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Inhibition of sphingosine-1-phosphate receptor 2 attenuated ligature-induced periodontitis in mice

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Abstract

Objectives: Periodontitis is an inflammatory bone loss disease initiated by oral bacterial inflammation. Herein, we determined whether inhibition of sphingosine-1-phosphate receptor 2 (S1PR2, a G protein-coupled receptor) by its specific antagonist, JTE013, could alleviate ligature-induced periodontitis in mice.

Materials and Methods: C57BL/6 mice were placed with silk ligatures at the left maxillary second molar to induce experimental periodontitis. Mice were treated with JTE013 or control vehicle (dimethyl sulfoxide, DMSO) oral topically on the ligatures once daily. After 15 days of treatment, RNA was extracted from the lingual mucosal tissues to quantify IL-1β, IL-6, and TNF mRNA levels in the tissues. Alveolar bone loss was determined by micro-computed tomography. Sagittal periodontal tissue sections were cut and stained by hematoxylin and eosin (H&E) for general histology, or stained by tartrate-resistant acid phosphatase (TRAP) for osteoclasts.

Results: Treatment with JTE013 attenuated ligature-induced alveolar bone loss compared with DMSO treatment. Treatment with JTE013 reduced IL-1β, IL-6, and TNF mRNA levels in murine gingival mucosal tissues, inhibited leukocyte infiltration in the periodontal tissues, and decreased the number of osteoclasts in the periodontal tissues compared with controls.

Conclusion: Oral topical administration of JTE013 alleviated periodontal inflammatory bone loss induced by ligature placement in mice.

KEYWORDS

bacteria, cytokine, osteoclast, periodontitis, sphingosine-1-phosphate receptor 2

1 | INTRODUCTION

Periodontitis is a prevalent chronic inflammatory bone loss disease affecting 47% of adults in the United States (Eke et al., 2012). It is initiated by formation of dysbiotic bacterial colonies on subgingival mucosa (Darveau, 2010; Hajishengallis & Lambris, 2012), resulting in release of inflammatory cytokines, such as IL-1β, IL-6, TNF-α, and RANKL. Enhanced levels of inflammatory cytokines subsequently recruit monocytes and macrophages to gingival tissues. These mononuclear cells can further differentiate and fuse to form multinucleated osteoclasts, resulting in destruction of alveolar bone tissues and subsequent tooth loss. Periodontitis can also influence systemic health, as severe periodontitis is associated with increased risk of other systemic diseases, such

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as atherosclerosis, diabetes, stroke, and compilations of pregnancy (Genco & Van Dyke, 2010; Jeffcoat et al., 2014; Pihlstrom et al., 2005).

Sphingosine-1-phosphate receptor 2 (S1PR2) is a G protein-coupled receptor, which is expressed in most tissues (Aarthi et al., 2011; Kluk & Hla, 2002). It is expressed on the plasma membrane and in the cytoplasm of mammalian cells (Aarthi et al., 2011; Kluk & Hla, 2002). S1PR2 couples with the G_i, G_q, and $G_{12/13}$ family of proteins, which regulate multiple signaling pathways, including Rho, Rac, phospholipase C, phosphoinositide 3-kinase (PI3K), NF-κB, and mitogen-activated protein kinases (MAPKs) (Aarthi et al., 2011; Kluk & Hla, 2002; Siehler et al., 2002; Takuwa, 2002; Yu, 2016).

Our previous studies showed that S1PR2 not only regulates S1P signaling, but also controls the inflammatory response stimulated by an oral bacterial pathogen, *Aggregatibacter actinomycetemcomitans* (*Aa*) (Hsu et al., 2019; Yu, 2016). Treatment with a specific S1PR2 shRNA or the S1PR2-specific inhibitor (JTE013) reduced IL-1β, IL-6, and TNF-α cytokine levels stimulated by *Aa* in murine bone marrow-derived monocytes and macrophages (BMMs) compared with controls (Hsu et al., 2019; Yu, 2016). Additionally, treatment with the S1PR2 shRNA or JTE013 inhibited cell chemotaxis stimulated by filter-sterilized cell culture media derived from BMMs infected with *Aa* (Hsu et al., 2019; Yu, 2016). Mechanistically, we determined that S1PR2 modulates PI3K, NF-κB, and MAPK protein kinases induced by *Aa*, subsequently affecting IL-1β, IL-6, TNF-α release, and cell chemotaxis response (Hsu et al., 2019; Yu, 2016).

S1PR2 not only controls inflammatory cytokine response initiated by bacterial pathogens, it also regulates bacterial phagocytosis. For instance, a previous study demonstrated that S1PR2 suppresses bacterial phagocytosis (Hou et al., 2015). They discovered that S1PR2 inhibits Rac1 activation induced by *Escherichia coli* (*E. coli*), subsequently leading to reduced filamentous actin (F-actin) polymerization and decreased bacterial phagocytosis (Hou et al., 2015). In addition, they showed that intratracheal instillation of the S1PR2-specific antagonist, JTE013 (4 mg/kg), improved survival rate in mice challenged by a lethal dose of *E. coli*. Treatment with JTE013 reduced the bacterial load in the blood and in bronchoalveolar lavage fluid compared with vehicle treatment. This study demonstrated that treatment with JTE013 alleviated bacterial infection by enhancing bacterial phagocytosis.

