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Anti-inflammatory effect of rosuvastatin decreases alveolar bone loss in experimental periodontitis

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Abstract: The effects of systemically administered rosuvastatin on alveolar bone loss (ABL), cytokine levels and oxidative status were investigated in rats with ligature-induced periodontitis. Rats were divided randomly into four groups: a non-ligated group (C); a non-ligated+rosuvastatin group (R); a ligated group (P); and a ligated+rosuvastatin group (PR). Ligatures were placed at the maxillary second molars, and rosuvastatin was administered for 14 days. After the rats had been euthanatized, histomorphometric and histological analyses were performed, and the serum levels of interleukin (IL)-1β, IL-10 and oxidant and antioxidant parameters (malondialdehyde [MDA], superoxide dismutase, glutathione, and glutathione peroxidase) were evaluted by enzyme-linked immunosorbent assay. Rosuvastatin significantly decreased the extent of ABL, inflammatory infiltration and osteoclasts in periodontitis, but increased the numbers of osteoblasts. Although rosuvastatin reduced the levels of IL-1β, they did not differ significantly between the PR and P groups. In the PR group, not only were IL-10 levels significantly higher but also the ratio of IL-1β to IL-10 was lower than in the P group. Although MDA levels were significantly increased in the P group relative to the C group, they did not differ significantly between the PR and C groups. The present data suggest that rosuvastatin decreases ABL in ligature-induced periodontitis, and that its anti-inflammatory effect is more remarkable than its antioxidant effect.

Keywords: rosuvastatin; alveolar bone loss; interleukin 1; interleukin 10; oxidative stress; periodontitis.

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Introduction

Periodontitis is a chronic inflammatory disease caused by infection with periodontopathogens, leading to alveolar bone loss, tooth mobility and early tooth loss (1). The major component of the tissue destruction results from activation of an inflammatory response to the bacterial challenge. This response is characterized by the production of inflammatory mediators, including cytokines and reactive oxygen species (ROS) (2). Interleukin (IL)-1β is a pro-inflammatory cytokine with immunological

functions, including activation of neutrophils, T- and B-lymphocytes, and osteoclasts during infection, acute-phase protein release and febrile responses (3,4). IL-10, on the other hand, is an anti-inflammatory cytokine that mediates immunological processes including pro-inflammatory immune responses and inflammation (5). It was reported that, in periodontitis, serum pro-inflammatory cytokines are increased or remain unchanged, whereas anti-inflammatory cytokines are increased (6-8). It has also been suggested that there is a relationship between the serum level of IL-1β and the severity of periodontal breakdown (9). An association between high pro-inflammatory to anti-inflammatory cytokine ratios, and clinical markers of periodontal breakdown has also been reported (10).

Recent studies of periodontitis and periodontal breakdown have suggested that ROS play a role in increased local and systemic variations in redox balance. ROS activate pro-inflammatory cytokines via nuclear factor kappa B (NF-kB) (11). Malondialdehyde (MDA), a final product of the peroxidation of polyunsaturated fatty acids in cells, is a marker of oxidative stress and and peroxidative tissue injury (12). Clinical studies have indicated that MDA levels are increased in serum, saliva, gingival crevicular fluid (GCF) and gingival samples from patients with periodontitis depending on ROS activity (13-15). At inflammatory sites, antioxidant systems protect tissue against the destructive effects of ROS and prevent or delay oxidation. Impairment of the balance between ROS and antioxidants creates oxidative stress and significantly contributes to many inflammatory diseases, including periodontitis (3). In patients with periodontitis, antioxidants (superoxide dismutase [SOD], glutathione [GSH] and glutathione peroxidase [GSH-Px], etc.) are significantly increased in comparison with periodontally healthy controls (16-18).

