Title

Local delivery of a CXCR3 antagonist decreases the progression of bone resorption induced by LPS injection in a murine model

Journal

Clinical Oral Investigations, 26(8)

ISSN

1432-6981

Authors

Lari, Soma
Hiyari, Sarah
de Araújo Silva, Davi Neto
et al.

Publication Date

2022-08-01

DOI

10.1007/s00784-022-04484-z

Peer reviewed

HHS Public Access

Author manuscript

Clin Oral Investig. Author manuscript; available in PMC 2023 February 01.

Published in final edited form as:

Clin Oral Investig. 2022 August ; 26(8): 5163–5169. doi:10.1007/s00784-022-04484-z.

Local Delivery of a CXCR3 Antagonist Decreases the Progression of bone resorption induced by LPS injection in a murine model

Soma Lari[1], **Sarah Hiyari**[1], **Davi Neto de Araújo Silva**[1,5], **Beatriz de Brito Bezerra**[1], **Makiko Ishii**[1,2], **Sepehr Monajemzadeh**[1], **Zhong-Kai Cui**[6], **Sotirios Tetradis**[3], **Min Lee**[4], **Flavia Q. Pirih**[1]

[1]School of Dentistry, Section of Periodontics, University of California, Los Angeles.

[2]Division of Periodontology, Department of Oral Biology and Tissue Engineering, Meikai University School of Dentistry.

[3]School of Dentistry, Section of Oral Radiology, University of California, Los Angeles.

[4]School of Dentistry, Section of Biomaterials Science, University of California, Los Angeles.

[5]Dentistry Department, Rio Grande do Norte Federal University, Natal, RN, Brazil.

[6]Department of Cell Biology, School of Basic Medical Sciences, Southern Medical University, Guangzhou, Guangdong, China

Abstract

Objectives—This experimental study was carried out to investigate the effects of locally delivered nanoparticles (AMG-487 NP) containing a CXCR3 antagonist in inhibiting the progression of LPS-induced inflammation, osteoclastic activity, and bone resorption on a murine model.

Materials and methods—Thirty, seven-week-old C57BL/6J male mice were used. Inflammatory bone loss was induced by *P.gingivalis*-lipopolysaccharide (*P.g.*-LPS) injections between the first and second maxillary molars, bilaterally, twice a week for six weeks (n=20). AMG-487 NP were incorporated into a liposome carrier and locally delivered on sites where *P.g.*-LPS was injected. Control mice (n=10) were injected with vehicle only. Experimental groups included: 1) Control, 2) LPS and 3) LPS+NP. At the end of 1- and 6-weeks mice were euthanized, maxillae harvested, fixed and stored for further analysis.

Results—Volumetric bone loss analysis revealed, at one week, an increase in bone loss in the LPS group (47.9%) compared to control (27.4%) and LPS+NP (27.8%) groups. H&E staining demonstrated reduced inflammatory infiltrate in the LPS+NP group compared to LPS group. At six weeks, volumetric bone loss increased in all groups, however, treatment with the CXCR3 antagonist (LPS+NP) significantly reduced bone loss compared to the LPS group. CXCR3

Corresponding author: Dr. Flavia Queiroz Pirih, University of California, Section of Periodontics, Los Angeles, CA, United States of America. fpirih@dentistry.ucla.edu.

CONFLICT OF INTEREST: The authors declare that they have no conflict of interest.

antagonist treatment significantly reduced osteoclast numbers when compared to LPS group at 1 and 6 weeks.

Conclusions—This study showed that local delivery of a CXCR-antagonist, via nanoparticles, in a bone resorption model, induced by LPS injection, was effective in reducing inflammation, osteoclast numbers, and bone loss.

Clinical relevance—CXCR3 blockade can be regarded as a novel target for therapeutic intervention of bone loss. It can be a safe and convenient method for periodontitis treatment or prevention applicable in clinical practice.

Keywords

AMG-487 nanoparticles; CXCR3 antagonist; Bone resorption; Inflammatory infiltrate; Osteoclast

INTRODUCTION

Periodontitis (PD) is a “chronic inflammatory disease associated with dysbiotic plaque biofilms and characterized by progressive destruction of the tooth-supporting apparatus [1,2].” PD affects 42.2% of the population over the age of 30 and 59.8% over the age of 65 [3]. According to the World Health Organization, PD is the major cause of tooth loss in adults [4], with *Porphyromonas gingivalis* (*P. gingivalis*) being one of the main pathogenic bacteria responsible for triggering a host immune response. [5–8]. PD pathogenesis consists of complex interactions between the biofilm bacteria, host’s genetic factors, and acquired environmental stressors such as smoking, poor oral hygiene, and systemic diseases like diabetes mellitus [9–12].

