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ORIGINAL ARTICALE

RESOLVIN E1 SUPPRESSES THE ALVEOLAR BONE LOSS AND INFLAMMATION IN EXPERIMENTAL PERIODONTITIS: IMMUNOHISTOCHEMICAL AND MOLECULAR GENETIC EVIDENCES

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Abstract

Background: Periodontitis is a chronic immune-inflammatory disease associated with microbial dysbiosis and dysregulated host response, leading to irreversible destruction of the periodontal apparatus and alveolar bone. Conventional treatments primarily target bacterial reduction but do not actively resolve inflammation or restore tissue homeostasis. Specialized pro-resolving mediators (SPMs), such as resolvin E1 (RvE1), derived from omega-3 fatty acids, have emerged as potent modulators of inflammation resolution through interaction with the chemerin receptor CHEMR23.

Objective: This study aimed to evaluate the effects of RvE1 on alveolar bone loss and inflammatory response in ligature-induced experimental periodontitis in rats, using histopathological, immunohistochemical, and molecular genetic approaches, and to identify CHEMR23-expressing cells as potential RvE1 targets.

Material and methods: Thirty male Wistar rats were randomly divided into five groups (n=6 per group): control (I), periodontitis (II), vehicle (III), early RvE1 treatment (IV; administration from Day 1), and late RvE1 treatment (V; administration from Day 7). Alveolar bone loss was assessed by measuring the cemento-enamel junction to alveolar bone crest distance. Histological evaluation and TRAP staining were used to assess inflammation and osteoclastic activity. Expression of CHEMR23 was examined immunohistochemically. Relative mRNA levels of TNF-α, IL-1β, RANK, and OPG in gingival tissues were determined by qPCR method.

Results: Ligature-induced periodontitis caused pronounced inflammatory infiltration, alveolar bone resorption, and increased numbers of TRAP+ osteoclasts, accompanied by up-regulation of TNF-α, IL-1β, and RANK. RvE1 administration significantly reduced the cemento-enamel junction to alveolar bone crest distance and inflammatory scores. Early RvE1 treatment showed stronger protection than late treatment, restoring near-normal bone architecture and cytokine expression profiles. CHEMR23 immunoreactivity was detected predominantly in osteoclasts, macrophages, and lymphocytes, suggesting these as primary RvE1-responsive cell populations.

Conclusion: Resolvin E1 effectively attenuates inflammation and bone resorption in experimental periodontitis, acting partly through CHEMR23-expressing osteoclasts and immune cells. Early intervention yields superior outcomes, highlighting the importance of timely modulation of inflammatory and osteoclastogenic pathways. These findings support RvE1 as a promising therapeutic candidate for host-modulatory management of periodontitis.

Keywords*:* Resolvin E1; Periodontitis; Rats; CHEMR23; Osteoclastogenesis; Inflammation; RANK/OPG signaling.

INTRODUCTION

Periodontitis is a chronic, multifactorial, immune-inflammatory disease characterized by microbial dysbiosis, unresolved inflammation, and progressive destruction of the tooth-supporting apparatus (i.e., alveolar bone, periodontal ligament, and cementum) [1]. In susceptible individuals, a shift in the oral microbial community triggers a dysregulated host immune response[2]. Instead of resolving, the inflammatory reaction persists, leading to tissue degeneration, periodontal pocket formation, and alveolar bone resorption. The pathogenic cascade involves neutrophil infiltration, release of reactive oxygen species and proteases, activation of osteoclastogenesis (via RANKL/OPG imbalance and proinflammatory cytokines such as IL-1β, TNF-α, IL-6, IL-17)[3,4]. Thus, periodontitis is currently considered not merely as an infectious disease but as an immune–microbial dysregulation associated with a progressive bone loss.

In conventional therapy, mechanical debridement (scaling and root planning) and antimicrobial agents aim to reduce microbial burden and suppress further progression. However, these interventions do not directly promote resolution of inflammation or stimulate bone regeneration. Over recent years, host-modulation approaches have emerged as a complementary strategy, seeking to balance the inflammatory microenvironment, favor resolution, and protect or restore the periodontium. Among these, lipid mediators of the resolution phase such as lipoxins, resolvins, protectins, and maresins have attracted considerable interest for their ability to actively orchestrate the resolution of the inflammation [5,6]. Unlike classical anti-inflammatory agents that inhibit pathways upstream (e.g. COX, LOX), these specialized proresolving mediators (SPMs) mediate the switching of local mediators toward resolution and actively involve immune clearance mechanisms (neutrophil apoptosis, efferocytosis, macrophage reprogramming) [7,8]. Among resolvins, resolvin E1 (RvE1), derived from eicosapentaenoic acid (EPA), is one of the most studied in the context of periodontal inflammation. RvE1 has been shown to reduce neutrophil infiltration, limit further leukocyte recruitment, promote macrophage-mediated clearance of apoptotic cells, and preserve tissue integrity [5, 9,10]. In preclinical models of periodontal disease, local administration of RvE1 has been reported to prevent alveolar bone loss or even to promote bone regeneration in ligature-induced periodontitis models [11,12]. In addition, some investigations suggest that RvE1 modulates the local microbiome composition indirectly (though not directly antimicrobial) in periodontitis models[9]. Differential effects of resolvins (e.g. RvD1 vs RvE1) in periodontal tissues (cementoblasts) have also been noted [13].

