RvE1 protects from local inflammation and osteoclast-mediated bone destruction in periodontitis

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SPECIFIC AIMS

Periodontitis is a well-appreciated example of leukocyte-mediated bone loss and inflammation that has pathogenic features similar to those observed in other inflammatory diseases such as arthritis. Resolvins are a new family of bioactive products generated from omega-3 fatty acids initiated by aspirin treatment that counter proinflammatory signals.

The aims of this study were: *1)* to evaluate the impact of 15-epi-16-parafluorophenoxy-LXA$_4$, an aspirin triggered lipoxin analog (ATLa), and a newly described EPA-derived Resolvin E1 (RvE1), on superoxide generation by human neutrophils from localized aggressive periodontitis (LAP) patients; *2)* to characterize specific binding of RvE1 on human neutrophils for radioligand binding experiments; and *3)* to evaluate the actions of RvE1 in regulation of neutrophil mediated tissue destruction and resolution of inflammation in a rabbit model of periodontitis using macroscopic (clinical and radiological), histologic, and histomorphometric analyses.

PRINCIPAL FINDINGS

1. Treatment of neutrophils from healthy donors with 50 nM ATLa blocked superoxide generation by ~90%, whereas neutrophils from LAP were less responsive to ATLa (response<5%)

2. Superoxide generation by PMN from LAP was blocked >90% by RvE1, similar to the inhibition obtained with healthy donors without periodontal disease, indicating that RvE1 is a potent regulator of PMN function

3. RvE1 displays specific binding sites on human neutrophils with an apparent K$_d$ of 47 nM and a B$_{max}$ of 9 fmol

4. Topical treatment with RvE1 at ~4 μg per tooth at the site of the ligature prevented periodontal disease associated tissue and bone loss by >95%.

The treatment was applied three times a week over a 6-wk period and RvE1 provided significant protection when compared with placebo group.

5. Radiographic evaluation revealed 4-fold protection from bone loss by RvE1; and the data support the clinical and morphometric observations

6. Histologic analysis demonstrated a prominent leukocyte infiltrate and bone loss in specimens from placebo treated animals, whereas essentially no neutrophil infiltration or tissue damage was noted in RvE1 treated animals

7. Tartrate resistant acid phosphatase (TRAP) stained sections revealed numerous osteoclasts in lacunae of resorbing bone from *Porphyromonas gingivalis*-infected animals (placebo), whereas RvE1 treated specimens contained few detectable TRAP positive cells

CONCLUSIONS AND SIGNIFICANCE

Excessive recruitment and activation of neutrophils in the periodontium contributes to the progression of

