Assessment of Vascular Endothelial Growth Factor and Matrix Metalloproteinase-9 in the Periodontium of Rats Treated With Atorvastatin

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Background: The aim of this study is to examine, for the first time, the role of systemic and local atorvastatin application on periodontium using histomorphometric and immunohistochemical analysis during and after experimental periodontitis induction with or without the presence of microbial dental biofilm.

Methods: One hundred ten male Wistar rats were used. Silk ligatures were placed around the cervical area of the mandibular first molars; rats in the healthy control group received no ligatures (n = 10). In experimental periodontitis groups (n = 90), systemic and local atorvastatin and saline were administered in three different periods; the control periodontitis group (n = 10) received no treatment. Histomorphometric analysis, which included alveolar bone area, alveolar bone level, and attachment loss, and immunohistochemical analysis, which included immunoreactivity of vascular endothelial growth factor (VEGF) and matrix metalloproteinase (MMP)-9, were performed after the rats were sacrificed at the end of the experimental procedure.

Results: There was a greater increase in alveolar bone area and VEGF immunoreactivity, as well as a greater decrease in alveolar bone and attachment loss and MMP-9 immunoreactivity, with systemic and local atorvastatin application during and after induction of experimental periodontitis. Local atorvastatin application showed better results on periodontium with regard to alveolar bone findings.

Conclusions: Systemic and local atorvastatin application showed beneficial effects on periodontium during and after induction of experimental periodontitis. Within the limits of this study, it can be concluded that atorvastatin, which is used for hypercholesterolemia treatment, can also be used as a protective and therapeutic agent for periodontal disease. *J Periodontol 2014;85:178-187.*

KEY WORDS

Atorvastatin; histology; immunohistochemistry; periodontal disease; rats.

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Periodontitis, an oral infectious disease caused by specific microorganisms or groups of microorganisms, is characterized by clinical attachment loss (AL), alveolar bone resorption, periodontal pocketing, and gingival inflammation.[1] Microorganisms and their products lead to the recruitment of inflammatory cells, generation of cytokines, release of proteolytic enzymes, and activation of osteoclasts. Thus, periodontal tissue breakdown, a characteristic feature of periodontitis, occurs.[2] Various regenerative procedures such as guided tissue regeneration, bone grafting, application of enamel matrix derivative, various polypeptide growth factors, and combinations of these therapies have been used for treatment of the destroyed periodontal tissues.[3] Although these techniques usually result

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in successful clinical outcomes, due to some practical difficulties and some mechanisms (biological and physicochemical characteristics of biomaterials and individual components), they have achieved limited success in the amount of periodontal architecture formed.[3]

Statins inhibit cholesterol synthesis in the liver. These agents block the conversion of 3-hydroxy-3-methylglutaryl-coenzyme A to mevalonate by blocking 3-hydroxy-3-methylglutaryl-coenzyme A reductase, which is the rate-limiting enzyme.[4,5] In addition to cholesterol-lowering activity, statins have pleiotropic effects, including promotion of vasculogenesis, expression of bone anabolic factors such as bone morphogenetic protein (BMP)-2, and anti-inflammatory, antioxidant, and immunomodulatory effects.[5] The anabolic effect of statins on bone is realized in two ways: 1) direct activation of BMP and 2) inhibition of bone resorption by decreasing the expression of receptor activator of nuclear factor κB ligand and cathepsin K.[6,7] Mundy et al.[8] first reported that statins stimulated bone formation in rodents and increased production of BMP-2 in bone cells. The use of statins such as simvastatin and atorvastatin has shown significant improvement in periodontal parameters (probing depth [PD], clinical attachment level [CAL], tooth mobility, and intrabony defect fill) in clinical studies and beneficial effects on alveolar bone loss (ABL) and bone growth in animal studies.[9-14] The above observations lead to the speculation that statin application could be beneficial for the periodontium by anti-inflammatory action.

Vascular endothelial growth factor (VEGF) is a multifunctional angiogenic growth factor, relatively specific for endothelial cells, which is expressed by many cells in both physiologic and pathologic processes. VEGF regulates vasculogenesis and angiogenesis, increases microvascular permeability, stimulates endothelial cell growth and differentiation, and induces migration of endothelial cells, monocytes, and osteoblasts.[15-17] Additionally, current studies suggest that VEGF also has an important role in the regulation of periodontal disease.[18-20] Matrix metalloproteinases (MMPs) are a family of metal-dependent proteolytic enzymes that degrade the proteinaceous components of the extracellular matrix and cleave signaling proteins.[21] The regulation of MMP activity occurs through transcriptional regulation of MMP genes, precursor activation, differences in substrate specificity, and MMP inhibitors (α2-macroglobulin and tissue inhibitors of MMPs).[22] MMPs participate in both physiologic (bone remodeling, wound healing, angiogenesis, apoptosis) and pathologic (cardiovascular disease, arthritis, cancer, periodontal disease) processes.[23] Evidence indicates that the amount of MMP-9 (gelatinase B) in gingival crevicular fluid is increased during periodontal disease and reduced after periodontal treatment.[24-28] As has been reported, both VEGF and MMP-9 play important roles in inflammation and wound healing; furthermore, neutrophil-derived MMP-9 was identified as a factor during angiogenic processes along with VEGF.[29,30] Although VEGF and MMP-9 have been widely studied in several diseases as well as periodontal disease, no data are available regarding the results of statin application on periodontium by detecting these markers.