Statins are inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoAR), the rate-limiting enzyme of the melavonate pathway, and are widely used to reduce the level of cholesterol (19). Clinical findings have suggested that the pleiotropic effects of statins may extend to mechanisms independent of their cholesterol-lowering effects. Recently, statins have attracted even more attention due to their antioxidant, anti-inflammatory, osteomodulatory and immunomodulatory effects (20). Rosuvastatin is a strong inhibitor of HMG-CoAR that decreases the level of low-density lipoprotein cholesterol to the greatest extent of all currently marketed statins. Rosuvastatin also reduces the levels of oxidative stress markers, upregulates antioxidant defense mechanisms, and prevents tissue damage (21). Data from animal studies have suggested some possible mechanisms underlying the beneficial role of various statins, such as simvastatin and atorvastatin, in preventing alveolar bone loss (ABL) (14,22-24). Moreover, recent clinical evidence indicates that statins may have beneficial effects on periodontal diseases (decreasing probing pocket depth and tooth loss, and increasing intrabony defect filling and clinical attachment) (25-29). In the present study, we investigated whether systemically administered rosuvastatin exerts any effect on ABL, the serum levels of pro-inflammatory and anti-inflammatory cytokines and oxidative status in rats with ligature-induced periodontitis.

Materials and Methods

Experimental protocols were conducted in accordance with ethical principles for the use of laboratory animals, as approved by the Ethics Committee on Animal Experimentation at Süleyman Demirel University (protocol no 2013-08). Thirty-nine adult male Wistar albino rats were housed at 24 ± 2°C under a 12:12-h light/dark cycle and received food and water *ad libitum*. Rats were weighed at the beginning of the study and immediately before sacrifice.

Experimental periodontitis model and treatment

Rats were randomly divided into four groups: a non-ligated group (C, $n = 9$); a non-ligated + rosuvastatin group (R, $n = 10$); a ligated group (P, $n = 10$); and a ligated + rosuvastatin group (PR, $n = 10$). At the baseline, in order to induce periodontitis, rats were first anesthetized with an intraperitoneal injection of ketamine (Ketalar, Istanbul, Turkey; 25 mg/kg) and xylazine (Xylazinbio 2%, Bioveta, Ivanovice na Hane, Czech Republic; 50 mg/kg). A silk ligature (3.0, Doğsan Sanayi, Istanbul, Turkey) was then placed around the cervix of the maxillary second molar for 14 days. Rosuvastatin (Crestor, Astra-Zeneca, Istanbul, Turkey; 20 mg/kg) was dissolved in water (30). The rosuvastatin, or water alone, was administered by oral gavage 1 h before ligation and then once daily for 14 days.

Analyses of serum samples

At the end of the study, the rats were euthanatized under general anesthesia, and 8 mL of blood was obtained from each. The blood samples were separated by centrifugation at 3,500 × g for 10 min, and serum samples were kept frozen at −80°C until analysis. The levels of IL-1β, IL-10, MDA, SOD, GSH, and GSH-Px were determined by enzyme-linked immunosorbent assay with specific commercial kits (East Biopharma, Hangzhou, China) in accordance with the manufacturer's instructions, and the

IL-1β/IL-10 ratio was also obtained.

Histomorphometric analysis

After sacrifice, the maxilla was split in half along the midline and between the central incisors. The right maxillary halves were placed in H_2O_2 for 24 h and then stained with 1% methylene blue to differentiate the cemento-enamel junction (CEJ). The extent of ABL between the CEJ and the alveolar bone crest was measured along the length of the exposed buccal root surfaces of the second molars in all groups. Three measurements (mesial-mild-distal) were made for each tooth. The total ABL was then expressed as the sum of the three measurements in millimeters. Morphometric analysis of ABL was performed using a digital camera (Leica MZ6, Leica, Wetzlar, Germany) with accompanying Leica software.

