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Effect of environmental tobacco smoke on COX-2 and SHP-2 expression in a periodontitis rat model

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Abstract

Objectives: To investigate the effects of environmental tobacco smoke (ETS) on the inflammatory process of periodontitis by evaluating bone loss and the expression of cyclooxygenase-2 (COX-2) and Src homology phosphotyrosine phosphatase 2 (SHP-2).

Materials and Methods: Eighty 6-month-old male *SD* rats were randomized into four groups (10 rats/group/per time point): (a) normal group, (b) ETS group, (c) ligature-induced periodontitis group, and (d) ligature-induced periodontitis + ETS group. After treatment with ligature and/or ETS for 8 and 12 weeks, the levels of alveolar bone resorption and the expressions of COX-2 and SHP-2 in periodontal tissue were analyzed using histology and immunohistochemistry.

Results: The ligature-induced periodontitis group displayed increased bone resorption and elevated expression of COX-2 and SHP-2 in periodontal tissues compared to the normal and ETS groups at 8 and 12 weeks. Furthermore, bone resorption and COX-2 and SHP-2 levels in the ligature-induced periodontitis + ETS group were significantly increased compared to those in the normal and ligature-induced periodontitis groups at both 8 and 12 weeks.

Conclusion: Environmental tobacco smoke increased alveolar bone loss in periodontitis with enhanced expression of COX-2 and SHP-2 in periodontal tissues. Further investigation is needed to explore the role of COX-2 and SHP-2 in ETS-associated periodontitis.

KEYWORDS

COX-2, environmental tobacco smoke, periodontitis, SHP-2

1 | INTRODUCTION

Periodontitis is a chronic disease characterized by inflammation and damage to the supporting tissues of the teeth, which can lead to the loss of the periodontal attachment of teeth (Offenbacher, 1996). Periodontal diseases are highly prevalent and may affect up to 90% of the world's population (Pihlstrom, Michalowicz, & Johnson, 2005). Even though a complex relationship between bacteria, host, and behavioral and environmental factors determines the development and progress of periodontitis, one of the most vastly studied risk factors is smoking (Bergstrom, 2003).

Smoking is associated with cardiovascular disease (El Fadl et al.., 2011), obesity (Nunn, 2003), Alzheimer's disease (Kamer et al., 2008), diabetes mellitus (Kudiyirickal & Pappachan, 2015), and diseases of the oral cavity. A systematic review concluded that smoking is related to approximately half of the population of periodontal disease (Bergstrom, 2006). However, compared to active-smoking persons, non-smoking individuals are exposed to the same environmental dust particles and various toxic substances to the same degree as active smokers through environmental tobacco smoke (ETS) (Johnson & Guthmiller, 2007). Environmental tobacco smoke, also known as passive smoking or secondhand smoke, is a combination of chemicals that results from the burning of a tobacco product and from smoke exhaled by a smoker. The research on ETS and periodontitis among U.S. non-smokers reveals that exposure to ETS or secondhand smoke is a risk factor for periodontitis in the lifetime in non-smokers (Sutton, Salas Martinez, & Gerkovich, 2017). Cigarette smoke affects the individual cells involved in the development of periodontal disease, such as those involved in inflammation, immunity, cell differentiation, apoptosis, and healing (Bagaitkar et al., 2009; Semlali, Chakir, Goulet, Chmielewski, & Rouabhia, 2011). Some previous epidemiological studies have investigated the impact of ETS on periodontitis (Akinkugbe, Slade, Divaris, & Poole, 2016; Sutton et al., 2017; Tanaka, Miyake, Hanioka, & Arakawa, 2013). However, few studies have focused on the inflammatory factors involved in the mechanism of bone loss in periodontitis associated with ETS.

Inflammation in the periodontal tissue and bone loss are the main characteristics of periodontitis (Offenbacher, 1996). Periodontal diseases are mediated by the host inflammatory response against bacteria in the periodontal tissue during the process of bone loss (Aoki-Nonaka et al., 2019). Complex inflammatory signals and molecules, including MMP, RANKL, IL-1β, IL-6, TNF-α, and PGE2, regulate osteoclastogenesis (Araújo et al., 2013). It is reported that cyclooxygenase-2 (COX-2) plays an important role in periodontitis by mediating inflammatory reactions in periodontal tissues and this process is closely related to PGE2 production (Schaefer et al., 2010; Yucel-Lindberg & Båge, 2013). Src Homology Phosphotyrosyl Phosphatase 2 (SHP-2), a tyrosine phosphatase, is highly correlated with multiple cell signaling processes, such as growth, survival, proliferation, differentiation, and apoptosis, and is particularly important in chronic inflammation (Coulombe & Rivard, 2016). Some studies reveal that SHP-2 is involved in the process of formation and differentiation of osteoblasts and osteoclasts to regulate bone formation and resorption (Zambuzzi, Milani, & Teti, 2010; Zhou et al., 2015). However, under ETS, the involvement and the expression of COX-2 and SHP-2 in the process of inflammation and bone loss in periodontitis remain to be investigated.

The aim of the present study was to investigate the effects of ETS on periodontal tissue and the possible molecular factors associated with this process by evaluating the alveolar bone loss and the expression of COX-2 and SHP-2 in periodontal tissue in rats under an ETS condition.

