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RESEARCH ARTICLE

RESOLVIN D1 (RvD1) REGULATES *PORPHYROMONAS GINGIVALIS* LIPOPOLYSACCHARIDE-INDUCED Del-1 AND CYTOKINE EXPRESSIONS IN HUMAN GINGIVAL FIBROBLASTS

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ABSTRACT

Purpose: To detect the effect of Resolvin D1 (RvD1) on Developmental endothelial locus-1 (Del-1) and cytokine expressions of human gingival fibroblast cells exposed to *Porphyromonas gingivalis* lipopolysaccharide (*P. gingivalis*-LPS).
Material and Methods: The effect of RvD1 on cell viability of human gingival fibroblasts exposed to *P. gingivalis*-LPS was determined by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Meanwhile, the effect of RvD1 on Del-1 and cytokine (IL-1β, IL-6, IL-8, IL-10, IL-17) expressions of human gingival fibroblasts exposed to *P. gingivalis*-LPS (1000 ng/mL) were studied by real-time PCR experiment, statistical analysis was performed using GraphPad Prism version 5 for Windows.
Results: Cell viability assay results demonstrated that RvD1 concentrations upregulated cell number compared to control group at 24 and at 72 h. While RvD1 reduced the IL-6, IL-8, and IL-17 mRNA expressions, the IL-10 and Del-1 mRNA expressions increased in a time- and dose-dependent manner. Also, IL-1β was not affected by RvD1 treatments.
Conclusion: The increased expression of Del-1 and IL-10 by RvD1 down-regulated the pro-inflammatory cytokine expressions induced by *P. gingivalis*-LPS in gingival fibroblast. Resolvin D1displayed regulatory effects on gingival inflammation in *P. gingivalis* LPS-induced cell culture experiment. In particular, results of this study show that Del-1 induced by RvD1 may have therapeutic potential to modulate periodontal inflammation.

Keywords: Cytokine, Del-1, gingival fibroblast, resolvin D1.

INTRODUCTION

Inflammation resolution is an active, well-coordinated mechanism that recovers tissue integrity and function, rather than the passive termination of inflammatory reactions by specialized pro-resolving lipid mediators (1,2). Lipoxins generated from arachidonic acid and resolvins and protectins derived from omega-3 polyunsaturated fatty acids are two examples of specific pro-resolving agonists (3). Docosahexaenoic acid (DHA) has a remarkable anti-inflammatory effect, which is attributed largely to its oxidation products such as resolvin, maresin, and

protectin (4). Resolvin D (RvD) is a group of lipoxygenase metabolites derived from DHA, with RvD1 and RvD2 receiving the most attention (5). The anti-inflammatory extracellular matrix protein known as Developmental Endothelial Locus-1 (Del-1) is released by endothelial cells and reduces inflammation in a range of organs, including the periodontium, brain, and lungs (6). Developmental endothelial locus-1 (Del-1) is an anti-inflammatory 52 kDa protein that is secreted by endothelial cells. It has three repeats that resemble epidermal growth factor (-EGF-) at the N-terminus (E1, E2, and E3) followed by two discoidin I-like domains (C1 and C2), or discoidin I-like domain 3 (EDIL3) (6). Furthermore, Del-1 specifically inhibits the expression of IL-17, which in turn inhibits the recruitment of neutrophils to the periodontium, reducing inflammation and bone loss. Del-1 also interacts with leukocyte 2 integrins, preventing the adhesion of ICAM-1 and lymphocyte function-associated antigen-1 (LFA-1, αLβ2, and CD11a/CD18) to inflammatory cells (7). The regulator role of Del-1 was displayed using young Del1 knocked-out (Edil3 -/-) mouse models, and the results show that Del-1 deficiency is associated with spontaneously high neutrophil infiltration and increased disease severity (8). Periodontal disease has been associated to gram-negative bacteria including Tannerella forsythia, Treponema denticola andPorphyromonas gingivalis. *Porphyromonas gingivalis* Lipopolysaccharide (*P. gingivalis*-LPS) is a crucial virulence factor and is critical in the onset and advancement of periodontal diseases. Moreover, *P. gingivalis*-LPS induces the expression of pro-inflammatory cytokines including tumor necrosis factor alpha (TNF-α), ınterleukin (IL)-1beta (β), and IL-6 in periodontal tissues (9,10). Del-1 deficiency caused severe inflammation and alveolar bone loss in animal periodontitis models, whereas local therapy with recombinant Del-1 reduced neutrophil infiltration and bone resorption (11). The expression of Del-1 is inhibited by the pro-inflammatory cytokine IL-17. Moreover, Del-1 is a necessary modulator of specialized pro-resolving mediators (SPMs)-dependent inflammation resolution that is essential for efficient synthesis of at least some key SPMs (RvD1 and RvE1).