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periodontal disease. Destruction of periodontal tissues appears akin to neutrophil-mediated tissue damage in the pathogenesis of arthritis and cardiovascular diseases. The results presented here are the first to demonstrate that specific RvE1 binding sites are present and distinct from LXA_4 receptors (ALX) on human neutrophils. In a human disease (LAP) characterized by excessive neutrophil activity (increased superoxide generation), RvE1 reduces neutrophil hyper-function. Neutrophils from LAP are otherwise refractory to modulation by LXA_4, further supporting the distinct sites of action for LXA_4 vs. RvE1 on human PMN (see full text and results). In a rabbit model of human periodontal disease, RvE1 prevents the initiation and progression of tissue destruction (**Figs. 1, 2**). Since neutrophil degranulation and superoxide anion release to the extracellular milieu contribute to tissue degradation in arthritis, periodontal ligaments and bone surrounding teeth, we further investigated the role of osteoclasts in bone resorption using histomorphometric analysis. Here, RvE1 treatment resulted in prevention of the emer-
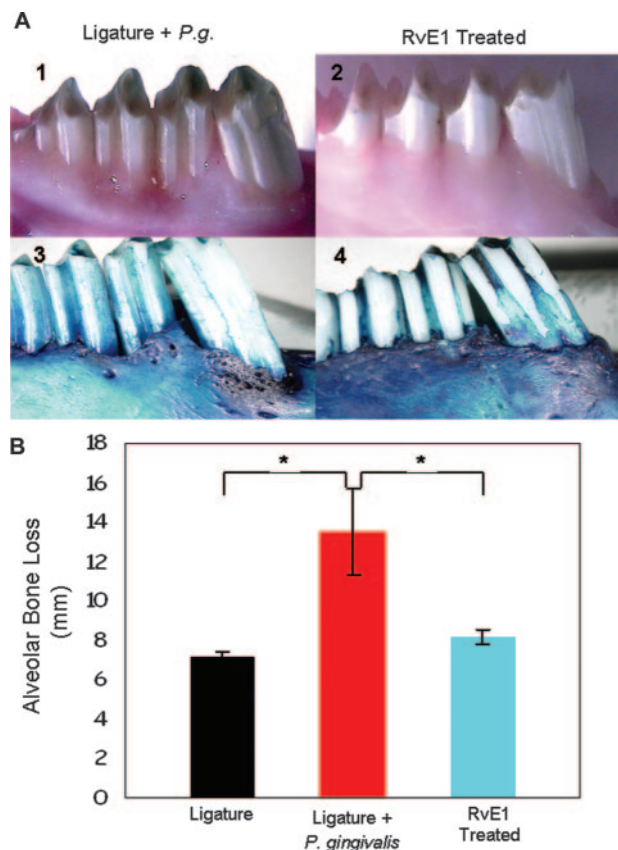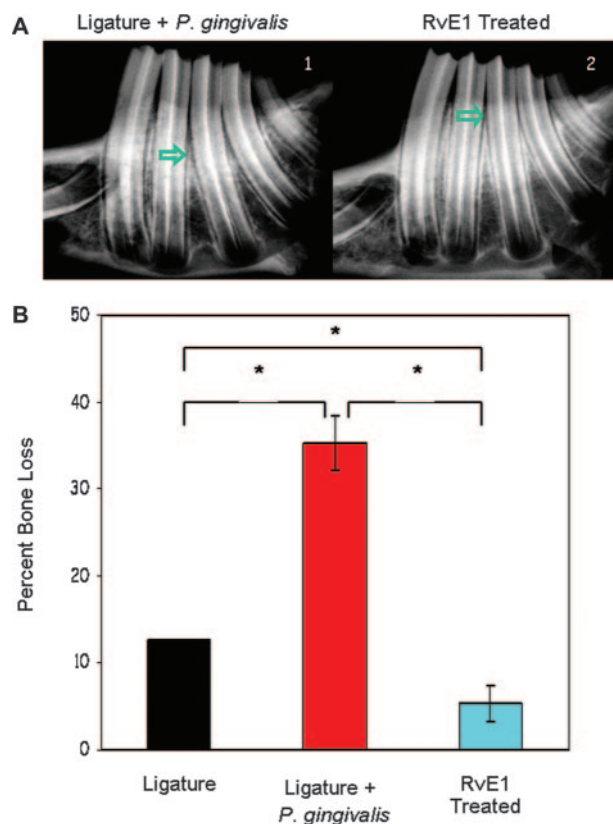

Figure 2. RvE1 treated periodontal lesions are protected from alveolar bone loss. Periodontitis was induced in New Zealand White Rabbits with 3-0 silk ligature and topical application of the human periodontal pathogen *P. gingivalis* for 6 wk. *A)* Representative radiographs of the placebo treated teeth reveal marked radiographic evidence of bone loss denoted by arrow (panel 1), whereas topical application of RvE1 inhibits bone loss (panel 2). Radiographic bone loss for all animals is quantified in *B. P.g.*-induced bone loss compared with ligature alone. RvE1 treatment prevented bone loss induced by *P.g.* as well as bone loss induced by ligature alone (*$P<0.05$).

gence of TRAP positive cells in bone indicating a direct inhibition of osteoclast mediated bone resorption. Together, these results indicate that resolvins, in this case RvE1, can act as a host response modulator in the control of the inflammatory diseases that also involve bone loss such as periodontitis and arthritis.

