Discuss the Mechanism by which Specialized Pro-resolving Lipid Mediators Lipoxins, Resolvins and Protectins can be used as treatments for Periodontal disease.

Introduction

Periodontal disease is a widespread chronic inflammatory disease affecting approximately 64.7 million adults in the USA alone (American Academy of Periodontology, 2019). Its painless symptoms mean patients often fail to seek early treatment, allowing the disease to progress into a more advanced stage characterised by irreversible hard and soft tissue damage. Despite non-surgical and surgical therapies existing, the multifactorial challenges surrounding the disease mean these methods are unsuccessful in 20-30% of chronic periodontitis cases (Shaddox and Walker 2010). Recently, the potent effects of pro-resolving mechanisms have been investigated as a means of treating chronic inflammatory diseases (Buckley et al. 2014). These novel approaches focus on utilising the active resolution coordinated by specialised pro-resolving lipid mediators, to regenerate the tissues lost to periodontitis.

The Inflammatory Response

Inflammation can be defined as the host's response to infectious tissue damage, with the aim being to restore tissue homeostasis. The inflammatory response is outlined in three distinct stages: the onset of inflammation, the onset of resolution and the return of tissue homeostasis, as depicted in Figure.1. Although there are several defining features of acute inflammation, it can be characterised in particular, by polymorphonuclear neutrophil (PMN) infiltration (Sugimoto et al. 2016). PMNs function in cell recruitment and phagocytosis, largely prevailing during the initial hours of inflammation (Selders et al. 2017). The release of specialised pro-resolving lipid mediators (SPMs) soon after, crucially mediate the removal of these PMNs, preventing the adverse effects of excessive inflammation. If SPM-led depletion of PMNs fails to occur however, it results in inadequate resolution and the development of a chronic inflammatory lesion as seen in periodontal disease (Sugimoto et al. 2016).

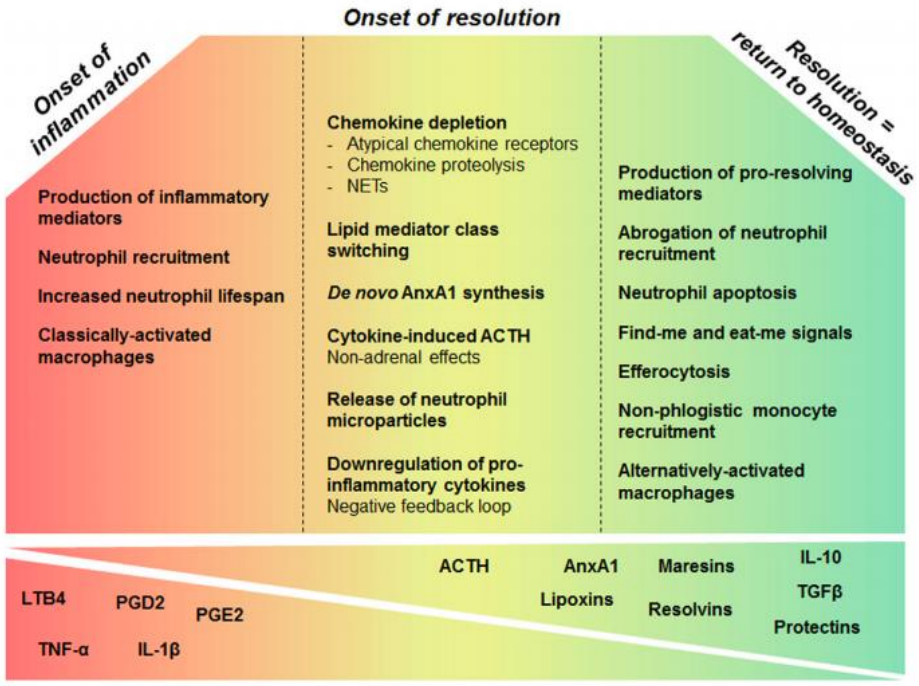

Figure 1. The cellular and molecular mechanisms by which the inflammatory and resolution processes occur. (Sugimoto et al. 2016)

Periodontal disease

Periodontal disease is a local inflammatory disease of periodontium, which occurs when a neutrophil-mediated injury progresses into a chronic immune lesion (Hasturk et al. 2007). The disease begins with gingivitis, an acute inflammatory disease of the gingiva which develops due to prolonged exposure to dental plaque. With gingivitis being reversible, removal of the plaque biofilm restores gingival health. That being said, failure to eradicate the biofilm causes an accumulation of dental plaque and an escalation in gingival inflammation (Van Dyke 2017). This alters the environment of the gingival sulcus, promoting the growth of gram-negative proteolytic bacteria such as Porphyromonas gingivalis (P.gingivalis) (Van Dyke 2017; Hasturk et al. 2007). Dysbiosis of the environment causes further influx of PMNs (Van Dyke 2017), and the release of additional inflammatory mediators which exacerbate damage to the periodontal tissues (Fig.1) (Pouliot et al. 2000). This excessive inflammation eventually leads to irreversible loss of attachment and destruction of alveolar bone (Highfield 2009), defining the shift from acute gingivitis into chronic periodontitis. Therefore, despite bacteria being a crucial aetiological factor, it is the exaggerated host response which determines whether periodontal disease advances.

