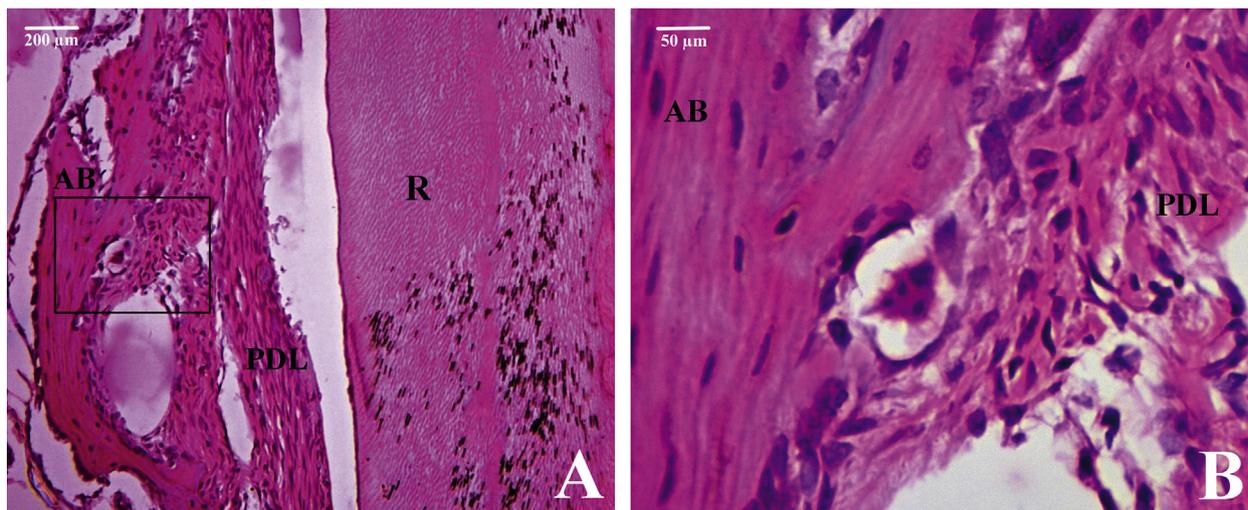


Fig 5. Light microscopic images of alveolar bone surface in group A, showing distinctive resorptive osteoclastic activity with multinuclear osteoclast within Howship lacunae. Hematoxylin and eosin staining.

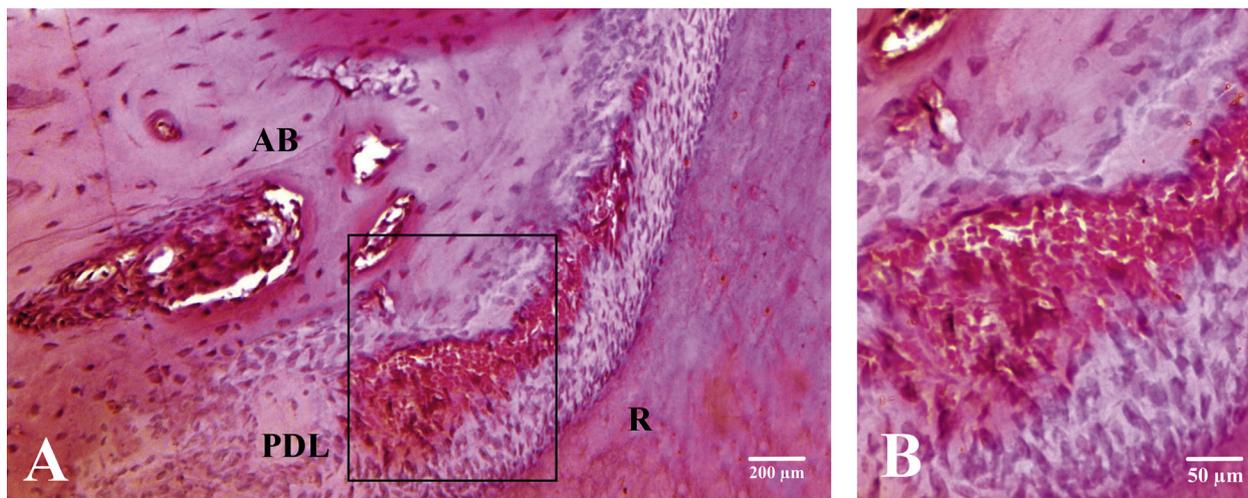


Fig 6. Light microscopic images of extensive extravasation of blood vessels observed along the mesial aspect of mandibular first premolar in group A. Hematoxylin and eosin staining.