Although many recent clinical and experimental works have concentrated on systemic and local statin treatments in periodontal disease, several issues still await clarification.[9-14] As periodontitis is an inflammatory and bone-destructive disease, and microbial dental biofilm accumulation is the primary etiologic factor for periodontal disease, detection of the effect of statin application may be useful owing to its anti-inflammatory action for periodontal disease prevention and therapy in the presence and absence of microbial dental biofilm. The aim of this study is to examine, for the first time, the role of systemic and local atorvastatin application on periodontium using histomorphometric and immunohistochemical analysis during and after experimental periodontitis induction with or without the presence of microbial dental biofilm.

MATERIALS AND METHODS

Animals

One hundred ten male Wistar albino rats (≈200 g) were used. The rats were housed separately in plastic cages with controlled room temperature (22°C ± 1°C) and humidity (50%). They were maintained in a 12-hour:12-hour light:dark cycle with food and water ad libitum. All animal care and experimental protocols were in compliance with guidelines approved by the Ethical Committee of Animal Research (protocol no. 2010/23).

Protocol of Experimental Periodontal Disease

Rats were divided into 14 groups (two control and 12 experimental). Experimental periodontitis was induced by tying 3.0 sterile silk ligatures around the cervical area of the mandibular first molars except in the healthy control group (n = 10). Ligatures were kept in position for 40 days to promote microbial dental plaque accumulation and inflammation.[31] In the experimental periodontitis groups (n = 90), systemic and local atorvastatin[‖] or saline was

‖ Sanovel, Istanbul, Turkey.

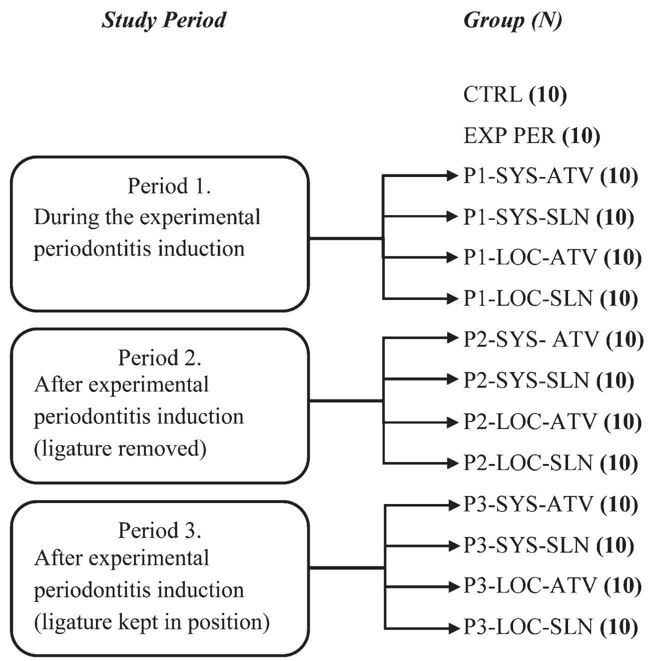

Figure 1.
Designation of experimental groups. CTRL = healthy control group; EXP PER = experimental periodontitis group; P = period; SYS = systemic; LOC = local; ATV = atorvastatin; SLN = saline.

administered daily in three different periods: period 1, atorvastatin and saline were administered during experimental periodontitis induction by ligatures (40 days); period 2, after experimental periodontitis induction (40 days), ligatures were removed, and atorvastatin and saline were administered (40 days); and period 3, after experimental periodontitis induction (40 days), ligatures were kept in position, and atorvastatin and saline were administered (40 days). The control periodontitis group (n = 10) received ligatures but no atorvastatin or saline. Designation of the experimental groups is shown in Figure 1.

Atorvastatin was administered systemically at a dose of 5 mg/kg in a volume of 0.5 mL and locally at a dose of 0.1 mg/kg in a volume of 0.05 mL.[9,32] Systemic application of atorvastatin and saline was performed by oral gavage.[30] Local application of atorvastatin and saline was performed subperiosteally in the buccal mucosa of the bottom right and left first molars, respectively. All rats were sacrificed at the end of the experimental period, and mandibular posterior teeth with the surrounding gingival tissues were removed carefully.

Histomorphometric Analyses

Samples were fixed in 10% neutral buffered formalin. Thee samples were decalcified in 8% formic acid (14 days) and embedded in paraffin. Serial paraffin sections (5 μm) were made from the mesio-distal aspects throughout the mandibular first molars. Three sections representative of the central area of each tooth were observed and stained with hematoxylin and eosin (H&E).