Histopathological analysis

The histological lesions were examined and scored by a pathologist (Ö.Ö.) who was blinded to the groups. The left maxillary halves were fixed in formalin solution, neutral-buffered (10%) and decalcified with ethylene-diaminetetraacetic acid solution (0.1 M) for 1 week. The specimens were then dehydrated through a graded ethanol series and embedded in paraffin. In order to obtain 5-µm-thick longitudinal sections, the paraffin blocks were serially cut in a mesio-distal direction along the long axis of each tooth. The specimens were stained with hematoxylin and eosin (HE). To assess the degree of ABL, the distances between the ABC and CEJ were measured. The lesioned areas were analyzed in each rat using a light microscope (Olympus CX41, Olympus Corporation, Tokyo, Japan). The Database Manual CellSens Life Science Imaging Software System (Olympus Corporation) was used for evaluation of inflammatory cell infiltration. The numbers of polymorphonuclear leukocytes (PMNLs) in the junctional epithelium and connective tissue subjacent to the epithelium and lymphocytes in the connective tissue (in a 0.05 × 0.05-mm area) were examined and counted at a magnification of ×40 (31). The numbers of osteoclasts and osteoblasts on the lesioned alveolar bone were counted in an area 1.23 mm square (×400 magnification) (32).

Statistical analyses

The findings were expressed as means with standard error. The variables were assessed using the Kolmogorov-Smirnov test if their distributions were normal. Data were analyzed statistically by one-way analysis of variance (ANOVA). When significant differences were found, post hoc LSD tests were performed. The Mann-Whitney *U* test was used for comparison of histopathological data among the groups. The SPSS software program (v. 11.0, SPSS, Chicago, IL, USA) was used for all statistical analyses at a significance level of $P < 0.05$.

The power was calculated with a statistical program (PASS 13. NCSS, LLC. Kaysville, UT, USA). The total sample of 39 rats (9, 10, 10, and 10 in the four groups) achieved 100% power to detect differences among the ABL means versus the alternative of equal means using an F test with a significance level of $P < 0.05$. The variation of the means was shown to be their standard deviation, which was 0.34. The common standard deviation within a group was assumed to be 0.03.

Results

All animals tolerated the experimental and treatment procedures well, and no complications were observed. At the baseline and at the end of the experiment, the body weights did not differ significantly among the groups ($P > 0.05$). Periodontitis was observed in all of the ligated teeth. When compared with the P group, ABL was significantly decreased in the PR group ($P < 0.05$) (Table 1, Fig. 1).

Figure 2 shows the main histopathological aspects. Periodontal inflammation and bone loss were not evident in the C and R groups. The PR group exhibited decreases in ABL and inflammatory cell infiltration relative to the P group. The numbers of PMNLs and lymphocytes differed among the groups, except between the C and R groups ($P < 0.05$) (Table 2). In the PR group, the numbers of PMNLs in the junctional epithelium and the connective tissue subjacent to the epithelium were lower than those in the P group ($P < 0.05$, $P < 0.001$), and the number of lymphocytes was also lower than that in the P group ($P < 0.001$). The P group showed the highest number of osteoclasts ($P < 0.001$) (Table 2). The PR group had fewer osteoclasts than the P group ($P < 0.001$). Osteoblasts were least numerous in the P group and most numerous in the PR group. There were no significant differences in the numbers of osteoblasts between the C and R groups, and between the PR and R groups ($P > 0.05$) (Table 2).

The levels of IL-1β, IL-10, MDA, SOD, GSH, and GSH-Px in serum are shown in Table 1. The IL-1β levels in the P and PR groups were higher than those in the C and R groups ($P < 0.05$). Although the IL-1β level was decreased in the PR group relative to the P group, the difference was not statistically significant ($P > 0.05$). The level of IL-10 was significantly higher in the PR group than in the P and R groups ($P < 0.05$). The IL-1β/IL-10 ratio did not differ between the control groups (C and

Table 1 Levels of parameters related to ABL, cytokines, and oxidant and antioxidant function