or 3rd week ($P = 0.220$). Similarly, a significant difference was reported in the average weekly rate of tooth movement between groups A and B ($P = 0.001$) showing significant reduction in group B.

Histologic changes along the mesial aspect of the mandibular first premolars in experimental groups A and B are illustrated in Figures 4-6.

In group A, receiving local administration of control vehicle solution, histologic sections along the mesial aspect of the root of the mandibular first premolar revealed multiple areas of bone resorption. Rough surface

of alveolar bone was observed with widespread intensive resorption foci (Fig 4, A). Multinuclear osteoclasts were seen forming Howship lacunae (Fig 5). Few monolayer osteoblasts were observed on the surface of alveolar bone. Discrete reduction of periodontal space in the direction of the movement was noted with irregular arrangement of periodontal ligament fibers. Extensive cellular infiltration and activity were observed adjoining the bone surface with increased fibroblastic proliferation in areas in close proximity to alveolar bone. Increased neovascularization was observed adjacent to the alveolar

Table IV. Results of histomorphometric analysis in groups A and B

Parameter	Group A	Group B	Intraclass correlation*
Number of osteoclasts [†]	6.07 ± 3.06	3.40 ± 2.10	0.902
Area of active bone-resorptive lacunae [‡]	0.28 ± 0.24	0.17 ± 0.07	0.879
Number of capillaries [†]	2.60 ± 1.35	1.87 ± 1.60	0.910

*Two-way mixed effects model using an absolute agreement definition; [†]Mean counts per mm² of alveolar bone surface; [‡]Mean values in mm² per mm² of alveolar bone surface.

Table V. Paired-samples *t* test comparing numbers of osteoclasts between groups A and B

Comparison	Paired differences						Significance (2-tailed)
	Mean*	SD	SEM	95% CI of difference			
				Lower	Upper		
Pair 1 Osteoclast A–osteoclast B	2.667*	3.697	0.955	0.619	4.714	0.014 [†]	

*Counts per mm² of alveolar bone surface; [†]The mean difference is significant ($P < 0.05$).

Table VI. Wilcoxon signed ranks test comparing areas of active bone-resorptive lacunae and numbers of capillaries between groups A and B

Group A			Group B			Wilcoxon signed ranks test	
Median	Min	Max	Median	Min	Max	Z	Asymptotic significance (2-tailed)
Area of active bone-resorptive lacunae*							
0.20	0.03	0.89	0.14	0.04	0.30	-0.238	0.017 [†]
Number of capillaries [‡]							
3	0	5	2	0	6	-1.872	0.061

*Values in mm² per mm² of alveolar bone surface; [†]The median difference is significant ($P < 0.05$); [‡]Counts per mm² of alveolar bone surface.

bone with extensive extravasation of blood vessels (Fig 6). New capillaries had irregularly formed endothelia and narrow lumen. Histologic sections revealed the absence of areas of severe inflammation, such as lymphocytic infiltration, in the periodontal tissues where repeated injections were performed.

Compared with those in group A, histologic sections along the mesial aspect of the root of the mandibular first premolar in group B, receiving local simvastatin, showed less striking osteoclastic activity (Fig 4, B). Alveolar bone surface showed a decrease in the number and extent of bone resorption lacunae. A reduction in the number of multinucleated osteoclasts was also observed. Alveolar bone exhibited a smoother surface, being uniform at times and cut at others. Arranged rows of osteoblasts with rounded profiles were observed on the alveolar bone surface. Bundles of dense fibers of periodontal ligament were noted with Sharpey fibers stretched into osteoid layers. Compared with group A, fibroblasts showed a more regular distribution throughout the periodontal ligament fibers.