In mouse models of periodontitis, genetic Del-1 loss promotes not only increased neutrophil infiltration but also IL-17-reliant inflammation-related tissue destruction. In contrast, deactivating of IL-17 signaling in Del-1-deficient animals, that is, mice with combined Del-1 and IL-17 receptor impairment, cures the Del-1 deficiency-related inflammatory diseases (12,13). Existing literature shows that RvD1 provides protection against IL-17-driven periodontal bone resorption in a Del-1-dependent manner via GSK-3b- and C/EBPb mechanism, whereas RvD1 disrupt this inhibitory pathway at the GSK-3b level by activating PI3K/Akt signalling (14).

The aim of this study was to clarify whether RvD1 affects cytokine and Del-1 mRNA expressions in human gingival fibroblasts. We hypothesized that RvD1 modulates the inflammatory response of P gingivalis LPS-induced HGF cells through cytokine and Del-1 expression.

MATERIAL AND METHODS

Cell Culture

Human gingival fibroblasts are the primary cell of gingival tissues and are involved in inflammation and immune responses as well as the synthesis and breakdown of connective tissue. Study was approved by Nigde Omer Halisdemir University, Non-Invasive Clinical Research Ethics Committee (Date: 24.03.2022, Decision Number: 2022/36).

Human gingival fibroblast (HGF) cells were thawed from our cell stock in cell culture media (DMEM; Gibco; Grand Island, NY, USA), 10% fetal bovine serum (FBS; Gibco), L-glutamine (600 mg/mL; Gibco;), penicillin (100 U/mL; Gibco), streptomycin (125 mg/mL; Gibco). Then, we maintained the cells in cell culture carbon dioxide incubator and observed the Hgf cells can be proliferation under inverted microscope. Cell line passage 3 (Hgf ǂ3) was used in the cell viability assay and the total RNA isolation experiments.

Experiments were performed twice in triplicate for each experiment for RNA isolation and three times in triplicate for cell viability.

Resolvin D1 (RvD1) Preparation

RvD1 was purchased from Cayman Chemicals (Ann Arbor, MI, USA). In line with previous studies on the effective dose of RvD1, a full range of RvD1 concentrations [Control (C), 1, 10, 100 ng/mL] was prepared, and added to the DMEM with 5% FBS (15,16).

Preparation of *Porphyromonas gingivalis* Lipopolysaccharide

Porphyromonas gingivalis Lipopolysaccharide product that is available for purchase was utilized in

its purest form (tlrl-ppglps-InvivoGen, SanDiego, USA). 1000 ng/mL of *P. gingivalis*-LPS was produced for use in cell viability and mRNA expression studies. Our earlier research revealed that cementoblasts and human gingival fibroblasts greatly reacted to *P. gingivalis*-LPS concentrations of 1000 ng/mL (9, 10).

Design of Study Groups

In order to determine the effect of RvD1 on *P. gingivalis*-LPS-induced cell viability and mRNA expression (cytokines, Del-1) of HGFs 5 study groups were planned including control (C), only 1000 ng/mL *P. gingivalis*-LPS (LPS) and 1 ng/mL of RvD1 combined with 1000 ng/mL *P. gingivalis*-LPS (LPS+R1), 10 ng/mL of RvD1 combined with 1000 ng/mL *P. gingivalis*-LPS (LPS+R10), 100 ng/mL of RvD1 combined with 1000 ng/mL *P. gingivalis*-LPS (LPS+R100) .