Parameters	Groups	Mean ± SE	95% CI for mean		Minimum	Maximum	Significant differences (*P* values)
			Lower bound	Upper bound			P-PR (0.042)
ABL (mm)	C	0.09 ± 0.01	0.06	0.13	0.03	0.17	P-C (0.000)
	R	0.08 ± 0.01	0.06	0.10	0.04	0.13	P-R (0.000)
	P	0.82 ± 0.05	0.71	0.93	0.52	1.07	PR-R (0.000)
	PR	0.70 ± 0.07	0.54	0.85	0.37	1.03	PR-C (0.000)
IL-1β (ng/L)	C	10.93 ± 1.31	7.91	13.94	5.96	15.64	P-C (0.000)
	R	12.30 ± 1.72	8.42	16.18	0.8	17.44	P-R (0.000)
	P	92.52 ± 14.30	60.17	124.87	10.4	167.6	PR-R (0.000)
	PR	76.58 ± 10.70	52.38	100.78	25.6	134.8	PR-C (0.000)
IL-10 (ng/L)	C	79.44 ± 4.44	69.20	89.69	75	115	P-PR (0.003)
	R	69.85 ± 2.60	63.97	75.72	54.84	79.71	PR-R (0.013)
	P	66.31 ± 5.01	54.99	77.63	31.72	75	
	PR	87.30 ± 6.26	73.14	101.45	59.26	126.5	
IL-1β/IL-10	C	0.14 ± 0.02	0.10	0.19	0.06	0.21	P-PR (0.044)
	R	0.18 ± 0.03	0.12	0.24	0.01	0.3	P-C (0.000)
	P	1.35 ± 0.19	0.92	1.77	0.33	2.23	P-R (0.000)
	PR	0.95 ± 0.18	0.53	1.37	0.28	2.27	PR-R (0.000)
							PR-C (0.000)
MDA (nmol/mL)	C	1.70 ± 0.09	1.49	1.90	1.34	2.26	P-C (0.027)
	R	1.56 ± 0.12	1.29	1.83	0.92	2.33	P-R (0.002)
	P	2.02 ± 0.10	1.80	2.24	1.41	2.44	PR-R (0.046)
	PR	1.84 ± 0.08	1.66	2.03	1.41	2.25	
SOD (ng/mL)	C	0.60 ± 0.02	0.56	0.67	0.53	0.74	
	R	0.58 ± 0.02	0.53	0.64	0.49	0.75	
	P	0.66 ± 0.03	0.58	0.74	0.54	0.85	
	PR	0.65 ± 0.04	0.56	0.75	0.44	0.90	
GSH-Px (U/mL)	C	193.08 ± 15.15	158.14	228.01	132.30	286.20	
	R	172.29 ± 9.60	150.57	194.01	107.50	203.70	
	P	188.2 ± 12.40	160.18	216.30	126.00	245.90	
	PR	182.72 ± 8.70	163.03	202.41	135.00	247.10	
GSH (ng/L)	C	4.32 ± 0.35	3.52	5.12	2.75	6.13	P-C (0.006)
	R	5.90 ± 1.06	3.50	8.29	1.84	11.13	PR-C (0.019)
	P	6.65 ± 0.59	5.31	7.99	4.08	9.85	
	PR	7.08 ± 0.31	6.39	7.77	5.78	8.63	

CI: Confidence interval, SE: Standard error, Significant difference ($P < 0.05$).

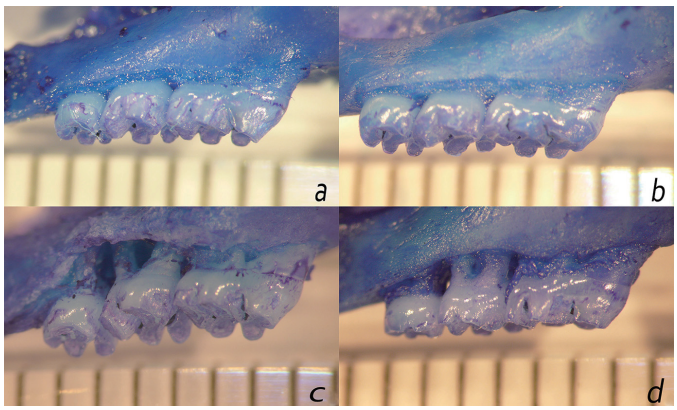

Fig. 1 Macroscopic aspects of maxillary halves from the C group (a), R group (b), P group (c), and PR group (d) (1% methylene blue dye, original magnification ×4).