At the receptor and signalling level, RvE1 acts via at least two known G-protein coupled receptors: ChemR23 (also known as CMKLR1) and the leukotriene B4 receptor BLT1, exerting agonist and ligand-biased effects [14,15]. Binding to ChemR23 leads to downstream signaling cascades that suppress NF-κB activation and proinflammatory cytokine expression (via predominantly β-arrestin–mediated bias)[16]. The concept of "biased agonism" at ChemR23 suggests that RvE1 may preferentially engage anti-inflammatory or resolution-promoting pathways (for instance via β-arrestin scaffolding) rather than classical G-protein pathways. In non-periodontal inflammatory models, activation of ChemR23 has yielded tissue-protective and anti-neutrophilic effects [17,18]. However, in the periodontitis, direct *in vivo* evidence elucidating these receptor-level mechanisms (especially β-arrestin bias in gingival/bony tissues) remains unclear.

Despite promising preclinical results, several research gaps remain in this area. First, many of the published studies focus on endpoint morphometric or histologic outcomes (bone loss measurement) without deep mechanistic analyses at the immunological or molecular level (e.g. receptor signaling, gene expression, pathway activation). Second, few studies have combined histopathology, immunohistochemistry for cell populations or molecular markers, and gene-level analysis (qPCR, RNA profiling) in a single experiment to elucidate the effects of RvE1 on both inflammation and bone metabolism. Third, the temporal kinetics of RvE1 action in periodontitis models remain underexplored.

Given these gaps, this study was designed to further investigate the effects of RvE1 on alveolar bone loss and inflammatory responses in a ligature-induced rat periodontitis model using histopathological assessment, immunohistochemical localization of relevant cellular and molecular markers, and molecular genetic analyses (gene mRNA expression profiling). The aim of the present study is to assess whether RvE1 administration can suppress alveolar bone loss and modulate inflammatory infiltrates and cytokine/chemokine expression in periodontal tissues.

MATERIAL AND METHODS

Animals and experimental model. Thirty male Wistar rats (*Rattus norvegicus*) weighing 180–200 g were used, with *ad libitum* access to food and water. The rats were randomly housed in standard polycarbonate cages in a temperature-controlled environment, maintained at 21–23ºC and 55–60% humidity, with 12 h of light and 12 h of darkness cycle. The rats were randomly (using a randomization table) allocated into five experimental groups: the control group (Group I) (n = 6), the periodontitis group (Group II) (n = 6), the periodontitis group with local administration of vehicle (Group III) (n =6), the periodontitis group with early local administration of Resolvin E1 (Group IV) (n = 6), and the

periodontitis group with late local administration of Resolvin E1 (Group V) (n = 6). After acclimatization of animals, rats from all experimental groups were anesthetized with a xylazine (1 mg/mL) solution. In the periodontitis group (Group II), periodontitis group with local administration of vehicle (Group III), and both periodontitis groups with RvE1 (Groups IV and V), the standard protocol for ligature-induced periodontitis was used [19, 20]. Briefly, to model the ligature-induced periodontitis, using surgical microforceps and needle holders, the 6–0 silk suture material was placed and knotted around 2nd upper molars bilaterally. The ligature was applied as close as possible to the gingival sulcus from the palatal surface. In the control group (Group I), animals were subjected to sham ligation without knotting a ligature around molars. Animals were monitored for the position of the applied ligature and the accumulation of dental plaque every other day up to Day 14 (Figure 1A).

Resolvin E1 was purchased from Cayman Chemical® (USA) (Cat# 10007848) in a bottle containing 25 µg dissolved in 500 µl of ethanol and stored before use in −80°C according to manufacturer's instructions (Figure 1B).

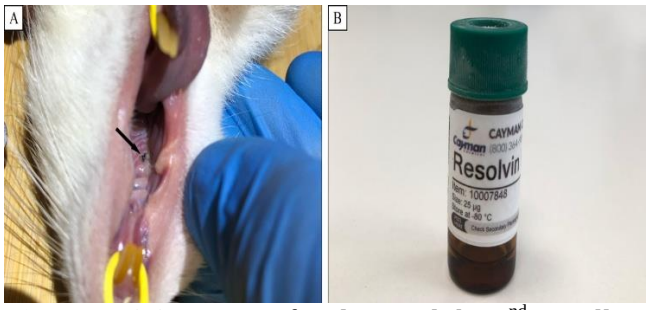

Figure 1. (A) Ligature fixed around the 2nd maxillae molar (arrow) on Day 14 of the experiment; Group II. (B) Resolvin E1 (25 µg) purchased from Cayman Chemical®.

Immediately before administration, RvE1 was aliquoted into 100 µl samples (50 ng/µl). Topical gingival administration of RvE1 was performed at Day 1 (Group IV) or at Day 7 (Group V) with a single dose of 10 µl per animal (500 ng). Animals from the Group III received the same dose of ethanol vehicle. The applied dose of RvE1 was based on the previously reported experimental studies [9,21].