Histomorphometric analysis was performed using a light microscope.[¶] Images were digitized with a camera[#] and analyzed with software[**] by a calibrated examiner (BA) with no prior knowledge of the experimental design.

In each section stained with H&E, the following parameters were assessed: 1) percentage of alveolar bone in furcation area, 2) ABL, and 3) AL. The percentage of alveolar bone area on each specimen was calculated as the ratio of the alveolar bone area to furcation area (Fig. 2A).[33] The alveolar bone area was determined as the combination of the trabecular bone area and bone marrow area in furcation. The level of the alveolar bone was determined by measuring the distance from the cemento-enamel junction (CEJ) to the alveolar bone crest (Fig. 2B).[31] AL was measured as the distance between the CEJ and the most coronal extent of connective tissue attachment to cementum (Fig. 2B).[19] ABL and AL values were measured at mesial and distal regions of mandibular first molars. The means of all measurements were used for data analysis.

Immunohistochemical Analyses

Sections (5 μm thick) were taken over poly-L-lysine coated slides, dried at room temperature, deparaffinized with xylene (2 × 15 minutes), rehydrated through graded alcohols, and washed with distilled water. Tissue sections were outlined with a hydrophobic pen. To reduce non-specific disclosing due to endogenous peroxidase, sections were washed with distilled water, incubated in 3% H_2O_2 (15 minutes), and washed (3 × 3 minutes) with phosphate-buffered solution (PBS, pH 7.4). The sections were preincubated with protein blocking solution[††] (10 minutes) at room temperature to block non-specific binding of second-step antibody. After the blocking phase, the sections were incubated for 60 minutes at room temperature with primary antibodies against VEGF[‡‡] and MMP-9.[§§] The sections were washed with PBS (3 × 3 minutes) and reacted with broad-spectrum secondary antibody[‖‖] to binding primer antibody for 10 minutes. The sections were washed with PBS (3 × 3 minutes) again and

¶ BX50, Olympus, Tokyo, Japan.
\# DP26 Digital Camera, Olympus.
** DP2-BSW, Olympus.
†† Ultra V Block, UltraVision Large Volume Detection System: Anti-Polyvalent, HRP; Thermo Scientific, Fremont, CA.
‡‡ VEGF Ab-1, Rabbit Polyclonal Antibody; Thermo Scientific.
§§ MMP-9 Ab-5, Mouse Monoclonal Antibody; Thermo Scientific.
‖‖ Biotinylated Goat Anti-Polyvalent Plus, Thermo Scientific.