Table 2 Histopathological data for the various groups

Groups	C	R	P	PR	(*P* values)
PMNL number in junctional epithelium					
(mean ± SE)	0.90 ± 0.17	1.00 ± 0.29	4.50 ± 0.40	3.60 ± 0.42	R-C (NS)
(min-max)	(0-2)	(0-2)	(3-7)	(2-6)	P-C (0.001)
					PR-C (0.001)
					P-R (0.001)
					PR-R (0.001)
					P-PR (0.05)
PMNL number in connective tissue					
(mean ± SE)	1.20 ± 0.78	1.70 ± 0.30	5.10 ± 0.50	3.20 ± 0.24	R-C (NS)
(min-max)	(1-2)	(0-2)	(3-7)	(2-4)	P-C (0.001)
					PR-C (0.001)
					P-R (0.001)
					PR-R (0.001)
					P-PR (0.001)
Lymphocyte number in connective tissue					
(mean ± SE)	0.77 ± 0.27	0.70 ± 0.26	31.70 ± 2.12	18.80 ± 1.28	R-C (NS)
(min-max)	(0-2)	(0-2)	(22-43)	(11-24)	P-C (0.001)
					PR-C (0.001)
					P-R (0.001)
					PR-R (0.05)
					P-PR (0.001)
Osteoclast number					
(mean ± SE)	5.88 ± 2.08	5.40 ± 1.26	14.70 ± 2.79	9.60 ± 1.77	R-C (NS)
(min-max)	(3-9)	(2-7)	(10-19)	(7-13)	P-C (0.001)
					PR-C (0.001)
					P-R (0.001)
					PR-R (0.001)
					P-PR (0.001)
Osteoblast number					
(mean ± SE)	10.11 ± 1.25	12.10 ± 1.91	6.40 ± 2.41	13.40 ± 2.45	R-C (NS)
(min-max)	(9-12)	(10-15)	(3-11)	(10-17)	P-C (0.001)
					PR-C (0.05)
					P-R (0.001)
					PR-R (NS)
					P-PR (0.001)

SE: Standard error, min-max: minimum-maximum, NS: no significant difference ($P > 0.05$).

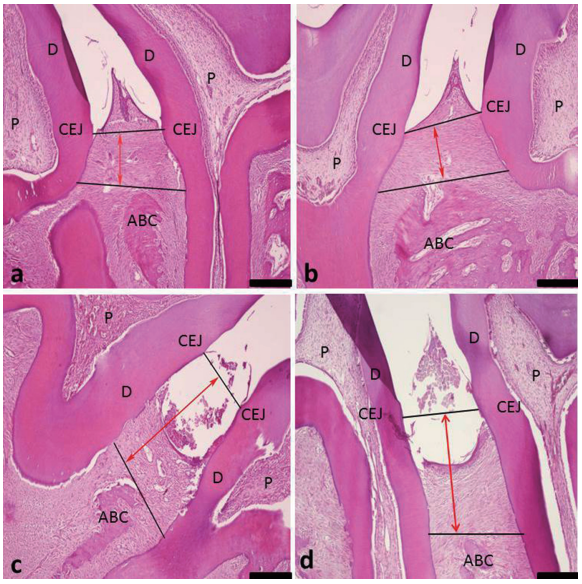

Fig. 2 Histopathological images of the various groups. a) C group: healthy periodontium; b) R group: no histopathological lesions in the periodontium; c) P group: almost complete loss of the gingival epithelial layer, marked alveolar bone loss and cementum resorption; d) PR group: reduced inflammation and alveolar bone loss, amelioration in connective tissue and gingival epithelial healing. P = pulp; D = dentin, CEJ = cement-enamel junction; ABC = alveolar bone crest. Arrows indicate distance between the CEJ and ABC. HE staining, Scale bars = 100 µm.