Biosynthesis and General Roles of Specialised Pro-resolving Lipid Mediators

The resolution of inflammation is an active process coordinated by lipoxins, resolvins and protectins, collectively named specialised pro-resolving lipid mediators (SPMs) (Sugimoto et al. 2016). These SPMs act as antagonists for specific G-protein coupled receptors (BLT1, ChemR23, ALX/FRP2, GPR18, GPR32) to inhibit the inflammatory response and allow the return of tissue homeostasis (Balta et al. 2017).

1. Lipoxins:

Lipoxins (LX) are endogenously expressed bioactive lipids with a vital role in downregulating neutrophil-mediated inflammation (Ali et al. 2019). Serhan and Levy (2018a) found that the onset of resolution is determined by a lipid-mediator-class-switch, where prostaglandin E2 (PGE2) and prostaglandin D2 (PGD2) released during the onset of inflammation (Fig.1) induce LX biosynthesis. More specifically, when human PMNs were exposed to PGE2, it triggered 15-lipoxygense (15-LOX) to produce LX in place of leukotriene B4 (LTB4). This crucial switch in phenotype prevented further PMN recruitment and induced macrophage efferocytosis, marking the early stages of resolution.

Importantly, lipoxin A4 (LXA4) has been discovered as a significant mediator of periodontal disease. Within the oral cavity, LXA4 and its isoform lipoxin B4 (LXB4) (Sommakia and Baker 2017) are biosynthesised by oxygenation of arachidonic acid (AA) via 15-LOX and 5-LOX, followed by enzymatic hydrolysis (Fig. 2) (Freire and Van Dyke 2013). The significance of LXA4 became evident after early investigations by Pouliot et al. (2000) revealed that lipoxin analogues prevented P.gingivalis-mediated leukocyte influx in vivo. Since then, LXA4 has been shown to induce bone regeneration in a swine model of periodontal disease and promote the proliferation of human periodontal stem cells (Sommakia and Baker 2017).

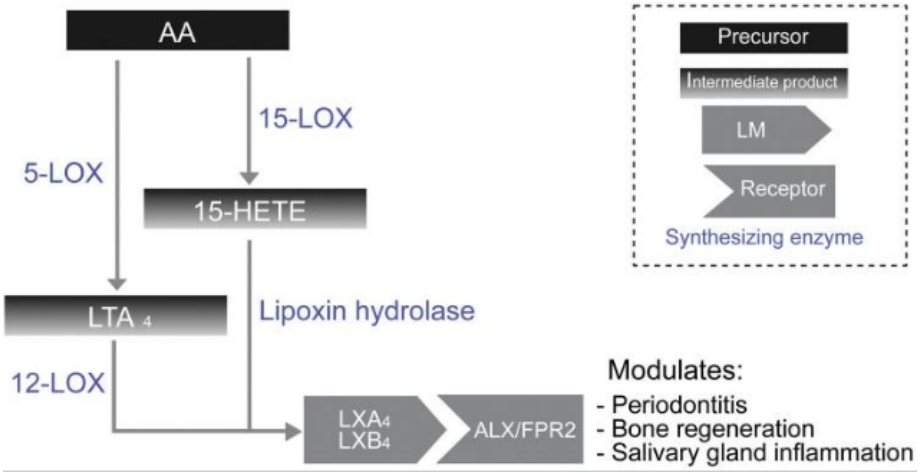

Figure 2. Biosynthesis of LXA4 and LXB4 from AA (Sommakia and Baker 2017).

2. Resolvins

Resolvins are divided into the D-series and E-series, which are derived from the dietary omega-3 polyunsaturated fatty acids (ω-3 PUFA) docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) respectively. (Sommakia and Baker 2017; Buckley et al. 2014)

D-series:

The D-series is formed via the hydroxylation of DHA, which occurs in two separate pathways. The first pathway involves 15-LOX mediated hydroxylation, and the second utilises aspirin-triggered COX-2 hydroxylation (Fig. 3A). Notably, RvD1 has several roles in resolving periodontal disease; examples include promoting wound closure and encouraging the proliferation of periodontal ligament fibroblasts. (Sommakia and Baker 2017).