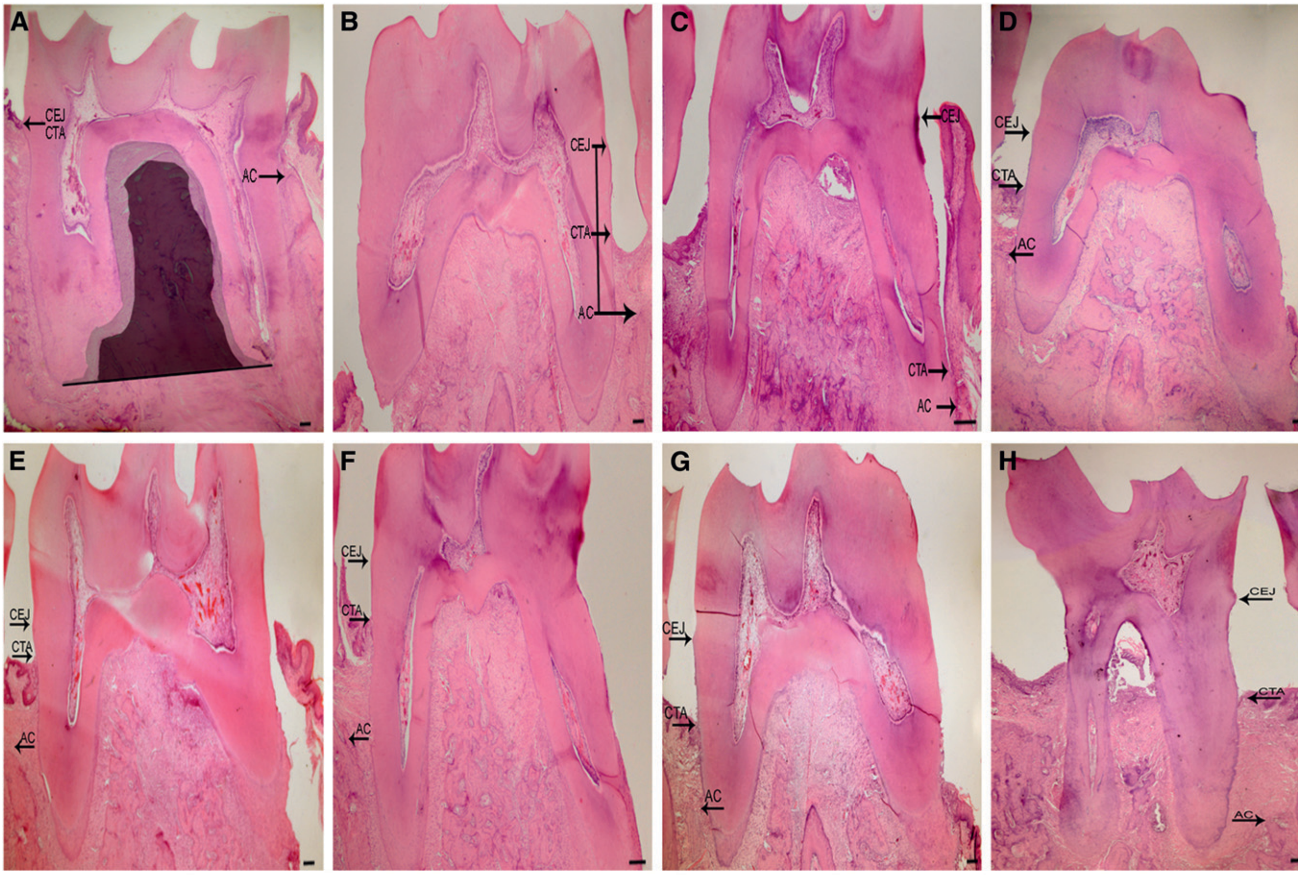

Figure 2.
Sections from the mesio-distal aspects throughout the mandibular first molars and reference areas for histomorphometric analysis. (H&E; original magnification ×4; bar = 100 μm.) CEJ = cemento-enamel junction, CTA = connective tissue attachment, AC = alveolar crest. ***A)*** *CTRL; calculation of alveolar bone area (furcation area = dark + light area, alveolar bone area = dark area).* ***B)*** *EXP PER, no atorvastatin; histometric measurement of alveolar bone and attachment loss.* ***C)*** *P1-SYS-ATV.* ***D)*** *P1-LOC-ATV.* ***E)*** *P2-SYS-ATV.* ***F)*** *P2-LOC-ATV.* ***G)*** *P3-SYS-ATV.* ***H)*** *P3-LOC-ATV.*

incubated in horseradish peroxidase–streptavidin complex.[¶¶] The slides were then immunolabeled with aminoethyl carbazole as a chromogen and Mayer's hematoxylin as the counterstain. Sections were mounted, dehydrated, and viewed under a light microscope.

The immunoreactivity of VEGF and MMP-9 proteins was scored by one researcher (BA), using a histologic scoring method (HScore).[34] Staining intensity was semiquantitatively scored according to the following categories: 0, absent; 1, weak; 2, moderate; and 3, intense. The formula was HScore = $\Sigma P_i\ (i + 1)$, where i represents the intensity scores, P_i is the percentage of stained cells, and 1 is the correction factor.

Statistical Analyses

The data were tested for normality using Kolmogorov-Smirnov test. Histomorphometric data were analyzed by Student t test for intraperiod comparisons and one-way analysis of variance and post hoc Tukey test for intergroup comparisons. Comparison of the immunohistochemical scores for VEGF and MMP-9 was analyzed using Kruskal-Wallis and Bonferroni-adjusted Mann-Whitney U non-parametric tests. All tests were performed using statistical software.[##]

RESULTS

Clinical and Radiographic Findings

Systemic and local application of atorvastatin were tolerated by all animals, and no complications occurred. Significant alveolar bone and attachment loss were detected in rats in the experimental periodontitis control group (EXP PER) compared with the healthy control (CTRL) group ($P = 0.000$).

Histomorphometric Findings

Alveolar bone area in the furcation region, alveolar bone level, and AL values are summarized in Table 1. Alveolar bone area was greater in all atorvastatin

¶¶ Streptavidin Peroxidase Plus, Thermo Scientific.
SPSS, v.16.0, IBM, Chicago, IL.

Table 1.

Percentage of Alveolar Bone in Furcation Area, Alveolar Bone Level, and AL (mean ± SD)

Group	Alveolar Bone Area (%)	Alveolar Bone Level (μm)	AL (μm)
CTRL	75.27 ± 1.39	623.72 ± 26.49	292.20 ± 43.88
EXP PER, no ATV	39.95 ± 0.46	1,540.32 ± 14.32	1,149.83 ± 62.62
P1-SYS-ATV	49.61 ± 3.01*	1,245.55 ± 19.46*	872.70 ± 121.51*
P1-SYS-SLN	40.33 ± 1.40†	1,542.12 ± 16.01†	1,151.83 ± 33.76†
P1-LOC-ATV	52.5 ± 1.22*†	1,177.77 ± 18.71*†	803.20 ± 51.59*
P1-LOC-SLN	39.03 ± 1.25‡	1,509.48 ± 26.83‡	1,122.67 ± 79.87‡
P2-SYS-ATV	55.57 ± 1.35	1,044.02 ± 20.21	607.87 ± 86.10
P2-SYS-SLN	43.69 ± 1.19§	1,363.80 ± 26.34§	956.43 ± 122.52§
P2-LOC-ATV	57.92 ± 1.24§	1,003.30 ± 24.96§	623.23 ± 45.68
P2-LOC-SLN	41.88 ± 1.60	1,417.00 ± 35.68	1,104.67 ± 103.39
P3-SYS-ATV	29.05 ± 1.66	1,719.23 ± 28.93	1,312.73 ± 24.54
P3-SYS-SLN	22.47 ± 1.83¶	1,968.10 ± 51.2¶	1,471.07 ± 45.61¶
P3-LOC-ATV	32.38 ± 1.02¶	1,675.90 ± 31.72¶	1,272.07 ± 44.73¶
P3-LOC-SLN	23.25 ± 1.53#	1,945.38 ± 39.89#	1,465.23 ± 47.53#