Cell Viability Assay

The 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) (Sigma, St. Louis, MO, USA) test was used to demonstrate the impact of RvD1 on cell viability at 24 and 72 hours of *P. gingivalis* LPS-induced HGFs (Sigma, St. Louis, MO, USA). In this experiment, third passage HGF cells were trypsinized, and 200 µL cell solution containing $2X10^4$ cells was added to each well. Following a 24-hour incubation period, HGFs were treated with only 1000 ng/mL *P. gingivalis* LPS (LPS), and [Control (C), 1 ng/mL RvD1 (LPS+R1), 10 ng/mL RvD1 (LPS+R10), 100 ng/mL RvD1 (LPS+R100)] ng/mL of RvD1 combined with 1000 ng/mL *P. gingivalis*-LPS. Resolvin D1 or *P. gingivalis*-LPS were not administered to the controls. Following the RvD1/*P. gingivalis* LPS treatment, MTT was applied to the wells and incubated for two hours. 200 µL of dimethyl sulfoxide were added to each well after incubation to obtain blue formazan, whose optical density was determined at 540 nm. Cell viability at 72 hours was also determined using this procedure.

Real-time Polymerase Chain Reaction (RT-PCR)

To determine the mRNA expressions of pro-/anti-inflammatory cytokines, total RNA from HGF cells was collected 24 or 72 hours following treatment with varied doses of RvD1 and 1000 ng/mL *P. gingivalis* - LPS. The EZ-RNA Total RNA Isolation Kit's recommended methodology for RNA extraction was followed (Kibbutz BeikHaemek, Israel). The A260/280 ratio, as determined by spectrophotometry, was maintained above 1.8 utilizing a complementary DNA (cDNA) synthesis kit, first-strand complementary DNA was produced from 1.0 µg of total RNA (Applied Biosystems High-Capacity RNA-to-cDNA kit, Foster City, USA). Using the Brilliant SYBR Green Q-PCR Master Mix (2X) (Thermo Scientific, Massachusetts, USA), real-time PCR was carried out using 1.0 µL of cDNA for a total volume of 25 µL.

In order to ascertain whether any nonspecific PCR amplifications were generated, melting curve investigations of the PCR products were carried out. The reference gene for normalization was GAPDH, which was unaffected by the research treatments.

Statistical analysis

Statistical analysis was performed using GraphPad Prism version 5 for Windows. mRNA expression, by calculating the ΔCt (Ct housekeeping gene - Ct gene

Table 1. Synthetic gene-specific oligonucleotide primers used in this research. All sequences are from human and listed 5'–3'

Primer	Forward	Reverse
IL-1β	CTGATGGCCCTAAACAGATGAA	TCGGAGATTCGTAGCTGGAT
IL-6	GGTACATCCTCGACGGCATCT	GTGCCTCTTTGCTGCTTTCAC
IL-8	ACTGAGAGTGATTGAGAGTGGAC	AACCCTCTGCACCCAGTTTTC
IL-10	CATCGATTTCTTCCCTGTGAA	TCTTGGAGCTTATTAAAGGCATTC
IL-17	CCCAGGGACCTCTCTCTAATC	ATGGGCTACAGGCTTGTCACT
Del-1	CCTGTGAGATAAGCGAAGC	GAGACTCGGTGAGTAGATG
GAPDH	ACCACAGTCCATGCCATCAC	TCCACCACCCTGTTGCTGTA