E-series:

The E-series is biosynthesised from EPA, as depicted in Figure 3B. Resolvin E1 (RvE1) is formed via oxygenation, mediated by either aspirin-triggered COX-2 or cytochrome P450 monooxygenase. RvE2 on the other hand, is formed via the hydroxylation of EPA-produced 18R-H(p)EPE. In particular, RvE1 is important within periodontal disease, due to its roles in reducing oral inflammation and alveolar bone loss. More recently, RvE1 has also been found to reduce the reactive oxygen species produced in P.gingivalis-elilcted periodontitis, and preserve bone loss within ligature-mediated periodontitis (Sommakia and Baker 2017).

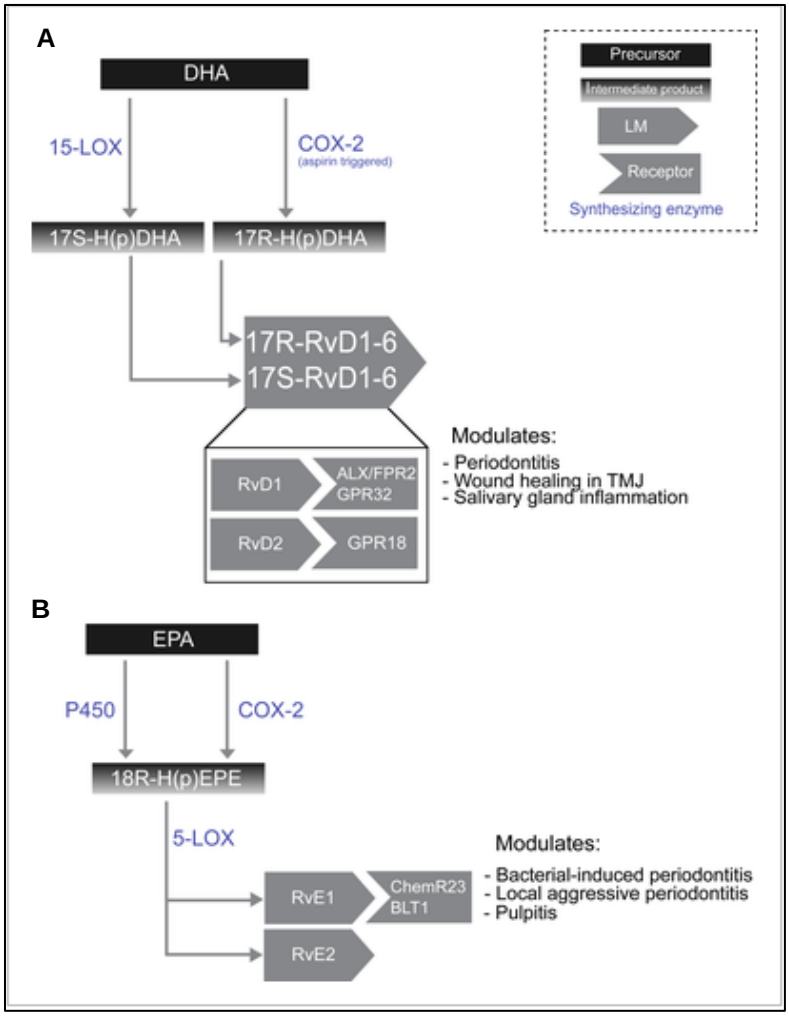

Figure 3. Biosynthesis of resolvins. A) The D series. B) The E-series. (Sommakia and Baker 2017).

3. Protectins

Similar to the D-series of resolvins, the biosynthesis of protectins occurs via a 17-lipoxygenation reaction mediated mostly by 15-LOX (Fig. 4A) (Serhan et al. 2018b). More specifically, protectin D1 (PD1) is synthesised from a 16(17)-epoxide intermediate produced by macrophages, PMNs and eosinophils. PD1 produced in neural systems, neuroprotectin D1 (NPD1), has protective roles in the retina and brain. Protectin DX, an isomer of PD1, is produced by sequential lipoxygenation (Fig.4B) (Serhan et al. 2019).

Recently, it has been discovered that peptide-conjugated SPMs have key tissue regeneration abilities. Within the protectin family, these are named protectin conjugates in tissue regeneration (PCTR). In particular, PCTR1, generated by human leukocytes, is known to enhance resolution and show an upregulated production by human M2 macrophages (Ramon, S. et al. 2016).