Furthermore, S1PR2 plays a key role in osteoclastogenesis and bone resorption stimulated by RANKL. Oral bacterial pathogens induce the generation of RANKL. RANKL, the critical cytokine for osteoclastogenesis, promotes mononuclear monocytes to fuse and form multinucleated osteoclasts, resulting in bone destruction and alveolar bone loss associated with periodontitis (Vaananen & Laitala-Leinonen, 2008). In the process of osteoclastogenesis, RANKL activates podosome components, which are cell adhesive units, consisting of F-actin, integrins, integrin-associated proteins, and protein kinases (PI3K, Src, and Pyk2) (Linder & Aepfelbacher, 2003). Increased levels of podosome components stimulated by RANKL lead to adhesion and fusion of mononuclear cells to form osteoclasts. Our previous study (Hsu et al., 2019) demonstrated that S1PR2 modulates podosome components induced by RANKL, subsequently influencing osteoclastogenesis and bone resorption. Murine bone marrow cells treated with either the S1PR2 shRNA or the S1PR2-specific inhibitor, JTE013, reduced these podosome components induced by RANKL. As a result, treatment with the S1PR2 shRNA or JTE013 inhibited osteoclastogenesis and suppressed bone resorption stimulated by RANKL (Hsu et al., 2019; Yu, 2016).

Herein, we used a ligature-induced experimental periodontitis model to determine whether treatment with the S1PR2-specific inhibitor, JTE013, in mice can reduce inflammatory cytokine release in gingival tissues, decrease leukocyte infiltration in the periodontal tissues, inhibit osteoclastogenesis, and alleviate alveolar bone loss induced by ligature placement compared with vehicle DMSO treatment in mice. This ligature model is widely used to initiate experimental periodontitis in different animals, including mice, rats, dogs, and non-human primates (Abe & Hajishengallis, 2013; Glowacki et al., 2013; Kajikawa et al., 2017; Weiner et al., 1979).

2 | MATERIALS AND METHODS

2.1 | Animals and reagents

The S1PR2-specific antagonist (JTE013) was obtained from Cayman Chemical and dissolved in DMSO (20 mg/ml). Eight-week-old male C57BL/6 mice were purchased from Jackson Laboratory. Mice were housed under a 12-hr light/12-hr dark cycle in specific pathogen-free conditions and had free access to food and water. All animal-related work was conducted in accordance with the guidelines laid down by the National Institute of Health (NIH) in the United States regarding the use of animals for experimental procedures, and approved by the Institutional Animal Care and Use Committee at the Medical University of South Carolina.

2.2 | Animal treatment

To induce inflammatory bone loss, a 5.0 silk suture (Roboz Surgical Instrument Co.) was tied around the cervical region of left maxillary second molars of all the mice under isoflurane anesthesia. The right maxillary teeth were untreated to serve as a baseline control for bone volume measurements. Animals were divided into two groups (10 mice/group). Previous studies (Hou et al., 2015; Ishii et al., 2010) used 3–4 mg/kg of JTE013 in mice. Considering that JTE013 would be diluted in the oral cavity as a result of mice drinking water, we chose a 5 mg/kg dose. In the experimental group, mice were administered the S1PR2-specific antagonist (JTE013, 5 mg/kg, 20 mg/ml, 13–16 μl) topically on the ligature under isoflurane anesthesia once daily. In the control group, mice were treated in the same way with an equal volume of vehicle (DMSO). The ligatures were checked daily and remained in place in all mice during the experimental period.

To promote persistent inflammation and osteoclastogenesis, we replaced all the ligatures on day 7 and day 11 to induce minor tissue injury and persistent inflammatory bone loss response.

After treatment with JTE013 or DMSO for 15 days, mice were euthanized. Both left and right lingual oral mucosal tissues (5 mice/group) were harvested and stored in TRIzol reagent (Thermo Fisher Scientific). Both sides of maxillary tissues (10 mice/group) were fixed in 10% buffered formalin solution for 48 hr. After fixation, the tissues were stored in 70% ethanol.

2.3 | Micro-computed tomography (μ-CT) scanning and alveolar bone loss assessment

Both sides of animal maxillae were scanned by a cone-beam μ-CT40 system (Scanco Medical AG, Switzerland). Three-dimensional μ-CT images were analyzed by the GE Healthcare MicroView software. The alveolar bone volume around the second molar was calculated within a region of interest (ROI). The width of the ROI was equal to the length between the cementoenamel junction (CEJ) of the 1st molar and the 3rd molar. The height of ROI was equal to the distance from molar cusp tips to root apices of the 2nd molar. The depth of ROI was equal to the buccal–lingual width of the 2nd molar plus 0.3 mm^3. To determine the bone volume in the ROI, bone volume fraction (BVF) was calculated as follows: BVF = bone volume ÷ total volume of ROI.