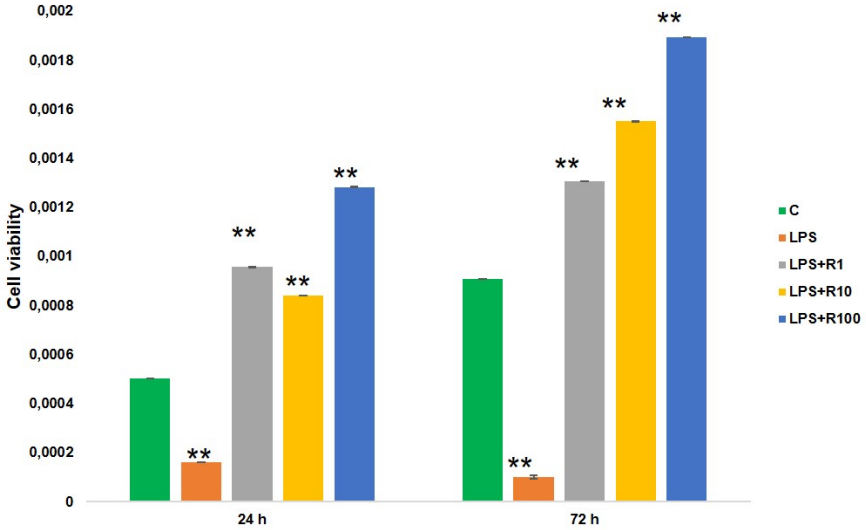

Figure 1. The effect of RvD1 on the *P. gingivalis*-LPS-induced cell viability in HGFs. The HGFs were treated with only 1000 ng/mL *P. gingivalis*-LPS and the combination of 1000 ng/mL *P. gingivalis*-LPS and 1 ng/mL RvD1 (LPS+R1), 10 ng/mL RvD1 (LPS+R10), 100 ng/mL RvD1(LPS+100) at 24 h and 72 h. *p<0.05, compared with the control (C).

of interest), and the expressions of different genes were expressed as 2-ΔCt. Triplicates were performed for each experimental point.The comparative Ct method was used for Q-PCR results (17). A one-way analysis of variance (ANOVA) with Dunnett's test and Tukey's HSD test was used to assess the cell viability and RT-PCR results. Differences between groups were considered significant when the confidence interval exceeded 95 % (P < 0.05).

RESULTS

RvD1 Effects on Viability of *P. gingivalis*-LPS Treated HGF Cells

Only 1000 ng/mL *P. gingivalis*-LPS (LPS)significantly decreased HGF cell viability (p<0.05) in a time-dependent manner compared to the control group (C) (Figure 1). Resolvin D1 counteracted the detrimental effects of *P. gingivalis*-LPS on cell viability when 1000 ng/mL *P. gingivalis* LPS and RvD1 were administered concurrently. Treatments with 1, 10 and 100 ng/mL RvD1 completely recovered the viability in a time- and dose-dependent manner compared to the levels found with 1000 ng/mL *P. gingivalis*-LPS alone (p<0.05). The highest increase in *P. gingivalis*-LPS-stimulated cell viability was achieved with 100 ng/mL RvD1, and it was 94% of the control after 24 and 72 hours (p<0.005) (Figure 1).

RvD1 Effects on HGFs Cytokines and DEL-1 Expressions Linked to Inflammation

Only 1000 ng/mL *P. gingivalis*-LPS (LPS) application, when compared to the control group (C), importantly improved the pro-inflammatory cytokine expressions (IL-1β, IL-6, IL-8, and IL-17) and reduced the anti-inflammatory cytokine expression (IL-10) at 24 and 72 h (p<0.05) (Figure 2). Additionally, 1000 ng/mL *P. gingivalis*-LPS combined with 1, 10, and 100 ng/mL RvD1 concentrations (LPS+R1, LPS+R10, LPS+R100) did not affect the expression of IL-1β compared to the control group at 24 and 72 hours (p>0.05). Conversely, treating cells with 1000 ng/mL *P. gingivalis* LPS and RvD1 concentrations (1, 10, and 100 ng/mL), when compared to the control group(C), reduced the expression of IL-6, IL-8, and IL-17 mRNA levels at both time periods (p<0.05). Resolvin D1 administration at 1, 10, 100 ng/mL dramatically upregulated the anti-inflammatory cytokine IL-10 in comparison to the control group (p<0.05). Additionally, the treatment with all RvD1 applications enhanced the expression of IL-10 that is suppressed by *P. gingivalis*-LPS to levels higher than those of the control group (p<0.05), with the rise becoming more noticeable at 72 h (Figure 2). The Del-1 mRNA expression levels between the HGF control and RvD1-treated groups displayed a important difference in RT-PCR results (p<0.05).