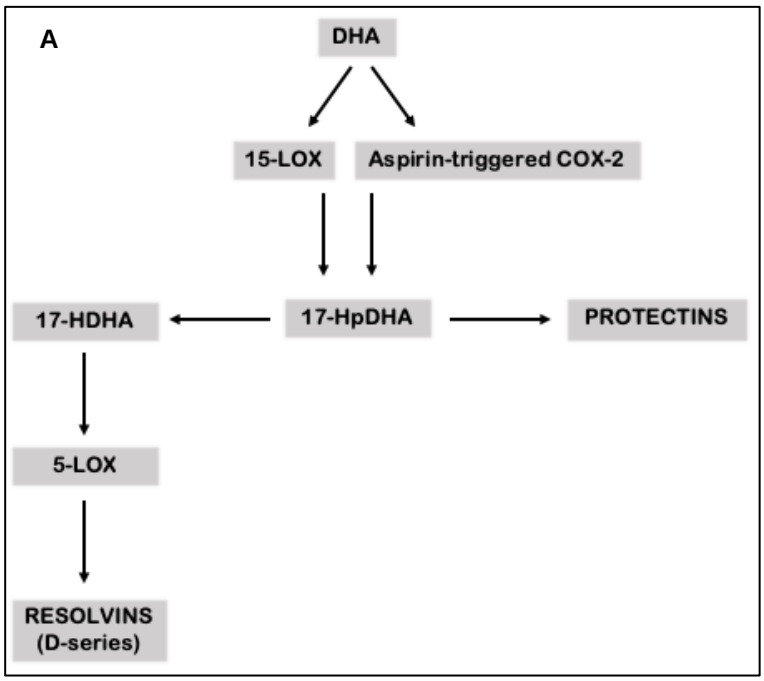

Figure 4A. Similarities in the biosynthesis of protectins and the D-series of resolvins.

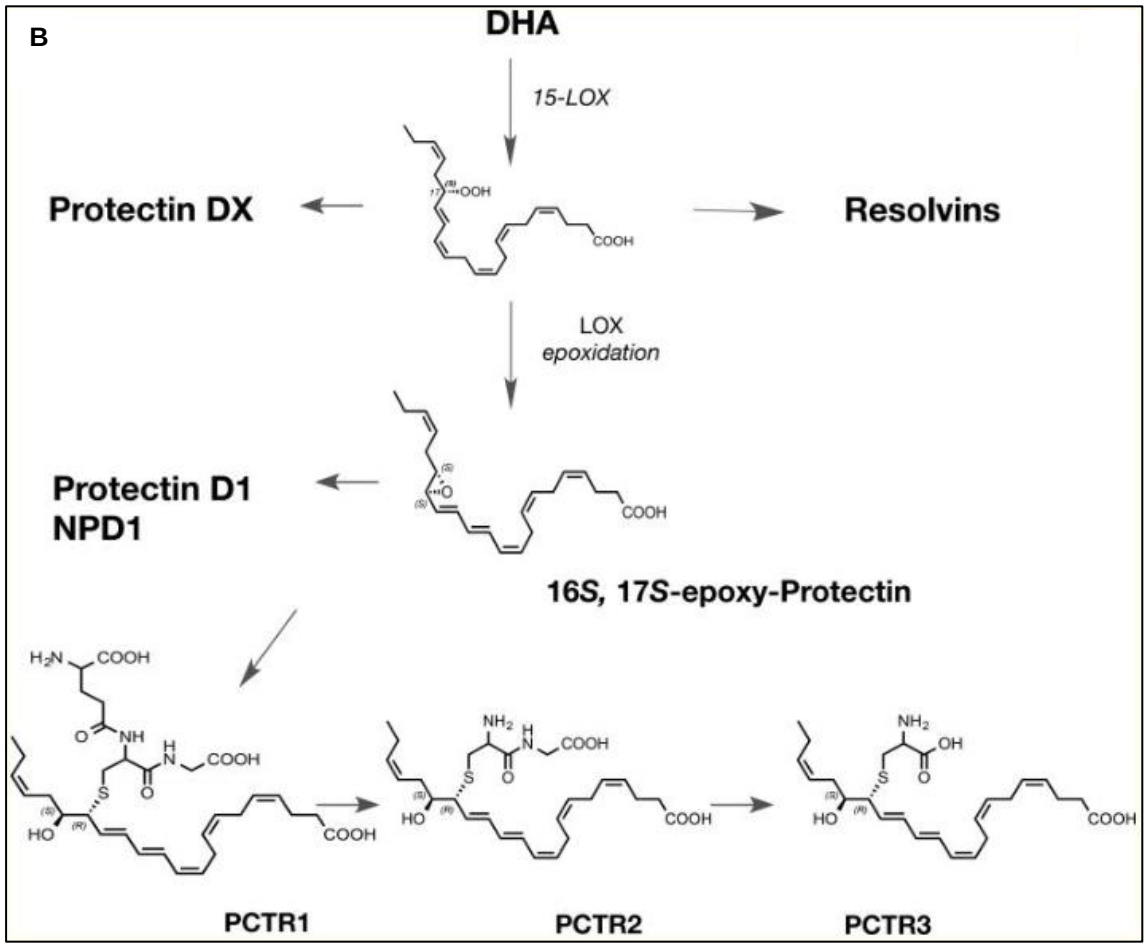

Figure 4B. Biosynthesis of PD1, NPD1 and PCTR1-3. (Serhan et al. 2018b)