2.4 | Tissue processing, staining, and pathological assessment

Maxillary bone tissues were decalcified in a 20% EDTA solution for four weeks followed by paraffin embedding. Five-micrometer sagittal paraffin tissue sections were cut, stained with hematoxylin and eosin (H&E) for general histology, or stained by tartrate-resistant acid phosphatase (TRAP) for osteoclasts as previously described (Yu et al., 2016). Inflammation in the periodontal tissues was scored by an experienced pathologist who was blinded to the treatment groups. Inflammatory criteria were defined as follows: 0, there was an absence of inflammatory cells in the periodontal tissues, and no indication of fibroblast proliferation or alveolar bone destruction; 1, inflammatory cells were focally infiltrated in the periodontal tissues and the total number of inflammatory cells was less than 100 per section, mild fibroblast proliferation and bone destruction were present; 2, inflammatory cells were diffusely infiltrated in the periodontal tissues, with a total number of 100–200 inflammatory cells per section, and moderate fibroblast proliferation and bone destruction were present; and 3, inflammatory cells were diffusely infiltrated in the periodontal tissues, with the total number of inflammatory cells above 200 per section, with profound fibroblast proliferation and bone destruction present. The number of multinucleated TRAP-stained osteoclasts surrounding alveolar bone was also evaluated by the pathologist.

2.5 | RNA extraction and real-time PCR

Oral tissues were homogenized in TRIzol by a bullet blender tissue homogenizer (Next Advance, Inc.) with RNase-free stainless steel beads. Total RNA was extracted, and complementary DNA was synthesized by a TaqMan Reverse Transcription Reagent (Applied Biosystems). The real-time PCR was conducted using a StepOnePlus Real-Time PCR System (Applied Biosystems) as previously described (Yu et al., 2011). The following primers were purchased from Applied Biosystems: IL-1β (Mm00434228_m1), IL-6 (Mm00446190_m1), TNF (Mm00443258_m1), and GAPDH (Mm99999915_g1). Sample mRNA levels were normalized to control GAPDH expression and were expressed as fold changes as compared with control groups.

2.6 | Statistical analysis

Mucosal inflammatory cytokine levels, micro-CT alveolar bone volume fraction, and the number of osteoclasts in periodontal tissue sections were analyzed by the paired *t* test to compare ligature side with control side in the same group of animals. We also analyzed these parameters using the unpaired *t* test to compare the JTE013-treated group with the DMSO-treated group in different animals. Inflammation scores were analyzed using Wilcoxon matched-pairs signed rank test to compare ligature side and control side in the same group of animals. The Mann–Whitney test was used to compare the inflammation score between the JTE013-treated group and the DMSO-treated group in different animals. Data were expressed as means ± standard deviation (*SD*). *p*-values less than 0.05 were considered significant.

3 | RESULTS

3.1 | Treatment with JTE013 attenuated alveolar bone loss induced by ligature placement in mice

To evaluate the potential for JTE013 treatment to protect against inflammatory bone loss in mice, the volume of alveolar bone was quantified from the μCT scans. In the absence of ligature placement, there was no significant bone loss observed in animals treated with either DMSO or JTE013 (Figure 1a). By contrast, severe alveolar bone loss was observed when ligatures were placed in mice and treated with DMSO, focused mainly around the 2nd molar and some in the 1st and 3rd molar regions. Since the alveolar bone loss affected the alveolar bone from the 1st molar to the 3rd molar, we created a standard box (Figure 1b) to evaluate alveolar bone loss in the standard box using bone volume fraction (BVF). In animals treated with DMSO with ligature placement, there was an average reduction of BVF of 0.1. In contrast, in animals treated with JTE013 with ligature placement, there was an average reduction of BVF of 0.06, a 40% of decrease of alveolar bone