14 days after the ligation or sham procedure, experimental animals were sacrificed by cervical dislocation under anesthesia, followed by decapitation. Samples of gingival tissue and jaw segments were collected bilaterally.

The scheme of the experiment is shown on the Figure 2.

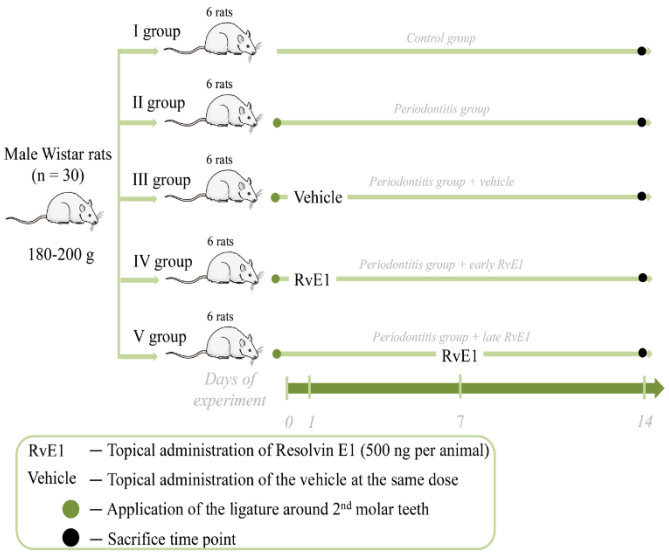

Figure 2. Scheme of the experiment

Gross description and assessment of the alveolar bone loss.

Maxillae were dissected and gingival tissues were removed. At the time of dissection, the inflammatory changes (edema, hyperemia) of the gingival tissue and pathological mobility of the molars were evaluated. Maxillae were treated with 30% hydrogen peroxide for 24 hours to completely remove any remaining soft tissue, washed in running water and dried. Alveolar bone level was measured on the palatal surface of the maxillae on digital images with scale using ImageJ software. Alveolar bone loss was calculated as mean linear values of a distance between cementoenamel junction (CEJ) to the alveolar bone crest (ABC), which was measured at palatal (2 to 3 sites) area at the level of the maxillary second molar. All measurements were performed by one examiner who was blinded to the specimen's group.

Real-Time qPCR.

Gingival tissues were homogenized for RNA extraction. Total RNA was extracted using the RNA-Extran Kit (Syntol®) following the manufacturer's instructions. The quantity and purity of the RNA were determined from the absorbance at 260/280 nm using a Nano-500 spectrophotometer (Helicon®). 20 ng of total RNA was reverse transcribed into cDNA using the MMLV RT kit (Syntol®) in accordance with the manufacturer's protocol. The CFX 96 TOUCH Real-Time PCR system (Bio-Rad®) and real-time PCR kit were used based on the manufacturer's instructions. Polymerase chain reaction (PCR) thermal cycling included three steps: step 1 at 50°C lasting 2 minutes, step 2 at 95°C lasting 10 minutes, and a final step 3 with 40 cycles at 95°C lasting 15 seconds and at 60°C lasting 1 minute. The relative mRNA expression levels of IL-1β (interleukin-1 beta), TNF-α (tumor necrosis factor alpha), RANK (receptor activator of nuclear factor kappa beta), and OPG (osteoprotegerin) (Table 1) were assessed. Fold changes in the gene expression were calculated by the ΔΔCt method, where ΔCT is the difference between the CT value of a target gene and the CT value of the internal control gene. ΔΔCT

is the difference between the ΔCT value of a target sample and the ΔCT value of a control sample [22]. The $^{2-}$ΔΔCT is the fold change of the target gene expression in a target sample relative to a control sample. An analysis of the results of tissue mRNA expression was made by a blinded observer who had no knowledge of the identity of the experimental groups.

Table 1. Primers used for RT-qPCR

mRNA	Forward (F) and Reverse (R) Primer Sequences
β-actin	F: AAGTACCCCATTGAACACGG
	R: ATCACAATGCCAGTGGTACG
IL-1β	F: TTGAGTCTGCACAGTTCCCC
	R: GTCCTGGGGAAGGCATTAGG
TNF-α	F: CCAGGTTCTCTTCAAGGGACAA
	R:CTCCTGGTATGAAATGGCAAATC
RANK	F: GTACCATGATCGAGGCTGGG
	R: GATAGTCCGCAGGTACGCTC
OPG	F: ACACACCAACTGCAGCTCAC
	R: TGTCCACCAGAACACTCAGC

Histological analysis.

Samples of the jaw segment were fixed in neutral buffered 10% formalin for ~24 hours, followed by decalcification in 10% EDTA (changed every 3 to 4 days) for 2 weeks, with subsequent histological processing using the Logos Hybrid Histological Processor (Milestone Medical®, Italy) and the Leica EG1150 Modular Tissue Embedding Center (Leica Biosystems®). Histologic sections (~ 4 μm) were obtained from 10% formalin-fixed paraffin-embedded blocks using an automatic rotary microtome Leica RM2255 (Leica Biosystems®) and stained with hematoxylin and eosin (H&E) for the descriptive histopathological evaluation. In addition to the descriptive histopathological analysis, the degree of inflammatory response and alveolar bone resorption was assessed semi-quantitatively using the following scale: 0 – absence of inflammatory infiltration and no signs of bone resorption; 1 – slight or moderate inflammatory infiltration without signs of bone resorption; 2 – moderate focal or diffuse inflammatory infiltration with initial signs of bone resorption; 3 – pronounced inflammatory infiltration with evident lacunar bone resorption; 4 – severe inflammatory infiltration with extensive bone destruction or osteolysis [23].