LAP is a unique model for neutrophil mediated local inflammation that usually resulted in severe tissue and bone destruction and a rapid tooth loss, especially in adolescents and young adults. Early loss of teeth leads to functional and aesthetic problems, which may result in psychological and social problems in this vulnerable population. Current treatment methods for periodontitis focus on control of the microflora with antibiotics and mechanical therapy with limited results and are ineffective for the treatment of LAP.

In the context of osteoclast-mediated diseases, such as periodontitis and arthritis, our results indicate that pro-resolving molecules (i.e., RvE1) prevent tissue destruction and bone resorption, and thus may provide the basis for new molecular therapeutic approaches to the treatment of inflammation-related bone disorders. [FJ]

Figure 1. RvE1 protects from soft tissue destruction and bone resorption in rabbit periodontitis. Periodontitis was induced in New Zealand White Rabbits with 3-0 silk ligature and topical application of the human periodontal pathogen *P. gingivalis* (*P.g.*) for 6 wk. *A)* Topical application of ~4 µg of RvE1 three times per wk prevented soft tissue inflammation and destruction (panel 1 vs. panel 2) and bone loss (panel 3 vs. panel 4). Direct measurements of alveolar bone loss for all animals were quantified in *B*. RvE1 treatment significantly inhibited bone loss (*$P<0.05$).

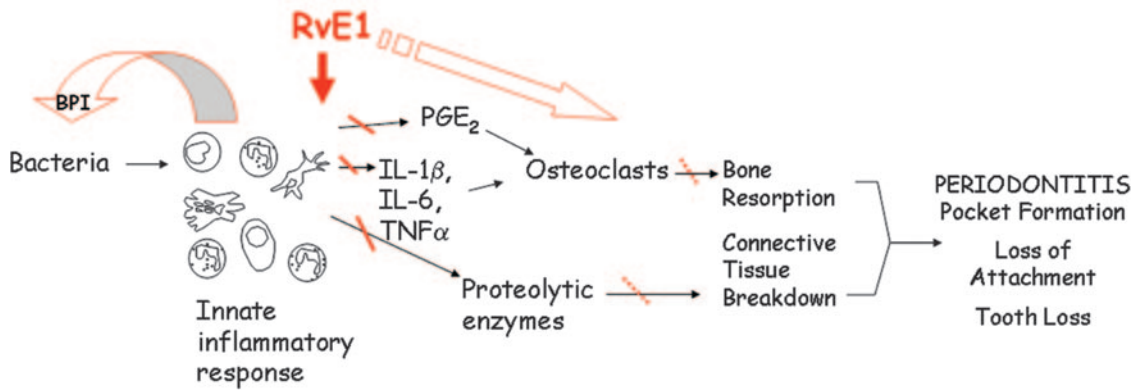# RvE1 Prevents Onset and Progression of Periodontitis

Figure 3. RvE1 prevents onset and progression of periodontitis. The oral biofilm comprised of pathogenic bacteria initiates the innate inflammatory response leading to innate and acquired immune tissue damage characteristic of periodontitis. It is the chronic nature of the inflammatory lesion that, like other bone destructive inflammatory lesions, contributes to the pathogenesis of tissue injury. As represented here, bacteria initiate the innate immune response, which leads to secretion of prostaglandins and cytokines. These in turn modify the phenotype of inflammatory and stromal cells to generate osteoclasts and release proteolytic enzymes that leads to soft tissue degradation and bone resorption. Eventually, this becomes severe enough to present as signs of periodontitis, namely, pocket formation and loss of connective tissue and bone attachment. RvE1 promotes resolution of inflammation through direct limitation of PGE_2 and cytokine secretion (solid red arrow). Indirect effects include blocking of osteoclast formation and secretion of antibacterial peptides by resident cells (open red arrows).