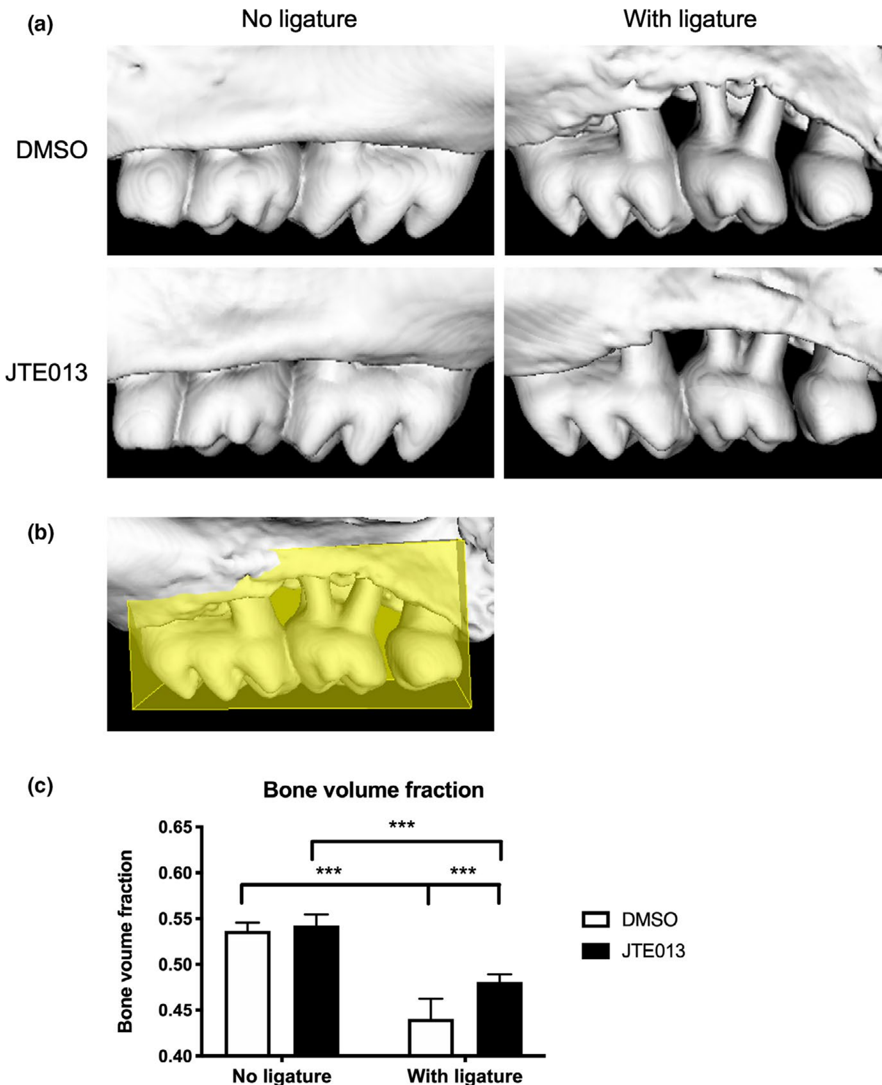


FIGURE 1 Treatment with JTE013 significantly suppressed alveolar bone loss induced by ligature placement compared with DMSO treatment in mice. C57BL/6 mice were placed with ligatures at the left maxillary 2nd molar for 15 days. JTE013 (5 mg/kg) or DMSO was administered oral topically at the ligature site once a day for 15 days. (a) Images of micro-computed tomography (μ-CT) scanning of maxillary alveolar bone tissues were displayed. (b) A standard box was created in the micro-CT image to calculate bone volume fraction in the total volume of standard box. (c) Maxillary alveolar bone volume loss was calculated by bone volume fraction (BVF = bone volume ÷ total volume of standard box, $n = 10$, $^{***}p < .001$) [Colour figure can be viewed at wileyonlinelibrary.com]

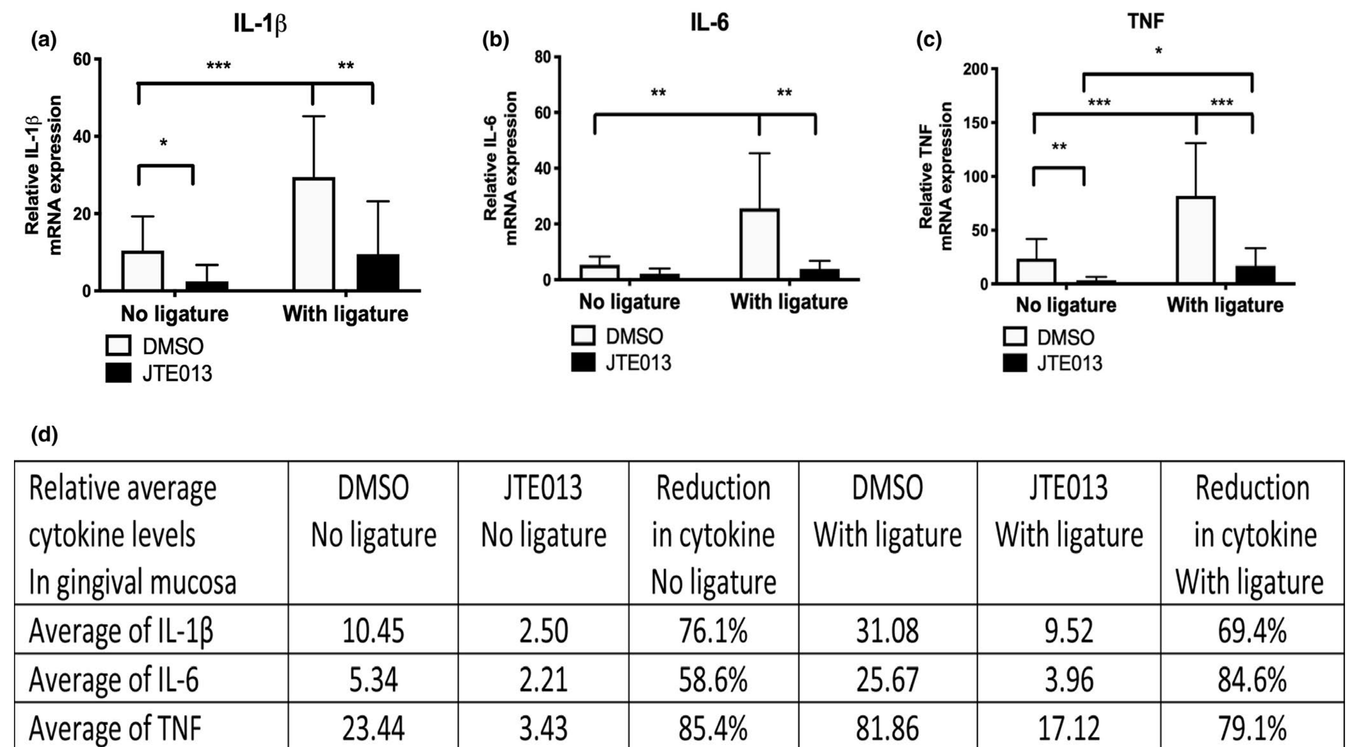
loss induced by ligature placement compared with vehicle treatment. Oral topical administration of JTE013 once daily for 15 days significantly attenuated alveolar bone loss compared with DMSO treatment ($n = 10$, $^{***}p < .001$).