SPMs in the Treatment of Periodontal Disease

Lipoxin A4

Introduction:

Investigations by Van Dyke (2015), revealed that the use of a pro-resolving nanomedicine is able to regenerate tissues lost to periodontitis. Microparticles from human neutrophils were used to create a nano-proresolving medicine (NPRM) incorporating a lipoxin analogue (benzo-lipoxin A4, bLXA4). The extent of regeneration was analysed following the use of this NPRM-bLXA4 to treat chronic periodontitis in Hanford miniature pig models.

Materials and Methods:

Interproximal periodontal defects were generated using a bur in the second and fourth premolars of two adult female swine. The defects were 6mm deep from the cemento-enamel junction (CEJ) and extended buccolingually to the interproximal surface. Ligatures were placed into these defects, and the pigs were fed a soft diet to induce plaque formation. At baseline, blood samples were collected, and defects and root surfaces were debrided. A notch was placed at the base of the defects; changes in bone volume were dictated by the vertical distance from the CEJ to the root notch. Defects were treated with bLXA4 alone, NPRM alone or NPRM-bLXA4, with surgery alone as the negative control. (Van Dyke 2015)

Results:

Three-dimensional (3D) bone analysis using micro-computed tomography (µCT) showed that NPRM-bLXA4 led treatment resulted in the greatest bone regeneration (Fig. 5A). This was confirmed by linear histomorphometric measurements which showed the distance from the root notch to the bone crest was significantly lower through utilisation of NPRM-bLXA4 (Fig. 5B). Crucially, this NPRM-bLXA4 led regeneration was not exclusive to hard tissues, as shown by the redeveloped supracrestal connective tissue fibres and cementum in Figure 5C. (Van Dyke 2015)