Statistically significant difference from *EXP PER group ($P = 0.000$); †P1-SYS-ATV group ($P < 0.05$); ‡P1-LOC-ATV group ($P = 0.000$); §P2-SYS-ATV group ($P < 0.01$); P2-LOC-ATV group ($P = 0.000$); ¶P3-SYS-ATV group ($P < 0.05$); #P3-LOC-ATV group ($P = 0.000$).

groups compared with control groups ($P = 0.000$). The amount of alveolar bone in the furcation area was significantly greater in local atorvastatin groups compared with systemic atorvastatin groups (intraperiod comparisons $P < 0.05$). Significant decreases in ABL and AL were observed in atorvastatin groups compared with control groups ($P = 0.000$). A significant decrease in ABL was observed in local atorvastatin groups compared with systemic atorvastatin groups in all periods ($P < 0.01$). There was no significant difference in AL between the systemic (P1-SYS-ATV and P2-SYS-ATV) and local (P1-LOC-ATV and P2-LOC-ATV) atorvastatin groups in the first and second periods ($P > 0.05$). Histologic appearances are shown in Figure 2.

Immunohistochemical Findings

Periodontal ligament VEGF and MMP-9 immunoreactivity findings are given in Table 2. There was a greater increase in VEGF immunoreactivity in atorvastatin groups compared with control groups ($P = 0.000$). VEGF immunoreactivity was greater in the local atorvastatin group (P1-LOC-ATV) compared with the systemic atorvastatin group (P1-SYS-ATV) in the first period ($P = 0.000$), whereas a significant increase in VEGF immunoreactivity was observed in the systemic atorvastatin group (P2-SYS-ATV) compared with the local atorvastatin group (P2-LOC-ATV) in the second period ($P = 0.000$). There was no significant difference in VEGF immunoreactivity between the systemic (P3-SYS-ATV) and local (P3-LOC-ATV) atorvastatin groups in the third period ($P > 0.05$).

A significant decrease in MMP-9 immunoreactivity was observed in atorvastatin groups compared with control groups ($P = 0.000$). There was no significant difference in MMP-9 immunoreactivity between systemic (P1-SYS-ATV) and local (P1-LOC-ATV) atorvastatin groups in the first period ($P > 0.05$). There was a greater decrease in MMP-9 immunoreactivity in the local atorvastatin group (P2-LOC-ATV) compared with the systemic atorvastatin group (P2-SYS-ATV) in the second period ($P = 0.000$), whereas a significant decrease in MMP-9 immunoreactivity was observed in the systemic atorvastatin group (P3-SYS-ATV) compared with the local atorvastatin group (P3-LOC-ATV) in the third period ($P < 0.05$). Immunohistochemical appearances are shown in Figures 3 and 4.

DISCUSSION

This study is designed to investigate the effectiveness of atorvastatin application on periodontium using histomorphometric and immunohistochemical analysis in three different periods. To the best

Table 2.

HSCORE Values of VEGF and MMP-9 Immunoreactivity (mean ± SD)

Group	VEGF Immunoreactivity	MMP-9 Immunoreactivity
CTRL	108.87 ± 1.46 (6.30)	100.52 ± 2.10 (5.60)
EXP PER	112.28 ± 1.25 (18.45)	213.15 ± 1.63 (117.65)
P1-SYS-ATV	123.33 ± 1.12 (55.00)*	150.41 ± 2.31 (49.35)*
P1-SYS-SLN	113.37 ± 1.65 (23.00)†	215.28 ± 1.73 (123.35)†
P1-LOC-ATV	137.39 ± 0.77 (115.40)*†	151.52 ± 2.67 (52.25)*
P1-LOC-SLN	131.44 ± 0.88 (96.10)‡	176.28 ± 1.80 (94.70)‡
P2-SYS-ATV	150.70 ± 1.78 (135.50)	121.26 ± 2.09 (35.50)
P2-SYS-SLN	120.31 ± 2.02 (45.85)§	156.23 ± 1.62 (64.90)§
P2-LOC-ATV	144.07 ± 1.46 (125.50)§	105.68 ± 2.09 (24.80)§
P2-LOC-SLN	134.06 ± 1.52 (104.55)	109.28 ± 1.37 (16.10)
P3-SYS-ATV	128.93 ± 0.93 (81.65)	170.65 ± 1.85 (77.75)
P3-SYS-SLN	116.13 ± 1.50 (34.60)¶	230.69 ± 2.12 (135.50)¶
P3-LOC-ATV	128.72 ± 0.94 (79.70)	172.70 ± 1.67 (84.05)¶
P3-LOC-SLN	126.23 ± 0.82 (65.40)#	196.44 ± 2.08 (105.50)#