3.2 | JTE013 decreased IL-1β, IL-6, and TNF mRNA levels in periodontal mucosal tissues induced by ligature placement in mice

Since oral bacterial pathogens stimulate the release of inflammatory cytokine (such as IL-1β, IL-6, and TNF), resulting in periodontal inflammation and subsequent alveolar bone loss, we determined whether inhibition of S1PR2 by JTE013 could reduce these inflammatory effects. Placement of ligature at the left maxillary 2nd molar significantly increased the mRNA levels of IL-1β, IL-6, and TNF compared with the right side without ligature placement (Figure 2a–c). The fold increases were 3.0 for IL-1β, 4.8 for IL-6, and 3.5 for TNF, respectively. Treatment with JTE013 significantly reduced these mRNA levels compared with DMSO treatment in mice both with and without the ligature placement. In the left lingual mucosal tissues with ligature placement, JTE013-treated animals reduced IL-1β by 69.4%, IL-6 by 84.6%, and TNF by 79.1%, respectively, compared with DMSO-treated animals (Figure 2d). In the right lingual mucosal tissues without ligature placement, JTE013-treated animals decreased IL-1β by 76.1%, IL-6 by 58.6%, and TNF by 85.4%, respectively, compared with DMSO-treated animals.

3.3 | Treatment with JTE013 reduced periodontal inflammation in periodontal tissues induced by ligature placement in mice

Since inflammatory cytokines chemoattract leukocytes in the periodontal tissues, we also determined whether JTE013 treatment affected periodontal leukocyte infiltration after ligature placement. Pathological evaluation of hematoxylin and eosin (H&E) staining of maxillary periodontal tissues revealed that the alveolar bone surface was smooth, with no indication of inflammation in periodontal tissues of mice treated with either DMSO or JTE013 without ligature



Relative average cytokine levels In gingival mucosa	DMSO No ligature	JTE013 No ligature	Reduction in cytokine No ligature	DMSO With ligature	JTE013 With ligature	Reduction in cytokine With ligature
Average of IL-1β	10.45	2.50	76.1%	31.08	9.52	69.4%
Average of IL-6	5.34	2.21	58.6%	25.67	3.96	84.6%
Average of TNF	23.44	3.43	85.4%	81.86	17.12	79.1%

FIGURE 2 Treatment with JTE013 significantly decreased IL-1β, IL-6, and TNF mRNA levels in oral mucosal tissues induced by ligature placement compared with DMSO treatment in mice. C57BL/6 mice were placed with ligatures at the left maxillary 2nd molar for 15 days. JTE013 (5 mg/kg) or DMSO was administered oral topically at the ligature site once a day for 15 days. (a) IL-1β mRNA, (b) IL-6 mRNA, and (c) TNF mRNA levels were measured by real-time PCR and normalized by GAPDH expression ($n = 5$, $^{**}p < .01$, $^{***}p < .001$). (d) Relative average cytokine mRNA levels and percentage of cytokine reduction between JTE013-treated group and control DMSO-treated group were calculated