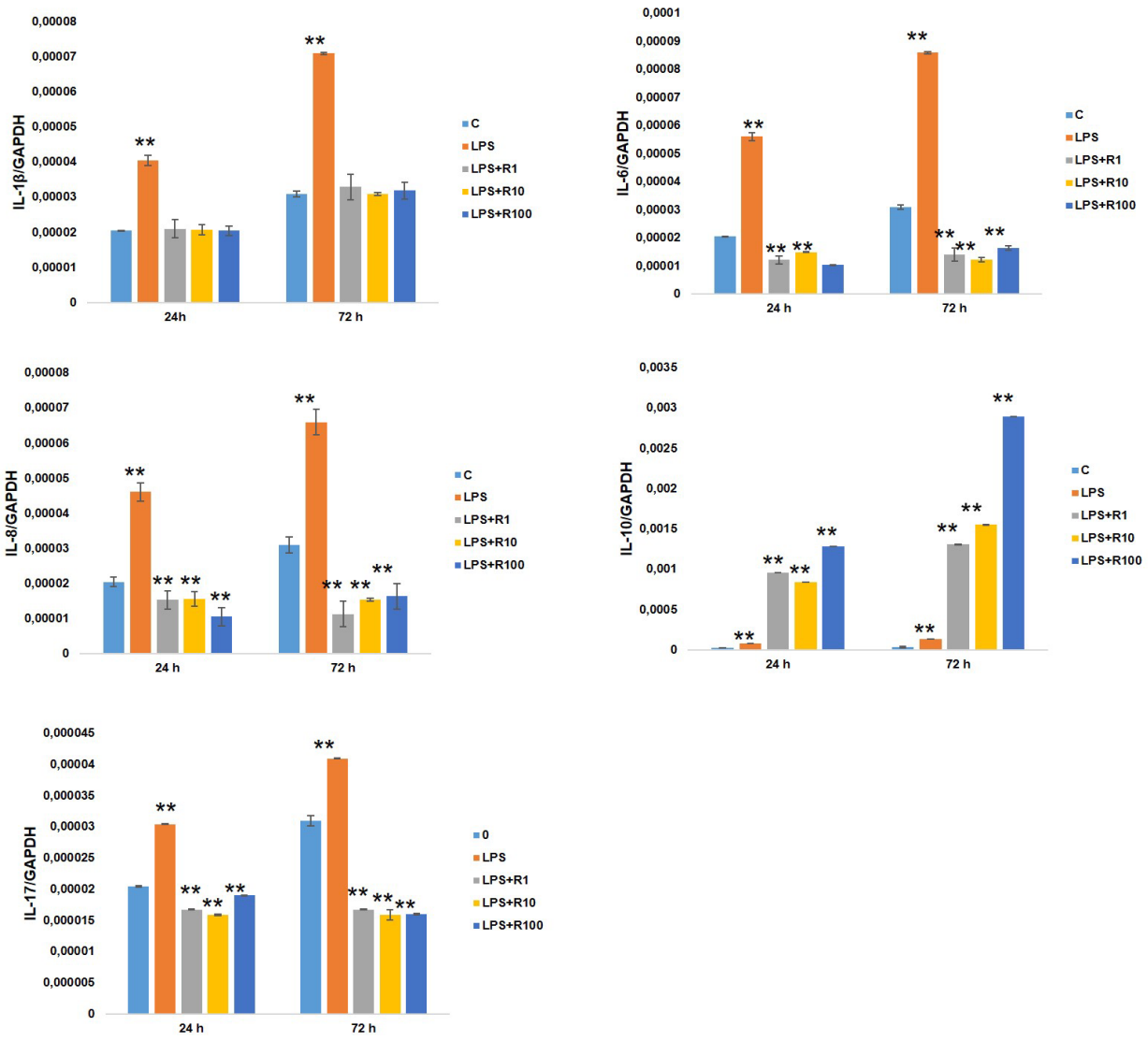

Figure 2. RvD1 affected the mRNA expressions of pro/anti-inflammatory cytokines of *P. gingivalis*-LPS-treated HGFs. The expression of pro/anti-inflammatory cytokines in HGFs from each group detected by quantitative RT-PCR (target genes were normalized to the housekeeping gene GAPDH). The cells were treated with only 1000 ng/mL *P. gingivalis*-LPS (LPS) and the combination of 1000 ng/mL *P. gingivalis*-LPS and 1 ng/mL RvD1(LPS+R1), 10 ng/mL RvD1 (LPS+R10), 100 ng/mL RvD1 (LPS+R100) at 24 h and 72 h. A.IL-1β, B. IL-6, C. IL-8, D. IL-10, E. IL-17 mRNA expressions of HGFs applied by only *P. gingivalis*-LPS, and the combination of *P. gingivalis*-LPS and RvD1. *p <0.05, compared with the control (C).