For immunohistochemical analysis, staining was performed using a BondMax Semiautomatic Immunohistostiner (Leica Biosystems®). The staining protocol included dewaxing, heat antigen unmasking using Bond Epitope Retrieval 2 solution (Leica Biosystems®) at pH = 9 for 20 minutes at 96°C, blocking peroxidase activity, incubation with the antibody for 15 minutes at room temperature and visualization using the Bond Polymer Refine Detection system (Leica Biosystems®). For better visualization, counterstaining was performed with hematoxylin according to the standard technique. The following primary antibodies were used: TRAP (Tartrate Resistant Acid Phosphatase) (Cloud-Clone®, Cat. #PAA902Ra01) and CHEMR23 (CMKLR1) (Affinity®, Cat. #DF3548). Quantitative analysis of TRAP-positive cells was performed on 3 to 5 high-power fields (HPF) of interradicular bone trabeculae per each histological slide. Histological slides were scanned using an Aperio CS2 Digital Pathology Slide Scanner (Leica Biosystems®), followed by digital image analysis using the Aperio ImageScope and ImageJ software [24]. Histopathological evaluation was performed by a blinded observer who had no knowledge of the identity of the experimental groups.

Statistical analysis.

Statistical analyses of the morphological data and RT-PCR results were performed for each IHC marker and target mRNA, and the data were expressed as the geometric mean ± SEM. Significant differences were determined using the Mann-Whitney U test. Values of $P < 0.05$ were considered to be significant. Variables were analyzed by statistics software (Statistica, StatSoft, v.10.0).

Ethics statements.

All procedures were performed under the recommendations of the Guide for the Care and Use of Laboratory Animals [25], ARRIVE guidelines (Animal Research: Reporting of In Vivo Experiments) [26] and were approved by the local Institutional Ethics Committee (A16-116072810135-5). The experimental part of the study was carried out in specialized premises of the vivarium of the Medical Institute named after S.I. Georgievsky, which are intended for keeping laboratory animals in standard and appropriate conditions to meet the physiological and environmental needs of experimental rats. The morphological part of the study and real-time qPCR analysis were carried out in the Central Research Laboratory of the Medical Institute named after S.I. Georgievsky using certified equipment.

RESULTS

RvE1 administration prevents alveolar bone loss.

In the control group (Group I), gingival tissues appeared healthy, with no signs of inflammation or tooth mobility. In contrast, rats with ligature-induced periodontitis (Group II) exhibited gingival edema, hyperemia, as well as molar mobility due to periodontal destruction and alveolar bone loss. The vehicle-treated group (Group III) showed comparable gross changes, indicating no effect of the vehicle alone. In animals treated with Resolvin E1, inflammatory alterations were visibly reduced. Early RvE1 administration (Group IV) led to near-normal gingival appearance, minimal edema, and absence of tooth mobility, while late administration (Group V) partially improved tissue appearance compared to untreated periodontitis. After soft tissue removal, gross evaluation of the maxillae demonstrated pronounced alveolar bone loss in Groups II and III, with irregular and

apically displaced bone crests. In RvE1-treated groups, the bone contour was better preserved, especially with early intervention (Group IV) (Figure 3). Quantitative analysis confirmed these findings: the CEJ–ABC distance was significantly increased in periodontitis (1.29 ± 0.25) and vehicle (1.15 ± 0.21) groups compared with controls (0.34 ± 0.08) ($p < 0.001$), whereas both early and late RvE1 treatments markedly reduced bone loss. These results demonstrate that local administration of Resolvin E1 effectively attenuates alveolar bone loss and improves periodontal integrity, with a stronger protective effect when applied at the early stage of inflammation.

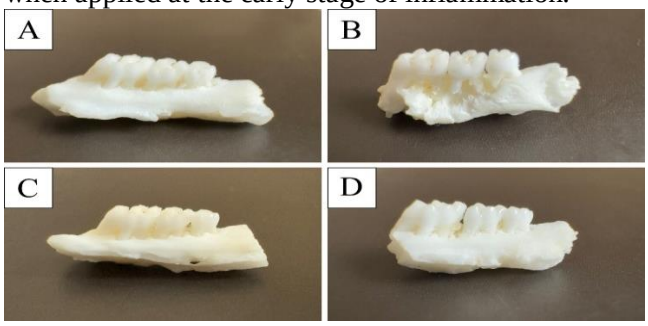

Figure 3. Gross appearance of the experimental rats' maxillae. (A) Control group (I) with no alveolar bone loss. (B) Ligature-induced periodontitis group (II) with severe bone destruction up to complete loss of the cortical plate. (C) Group of treated animals with early administration of RvE1 (IV) with minimal bone resorption. (D) Group of treated animals with late administration of RvE1 (V) with mild alveolar bone loss.

RvE1 reduces histopathological signs of inflammation and bone resorption.