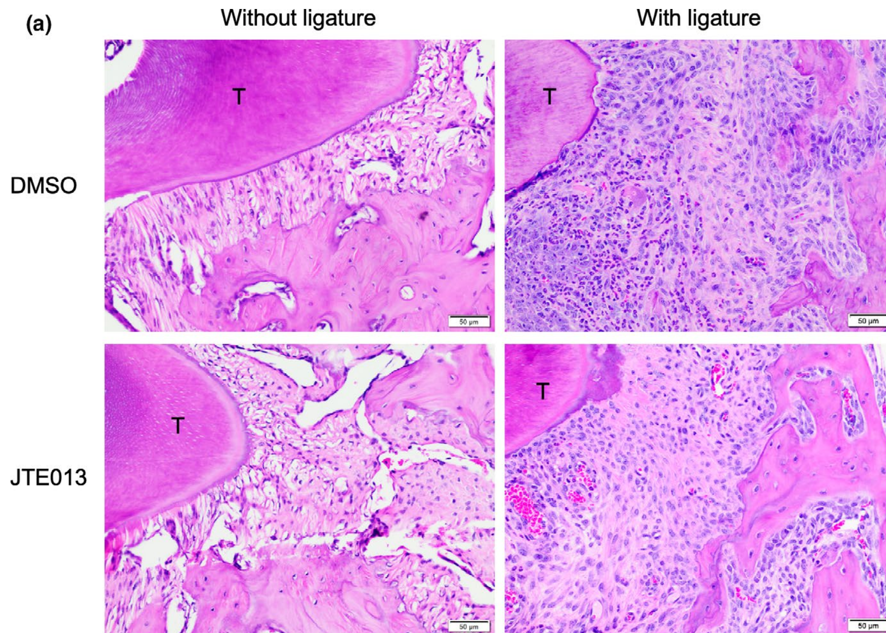
placement (Figure 3). By contrast, in mice with ligature placement and treated with DMSO, infiltration of polymorphonuclear leukocytes and mononuclear leukocyte was observed in the periodontal tissues. In addition, many proliferative fibroblasts were observed in stromal tissues, and the alveolar bone appeared as irregular pieces. The average inflammation score in tissues with ligature placement and treated with DMSO was 2.47. In contrast, in mice with ligature placement and treated with JTE013, there was a significant reduction in leukocyte infiltration of the periodontal tissues. The average inflammation score in these tissues was 1.70, a significant reduction compared with DMSO control ($n = 10$, $^{*}p < .05$).

3.4 | Treatment with JTE013 decreased the number of osteoclasts in periodontal tissue induced by ligature placement in mice

To evaluate whether JTE013 treatment could impact osteoclastogenesis in periodontal tissues derived from the experimental mice, we stained tartrate-resistant acid phosphatase (TRAP) for osteoclasts in maxillary tissues. In mice without ligature placement, no TRAP-stained osteoclasts were observed in periodontal tissues treated with JTE013 (Figure 4) and few osteoclasts (1–3 per section) were observed in periodontal tissues treated with DMSO. In mice treated with DMSO and with ligature placement, many TRAP-stained osteoclasts were observed (average 78 osteoclasts per section) surrounding the alveolar bone tissues. In contrast, for mice treated with JTE013 with ligature placement, a significant reduction of the number of osteoclasts (average 41 osteoclasts per section, a 52.6% reduction) surrounding the alveolar bone tissues was observed ($n = 10$, $^{*}p < .05$).

4 | DISCUSSION

Herein, we demonstrate the efficacy of a S1PR2-specific inhibitor, JTE013, for alleviating inflammatory bone loss in vivo. Oral topical administration of JTE013 (5 mg/kg) once daily for 15 days significantly reduced alveolar bone loss induced by ligature placement compared with control vehicle (DMSO) treatment. Treatment with JTE013 decreased IL-1β, IL-6, and TNF mRNA levels in the oral mucosa tissues; alleviated the degree of periodontal inflammation; and decreased the number of osteoclasts in periodontal tissues compared with DMSO treatment. These results are in accordance with our previous in vitro studies, which showed that JTE013 inhibited IL-1β, IL-6, and TNF protein levels stimulated by the oral pathogen *Aa*, as well as suppressed osteoclastogenesis and bone resorption induced by RANKL in murine bone marrow cells (Hsu et al., 2019;



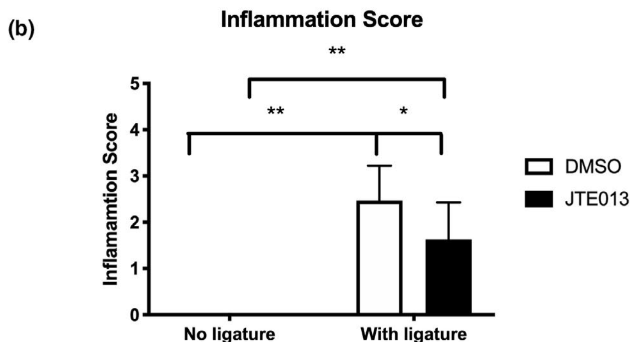


FIGURE 3 Treatment with JTE013 reduced leukocyte infiltration induced by ligature placement in the periodontal tissues compared with DMSO treatment in mice. C57BL/6 mice were placed with ligatures at the left maxillary 2nd molar for 15 days. JTE013 (5 mg/kg) or DMSO was administered oral topically at the ligature site once a day for 15 days. (a) Images of hematoxylin and eosin (H&E) staining of periodontal tissue sections (magnification, 200x) were displayed. The letter T stands for tooth. (b) Inflammation score in the periodontal tissues was evaluated ($n = 10$, *$p < .05$, ***$p < .001$) [Colour figure can be viewed at wileyonlinelibrary.com]

Yu, 2016). These findings are also similar to a previous study, which demonstrated that mice pretreated with JTE013 (1.2 mg/kg) reduced serum IL-1β and IL-18 levels induced by LPS (Skoura et al., 2011). Furthermore, since the previous study (Hou et al., 2015) revealed that JTE013 promotes bacterial phagocytosis and reduces bacterial burden of *E. coli*, JTE013 might reduce the amount of bacterial colonization around the ligatures, and attenuate IL-1β, IL-6, and TNF in the oral mucosa. Our study results are also in consistent with another previous study (Ishii et al., 2010), which demonstrated that JTE013 (3 mg/kg) treatment alleviated osteoporosis in mice induced by RANKL.