The estimated ICCs for the repeated measurements of histomorphometric parameters were 0.902 for numbers of osteoclasts, 0.879 for the areas of bone-resorptive lacunae, and 0.910 for numbers of capillaries, revealing high reliability between the repeated measurements (Table IV).

Mean values and standard deviations were calculated for the histomorphometric parameters analyzed on the pressure side along the mesial aspect of the root of the mandibular first premolar in groups A and B (Table IV). The normality tests confirmed the assumption of normality for numbers of osteoclasts only. However, the normality assumption was not verified for the areas of active bone-resorptive lacunae and numbers of capillaries.

Results of paired-samples *t* tests, presented in Table V, revealed that the number of osteoclasts on the mesial side of mandibular first premolar in group A was significantly higher than in group B ($P = 0.014$). Results of Wilcoxon signed ranks tests, presented in Table VI, showed that the area of active bone-resorptive

lacunae in group A was significantly higher than in group B ($Z = -2.387$; $P = 0.017$). However, regarding the number of capillaries, no significant difference was recorded between the groups ($Z = -1.872$; $P = 0.061$).

DISCUSSION

This study aimed to clarify the possible effects of local administration of simvastatin as a potential pharmacologic strategy aimed at inhibiting orthodontic tooth movement. Histologic and histomorphometric analyses were performed to investigate the possible effect of local simvastatin administration on the alveolar bone modeling related to experimental tooth movement. An animal model was selected to test the hypothesis of this study. Rats and rabbits are considered to be ideal animals to obtain a clear picture of bony changes under stress.²⁹ Furthermore, rabbits have sufficient periodontal width for the intraligamentous injection required for the present study.³⁰ Compared with other species, such as primates and some rodents, the rabbit model has faster bone turnover.³¹

Although Mundy et al³² reported a quick response of bone morphogenetic protein 2 to statins within 3-5 days in vivo and in vitro, beneficial effects of statins on fracture healing were extended to 5-14 days of local statin exposure to the fracture site,³³ suggesting that statins cause a delayed onset of endogenous bone morphogenetic protein 2 production.³⁴ Therefore, extending the experimental tooth movement phase in the present study to 21 days allowed sufficient time for the effect of simvastatin on the periodontium. This period of active tooth movement was in accordance with previous studies evaluating tooth movements in rabbits.^{35,36}

The orthodontic appliance used in the present study exerted an orthodontic force of ~ 100 cN. This was in accordance with the force level previously used for inducing orthodontic movement of mandibular premolars in a rabbit model.³⁵ Furthermore, the local simvastatin dosage of 0.5 mg was selected based on previous animal studies recommending a dose of 0.1-0.5 mg of simvastatin as the optimal local dose for stimulating maximum bone regeneration without inducing local inflammation.³⁷

Pluronic F127 exhibits the unique property of reversible thermal gelation by transforming from low-viscosity solutions at a temperature $\leq 10^\circ\text{C}$ to clear semisolid gels at normal physiologic body temperature, above its transitional temperature (21°C). Moreover, the drug release from such a gel occurs over a period of up to 1 week. Such gels can be localized near the injection site and widespread distribution of the drug can be minimized.³⁸ These properties rendered Pluronic F127 an attractive

vehicle for controlled release. In the present study, both intraligamentous and submucosal injections were performed to ensure adequate coverage of the experimental site.³⁰

Tissue reactions to orthodontic forces in adult humans start within 2 days after force application, whereas in rodents, tissue reactions start within 30 minutes of force application.^{39,40} Kilic et al³⁶ showed that tooth movement in rabbits occurred in 3 phases: initial phase, arrest or lag phase, and acceleration or progressive movement phase.