HGFcells induced by *P. gingivalis* LPS were treated with RvD1 concentrations (1, 10, 100 ng/mL) and total RNA was isolated from HGFs at 24 and 72 h with or without RvD1 concentrationsto determine the effects of RvD1 on Del-1 mRNA expression levels. Figure 3 shows that after the application of RvD1, the expression levels of Del-1 in the HGFs were significantly increased in a time- and dose-dependent manner at 24 and 72 h ($p<0.05$). as compared to the control group.

DISCUSSION

Resolvin D1, an omega-3 polyunsaturated fatty acid metabolite, has been demonstrated to reduce inflammation in vivo and decrease polymorphonuclear neutrophil (PMN) accumulation in tissue by preventing human PMN transendothelial migration and improving macrophages potential to phagocytose PMNs (16). We demonstrated earlier that *P. gingivalis* LPS-induced modification of phenotypic and inflammatory characteristics in HGF

could potentially be a pathogenic mechanism underlying tissue destruction. Therefore, we hypothesized that RvD1 modulates the inflammatory response of *P. gingivalis* LPS-induced HGF cells through cytokine and Del-1 expressions.

The results of this study demonstrate that RvD1 modulates cell viability and pro-/anti-inflammatory cytokine profiles andDel-1 expression of *P. gingivalis*-LPS-induced in HGFs. We used optimum dosage (1000 ng/mL) of *P. gingivalis*-LPS according to our previous studies (9,10. Resolvin D1reduced the pro-inflammatory cytokine expressions, whereas anti-inflammatory and Del-1 expressions increased in a time- and dose-dependent manner. In our previous study, *P. gingivalis*-LPS suppresses cell viability and proliferation in HGFs (9). Additionally, report we reported in our earlier study that 1000 ng/mL *P. gingivalis*-LPS significantly reduced cell viability in cementoblasts (10). In the literature we found another study performed by Khaled et al., in which they analyzed the effects of RvD1 using different concentrations (0- 1000 ng/mL) on HGFs and they determined that RvD1 both had no cytotoxic effects and importantly supressed the toxic effects of 13.5% (v/v) *P. gingivalis* supernatant on HGFs (16). Our findings are consistent with the findings of the Khaled et al. study. Cao et al. reported that RvD1 (50, 100, and 200 nM) human osteoblastic osteosarcoma cell line (MG-63) cell viability did not affect, but cell viability importantly decreased by 1000 ng/mL Escherichia coli-LPS application, and these reductions were significantly reversed by RvD1 treatment (18). Also, another study revealed that high-dose *P. gingivalis* LPS (≥ 50 µg/mL) importantly suppressed cell viability, while low-dose *P. gingivalis* LPS (≤ 10 µg/mL) did not importantly affect HGF viability (19).

Gingival fibroblasts are abundant in gingival tissue and have been reported to contribute in immune response and inflammatory events (20). Additionally, the cells react to different inflammatory cytokines and growth hormones (21). Cytokines are essential for regulating the inflammatory system. In addition to immune cells including lymphocytes, monocytes, macrophages, and granulocytes, they are also generated by epithelial, endothelial, and fibroblast cells. We analyzed the pro-/anti-inflammatory cytokine expressions of HGFs induced with 1000 ng/mL *P. gingivalis*-LPS and incubated with control, 1, 10, 100 ng/mL RvD1 at 24 and 72 h. Also, we applied to HGFs only 1000 ng/mL *P. gingivalis*-LPS for cytokine expressions at 24 and 72 h. As compared to the control, no significant difference in the IL-1β mRNA expression of *P. gingivalis*-LPS-induced-HGFs treated with any of RvD1 but only *P. gingivalis* LPS treated HGFs significantly increased IL-1β mRNA expression at 24 and 72 h . When cells were stimulated with only *P. gingivalis*-LPS IL-6, IL-8, and