Despite being a multifactorial disease, and strongly associated with periodontopathogenic bacteria, it is approximately 50% heritable[13]. Our laboratory performed Genome-Wide Association Studies (GWAS) in LPS-induced periodontitis (*Porphyromonas gingivalis* lipopolysaccharide) with the Hybrid Mouse Diversity Panel (HMDP) and identified genes in the CXC chemokine family (CXCL9 and CXCL10) as highly associated with the periodontal bone loss phenotype. Moreover, CXCL9 and CXCL10 mRNA and protein levels were upregulated following *P. gingivalis* LPS-induced-periodontal inflammation [14]. Genetic deletion of the CXCR3 receptor (the main CXCL9 and 10 receptors [15,16]) in mice, demonstrated a ~50% reduction in bone loss compared to wildtype-LPS treated mice [14]. CXCL family members CXCL9 (monokine-induced gamma interferon, MIG) and CXCL10 (interferon gamma-induced protein 10, IP-10) are pro-inflammatory cytokines secreted by many cell types, including monocytes, endothelial cells, and fibroblasts [17]. CXCL9 and 10 are important for cell migration, differentiation, and adhesion. However, their main role is believed to be the propagation of inflammation [18–22].

The most common mechanism of action for CXCL9 and 10 is through the CXCR3 receptor [18]. In the periodontium, increased CXCL9 and 10 expression levels correlate with PD. Moreover, CXCL9 and 10 expression levels are not only higher in periodontally diseased tissues, but also in the serum of these patients [23,24–27]. A recent publication identified CXCL10 among the top 200 most expressed genes in PD; furthermore, the same study

identified CXCL10 as having one of the highest degrees of interaction in a protein-protein network [28]. We have also demonstrated that blocking the CXCR3 receptor by systemic administration of a CXCR3 antagonist, AMG487, significantly reduced LPS-induced bone loss [14,29] pointing to CXCL9 and 10 as strong candidates for modulating the host response to PD. An innovative possibility is the delivery of CXCR3 in nanoparticles that present, among other advantages, high stability, better access to deep periodontal pockets, carrying capacity and differential drug incorporation [42,43]. The nanoparticulate systems used for local drug delivery include polymeric nanoparticles, nanofibers, and liposomes, all showing different properties when targeting specific bacteria and host cells [41]. In our current study we propose to evaluate the effect of a locally delivered CXCR3 antagonist on inflammation, osteoclastic activity, and bone loss in a murine model of LPS-induced periodontitis [30,31,32,33].

MATERIALS AND METHODS

Mice

Thirty, seven-week old, C57BL/6J male mice were used according to the Chancellor's Animal Research Committee of the University of California, Los Angeles, and the Animal Research: Reporting In Vivo Experiments (ARRIVE) protocols for the submission of animal studies were followed [34]. Mice were purchased from Jackson Laboratory (Bar Harbor, ME, USA) and maintained at UCLA for the duration of the study in a temperature- and light-controlled environment, and fed a standard chow (Bio Serve, Frenchtown, NJ, USA).

Model of bone resorption induced by LPS injection.

Inflammatory bone loss was performed as previously described [35]. In brief, mice received 2μL (10μg/mL) of *Porphyromonas gingivalis*-lipopolysaccharide (*P.g.*-LPS) (# tlrl-ppglps, InvivoGen, San Diego, CA, USA) injections in the interdental papilla between the first and second maxillary molars on both the right and left sides using a 10μL Hamilton syringe with a 0.33-gauge needle (Hamilton Company, Reno, NV, USA). Mice received injections twice a week for 6 weeks. Control mice did not receive *P.g.*-LPS injections as previously described once there was no statistical difference in bone levels between noninjected and vehicle-injected groups [35]. While treatment mice did not exhibit signs of overt soft tissue damage or inflammation.

CXCR3 antagonist nanoparticle treatment

Nanoparticles of CXCR3 antagonist named AMG-487 NP (Tocris, R&D Systems, MN, USA) were used to block CXCR3 in vivo. AMG-487 NP was incorporated into a liposome carrier. The liposome nanoparticles were created via self-assembly of palmitic acid and cholesterol, as previously described[36]. The nanoparticles were locally delivered with a Hamilton syringe (33-gauge needle) at a concentration of 0.5 μM in the site where LPS-induced bone resorption was developed. Mice received the injection twice a week for 6 weeks. The injections were performed one day before LPS treatment. Mice were divided in three groups: 1) Control group: local Tris-phosphate buffer injection; 2) LPS group: local Tris-phosphate buffer injection + *Pg*-LPS and 3) LPS+NP group: *Pg*-LPS + AMG-487 NP. The person performing the injections was blinded for the treatment. At the end of one

and six weeks, mice were euthanized, maxillae harvested, fixed for 48h in 10% buffered formalin for 48 hours, and subsequently stored in 70% EtOH for further analysis.