Data mean ± SD (mean rank).
Statistically significant difference from *EXP PER group (P = 0.000); †P1-SYS-ATV group (P = 0.000); ‡P1-LOC-ATV group (P = 0.000); §P2-SYS-ATV group (P = 0.000); P2-LOC-ATV group (P = 0.000); ¶P3-SYS-ATV group (P <0.05); #P3-LOC-ATV group (P = 0.000).

of our knowledge, this is the first trial examining the effect of systemic and local atorvastatin application on periodontium using histomorphometric analysis and VEGF and MMP-9 by immunohistochemical methods in an experimental periodontitis model.

This study uses ligature-induced experimental periodontitis, which is a well-established model of experimental periodontitis in rats and a simple and reliable technique for disease induction. Rat models are commonly used in experimental periodontitis studies, due to the similarities in structure and organization of the periodontal tissues to those of humans.[35-37]

Several animal studies have explored the use of statins in the treatment of periodontal defects or periodontitis-induced bone loss.[9,10,32,38-40] Twice-weekly injections of simvastatin have a protective effect on attachment apparatus and alveolar bone during experimental periodontitis induction in rats.[9] A study by Seto et al.[10] suggested that topically administered simvastatin (0.2 mg) after experimental periodontitis induction has a significant stimulatory effect on alveolar bone formation. The histomorphometric analysis performed in the present study demonstrates that systemic and local application of atorvastatin prevents ligature-induced alveolar bone loss in the first and third periods and improves recovery of alveolar bone loss in the second period. The beneficial effect of systemic and local atorvastatin on alveolar bone in the furcation area during and after experimental periodontitis induction was observed in this study. Similar to these systemic atorvastatin treatment findings, it has been found that systemic atorvastatin (9 mg/kg/day) and simvastatin (10 and 30 mg/kg/day) applications starting 30 minutes and 1 hour, respectively, before ligature placement prevent ABL in experimental periodontitis.[13,32]

Although no beneficial effect of local simvastatin application alone on periodontal defects in animal models was reported in two studies,[38,40] significant intrabony defect fill with scaling and root planing (SRP) plus subgingivally delivered simvastatin (1.2 mg/0.1 mL) in patients with chronic periodontitis was shown in two clinical studies.[12,41] Significant improvements in alveolar bone height and tooth mobility at sites treated with SRP plus systemic atorvastatin (20 mg) versus SRP alone have been found in another clinical study.[11] In a recent report, local delivery of atorvastatin into intrabony defects was observed to increase PD reduction, CAL gain, and improved bone fill compared with placebo gel as an adjunct to SRP.[14] Systemic and local atorvastatin applications in the present study are protective against the impact of periodontitis on attachment apparatus in all three periods.

Moreover, this study examines the effect of systemic and local atorvastatin on VEGF and MMP-9 in periodontal ligament by immunohistochemical methods. A direct comparison with other studies is not possible, as there have been no studies that examined the effect of systemic and local atorvastatin on VEGF and MMP-9 immunoreactivity. VEGF is a multifunctional angiogenic growth factor and has a critical role in both physiologic and pathologic processes. Studies in the periodontal literature on the role of VEGF have shown conflicting results.[19,42,43] In the present study, increased VEGF immunoreactivity as well as reduction in attachment and alveolar bone loss is observed in the groups treated with local and