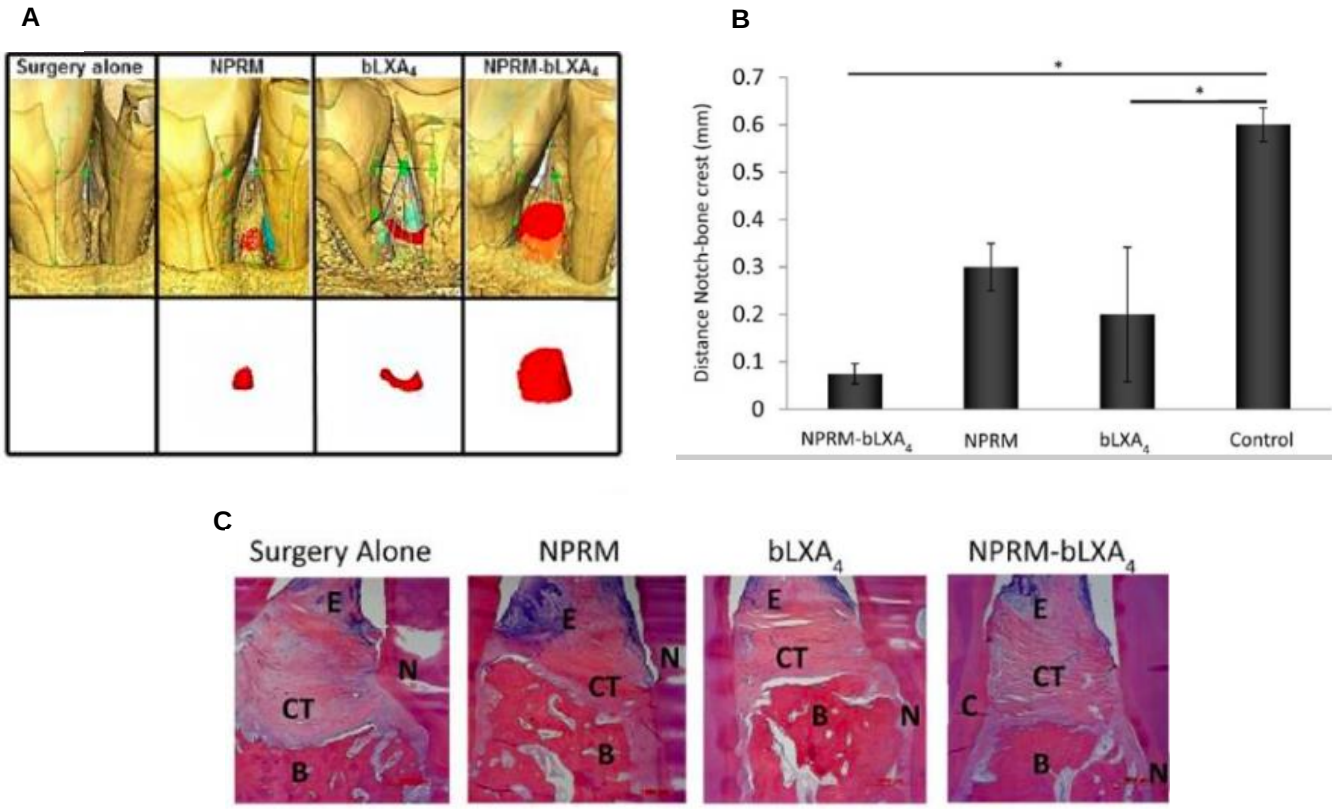

Figure 5. NPRM-bLXA4 mediated hard and soft tissue regeneration. A) 3D µCT images of the 4 treatments; red region = new bone. B) Regeneration of the periodontium quantified by the distance of the bone crest from the root notch. C) Regeneration of the periodontal organ (B = bone, CT = connective tissue, E = epithelium, N = notch, C = cementum). (Van Dyke 2015)

Furthermore, histological analysis at 12 weeks revealed the presence of a dense inflammatory infiltrate in the interproximal and subepithelial areas of the periodontal lesion treated by surgery-alone. In contrast, NPRM-bLXA4 or bLXA4 mediated treatment showed minimal infiltration of inflammatory cells, yet with a greater density of fibroblasts. Additionally, analysis of systemic lipid mediator levels post-treatment revealed that bLXA4 greatly up-regulated the expression of the arachidonic acid and ω-3 PUFA derived pro-resolving mediators, whilst downregulating the production of pro-inflammatory prostaglandins. Albeit PGE2 levels reducing from week 0-8, a sudden increase was shown from week 8-12. Significantly, this correlated with the dramatic surge in PGF2α levels (Fig. 6). (Van Dyke 2015)

	Week 0		2		4		8		12	
Prostaglandin levels (pg/mL of serum)	Pig 1	Pig 2	Pig 1	Pig 2	Pig 1	Pig 2	Pig 1	Pig 2	Pig 1	Pig 2
PGE2	18.9	7.6	2.8	7.3	5.8	7.2	7.8	3.4	19.2	15.8
PGF2α	1646.7	494.0	45.1	327.6	41.1	334.2	93.5	20.4	1180.0	1086.1

Figure 6. PGE2 and PGF2α levels from week 0-12. Red = the fluctuations from week 0-8 and week 8-12. Numerical values taken from Van Dyke (2015).

Discussion:

NPRM-bLXA4 induces several mechanisms allowing the regeneration of hard and soft tissue. Firstly, the reduction in PGE2 levels prevents further tissue damage, by limiting PGE2's adverse effects of stimulating of alveolar bone resorption and disrupting connective tissue metabolism (Pouliot et al. 2000). Secondly, the indirectly-induced increase in PGF2α levels contributes to the reversal of alveolar bone loss, due to PGF2α's potency in bone remodelling (Kuroyanagi et al. 2016). More specifically, PGF2α regulates the synthesis of osteoprotegerin (OPG), a decoy receptor for RANKL. OPG prevents the binding of RANKL to RANK, inhibiting osteoclast differentiation and subsequent osteoclastic alveolar bone resorption (Kuroyanagi et al. 2014).

Thirdly, the NPRM-bLXA4 mediated fibroblast surge permits the regeneration of supracrestal connective tissue fibres, due fibroblasts' powerful role in wound healing. Within the periodontium, they function to induce the synthesis of a collagenous network to connect the alveolar bone and gingiva to the cementum (Smith et al. 2019). Lastly, with NPRM-bLXA4 or bLXA4 led treatment reducing the inflammatory infiltrate, it demonstrates the ability of bLXA4 to switch the pro-inflammatory response into the pro-resolving response – a fundamental concept in treating periodontal disease.

Crucially, the enhanced potency of NPRM-bLXA4, compared to bLXA4 alone, can be explained by two concepts. Firstly, NPRM's exhibition of anti-inflammatory effects via the bLXA4 receptor ALX/FRP2; and secondly, NPRM's ability to enhance pro-resolving pathways without activating dendritic cells and causing nanotoxicity, like other nanoparticle systems. (Van Dyke 2015)

RvE1

Introduction:

Studies on six-week old Wistar rats by Lee et al. (2016) showed the topical application of RvE1 can regenerate alveolar bone.