Micro-computed tomography analysis

Micro-computed scanning and analysis was performed as previously published [14,37]. In brief, maxillae were scanned using a μ-computed tomography (μ-CT) scanner (SkyScan 1172; SkyScan, Aartelaar, Belgium) at 10 μm voxel size. To assess volumetric bone levels, samples were oriented using DataViewer (V.1.5.2 Bruker, Billerica, MA). The long axis of the tooth was parallel to the sagittal and coronal axes and perpendicular to the axial axis. The CEJ of the second molar was parallel in the sagittal and coronal planes. For all teeth, circumferential volumetric bone levels were assessed in the axial plane. Using CTAn (V.1.16 Bruker, Billerica, MA), volumetric measurements started 20 slices below the CEJ going towards the teeth apices, which represented healthy bone level distances. Volumetric measurements were taken until the alveolar bone crest was first identified. A single blinded examiner oriented the images and performed volumetric analysis. Data from each group was averaged to determine the amount of circumferential bone loss per group.

Histology

Maxillae were decalcified in 15% EDTA for 4 weeks. Following decalcification, 5μm thick sections were cut in the coronal plane using a microtome (McBain Instruments, Chatsworth, CA, USA). Sections were stained with hematoxylin and eosin (H&E) using standard protocols. For osteoclast analysis, tartrate-resistant acid phosphatase (TRAP) staining was performed using a leukocyte acid phosphatase system (Sigma-Aldrich, MO, USA) according to manufacturer's protocol [38]. TRAP+ multinucleated cells (osteoclasts), in contact with the bone surface, were considered as osteoclasts (OC) [39]. OCs were counted along the buccal and palatal alveolar bone on the distal root of 1st molar and mesial root of 2nd molar and then averaged from a total of 5 sections per animal. Averaged OC numbers were normalized to the respective controls as well as bone length. Bone length represented the average of the distance between the most coronal portion of the alveolar bone to the apex of the distal root on the 1st molar and mesial root of 2nd molar. Slices were digitally imaged using Aperio ImageScope model V11.1.2.752 (Leica Biosystems, Vista, CA, USA).

Statistics

All statistical analyses were performed using Prism5 (GraphPad, La Jolla, CA, USA). For bone loss analysis, measurements were averaged per animal and subsequently averaged per group to create a mean bone loss value per group (mean ± standard error of mean). For quantification of TRAP staining, 5 sections per animal were stained and OC numbers were averaged per animal, subsequently mean OC per animal, per group was averaged to create a mean number of OC per group (mean ± standard error of the mean). Statistical significance between groups was assessed using one-way ANOVA, and Student *t* tests. Significance levels were as follows: $p \leq 0.01$; $p \leq 0.001$; $p \leq 0.0001$.

RESULTS

CXCR3 antagonist reduces periodontal bone loss

Previous studies have demonstrated that CXCR3 knockout mice treated with LPS injections exhibited reduced bone loss and osteoclast numbers compared to wild-type mice. In addition, systemic administration of a CXCR3 antagonist demonstrated reduction in bone loss and number of osteoclasts [14]. We and others have not observed any complications with the CXCR3 antagonist locally and systemically[14,40–42].

Therefore, to investigate if local delivery of an antagonist could reduce bone loss induced by *P.g.*-LPS we employed the delivery of the CXCR3 antagonist via nanoparticles.

Following radiographic assessment of bone loss through unidimensional and 3D images, it was revealed that the LPS+NP group had reduced bone loss when compared to the LPS groups at 6 weeks (Fig. 1). These changes were observed as early as 1-week (Fig.2a), where the LPS group (47.9%) presented a 1.7-fold increase on bone loss compared to control (27.4%) and LPS+NP (27.8%) groups ($p \leq 0.001$). At six weeks (Fig. 2b), volumetric bone loss increased in all groups, however, treatment with CXCR3 antagonist (LPS+NP) significantly reduced this bone loss compared to LPS only group ($p=0.01$). The percentage of bone loss on the LPS+NP group was comparable to the control group (ns).

CXCR3 antagonist inhibits osteoclast induction

Specimens were further analyzed for histological features. At 1 week, H&E staining demonstrated mild infiltration of inflammatory cells into the connective tissues consistent with LPS treatment when compared to control group. At 6 weeks, LPS treatment led to an increase in inflammatory infiltrate, whereas treatment with CXCR3 antagonist visibly reduced the inflammatory response. (Fig 3).

Osteoclast numbers were evaluated through tartrate resistant acid phosphatase (TRAP) staining (Fig. 4a). Treatment with LPS led to a statistically significant increase in osteoclast numbers on weeks 1 and 6. Moreover, local delivery of the CXCR3 antagonist resulted in a reduction in the numbers of osteoclasts when compared to LPS alone at 1 and 6 weeks ($p=0.001$). These levels were comparable to those observed in control mice (ns) (Fig. 4b).

DISCUSSION

Studies have shown different and important roles for chemokine receptors and some selective CXCR3 antagonists, such as AMG487, in face of inflammatory conditions. In addition, the progression of these conditions seems to be influenced by blocking these receptors [23,43]. Given that our laboratory demonstrated that by deleting the CXCL9 and 10 receptor (CXCR3) or by blocking CXCR3 receptor with systemic administration of a CXCR3 antagonist led to a considerable decrease in LPS-induced periodontal bone loss in this study, we sought to determine if sustained-release of the local administration of a CXCR3-antagonist, AMG487, would also lead to a decrease in LPS-induced bone loss.