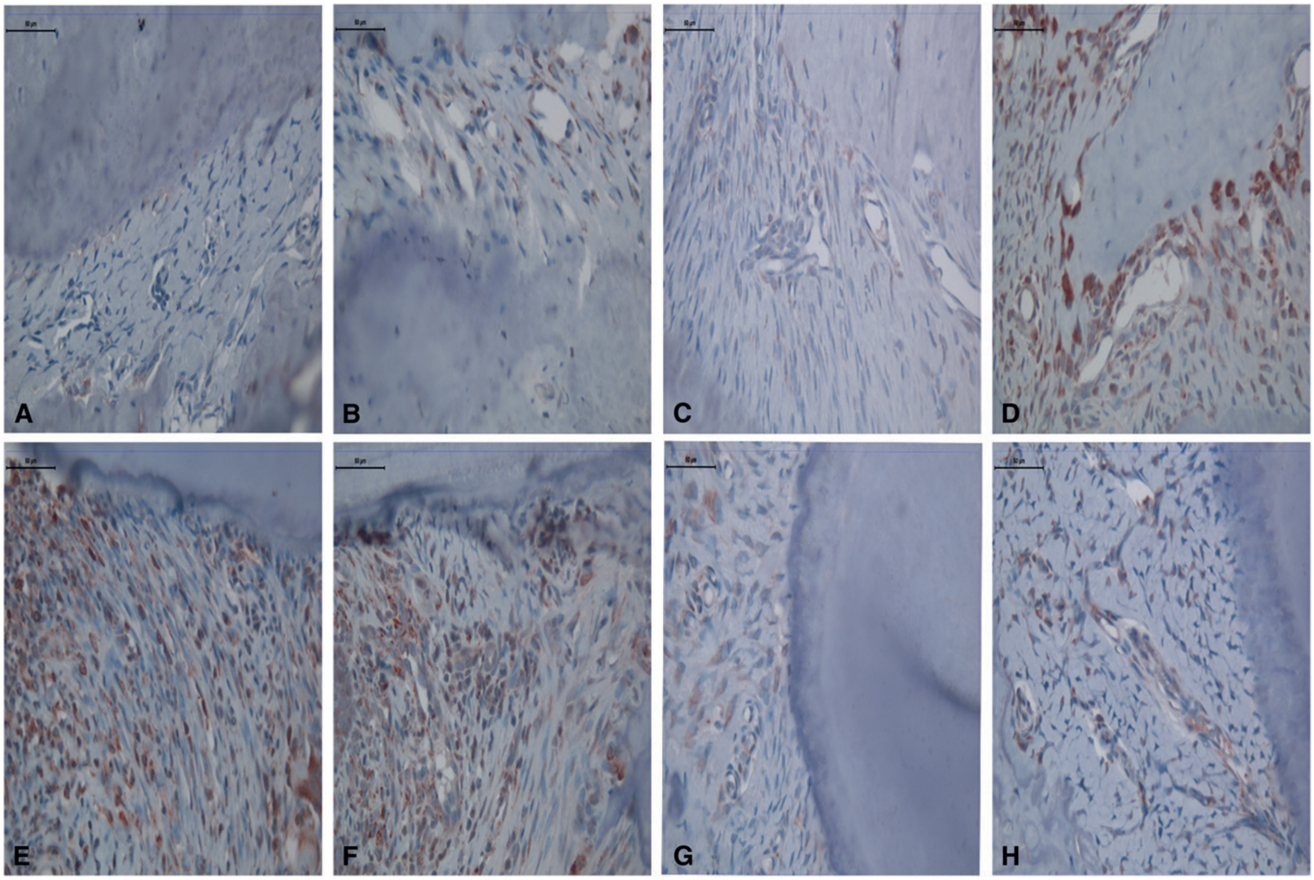

Figure 3.
VEGF immunoreactivity in periodontal ligament. (aminoethyl carbazole was added for color development as a chromogen; original magnification ×100.) ***A)*** *CTRL.* ***B)*** *EXP PER, no ATV.* ***C)*** *P1-SYS-ATV.* ***D)*** *P1-LOC-ATV.* ***E)*** *P2-SYS-ATV.* ***F)*** *P2-LOC-ATV.* ***G)*** *P3-SYS-ATV.* ***H)*** *P3-LOC-ATV.*

systemic atorvastatin. VEGF levels in serum and gingival crevicular fluid decreased after Phase I periodontal therapy, inconsistent with these VEGF findings.[43] Considering the histomorphometric findings, increased VEGF immunoreactivity is probably related more to the healing stage of periodontal disease than to the destruction stage of the lesion, as reported in the present authors' previous study.[19]

MMP-9, predominantly derived from neutrophils, is a proteolytic enzyme and diagnostic marker of periodontal disease activity.[24,28] Several clinical studies have suggested an association between increased MMP-9 activity and periodontal disease sites.[25,26,44] Also, MMP-9 significantly decreases after periodontal therapy.[27,28] The present study shows that local and systemic atorvastatin application decreases MMP-9 immunoreactivity in all three periods. This is consistent with the previous studies on MMP-9, periodontal disease, and periodontal therapy. Considering the histomorphometric findings, decreased immunoreactivity of MMP-9, which is predominantly secreted by inflammatory cells following atorvastatin application, is probably related more to periodontal breakdown than the healing of periodontium.