Histological examination of H&E-stained sections revealed distinct differences in the structure of the periodontal tissues among experimental groups (Figure 4). In the control animals (Group I), the gingiva and alveolar bone displayed normal architecture with intact epithelial layer, connective tissue, and well-organized interdental and interradicular trabecular bone. No inflammatory cell infiltration was observed.

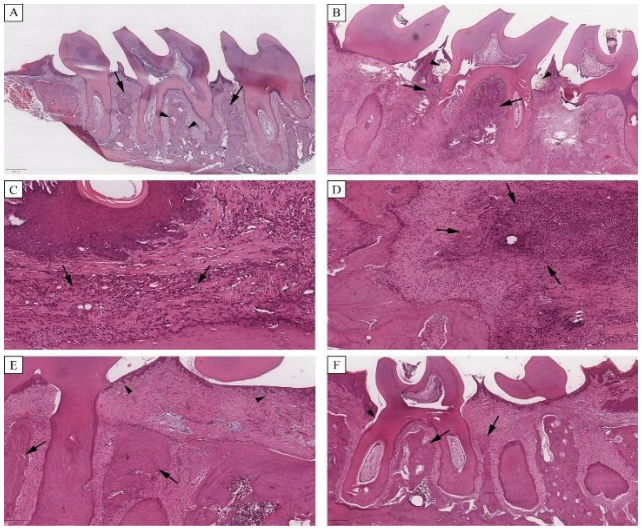

Figure 4. *(A)* Normal histological structure of the tooth-alveolar segment in a rat of the control group (I) with interdental (arrows) and interradicular (arrowheads) trabecular bone; H&E, x100 (B*)* Ligature-induced periodontitis (group II) with the pronounced inflammatory reaction and almost totally lysed interdental and interradicular bone trabeculae (arrows); remnants of the ligature (arrowheads); H&E, x100 (C) Severe mixed cellular inflammatory infiltration (arrows) at the subepithelial area; ligature-induced periodontitis (group II); H&E, x600. (D) Focal pronounced inflammatory infiltration at the proper connective tissue gingival plate (arrows); ligature-induced periodontitis (group II); H&E, x400. (E) Minimal osteolytic changes in interdental and interradicular bone trabeculae (arrows) with mild subepithelial inflammation (arrowheads); periodontitis group with early local administration of RvE1 (group IV); H&E, x200. (F) Partial resorption of the bone trabeculae (arrows) along with increasing of periodontal pockets (arrowhead); periodontitis group with late local administration of RvE1 (group V); H&E, x100.

In the ligature-induced periodontitis group (Group II), severe tissue destruction and inflammatory alterations were evident. The gingival epithelium was hyperplastic, with ulceration in some areas and intense mixed inflammatory infiltrate extending into the subepithelial and connective tissue layers. Interdental and interradicular bone trabeculae exhibited extensive osteolysis, with almost complete resorption in several specimens. Similar histopathological features were observed in the vehicle-treated group (Group III), indicating no therapeutic effect of the vehicle alone.

In contrast, animals treated with RvE1 showed a marked attenuation of inflammatory and destructive changes. Early RvE1 administration (Group IV) resulted in nearly normal gingival morphology with only mild inflammatory infiltration and preservation of most interdental and interradicular bone trabeculae. The alveolar crest appeared continuous, and osteolytic changes were minimal. In the late RvE1 group (Group V), moderate inflammatory infiltration and partial bone resorption persisted; however, these alterations were clearly less pronounced than in untreated periodontitis.

Statistical analysis confirmed a significant increase in inflammatory and resorptive scores in Groups II (3.68 ± 0.22) and III (3.55 ± 0.27) compared with the control (0.25 ± 0.11) (both $p < 0.001$), while both RvE1-treated groups exhibited significantly lower scores (1.24 ± 0.19 and 2.03 ± 0.21, respectively) ($p < 0.01$). The early RvE1 administration was particularly effective, showing nearly a two-fold reduction in histological severity compared with untreated periodontitis.

The differences in the severity of lacunar bone resorption observed during histopathological examination were confirmed by changes in osteoclastic activity, as determined by TRAP (tartrate-resistant acid phosphatase) staining (Figure 5A-D). In the control group (I), only a

few TRAP-positive multinucleated cells were observed along the bone surface (2.1 ± 0.4 cells per HPF), indicating a physiological baseline level of bone remodeling. In the ligature-induced periodontitis group (II), there was a marked increase in the number of TRAP-positive osteoclasts (11.8 ± 2.8 cells per HPF; $p < 0.001$ compared to control group) located at the resorptive lacunae of interdental and interradicular bone trabeculae, corresponding to the areas of pronounced bone destruction. The vehicle-treated group (III) displayed a similar pattern (10.9 ± 2.4 cells per HPF; $p < 0.001$ compared to control group) confirming that the vehicle itself did not influence osteoclastic activity. In contrast, RvE1 treatment resulted in a clear attenuation of osteoclast formation and activity. In the early RvE1 group (IV), the number of TRAP+ cells was substantially reduced compared to the untreated periodontitis (4.6 ± 0.8 cells per HPF; $p < 0.05$ compared to control group), and osteoclasts were mainly localized as isolated small clusters along preserved bone surfaces. The late RvE1 group (V) also showed fewer TRAP+ cells than the untreated periodontitis group, though osteoclastic activity remained moderately elevated compared with controls (8.7 ± 1.9 cells per HPF; $p < 0.01$ compared to control group). A summary of the CEJ–ABC distance, inflammatory and bone resorption scores, and the number of $TRAP^+$ cells is presented in Table 2.