As noted in previous GWAS, CXC chemokine family genes CXCL9 and CXCL10 have been associated with periodontal bone loss phenotype in LPS-induced periodontitis. Besides that, systemic administration of a CXCR3 receptor antagonist demonstrated a beneficial effect on reduction of bone loss (~46%) and number of osteoclasts through systemic administration of AMG487 [14]. In our present study, the local administration of this antagonist showed a significant reduction (approx. 50%) in bone loss in the LPS+NP group compared to the LPS group. Furthermore, we observed significantly less osteoclasts in LPS+NP groups compared to LPS group, histologically. According to the radiographic and number of osteoclasts changes, the local administration of CXCR3 antagonist via nanoparticles showed similar efficacy as compared to CXCR3-antagonist systemic administration to mice [14], suggesting that local administration of a CXCR3-antagonist could be potentially utilized as a treatment for bone resorption.

CXCR3 plays an important role during leukocyte recruitment in the early host response. Under normal circumstances, low levels of the chemokine ligands for CXCR3 (such as CXCL9 and CXCL10) are expressed in the epithelium. However, the expression of CXCL10, an IFN-γ inducible chemokine, increases markedly in inflammatory processes. In addition, CXCR3 is expressed at high levels on tissue-infiltrating, activated T cells in several inflammatory systemic disorders [44, 45].

The CXCR3 antagonist utilized in this study was delivered in nanoparticles. The nanoparticle used in herein varied in size from 1nm-100nm [30]. This new small molecule nanoparticle may be the ideal method for effectively delivering treatment. Some of the various nanoparticulate systems available for local drug delivery include polymeric nanoparticles, nanofibers and liposomes, each playing a key role in achieving special properties to target bacteria and specific host cells [44]. Nanoparticles differ from traditional delivery systems not only by size, but also by their pharmacodynamic and pharmacokinetic properties. As drug carriers, nanoparticles must have conditions such as high stability, high carrier capacity, and incorporation of both hydrophilic and hydrophobic drugs, various routes of administration, controlled release or sustained release of the drug over time [45,46].

An advantage of utilizing nanoparticles is that nanoparticles could also access sites that are unreachable by other devices, such as deep periodontal pockets or furcation areas. Moreover, due to the small size of nanoparticles, their intracellular uptake efficiency is higher compared to microparticles, which improves and maintains the drug concentration for a prolonged period in the periodontal tissues. Through the local delivery of nanoparticles containing a CXCR3 antagonist, a decrease in the inflammatory infiltrate, bone loss and number of osteoclasts were observed in our study. These results suggest that CXCR3 small molecule antagonists may be an effective option for the therapeutic treatment of LPS-induced bone loss.

In this study, we assessed the effects of CXCR3 antagonist nanoparticles at two time points of disease induction (1 week and 6 weeks). Although similar changes were observed in both time points, there was a tendency to be more prominent in the 6-week group. In our previous study, we provided an observation period of 6 weeks after the systemic administration of CXCR3 antagonist nanoparticles. As a result, a reduction in bone loss and number of

osteoclasts was observed [14]. In the present study, equivalent results were obtained not only at 6 weeks, but also at the 1-week time point. Therefore, we can infer that such nanoparticles have a preventive effect on acute inflammation model even if they are administered in a relatively short period of time.

There have been no reports regarding the decrease in progression of bone resorption induced by LPS injection using CXCR3 antagonist nanoparticles. Given our positive results, it is important to determine the most effective CXCR3 antagonist dose as well as how long the antagonist is present and active in the area. Taken together, our results suggest that regulating the host immune response, and specially monitoring chemokine expression levels, could aid in our understanding of the pathogenesis of bone resorption, as well as serve as the foundation for more personalized patient treatment. Therefore, it is suggested that CXCR3 blockade can be regarded as a novel target for therapeutic intervention of bone loss and that it can be a safe and convenient method of periodontitis treatment or prevention applicable in general clinical practice.

FUNDING:

This work was supported by NIH/NIDCR DE023901-01 and the UCLA Faculty Development grant.