Materials and Methods:

18 rats were separated into four groups: no ligature (n=6; health status), ligature alone (n=6), ligature with vehicle (n=3; diseased status) and ligature with RvE1 (n=3). The ligatures were positioned subgingivally on the left and right upper second molars and RvE1 was topically applied on alternate days, in a 0.25 mg/ml dose. In the context of this study, the vehicle is the solution permitting the application of RvE1; it functions as a control, to identify if the treatment elicits adverse effects on the outcome. (Lee et al. 2016)

Results:

The results showed that RvE1 treatment reduced alveolar bone loss. More specifically, the area of exposed root surfaces and distance between the CEJ and alveolar bone decreased by 2.24mm^2 and 3.17mm, respectively (Fig. 7). These findings were confirmed as RvE1 treatment showed a 60% reduction in osteoclast density, in comparison to the vehicle group. RvE1 led treatment also significantly lowered the mean inflammatory cell count and reduced the gingival expression of the CXCL1 and MMP-13 genes, when compared to the vehicle group. (Lee et al. 2016)

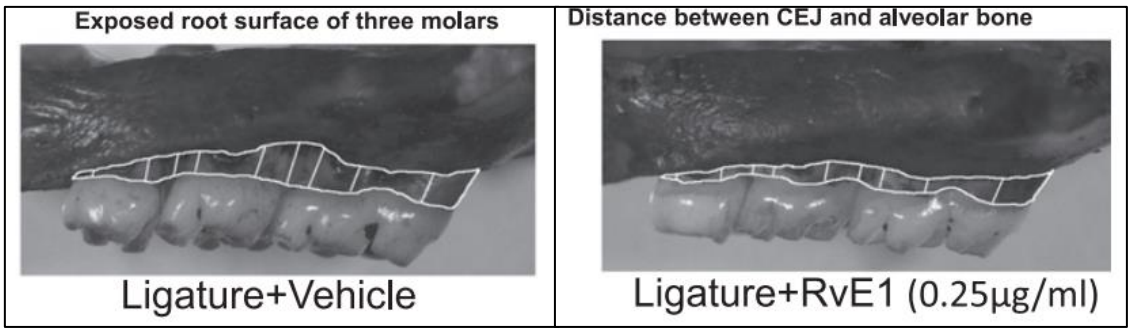

Figure 7. 30-40% alveolar bone regenerated with the topical application of RvE1. (Lee et al. 2016)

Discussion:

There are several mechanisms by which RvE1 functions to restore alveolar bone.
Firstly, in binding to its ChemR23 receptor on osteoblasts (OB), RvE1 is able to stimulate the OB-production of OPG, the significance of which has been previously discussed. Secondly, in binding to its BLT1 receptor on osteoclasts, RvE1 is able to inhibit RANKL-mediated osteoclast growth, preventing further osteoclastic alveolar bone resorption. RvE1 also lessens the osteoblast-lineage-cell mediated production of MMP-13. As a result, MMP-13's role of activating osteoclasts via the removal of non-mineralized collagen fibrils from alveolar bone, is made less potent. (Hernandez et al. 2006).

Secondly, RvE1 functions to remove PMNs from periodontal sites, crucially helping to resolve the chronic inflammation. The RvE1-mediated reduction in inflammatory infiltrate is the result of RvE1's ability to downregulate the expression of L-selectin and CD18 in PMNs. This impedes the ability of PMNs to bind to their counter-receptors, preventing their adhesion to the endothelium. This subsequently reduces leukocyte migration along the vascular wall and prevents their invasion into and destruction of periodontal lesions (Balta et al. 2017). Neutrophil migration is also limited by the RvE1-mediated reduction of CXCL1 expression, a gene encoding a CXC chemokine which would usually act as a chemoattract for neutrophils (National Center for Biotechnology Information, 2020).

PCTR1

Introduction:

PCTR1's pro-resolving roles in peritonitis, an inflammatory disease of the peritoneum (Spalding and Williamson 2008), may be transferable in the treatment of periodontal disease.

Materials and Methods:

Peritonitis was induced in 6-8-week-old male FVB mice by injection of Escherichia coli (E. coli). PCTR1 was then organically synthesised and injected into the mice 12 hours later, in a dose of

30ng/mouse. Exudates were collected via peritoneal lavage 48 hours after inducing infection. (Ramon et al. 2016).

Results:

Examination of exudates showed the levels of PCTR1 increased during the resolution phase, with the peak level equating 5pmol/L. The up-regulation of PCTR1 correlated with flow cytometry analysis, revealing a two-fold increase in peritoneal macrophages 48 hours after inflammation. Similarly, within 24 hours, PCTR1 was shown to enhance macrophage-led phagocytosis of E. coli by 60%, and lead to a 57% faster clearance-rate of PMNs. Significantly PCTR1 also reduced the exudate-levels of proinflammatory eicosanoids, namely PGE2, PGD2 and thromboxane B2 by 48%, 64% and 40% respectively.