Table 2. Quantitative assessment of alveolar bone loss, inflammatory response, and osteoclastic activity in experimental groups

	Control (I)	Periodontitis (II)	Periodontitis + Vehicle (III)	Periodontitis + early RvE1 (IV)	Periodontitis + late RvE1 (V)
CEJ-ABC distance (mm)	0.34 ± 0.08	1.29 ± 0.25*	1.15 ± 0.21*	0.48 ± 0.12†	0.71 ± 0.09*, †
Inflammatory and bone resorption score	0.25 ± 0.11	3.68 ± 0.22*	3.55 ± 0.27*	1.24 ± 0.19*, †	2.03 ± 0.21*, †
Number of TRAP+ cell per HPF	2.1 ± 0.4	11.8 ± 2.8*	10.9 ± 2.4*	4.6 ± 0.8*, †	8.7 ± 1.9*

*Notes: * $p < 0.05$ compared to the control group; † $p < 0.05$ compared to the vehicle group*

Immunohistochemical analysis of CHEMR23 receptor expression was performed to identify potential cellular targets of RvE1 within the periodontal tissues (Figure 5E-F). Although quantitative evaluation was not conducted, a consistent pattern of CHEMR23 immunoreactivity was observed across experimental groups. In control group, CHEMR23 expression was weak and limited to scattered mononuclear cells in the connective tissue without evidence of inflammatory activation. In contrast, animals with ligature-induced periodontitis (Groups II and III) exhibited marked CHEMR23 expression within multinucleated osteoclasts located along resorptive lacunae, as well as within macrophages and lymphocytes composing the inflammatory infiltrate. In RvE1-treated groups (Groups IV and V), the number of CHEMR23+ cells appeared reduced, consistent with the attenuation of inflammation and bone resorption observed histologically. The presence of CHEMR23 immunoreactivity in osteoclasts, macrophages, and lymphocytes supports their potential role as cellular targets of RvE1, given that CHEMR23 is a recognized receptor mediating the pro-resolving and anti-inflammatory effects of resolvins.

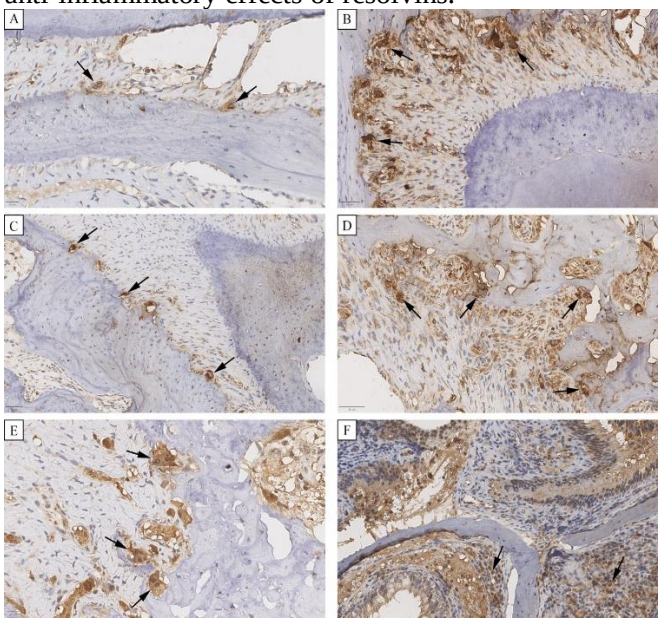

Figure 5. (A) Few TRAP+ cells (osteoclasts) (arrows) along the bone trabecula in control group (I); IHC, x200. (B) Massive presence of high-expression TRAP+ cells (arrows) within the lacunae of the resorbed bone in periodontitis group (II); IHC, x400. (C) TRAP+ cells (arrows) on the surface of the bone trabecula in early RvE1 group (IV); IHC, x200. (D) Area of active TRAP+ (arrows) bone resorption in late RvE1 group (V); IHC, x400. (E) Immunohistochemical expression of CHEMR23 receptor in osteoclasts (arrows); periodontitis group (II); IHC, x400. (F) CHEMR23-immunopositive lymphocytes (arrows) within the inflammatory infiltrate; periodontitis group (II); IHC, x400.