Our previous in vitro study (Hsu et al., 2019) showed that JTE013 (8 μM, about 3.2 μg/ml) reduced IL-1β by 90.2%, IL-6 by 80.8%, and TNF-α by 49.5% induced by the oral pathogen *Aa* compared with the vehicle-treated controls. JTE013 (8 μM) reduced the number of osteoclasts by 95.3% in bone marrow cells stimulated with RANKL and by 83.7% in cells co-cultured with both RANKL and *Aa*-stimulated cell culture media compared with the control vehicle group. In the current in vivo study, we administered JTE013 (20 mg/ml, 13–16 μl, about 260 to 320 μg) on the ligatures once daily, which reduced IL-1β by 69.4%, IL-6 by 84.6%, and TNF by 79.1%, respectively, induced by the ligature placement on the left side gingival mucosa compared with vehicle treatment. Treatment with JTE013 decreased the average number of osteoclasts by 52.6% and reduced the alveolar bone loss by 40% compared with vehicle treatment. Although we used a higher dose of JTE013 compared with the dose in our previous in vitro studies, JTE013 did not completely suppress alveolar bone loss induced by ligature placement. This might be caused by the following reasons: (a) JTE013 concentration in the oral cavity can be diluted by drinking water or eating food in mice, and (b) JTE013 was only administered once daily. Although ligatures might play a role in retention of JTE013 in the oral cavity, JTE013 concentration in the oral cavity could be diluted to below 8 μM in the oral cavity after a long period of time (such as several hours). There was a limitation in this study to determine the JTE013 concentration in the oral mucosa tissues of mice by chemical analysis. Future study is needed to determine the JTE013 concentration in the oral mucosa tissues, and if multiple oral topical administration of JTE013 could further decrease inflammatory cytokine production, inhibit osteoclastogenesis, and reduce alveolar bone loss induced by ligature placement in animals.

In the current study, low levels of IL-1β, IL-6, and TNF mRNA were detected in the right side of lingual mucosa tissues even without ligature placement. This could be because of the smaller spaces between teeth in mice compared to those in larger animals,