REFERENCES

1. Papapanou PN, Sanz M, Buduneli N, et al. Periodontitis: Consensus report of workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. J Periodontol. 2018;89 Suppl 1:S173–S182. [PubMed: 29926951]
2. Hajishengallis G, Chavakis T, Lambris JD. Current understanding of periodontal disease pathogenesis and targets for host-modulation therapy. Periodontol 2000. 2020;84(1):14–34. [PubMed: 32844416]
3. Eke PI, Thornton-Evans GO, Wei L, Borgnakke WS, Dye BA, Genco RJ. Periodontitis in US Adults: National Health and Nutrition Examination Survey 2009–2014. J Am Dent Assoc. 2018;149(7):576–588 e576. [PubMed: 29957185]
4. Petersen PE. The World Oral Health Report 2003: continuous improvement of oral health in the 21st century--the approach of the WHO Global Oral Health Programme. Community Dent Oral Epidemiol. 2003;31 Suppl 1:3–23. [PubMed: 15015736]
5. Hajishengallis G, Darveau RP, Curtis MA. The keystone-pathogen hypothesis. Nat Rev Microbiol. 2012;10(10):717–725. [PubMed: 22941505]
6. Sedghi L, DiMassa V, Harrington A, Lynch SV, Kapila YL. The oral microbiome: Role of key organisms and complex networks in oral health and disease. Periodontol 2000. 2021;87(1):107–131. [PubMed: 34463991]
7. Joseph S, Curtis MA. Microbial transitions from health to disease. Periodontol 2000. 2021;86(1):201–209. [PubMed: 33690926]
8. Abusleme L, Hoare A, Hong BY, Diaz PI. Microbial signatures of health, gingivitis, and periodontitis. Periodontol 2000. 2021;86(1):57–78. [PubMed: 33690899]
9. Jepsen S, Caton JG, Albandar JM, et al. Periodontal manifestations of systemic diseases and developmental and acquired conditions: Consensus report of workgroup 3 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. J Periodontol. 2018;89 Suppl 1:S237–S248. [PubMed: 29926943]
10. Wade WG. Resilience of the oral microbiome. Periodontol 2000. 2021;86(1):113–122. [PubMed: 33690989]
11. Darveau RP, Curtis MA. Oral biofilms revisited: A novel host tissue of bacteriological origin. Periodontol 2000. 2021;86(1):8–13. [PubMed: 33690952]

12. Hajishengallis G, Lamont RJ. Polymicrobial communities in periodontal disease: Their quasi-organismal nature and dialogue with the host. Periodontol 2000. 2021;86(1):210–230. [PubMed: 33690950]
13. Pihlstrom BL, Michalowicz BS, Johnson NW. Periodontal diseases. Lancet. 2005;366(9499):1809–1820. [PubMed: 16298220]
14. Hiyari S, Green E, Pan C, et al. Genomewide Association Study Identifies Cxcl Family Members as Partial Mediators of LPS-Induced Periodontitis. J Bone Miner Res. 2018;33(8):1450–1463. [PubMed: 29637625]
15. Loetscher M, Gerber B, Loetscher P, et al. Chemokine receptor specific for IP10 and mig: structure, function, and expression in activated T-lymphocytes. J Exp Med. 1996;184(3):963–969. [PubMed: 9064356]
16. Loetscher M, Loetscher P, Brass N, Meese E, Moser B. Lymphocyte-specific chemokine receptor CXCR3: regulation, chemokine binding and gene localization. Eur J Immunol. 1998;28(11):3696–3705. [PubMed: 9842912]
17. Luster AD, Unkeless JC, Ravetch JV. Gamma-interferon transcriptionally regulates an early-response gene containing homology to platelet proteins. Nature. 1985;315(6021):672–676. [PubMed: 3925348]
18. Groom JR, Luster AD. CXCR3 ligands: redundant, collaborative and antagonistic functions. Immunol Cell Biol. 2011;89(2):207–215. [PubMed: 21221121]
19. Groom JR, Luster AD. CXCR3 in T cell function. Exp Cell Res. 2011;317(5):620–631. [PubMed: 21376175]
20. Groom JR, Richmond J, Murooka TT, et al. CXCR3 chemokine receptor-ligand interactions in the lymph node optimize CD4+ T helper 1 cell differentiation. Immunity. 2012;37(6):1091–1103. [PubMed: 23123063]
21. Dufour JH, Dziejman M, Liu MT, Leung JH, Lane TE, Luster AD. IFN-gamma-inducible protein 10 (IP-10; CXCL10)-deficient mice reveal a role for IP-10 in effector T cell generation and trafficking. J Immunol. 2002;168(7):3195–3204. [PubMed: 11907072]
22. Ichikawa A, Kuba K, Morita M, et al. CXCL10-CXCR3 enhances the development of neutrophil-mediated fulminant lung injury of viral and nonviral origin. Am J Respir Crit Care Med. 2013;187(1):65–77. [PubMed: 23144331]
23. Igic M, Kostic M, Basic J, et al. Bleeding Index and Monocyte Chemoattractant Protein 1 as Gingival Inflammation Parameters after Chemical-Mechanical Retraction Procedure. Med Princ Pract. 2020;29(5):492–498. [PubMed: 32241014]
24. Garlet GP, Martins W Jr., Ferreira BR, Milanezi CM, Silva JS. Patterns of chemokines and chemokine receptors expression in different forms of human periodontal disease. J Periodontal Res. 2003;38(2):210–217. [PubMed: 12608917]
25. Gemmell E, Carter CL, Seymour GJ. Chemokines in human periodontal disease tissues. Clin Exp Immunol. 2001;125(1):134–141. [PubMed: 11472436]
26. Sakai A, Ohshima M, Sugano N, Otsuka K, Ito K. Profiling the cytokines in gingival crevicular fluid using a cytokine antibody array. J Periodontol. 2006;77(5):856–864. [PubMed: 16671879]
27. Aldahlawi S, Youssef AR, Shahabuddin S. Evaluation of chemokine CXCL10 in human gingival crevicular fluid, saliva, and serum as periodontitis biomarker. J Inflamm Res. 2018;11:389–396. [PubMed: 30464571]
28. Guo X, Wang Y, Wang C, Chen J. Identification of several hub-genes associated with periodontitis using integrated microarray analysis. Mol Med Rep. 2015;11(4):2541–2547. [PubMed: 25483140]
29. Guo YC, Chiu YH, Chen CP, Wang HS. Interleukin-1beta induces CXCR3-mediated chemotaxis to promote umbilical cord mesenchymal stem cell transendothelial migration. Stem Cell Res Ther. 2018;9(1):281. [PubMed: 30359318]
30. Mudshinge SR, Deore AB, Patil S, Bhalgat CM. Nanoparticles: Emerging carriers for drug delivery. Saudi Pharm J. 2011;19(3):129–141. [PubMed: 23960751]
31. Kumar AJ, Anumala N, Avula H. Novel and often bizarre strategies in the treatment of periodontal disease. J Indian Soc Periodontol. 2012;16(1):4–10. [PubMed: 22628955]