In the present experiment, local and systemic atorvastatin applications prevent alveolar bone resorption and induce alveolar bone formation via increasing VEGF immunoreactivity and decreasing MMP-9 immunoreactivity. This is consistent with reports that statins increase endothelial nitric oxide synthase and VEGF and also decrease MMPs.[45,46]

When the histomorphometric and immunohistochemical results are taken into consideration, local application of atorvastatin has greater positive effects on alveolar bone than systemic application in the three different periods. Similar to the alveolar bone findings, locally applied lovastatin was reported to have greater beneficial effect than systemic lovastatin on bone formation in rat femur and tibia.[47] Systemic and local atorvastatin application has beneficial effects on soft tissues of rat periodontium in this study; however, it is not

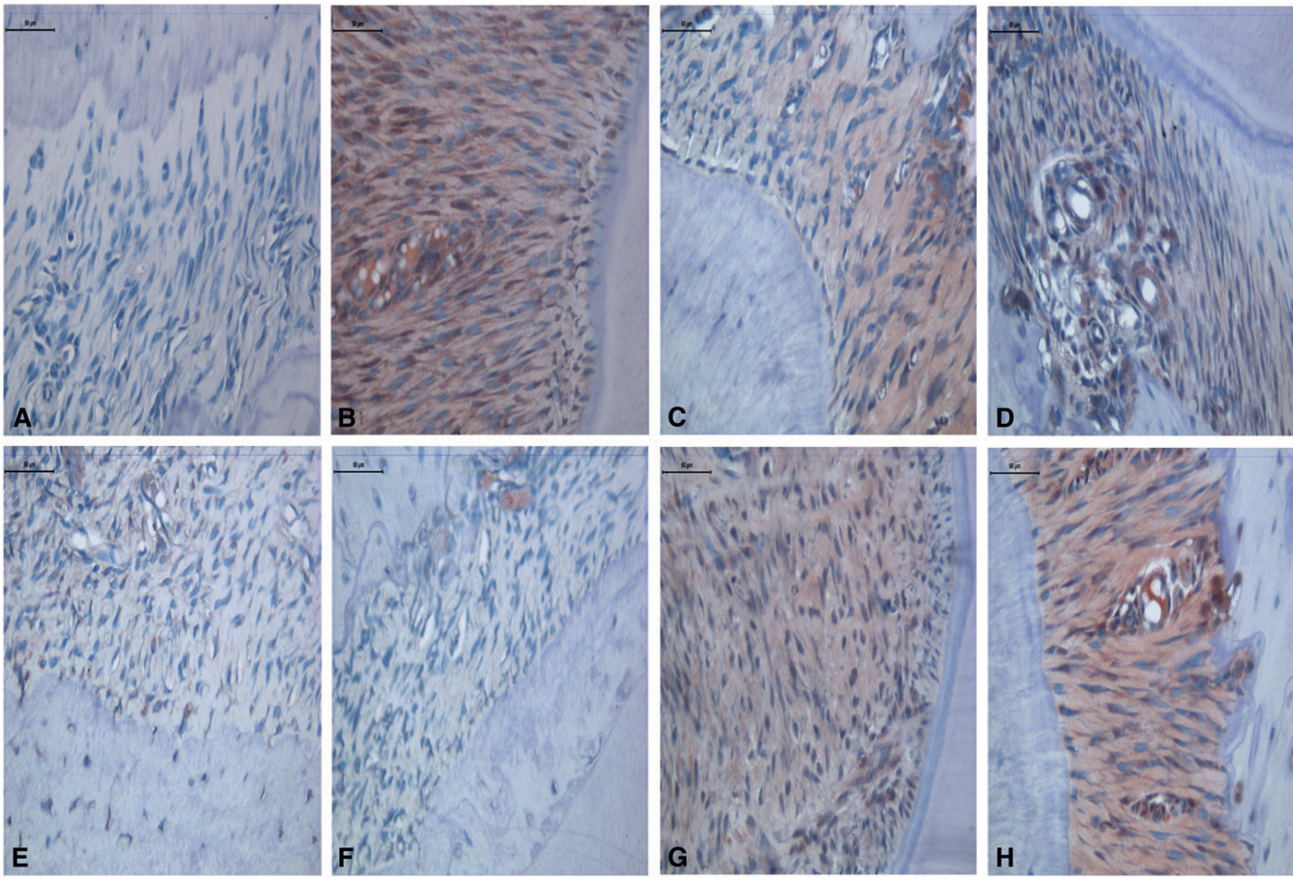

Figure 4.
MMP-9 immunoreactivity in periodontal ligament. (aminoethyl carbazole was added for color development as a chromogen; original magnification ×100.) ***A)*** *CTRL.* ***B)*** *EXP PER, no ATV.* ***C)*** *P1-SYS-ATV.* ***D)*** *P1-LOC-ATV.* ***E)*** *P2-SYS-ATV.* ***F)*** *P2-LOC-ATV.* ***G)*** *P3-SYS-ATV.* ***H)*** *P3-LOC-ATV.*

clear which application has a better effect on periodontal soft tissues.

CONCLUSIONS

There was a greater increase in alveolar bone area and VEGF immunoreactivity, and a decrease in alveolar bone and attachment loss and MMP-9 immunoreactivity, with systemic and local atorvastatin application during and after induction of experimental periodontitis.

In light of these findings, it is relevant to assume that atorvastatin, which is used for hypercholesterolemia treatment, can also be used as a protective and therapeutic agent for periodontal disease. Additional studies should be undertaken to further elucidate the role of systemic and local statin administrations on Phase I periodontal therapy as an agent to improve clinical parameters and on reconstructive periodontal surgery as a grafting material.

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