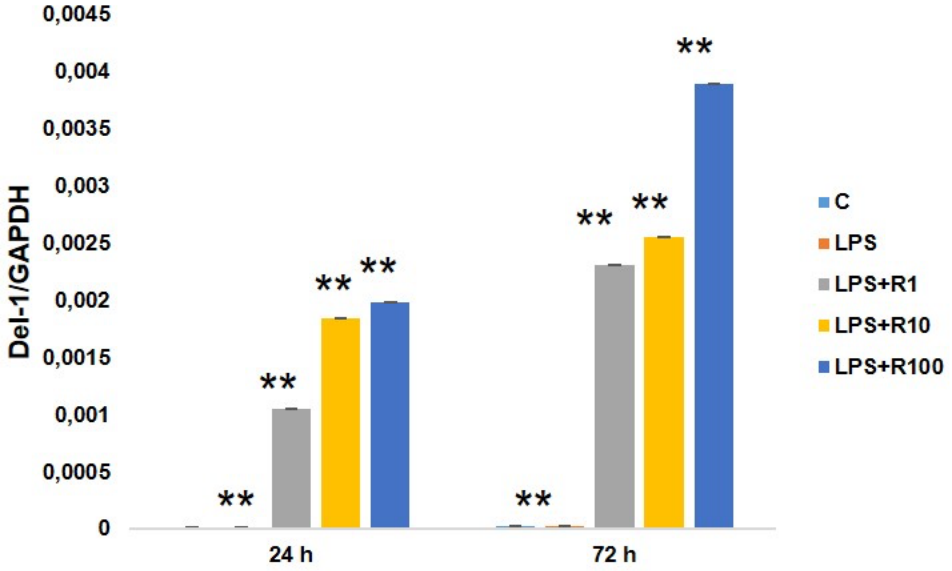

Figure 3. Demonstration of the effects of only *P. gingivalis*-LPS and the combination of 1000 ng/mL *P. gingivalis*-LPS (LPS) and 1 ng/mL RvD1 (LPS+R1), 10 ng/mL RvD1 (LPS+R100), 100 ng/mL RvD1 (LPS+R100) on Del-1 expression in HGFs at 24 h and 72h. *p <0.05, compared with the control (C).

IL-17 mRNA expressions dramatically increased, but RvD1 administration following *P. gingivalis*-LPS stimulation significantly decreased IL-6, IL-8, and IL-17 levels compared to the control group in a time- and dose-dependent manner. Our previous study demonstrated that higher concentrations (1000 and 3000 ng/mL) of *P. gingivalis*-LPS importantly stimulated IL-6 and IL-8 mRNA expressions of HGFs on days 3 and 8 (9). Another one of our studies had previously revealed that 1000 ng *P. gingivalis*-LPS dramatically increased the expression of IL-1β, IL-8, and IL-17 mRNA expression of cementoblasts at 16 and 24 hours. However, IL-8 and IL-17 expressions were decreased in the cementoblast cells induced with 1000 ng/mL *P. gingivalis*-LPS at 72 h. (10). The differences in the results between these studies may be due to the different origins of the cementoblast/HGF and the induction of both cells by *P. gingivalis*-LPS may result in different cytokine response in a time-dependent manner. Khaled et al. investigated the impact of RvD1 on *P. gingivalis* treatment on HGF expression of cytokines and they demonstrated that supernatant of *P. gingivalis* dramatically upregulated the mRNA expression of IL-6, IL-5, IL-17, IL-10, IL-8, monocyte chemoattractant protein (MCP)-1, MCP-2, and MCP-3. Also, the combination of *P. gingivalis* supernatant and RvD1 on HGFs showed that RvD1 significantly improved the TGF-β1 and importantly decreased MCP-1 and IL-6 levels (14). Our pro-inflammatory cytokine results in general are consistent with the literature (16). As reported in this study RvD1 dose-dependently increased IL-10 expression of *P. gingivalis*-LPS-induced HGFs at 24 and 72 h. The effects of 1000 ng/mL *P.gingivalis*-LPS on the expression of IL-10 mRNA expression was revealed in cementoblasts by our earlier study and our results demonstrated that 1000 ng/mL *P. gingivalis*-LPS increased the production of IL-10 in cementoblasts (10). Similarly, Khaled et al. reported that only *P. gingivalis* supernatant application to HGFs stimulated IL-10 mRNA expression (16). In this study, RvD1 concentrations significantly increased IL-10 expression in *P. gingivalis*-LPS-induced HGFs in a time- and dose-dependent manner.