32. Cui ZK, Sun JA, Baljon JJ, et al. Simultaneous delivery of hydrophobic small molecules and siRNA using Sterosomes to direct mesenchymal stem cell differentiation for bone repair. Acta Biomater. 2017;58:214–224. [PubMed: 28578107]
33. Kim S, Cui ZK, Koo B, Zheng J, Aghaloo T, Lee M. Chitosan-Lysozyme Conjugates for Enzyme-Triggered Hydrogel Degradation in Tissue Engineering Applications. ACS Appl Mater Interfaces. 2018;10(48):41138–41145. [PubMed: 30421603]
34. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG. Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. PLoS Biol. 2010;8(6):e1000412. [PubMed: 20613859]
35. Hiyari S, Atti E, Camargo PM, et al. Heritability of periodontal bone loss in mice. J Periodontal Res. 2015;50(6):730–736. [PubMed: 25581386]
36. Cui ZK, Bastiat G, Jin C, Keyvanloo A, Lafleur M. Influence of the nature of the sterol on the behavior of palmitic acid/sterol mixtures and their derived liposomes. Biochim Biophys Acta. 2010;1798(6):1144–1152. [PubMed: 20153720]
37. Hiyari S, Wong RL, Yaghsezian A, et al. Ligature-induced peri-implantitis and periodontitis in mice. J Clin Periodontol. 2018;45(1):89–99. [PubMed: 28921659]
38. Pirih FQ, Michalski MN, Cho SW, et al. Parathyroid hormone mediates hematopoietic cell expansion through interleukin-6. PLoS One. 2010;5(10):e13657. [PubMed: 21048959]
39. Chaichanasakul T, Kang B, Bezouglaia O, Aghaloo TL, Tetradis S. Diverse osteoclastogenesis of bone marrow from mandible versus long bone. J Periodontol. 2014;85(6):829–836. [PubMed: 24003963]
40. Bakheet SA, Alrwashied BS, Ansari MA, et al. CXCR3 antagonist AMG487 inhibits glucocorticoid-induced tumor necrosis factor-receptor-related protein and inflammatory mediators in CD45 expressing cells in collagen-induced arthritis mouse model. Int Immunopharmacol. 2020;84:106494. [PubMed: 32304993]
41. Bakheet SA, Ansari MA, Nadeem A, et al. CXCR3 antagonist AMG487 suppresses rheumatoid arthritis pathogenesis and progression by shifting the Th17/Treg cell balance. Cell Signal. 2019;64:109395. [PubMed: 31449849]
42. Walser TC, Rifat S, Ma X, et al. Antagonism of CXCR3 inhibits lung metastasis in a murine model of metastatic breast cancer. Cancer Res. 2006;66(15):7701–7707. [PubMed: 16885372]
43. Bakheet SA, Alrwashied BS, Ansari MA, et al. CXC chemokine receptor 3 antagonist AMG487 shows potent anti-arthritic effects on collagen-induced arthritis by modifying B cell inflammatory profile. Immunol Lett. 2020;225:74–81. [PubMed: 32590119]
44. Li Q, Yang G, Li J, et al. Stem cell therapies for periodontal tissue regeneration: a network meta-analysis of preclinical studies. Stem Cell Res Ther. 2020;11(1):427. [PubMed: 33008471]
45. Vane J Towards a better aspirin. Nature. 1994;367(6460):215–216. [PubMed: 8121485]
46. McDonald JS, Cavanaugh PF, Pavelic LJ, Limardi RJ, Gluckman JL, Pavelic ZP. Prostaglandin H synthase isoenzyme distribution in the gingival tissue of patients with periodontitis: pronounced expression adjacent to gram-positive bacteria. Inflammopharmacology. 1997;5(2):109–118. [PubMed: 17694359]