The experimental appliance used in the present study yielded an appreciable magnitude of tooth movement in both groups A (1.77 ± 0.15 mm) and B (1.04 ± 0.38 mm). The total magnitude of tooth movement reported in group B was significantly less than that reported in group A. The effect of local administration of simvastatin resulted in a percentage of inhibition of $39.8 \pm 22.6\%$ compared with the corresponding group with the local administration of control vehicle. This reduction in the magnitude of tooth movement in group B could be attributed to the local effects of simvastatin administration, with a reduction in osteoclastic activity and number and diminished cellular infiltrate, which are essential elements for orthodontic tooth movement.²² This burden can be a favorable outcome from the anchorage point of view. Absolute inhibition seems to be difficult to achieve because the tooth would be expected to move at least the width of the periodontal ligament. Moreover, the existence of some tooth movement regardless of simvastatin injection suggests that other pathways are possibly involved in orthodontic tooth movement.

In both groups A and B, the weekly incremental rate of tooth movement was highest during the 1st week, indicating a relatively high displacement. This finding was expected due to the initial deformation of dentoalveolar structures and stretching or compression of the periodontal ligament upon initial force application. After the 1st week, a significant reduction in the weekly incremental rate of tooth movement was reported during the 2nd week in group A. This corresponds, most probably, to the hyalinization processes that are produced at a rather early stage during orthodontic tooth movement.⁴¹ A further reason is that maximum dentoalveolar deformation had been reached, but bone modeling processes were not yet set into motion. Regional differences in bone morphology have been indicated: The alveolar bone mesial to the first premolar becomes more compact in the mesial direction, which means that in the course of mesial tooth movement, resistance in bone was not constant but increased and consequently the rate of tooth

movement would be expected to slow down.⁴² During the 3rd week in group A, the weekly incremental rate of tooth movement continued at the same weekly rate reported for the 2nd week, which might correspond to the linear phase of orthodontic tooth movement due to the active ongoing bone resorption processes.

In group B, receiving simvastatin injections, no significant differences were reported in the weekly incremental rates of tooth movement during the 3 weeks. This finding suggests that the incremental rate of tooth movement continued at almost the same rate throughout the entire experimental tooth movement phase. This finding might also be attributed to the possible early burden of simvastatin producing an initial reduction in orthodontic tooth movement that continued throughout the entire experimental tooth movement phase.²¹ This assumption was confirmed when pairwise comparisons of weekly incremental rates of tooth movement were conducted between groups A and B. The weekly incremental rate of tooth movement was significantly higher in group A during the 1st week only. This confirms the early effects of local administration of simvastatin in the reduction of orthodontic tooth movement by inhibiting bone resorption induced by orthodontic mechanical stress.

The initial increase in the weekly incremental rate of tooth movement during the 1st week, followed by a decrease in the weekly incremental rate of tooth movement during the 2nd week is in agreement with previous studies by Kirschneck et al⁴³ and Reitan and Kvam⁴¹ on rodents. However, the weekly incremental rates of tooth movement during the 3rd week in the present study do not conform to those earlier reports, denoting an increase in rate of tooth movement during the 3rd week. Moreover, the results of the present study do not coincide with those reported by Kilic et al.³⁶ In their study on rabbits, the initial phase of tooth movement lasted for 0-4 days, followed by arrest or lag phase for 5-10 days, and finally followed by acceleration phase for 11-20 days.

Despite the significant reduction in the weekly incremental rate of tooth movement being recorded only during the 1st week of experimental tooth movement, pairwise comparisons of the average weekly incremental rates of tooth movement between groups A and B revealed a significant difference. Local administration of simvastatin resulted in a significant reduction of the average weekly incremental rate of tooth movement throughout the entire experimental period. Interpreting these results could yield the assumption that the initial effect of simvastatin administration could affect the average weekly incremental rate throughout the entire tooth movement phase. Furthermore, the maintenance

of an optimum concentration of the drug locally for a long duration might have been responsible for enhancing the effect of simvastatin.