R) ($P > 0.05$), but was lower in the PR group than in the P group ($P < 0.05$). The MDA level was higher in the P group than in the C group ($P < 0.05$), but did not differ significantly between the PR and C groups ($P > 0.05$). The levels of SOD and GSH-Px showed no significant differences among the groups ($P > 0.05$). The P and PR groups had higher GSH levels than the C group ($P < 0.05$).

Discussion

This study examined the effects of rosuvastatin on ABL in rats with ligature-induced periodontitis in relation to the levels of cytokines and oxidative stress. While rosuvastatin decreased the extent of ABL, its anti-inflammatory effect seemed to be more pronounced than its amelioration of oxidative stress. Various experimental animal models of periodontitis have been investigated (33-35). Ligature-induced periodontitis in rats is a widely used model, being easy to perform, well characterized, and highly reproducible. Kuhr et al. (36) have recommended this model only for short observation periods (≤15 days). The maximum ABL in this model has been reported to occur between days 7 and 11 after ligature placement (37). Thus, we examined the ligature-induced periodontitis after a period of 14 days.

Statins have additional pleiotropic effects independent of their serum cholesterol-lowering properties, including anti-inflammatory, antioxidant, and immunomodulatory effects (20). Some studies have investigated the effects of various doses of different statins for prevention of ABL (22,23). In a study of ligature-induced periodontitis, Goes et al. (22) reported that a high dose of atorvastatin (9 mg/kg/day) reduced ABL by over 47% in comparison to saline, but not at low doses (1 or 3 mg/kg/day). Dalcico et al. (23) reported that simvastatin at 3 mg/kg/day had no positive effect on periodontal disease. Hughes et al. (30) reported that rosuvastatin at 2 or 20 mg/kg/day prevented bone loss induced by ovariectomy. Statins at low doses not only decrease osteogenesis but also increase bone resorption, and only high doses stimulate bone formation (38). It has been reported that the antioxidant effect of rosuvastatin starts on day 3 (39), and that the 90% lipid-reducing dose of rosuvastatin is 20 mg/kg at day 14 (40). Animal studies have shown that orally administered statins produce effects equivalent to those obtained via subcutaneous or intraperitoneal administration only at higher doses (38). Statins can have side effects including muscle symptoms, diabetes, depression, organ-specific and systemic (liver, kidney, lung and central nervous system) effects. Such side effects are especially associated with higher doses, drug interactions and endocrine problems (41,42). In the present study, we administered rosuvastatin at 20 mg/kg/day via oral gavage for 14 days to rats with experimentally induced periodontitis. No drug-related complications were observed in the rats.

Recently, it was reported that rosuvastatin gel reduced gingival inflammation, increased clinical attachment gain and resulted in significantly greater clinico-radiographic improvement of intrabony defects in comparison with atorvastatin gel, as an adjunct to nonsurgical periodontal treatment (28,29). When the histomorphometric and histopathological findings were taken into consideration, systemically administered rosuvastatin reduced ABL and inflammatory inflammation in rats with experimental periodontitis. Infiltration of PMNLs and lymphocytes in the PR group was less than that in the P group. Also, increases in the number of lymphocytes in periodontitis groups supported that in experimental models of periodontitis characterized by chronic inflammatory cell infiltration, lymphocytes in the connective tissue have been the predominant cell type (37,43,44).

Statins reduce NF-kB ligand-induced osteoclast differentiation and the number of osteoclasts and inhibit osteoblast apoptosis (45,46). In the present study, rosuvastatin decreased the number of osteoclasts in rats with experimental periodontitis; at the same time, the number of osteoblasts was highest in the PR group and lowest in the P group. It has been suggested that rosuvastatin, rather than increasing bone formation, inhibits bone resorption by osteoclasts and reduces the production of IL-6 by osteoblasts (30,47). In the present study, although osteoclasts and osteoblasts in sampled tissues were counted, the cytokines or proteins related to osteoblast apoptosis (transforming growth factor β, Smad protein 3) and the cytokines exerting indirect inhibitory effects on osteoclast function were not evaluated (38,45,47). However, the fact that the osteoblast count in the PR group did not differ from that in the control groups suggests that rosuvastatin may be able to enhance osteoblast differentiation or prevent osteoblast apoptosis.