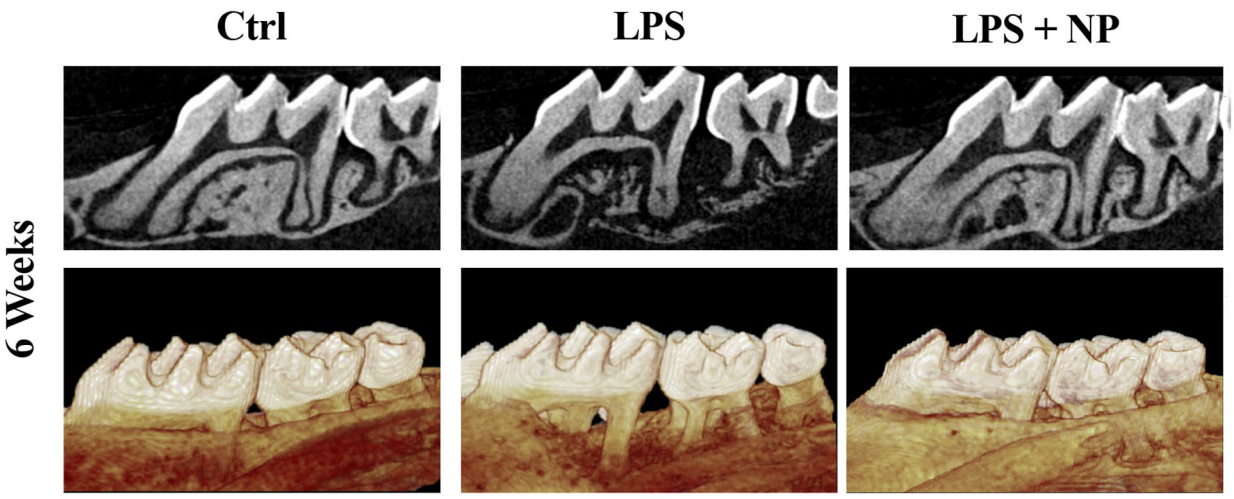

Fig. 1.

CXCR3 treatment reduced bone loss at 6 weeks. Representative sagittal radiographic images and 3D images of control (ctrl), LPS and LPS+NP groups. Note the reduction in alveolar bone (between the first and second molars) in the LPS group

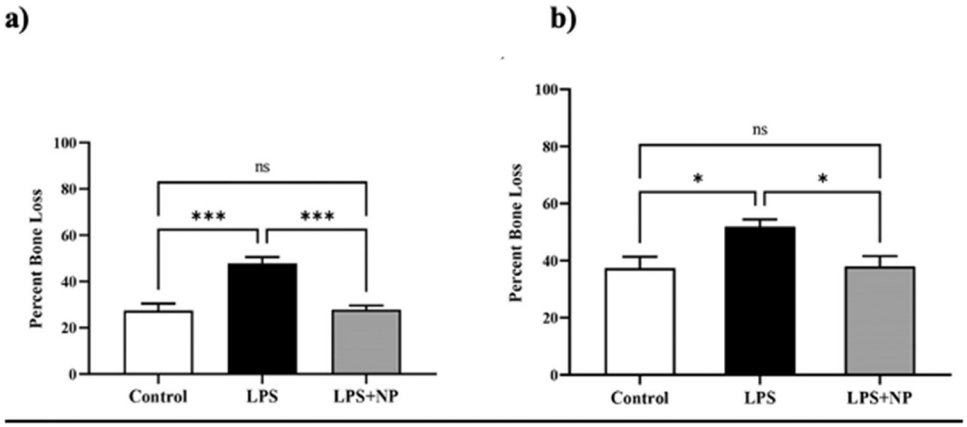

Fig. 2.

Graphs representing the averaged bone levels in control (Ctrl), LPS and LPS+NP groups at 1 (A) and 6 weeks (B) in volumetric bone loss. For both graphs significance was compared using a Tukey HSD test. n=5 mice/group, p=0.01*; $p \leq 0.001$***. Data represented as mean ± standard error of the mean (SEM)