Developmental endothelial locus-1 (Del-1) has been discovered as a new antagonist of the leukocyte LFA-1 integrin (CD11a/CD18), which restricts the movement of human neutrophils through endothelial cells (22). It modulates neutrophil recruitment to the periodontium and prevents inflammation and bone loss. Moreover, Del-1 prevents periodontal inflammation by inhibiting LFA-1 integrin-dependent neutrophil recruitment and IL17-mediated inflammation (7). In the present study, Del-1 expression was inhibited by only *P. gingivalis*-LPS, whereas IL-17 expression was upregulated by only *P. gingivalis*-LPS. While the level of Del-1 was increased by the combination of *P. gingivalis*-LPS and RvD1 as a time and dose-dependent manner, IL-17 expression was decreased in similar *in vitro* experiment conditions. Interleukin-17 is a pro-inflammatory cytokine that stimulates granulopoiesis and regulates neutrophil function, survival, and recruitment (23) andIL-17 suppresses the expression of Del-1 (12). When compared to young mice, the periodontium of old mice exhibits lower levels of Del-1 expression, which is correlated with high neutrophil recruitment and inflammatory bone loss that is IL17A-dependent in old mice in vivo models (12). Inonu et al. compared that salivary IL-17, LFA-1, and Del-1 were measured in individuals having gingivitis (G), chronic periodontitis (CP), and generalized aggressive periodontitis (GAP). It was determined that the CP and GAP groups had higher amounts of IL-17 and lower levels of Del-1 than the G and H groups (24). In addition, Maekawa et al. reported that IL-17 decreases Del-1 expression in human umbilical vein endothelial cells (HUVEC) by the GSK-3b- and C/EBPb-dependent pathway. Additionally, RvD1 reverses IL-17-stimulated Del-1 downregulation in vivo (14). Our results support an inverse relationship between Del-1 expression and IL-17 expression resulting from RvD1 in HGFs. Indeed, the periodontal tissue production of IL-17 is inhibited by Del-1, which is act as a gatekeeper of leukocyte recruitment and inflammation.

The lack of evaluation of Del-1 and cytokines (IL-1β, IL-6, IL-8, IL-10, and IL-17) protein levels was a limitation of this study. Quantification of protein levels would substantially improve our data and confirm the changes in mRNA expression in the HGF cells treated with a combination of *P. gingivalis*-LPS and RvD1.

CONCLUSION

Based on the findings of this study, *P. gingivalis*-LPS stimulates pro-inflammatory cytokines production in HGFs. Furthermore, combination of RvD1 and *P. gingivalis*-LPS decreased the pro-inflammatory cytokine expressions, while upregulating the anti-inflammatory cytokine expression and Del-1 levels in

a time- and dose-dependent manner. These findings seem to be consistent with the hypothesis that RvD1 modulates the gingival fibroblast from the *P.gingivalis*-LPS-stimulated inflammation by acting on pro-/anti-inflammatory cytokines of this damage. Also, RvD1 differentially affected IL-17 and Del-1 expressions in *P. gingivalis*-LPS-stimulated HGFs. RvD1 can thus provide a promising platform for IL-17-driven pathological inflammation including periodontal disease in a Del-1-dependent manner.

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Author contributions: SBB: Conception, design and finansman for research, literature review, writing and critical review for manuscript. SSH: Conception, design, supervision, critical review for manuscript.

Conflict of interests: No conflicting relationship exists for any author in this study.

Ethical approval: Study was approved by Nigde Omer Halisdemir University, Non-Invasive Clinical Research Ethics Committee (Date: 24.03.2022, Decision Number: 2022/36).

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