RvE1 treatment affects RANK/OPG ratio and mRNA expression of proinflammatory cytokines. Quantitative real-time PCR analysis was performed to evaluate the expression of inflammatory (TNF-α, IL-1β)

and osteoregulatory (RANK, OPG) genes in gingival tissues (Figure 6). The control group (I) showed basal transcriptional levels for all investigated genes. In the ligature-induced periodontitis group (II), there was a marked up-regulation of TNF-α and IL-1β mRNA compared with the control, reflecting activation of the local inflammatory response. The RANK transcript, which promotes osteoclast differentiation, was also significantly elevated, whereas the anti-resorptive regulator OPG was down-regulated, resulting in a strongly increased RANK/OPG ratio indicative of increase in number of TRAP+ cells and osteoclastogenic activation. The vehicle group (III) showed a similar expression profile, confirming that the vehicle did not affect gene expression. Treatment with Resolvin E1 (RvE1) substantially normalized the inflammatory and bone-related gene expression. In the early RvE1 group (IV), TNF-α and IL-1β mRNA levels were markedly reduced, approaching near-control values, and the RANK/OPG ratio was significantly decreased, consistent with suppression of osteoclasts. The late RvE1 group (V) also showed a significant down-regulation of inflammatory cytokines and partial recovery of OPG expression, though the effect was less pronounced than with early treatment.

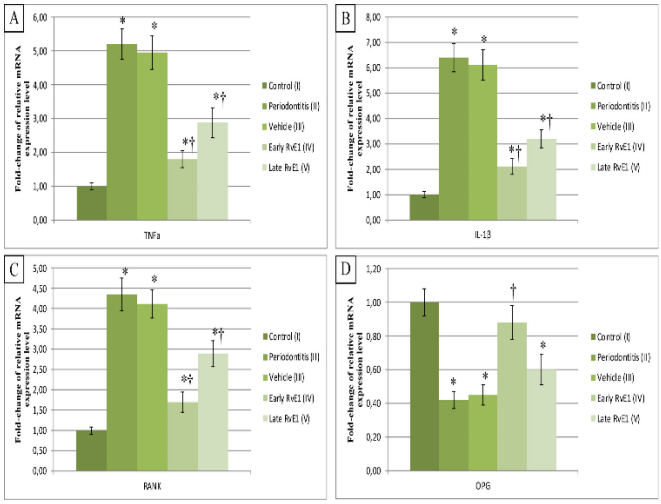

Figure 6. Fold-change of relative mRNA expression of TNF-α (A), IL-1β (B), RANK (C) and OPG (D) in experimental groups. Notes: * $p < 0.05$ compared to the control group; † $p < 0.05$ compared to the vehicle group.

DISCUSSION

The present study demonstrated that topical administration of resolvin E1 (RvE1) markedly attenuates periodontal inflammation and alveolar bone resorption in a ligature-induced periodontitis model in rats.

Comprehensive histological, immunohistochemical, and molecular analyses consistently indicated that RvE1 exerts a strong protective effect on periodontal tissues, particularly when administered at the early stage of disease development.

Gross morphometric examination has revealed that RvE1 treatment significantly reduced the CEJ–ABC distance compared with untreated periodontitis, confirming effective prevention of alveolar bone loss. These findings are in agreement with previous reports demonstrating that topical or systemic administration of RvE1 prevents or reverses bone resorption in experimental periodontitis, both in rats [9,12] and rabbits [27]. Consistent with these studies, the present work showed that RvE1 normalized the histopathological architecture of the gingiva, reduced inflammatory infiltration, and preserved alveolar bone, indicating that RvE1 promotes active resolution rather than simple suppression of inflammation.

The semi-quantitative scoring of inflammation and TRAP immunostaining further supported the anti-resorptive activity of RvE1. The significant decrease in the number of TRAP-positive osteoclasts and the normalization of the RANK/OPG mRNA ratio suggest that RvE1 limits osteoclastogenesis, consistent with previous reports that resolvins interfere with RANKL-induced osteoclast differentiation through inhibition of NF-κB and MAPK signaling pathways [28,29]. At the cytokine level, RvE1 markedly reduced gingival TNF-α and IL-1β expression, key mediators of osteoclast activation and connective-tissue destruction. These data agree with prior findings that RvE1 suppresses pro-inflammatory cytokines in tissues, thereby accelerating resolution and tissue repair [30-32]. The normalization of RANK and OPG transcription observed here further indicates restoration of the bone remodeling balance, which is a hallmark of successful resolution in periodontitis models.

The present study also provides immunohistochemical evidence supporting the role of the chemerin receptor 1 (CHEMR23, also known as CMKLR1) as a probable cellular target mediating the action of RvE1 in the periodontium. CHEMR23 is a G protein–coupled receptor capable of ligand-dependent biased signaling, producing either pro-inflammatory or pro-resolving effects depending on the activating ligand [33]. While chemerin stimulates classical G-protein pathways and promotes inflammation[34], specialized pro-resolving mediators (SPMs) such as resolvins, as well as synthetic agonists and monoclonal antibodies, preferentially activate β-arrestin–dependent cascades that suppress inflammation, enhance efferocytosis, and promote tissue repair [35-37]. Over the past decade, *in vitro* and *in vivo* studies have confirmed that resolvin-mediated CHEMR23 activation accelerates resolution of inflammation and protects against chronic immune-mediated injury.

At the cellular level, resolvins reprogram macrophages from a pro-inflammatory (M1) phenotype toward a unique “resolution-phase” state characterized by high IL-10 production, increased phagocytic activity, and down-regulation of TNF-α and IL-6 [36,37]. RvE1 and

Protectin D1 were also shown to enhance clearance of apoptotic neutrophils and shorten the inflammatory phase[38]. In addition, RvE1 limits neutrophil transmigration and infiltration in a CHEMR23-dependent manner [15], while other resolvins such as RvD1 and RvD2 exert similar anti-neutrophilic and macrophage-modulating effects [39-41].