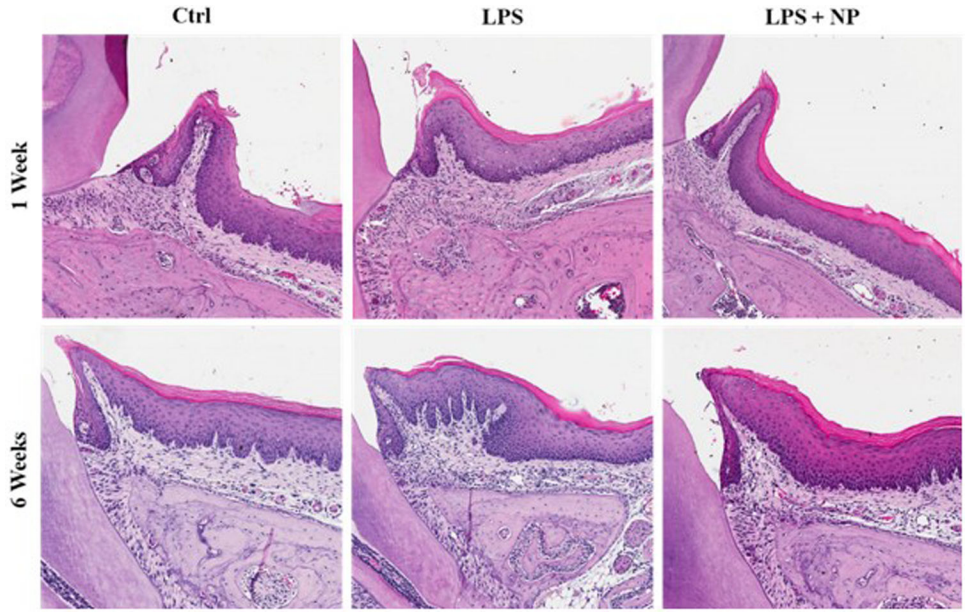

Fig. 3.

Histological assessment of hematoxylin and eosin (H&E) staining. Representative coronal images of control (Ctrl), LPS and LPS+NP group. Note the increased inflammatory infiltrate in LPS groups at 1 and 6 weeks. On the other hand, LPS+NP group presented a mild inflammatory infiltrate at both 1 and 6 weeks. 20X magnification

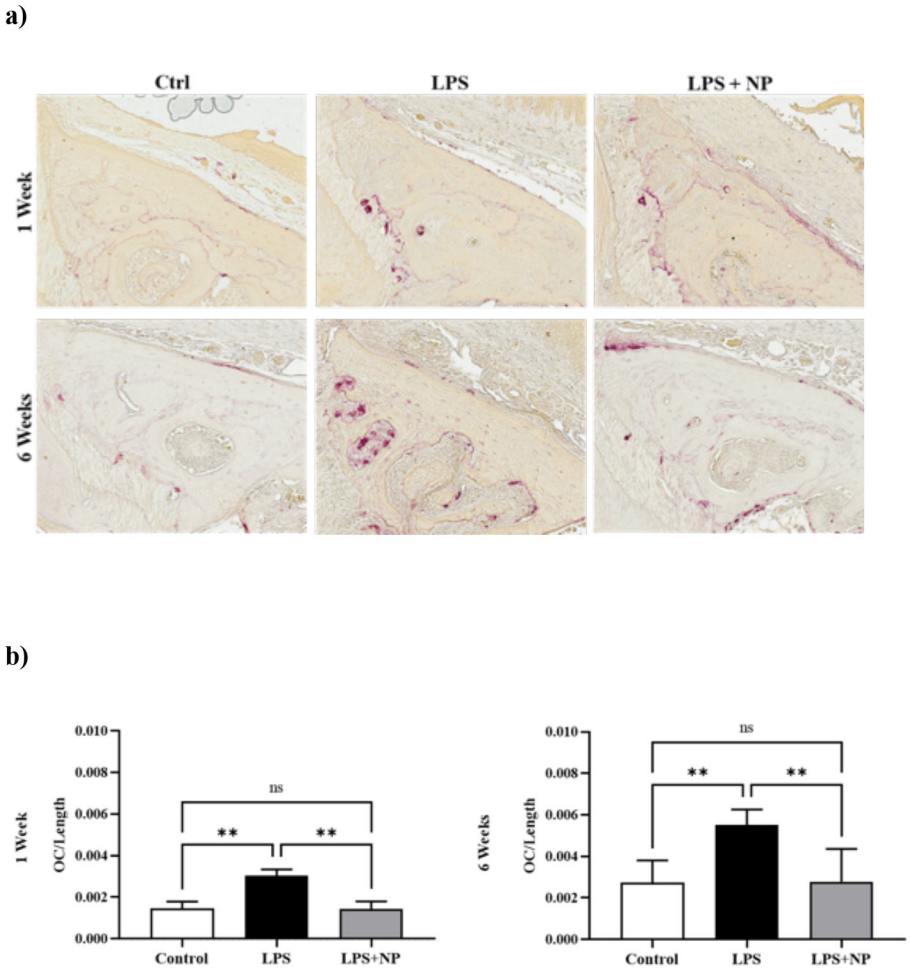

Fig. 4.

Histological assessment of osteoclast numbers. Representative coronal Tartrate Resistant Acid Phosphatase (TRAP) staining images of control (Ctrl), LPS and LPS+NP group (A). Note the increased number of osteoclasts in the LPS group. However, osteoclasts tended to be less in LPS+NP groups at both 1 week and 6 weeks groups. 20X magnification. Graph representing the averaged osteoclast number divided by the alveolar bone length considered in analysis control (Ctrl), LPS and LPS+NP groups (B). Significance was compared using a Tukey HSD test. n=5 mice/group, p=0.001**. Data represented as mean ± standard error of the mean (SEM)