MDA is a well-known marker of oxidative stress and peroxidative tissue injury (12), and an increase in its level is a result of direct interaction of PMNLs with pathogens or an indirect effect of activation of redox-sensitive transcription factors (e.g., NF-κB) (11). Ersoy et al. (48) observed that rosuvastatin treatment significantly diminished lipid peroxidation in the brain of rats with noise-induced oxidative stress. Otto et al. (49) reported that rosuvastatin protected against nitrate-induced oxidative stress. In the present study, however, MDA levels were significantly higher in the P group than in the C group; the difference in the levels of MDA between the

PR and C groups was not significant.

The antioxidant function of GSH plays a significant role in its direct scavenging of ROS (50). It has been reported that serum GSH levels are increased in experimental periodontitis (14). Schupp et al. (51) reported that GSH was increased in human promyelocytic cells incubated with/without rosuvastatin, inducing the formation of ROS, and that this effect was independent of HMG-CoAR inhibition. In the present study, GSH levels were significantly higher in the periodontitis groups than in the C group.

Some previous studies have reported significant increases, significant decreases and non-significant changes in SOD and/or GSH-Px levels in patients with periodontitis relative to healthy subjects (14-18). In the present study, the levels of SOD and GSH-Px did not differ significantly among any of the groups. There are currently no data about the effects of rosuvastatin on SOD and GSH-Px in periodontitis.

The IL-1β/IL-10, IL-1β/IL-4, IL-1β/IL-1 receptor antagonist and IL6/IL-10 ratios are known to be closely associated with the inflammatory response and the severity of periodontal disease (10,23,52). It has been suggested that statins may be able to influence periodontal breakdown through a decrease of inflammatory cytokines (53). Many studies have reported that statins reduce the level of IL-1β (14,23,54), and increased or do not change the level of IL-10 in experimental periodontitis (14,23). While T helper (Th)1 cells produce mainly pro-inflammatory cytokines, inducing a cellular immune response, Th2 cells produce mainly anti-inflammatory cytokines, inducing a humoral immune response (55). Periodontitis is associated with a Th2-type response rather than a Th1-type response (7). IL-10 is produced mainly by Th2 cells and usually inhibits the differentiation of Th1 cells (55). In the present study, although rosuvastatin reduced the level of IL-1β in the PR group, the effect was not significantly different from that in the P group. Higher IL-10 levels were found in the PR group than in the P group. Moreover, the IL-1β/IL-10 ratio in the PR group was lower than that in the P group. These findings show that rosuvastatin significantly increased the levels of IL-10 in rats with periodontitis. The different effects of rosuvastatin on various Th subsets could bring about a change in the inflammatory cell profile and the ratios of pro-inflammatory/anti-inflammatory cytokines. It has been reported that simvastatin improves IL-17/IL10 imbalance in chronic obstructive pulmonary disease (56). The anti-inflammatory effects of some therapeutics may be attributable to pre-resolving lipid mediators such as lipoxins (57,58). In inflammation, statins also increase lipoxins, thus reducing PMNLs and increasing IL-10 (59).

In conclusion, the findings of the present study suggest that rosuvastatin decreases ABL in rats with experimentally induced periodontitis. Although rosuvastatin decreases oxidative stress, its anti-inflammatory effects appear to be more remarkable than amelioration of oxidative status, perhaps through agonistic resolution of inflammation via receptor-mediated cellular signals, rather than through antagonistic mechanisms (57). Although the limitations of our study need to be taken into consideration, various dosages of rosuvastatin should be used when examining its protective effects against ABL in periodontitis, and it would be interesting to analyze changes in related parameters in the gingiva. The present findings may provide a useful basis for further studies designed to examine the antioxidant and anti-inflammatory properties of rosuvastatin as a host-modulatory agent for prevention of periodontal disease or regenerative therapy.

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Conflict of interest

The authors have no potential conflict of interest to declare with respect to this study.

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