Orthodontic tooth movement is mediated by a coupling of bone resorption on the compressed side of the periodontal ligament and bone formation on the tension side.^{41,44} Histologic sections in experimental group A along the mesial aspect of the mandibular 1st premolar revealed classic changes corresponding to orthodontic tooth movement, including the widespread of osteoclasts forming resorption foci along the alveolar bone surface, irregular arrangement in periodontal ligament fibers, and increase in vascularization.

Statistical comparisons revealed a significant increase in the number of osteoclasts in group A compared with group B. Moreover, a significant increase in the areas of bone-resorptive lacunae was seen in group A. Because the number of osteoclasts showed a significant reduction in group B, local administration of simvastatin might have either inhibited the recruitment of osteoclasts, promoted osteoclast apoptosis, or both.⁴⁵ This could explain the reduced number of osteoclasts along the alveolar bone surfaces toward the periodontal ligament in the group receiving simvastatin.

This significant effect on numbers of osteoclasts and areas of bone resorptive lacunae could be attributed to the diffusion of the intraligamentous and submucosal simvastatin solutions within the periodontium, from where it passed subperiosteally at the level of the alveolar crest and proceeded along the vascular canals to reach and act directly on the bone. Statins were reported to interfere with the generation of isoprenoids leading to disruption of vesicular fusion and ruffled border formation of osteoclasts, which are essential for their bone-resorbing activity.^{45,46} As a result, osteoclast inactivation occurs and bone resorption is inhibited. These histologic findings conform to those reported by Pytlík et al⁴⁷ and Abdelkawy et al⁴⁸ confirming the effect of simvastatin in hindering bone resorption processes and intensifying bone formation processes.

A weekly injection of simvastatin solution was performed in the present study. This was in accordance with the study by Masuzaki et al⁴⁹ revealing that a single injection of statin safely and successfully increased the mechanical properties of bone and enhanced new bone formation for up to 2 weeks. Another study, by Yazawa et al,¹⁴ reported increased alkaline phosphatase and osteopontin levels in periodontal cells for 7 days as well as increased calcium content for 21 days with simvastatin exposure. Furthermore, a study by Park et al⁵⁰ showed that simvastatin-loaded hydrogel provided a complete osteogenic differentiation for 14 days with no detectable cytotoxicity. The observed

elevation in matrix metalloproteinase 13 expression, fibronectin, and procollagen gene activation fostered increased collagen synthesis and osteoblast recruitment.

The aforementioned studies confirmed the prolonged biologic effect of simvastatin up to 14 days even after a brief exposure. Moreover, a single injection per week allowed the avoidance of possible tissue damage caused by frequent local injections, thus lengthening the interval between successive injections as recommended by Venkataramana et al.⁵¹

Correlating the histologic and tooth movement findings regarding experimental orthodontic tooth movement, the present study demonstrates that the local administration of simvastatin could yield a relative reduction in the number of osteoclasts and area of active bone-resorptive lacunae hindering bone resorption processes. This could provide a reasonable explanation for the reduction of tooth movement magnitudes reported in the group receiving local injections of simvastatin.

A limitation of this study was the possible physiologic distal drift causing underestimation of the experimental tooth movement. In addition, histologic analysis of tissues at multiple time points during orthodontic tooth movement was not performed. Furthermore, the experimental tooth movement phase in the present study was confined to 21 days. Future studies are required to determine whether the reduction of orthodontic tooth movement requires the sustained release of simvastatin throughout the entire duration of orthodontic tooth movement, or if transient exposure during a critical time-limited period is sufficient.

On the other hand, the method of local administration of simvastatin used in the present study was simple, safe, and noninvasive. No appreciable macroscopic changes, such as edema, redness, or erosion, were noted at the local injection sites, suggesting a possible adequate method for use in the orthodontic field. Although this method seems to be promising, careful long-term evaluation should be conducted in other animals before its use in humans.

CONCLUSIONS

Within the limitations of the present study, the following could be concluded:

1. Local administration of simvastatin can reduce the rate and magnitude of orthodontic tooth movement.
2. Local administration of simvastatin diminishes bone-resorption processes associated with orthodontic tooth movement, reducing the number of os-

teoclasts and the subsequent area of active bone resorption.

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