Further investigations incubating 0.001-10nmol/L PCTR1 with human macrophages treated with a serum-treated zymosan (a repeating chain of β-1,3 linked glucose in yeast cell walls, inducing an inflammatory response in macrophages (Collins English Dictionary, 2014)), reduced levels of tumour necrosis factor-α (TNF-α), interleukin-3 (IL-3), IL-6 and IL-8, and amplified levels of matrix metalloprotease-3 and transforming growth factor-β (TGF-β). (Ramon et al. 2016).

Discussion:

PCTR1's pro-resolving mechanisms may be useful in treating periodontal disease. Firstly, PCTR1's ability to enhance nonphlogistic macrophage recruitment and efferocytosis of apoptotic PMNs, would aid in initiating resolution (Ramon et al. 2016). Secondly, PCTR1's role of increasing TGF-β, an inhibitor of TNF-α, would promote periodontal repair. Within inflamed periodontal tissues, increased levels of the pro-inflammatory cytokines IL-6 and TNF-α are expressed. TNF-α is released from macrophages after the detection of oral bacteria, causing the subsequent release of collagenases from gingival fibroblasts. These function in collagen destruction and alveolar bone resorption (Noh et al. 2013). Since PCTR1 reduces TNF-α levels, it prevents these adverse effects, allowing for bone regeneration. PCTR1's ability to reduce IL-6 also discourages alveolar bone resorption, due to inhibiting IL-6 mediated osteoclast formation. Likewise, PCTR1-led

reduction in PGE2 levels would promote soft tissue formation, by preventing PGE2's roles in the inhibition of fibroblast-led collagen synthesis. (Tawfig 2016).

Conclusion

In conclusion, the evidence discussed gives an insight into the mechanisms by which SPMs can be used in the treatment of periodontal disease. All of the chosen studies were published within the last 5 years, making the results relevant to current concerns on the need for novel treatment options.

In using miniature swine models, the investigations on NPRM-bLXA4 provided promising evidence that the treatment could succeed in humans. To be specific, swine have a greater anatomical and physiological similarity to humans than any other laboratory animal. Since the omnivorous diet, masticatory movements and periodontal lesion imitated human periodontitis, the experiment gave an accurate insight into the likely human response (Van Dyke 2015).
Furthermore, the collected evidence was effectively represented by using 3D μCT images coupled with histological analysis. This gave two different, but equally comprehensive, means of visualising the tissue regeneration. Bias was eliminated from these results, as all measurements were conducted under blind conditions by trained examiners. Despite these factors, additional experimental evidence must be collected before implementation into humans, as two swine insufficiently represent the success of this treatment in all types of periodontal disease.

A suitable experimental method was also used to investigate RvE1. Firstly, the validity of the method was evident through the application of a 4-μl vehicle, which ensured the results were solely as a consequence of RvE1 treatment. Secondly, the rats were sedated under ketamine for all procedures, and despite the sacrifices made to dissect the maxillae, the method was ethically approved by the Institutional Animal Care and Use Committee of The Forsyth Institute.
In addition to this, the similarities in the pattern of differential expression in rat periodontal health and disease, suggests that the success of RvE1 could be transferable to humans. That being said, although RNA sequencing revealed RvE1's role in enhancing bone-regeneration pathways, Kyoto

Encyclopedia of Genes and Genomes (KEGG) analysis proved these pathways to be somewhat insignificant for the regeneration of alveolar bone. As a result, there is need for further research to be conducted regarding the specific role RvE1 plays in tissue regeneration, before it can be addressed as a viable treatment method for human periodontal disease. (Lee et al. 2016)

The experiment investigating the role of PCTR1 in treating peritonitis, is less able to answer the question at hand. Firstly, the infection was induced via E. coli, a bacterium which is not known to correlate with periodontal disease. As a result, we cannot be assured that PCTR1 would permit the healing of tissues infected with periodontitis-specific bacteria. Similarly, the results obtained were specific to peritoneal exudates, and despite the obvious similarities between inflammatory mediators in peritonitis and periodontitis, it is not certain these findings would coincide with treating periodontal disease. Crucially however, the study gave an invaluable insight into the possibility for PCTR1 to serve as an endogenous treatment method in periodontal disease, considering its potent role in resolving inflammation.

In summary, despite the potential for SPMs to be used in the treatment of periodontal disease, greater research must be conducted to allow its common use in humans. Other effective treatment approaches such anticytokine therapy, which instead targets pro-inflammatory cytokines in the hope of eliminating chronic inflammation (Waykole et al. 2009), create competition for SPM-led treatment. Novel research must therefore regard SPMs the superior treatment option, in order for their success.

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