In periodontal models, resolvin-mediated CHEMR23 activation has been directly linked to inflammation resolution and bone protection. In classical ligature- and *P. gingivalis*-induced models, local RvE1 application reduced neutrophil infiltration, TRAP+ osteoclast numbers, periodontal pocket depth, and alveolar bone loss [11,27]. In rats, RvE1 downregulated over 20 inflammatory and bone-resorption–related genes, including *Il1b*, *Mmp3*, *Mmp13*, and *Acp5*, confirming broad transcriptomic modulation via CHEMR23 [9]. Other SPMs, such as RvD2, have shown comparable benefits, decreasing the RANKL/OPG ratio and shifting immune responses toward an anti-inflammatory phenotype [42].

Beyond this, CHEMR23 signaling has been recognized as a key modulator of bone homeostasis. Pro-inflammatory activation by chemerin enhances osteoclastogenesis and inhibits osteoblast differentiation [43-45], whereas CHEMR23 engagement by resolvins produces the opposite, osteoprotective outcome. In models of TNF-α–induced calvarial osteolysis, RvE1 administration reduced osteoclast number and size, normalized the RANKL/OPG ratio, and suppressed NF-κB and PI3K–AKT pathway activation[46]. Likewise, RvD1 restored *OPG* expression and increased osteoblast differentiation markers such as *RUNX2* and *OSX*, while lowering RANKL, IL-1β, and TNF-α expression [47,48].

A notable aspect of the present experimental study is the comparison of two treatment schedules. Early administration of RvE1 (Day 1) was more effective than late administration (Day 7) in reducing inflammatory infiltration, osteoclast number, and bone loss. This finding emphasizes that the timing of pro-resolving mediator delivery is critical: administration during the initial phase of inflammation likely halts the cascade before irreversible tissue damage occurs. Similar kinetics-dependent effects were reported in other models where early RvE1 treatment led to sustained resolution, while delayed treatment achieved only partial repair [12,49]. The enhanced efficacy of early RvE1 may relate to its ability to shift macrophage phenotype toward pro-resolving M2 subsets, limit neutrophil recruitment, and rapidly restore the RANK/OPG balance before full activation of osteoclastogenic pathways.

Several limitations should be acknowledged. First, the study was conducted in a relatively small sample of rats, and quantitative assessment of CHEMR23 expression was not performed, limiting detailed receptor-level analysis. Second, the use of a single RvE1 dose and local application route precludes direct extrapolation to clinical dosing regimens. Also, only a limited panel of genes (TNF-α, IL-1β, RANK, OPG) was evaluated, and future transcriptomic or proteomic studies could provide a more comprehensive insight into RvE1-regulated signaling pathways.

In summary, this study provides new experimental evidence that resolvin E1 exerts potent protective and pro-resolving effects in experimental periodontitis through suppression of inflammatory cytokine expression, inhibition of osteoclastogenesis, and modulation of CHEMR23-expressing cells within the periodontal microenvironment. The findings reinforce the concept of RvE1 as a promising candidate for host-modulation therapy in periodontitis and highlight the therapeutic importance of early intervention to achieve maximal resolution of inflammation and preservation of alveolar bone.

CONCLUSION

This experimental study demonstrated that local administration of RvE1 suppresses periodontal inflammation and alveolar bone loss in a ligature-induced periodontitis model in rats. Comprehensive morphological, immunohistochemical, and molecular findings confirmed that RvE1 downregulates key pro-inflammatory mediators (TNF-α, IL-1β), limits osteoclastogenesis by normalizing the RANK/OPG ratio, and preserves alveolar bone integrity. Immunohistochemical localization of CHEMR23 receptors in osteoclasts, macrophages, and lymphocytes indicates that these cell populations are potential cellular targets of RvE1 within the periodontal microenvironment. Comparative analysis of treatment timing revealed that early administration of RvE1 (initiated at Day 1 after ligature placement) produced more pronounced protective effects than late administration (Day 7), emphasizing the therapeutic importance of early modulation of the inflammatory response to prevent irreversible bone destruction. Overall, these results strengthen the evidence that RvE1 represents a promising host-modulatory agent for the prevention and treatment of inflammatory bone loss in periodontitis. Further studies are warranted to explore dose–response relationships, receptor-level mechanisms, and translational applicability in clinical models.

DECLARATIONS

Authors' contributions

IGR and KMA conceptualized the experimental study, coordinated the overall manuscript structure. AAB and KMA performed histopathological and immunohistochemical analysis. OAN, OLI, ASK and MOT contributed to literature analysis, mRNA expression analysis, and drafted the sections. IGR, OAN and KMA critically revised the manuscript and ensured consistency across all sections. All authors reviewed, edited, and approved the final version of the manuscript.

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Declared none.

Ethics

The study adhered to the principles of the Declaration of Helsinki and the protocol was approved by the Institutional Ethical Committee (No. AAAA-A20-120061990017-6).

Data Availability Statement

The data used and/or analyzed during the current study are available from the corresponding author.

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Conflict of interest

The authors declare no conflict of interest.

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