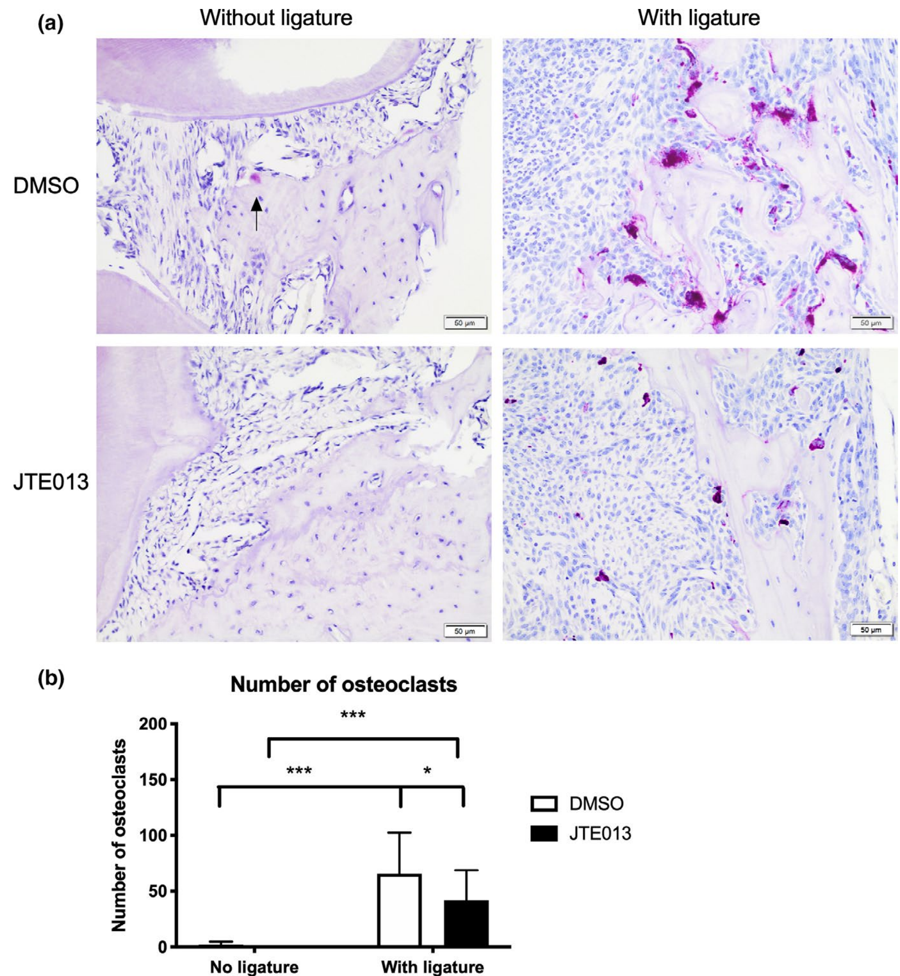


FIGURE 4 Treatment with JTE013 decreased the number of osteoclasts induced by ligature placement in the periodontal tissues compared DMSO treatment in mice. C57BL/6 mice were placed with ligatures at the left maxillary 2nd molar for 15 days. JTE013 (5 mg/kg) or DMSO was administered oral topically at the ligature site once a day for 15 days. (a) Images of tartrate-resistant acid phosphatase (TRAP) staining of periodontal tissue sections (magnification 200x) were displayed. (b) The number of TRAP-stained osteoclasts surrounding alveolar bone per tissue section was calculated ($n = 10$, $^{*}p < .05$, $^{***}p < .001$). The arrow points to a TRAP-stained osteoclast in the periodontal tissue without ligature placement in DMSO-treated group [Colour figure can be viewed at wileyonlinelibrary.com]

such as rats. Therefore, ligature placement in mice causes mucosa damage, bacterial colonization, and severe inflammation, which can impact the cytokine levels in the contralateral lingual mucosa tissues. Inhibition of sphingosine-1-phosphate receptor 2 by JTE013 significantly suppressed the inflammatory cytokine production on both sides (Figure 2). A previous study (Marchesan et al., 2018) showed that long-term (12 to 18 days) ligature placement significantly enhanced alveolar bone loss compared with short-term (3 to 6 days) ligature placement. However, the number of osteoclasts and inflammatory cells in the periodontal tissues was significantly reduced in the long-term ligature placement groups compared with short-term ligature placement (Marchesan et al., 2018). In the current study, we replaced all the ligatures at day 7 and day 11, which resulted in mild mucosal injury, bacterial colonization, and persistent chronic inflammatory response in the periodontal tissues. Therefore, infiltration of leukocytes and osteoclasts can be observed in the periodontal tissues 15 days after ligature placement.

Current treatments for patients with periodontitis include scaling and root planing to remove the bacterial plaque and calculus; antibiotic treatment to reduce or eliminate microbial pathogens; and in severe cases, surgical procedures to reduce or eliminate periodontal pockets. Despite the current treatment modalities, periodontitis is still the major causes of alveolar bone loss and tooth loss in adults (Batista et al., 2012; Eke et al., 2012). Additionally, long-term antibiotic treatment not only kills oral pathogens, but also eradicates good bacteria, which sometimes leads to severe effects, such as pseudomembranous colitis.

Oral topical administration has several advantages for patients. First, it is easier to administer drugs by oral topical administration than any parenteral administration (including digestive tract administration, intravenous injection, intramuscular injection, and subcutaneous injection). Second, drugs administered by the oral topical route primarily elicit local effects at the site of application, avoiding systemic side effects that might harm the liver or kidneys. Since JTE013 acts on multiple mechanisms, including promoting bacterial phagocytosis, reducing inflammatory cytokine release, and inhibiting osteoclastogenesis, it has the advantage over administration of any inhibitor which acts on only one mechanism (such as only reducing inflammatory cytokine release) (Rogers et al., 2007) or an antibiotic (Tonetti et al., 2012) because antibiotics can cause antibiotic resistance and side effects associated with using the antibiotics (Bonnetblanc, 2002). Interestingly, another study demonstrated that treatment with JTE013 in the food in type 2 diabetes obese *ob/ob* mice increased insulin sensitivity and reduced blood glucose compared with

vehicle treatment (Kitada et al., 2016). Since type 2 diabetic patients increased prevalence, extent, and severity of periodontal disease (Preshaw & Bissett, 2013; Taylor & Borgnakke, 2008), JTE013 might be beneficial to treat type 2 diabetes-associated periodontitis. Currently, JTE013 has only been used in animal studies. In the current study, both groups of mice maintained similar body weight after ligature placement. There was no significant difference in animal body weight between JTE013-treated group and vehicle-treated group (data not shown). Future studies are needed to determine cytotoxicity, stability, and dissolution of JTE013 to be used in future clinical trial, and if JTE013 could be administered oral topically at the gingivitis stage in patient to prevent disease progression to periodontitis and subsequent tooth loss.

It is important to note that this study was limited to the prevention of periodontal disease progression. It is unknown how S1PR2 and JTE013 may influence bone regeneration after inflammatory bone loss. Future studies are needed to determine whether treatment with JTE013 can enhance bone regeneration after periodontal bone loss.

In summary, we demonstrate that oral topical administration of a S1PR2-specific antagonist, JTE013, attenuated inflammatory bone loss in mice with periodontitis induced by ligature placement. Treatment with JTE013 inhibited gingival inflammatory cytokines release, suppressed periodontal inflammation, reduced osteoclastogenesis, and alleviated alveolar bone loss.

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CONFLICT OF INTEREST

All authors declare that there are no conflicts of interest.

AUTHOR CONTRIBUTIONS

Marquies Snipes: Data curation; Investigation; Writing-original draft. **Chao Sun:** Data curation; Formal analysis; Writing-review & editing. **Hong Yu:** Conceptualization; Data curation; Funding acquisition; Investigation; Supervision; Writing-review & editing.

PEER REVIEW

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