2 | MATERIALS AND METHODS

2.1 | Animals

The experiments were performed using 80 male *SD* rats (6 months old, 280–320 g) housed in standard conditions (12-hr light/dark cycle and 22 ± 0.1℃), with access to food and water ad libitum. Animals were provided by the Center of Laboratory Animal Science, Hebei Medical University (license: SCXK(JI)2018-004, ID:1810103). The protocol for experimental procedures and animal treatments were approved by the Animal Ethics Committee of Stomatology School of Hebei Medical University, China. Eighty rats were randomly divided into four groups (10 rats/group at each time point): normal group (N group, control group), environmental tobacco smoke group (S group), ligature-induced periodontitis group (L group), and ligature-induced periodontitis plus environmental tobacco smoke group (LS group).

2.2 | Experimental models of periodontitis and environmental tobacco smoke

Animals were anesthetized by intraperitoneal injection of 10% chloral hydrate (0.3 ml/100 g body weight) on day 1. In L and LS group, a stainless wire with a diameter of 0.2 mm was placed around the cervical area of the bilateral maxillary first molar in each rat. The knots were placed at the palatal surface of the teeth. Rats of S and LS groups were put in the EST box as described below.

As shown in Figure 1A, a plastic box (volume 0.1 m^3) designed according to the device reported by Shen et al. (2016) with minor modifications, was used for the environmental smoke environment. Two holes with a diameter of 1.0 cm were opened on the left side toward the top of the box and two holes with a diameter of 1.0 cm were opened on the right side toward the bottom of the box. The smoke produced by four cigarettes (tar content 17 mg, nicotine 1.2 mg, CO 13 mg per cigarette) penetrated the box using a static inhalation method without applying any extra flow rate. The concentration of $PM_{2.5}$ (particulate matter with aerodynamic diameter < 2.5 μm), which is considered as one of the most representative and reliable particulate matters of smoke, was measured by using Aerosol Detector DUSTTRAKTM II-Model 8,530 (TSI Instrument Co., Ltd) and the average concentration of $PM_{2.5}$ was 220.7 mg/m^3. Rats (5 at a time) of S and LS groups were placed in the "simulative ETS box" as mentioned above for 20 min twice a day with an interval of 6 hr.

All animals from each group were treated for 8 and 12 weeks, respectively. At 8 or 12 weeks, the maxilla and lung tissues from each group were harvested after the rats were euthanized. The maxillae were dissected into two parts for general observation and histological examination. Lung tissues were used to evaluate the effect of ETS on the animals in accordance with histological observation.

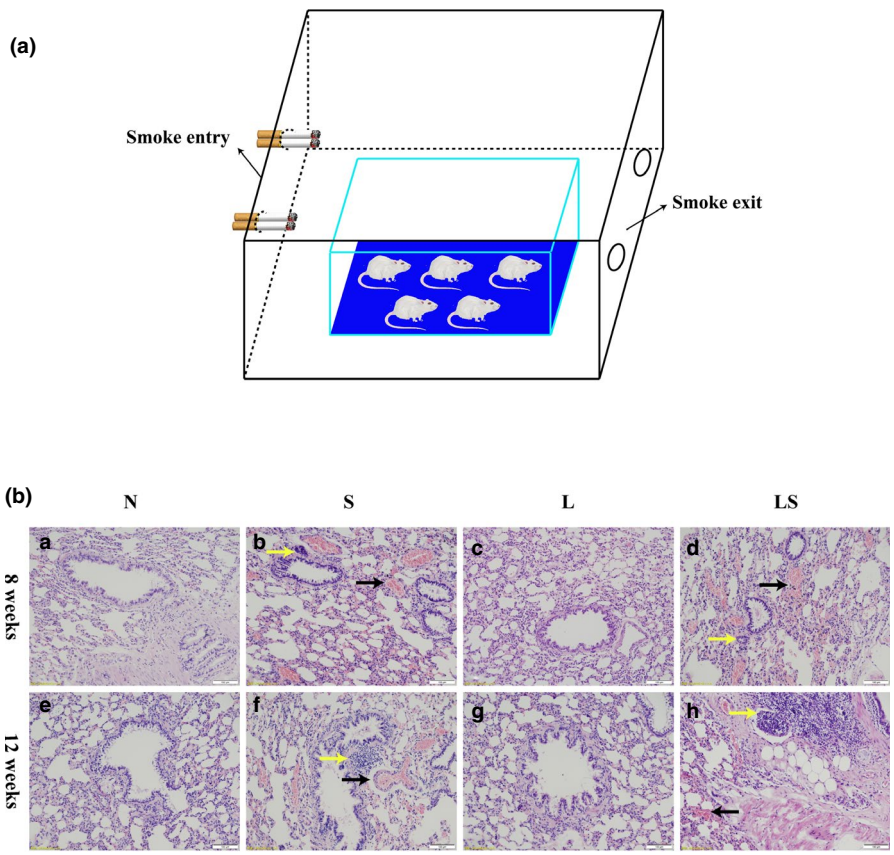


FIGURE 1 (A) Sketch map of a self-made plastic box used for the exposure to environmental tobacco smoke. The smoke produced by four cigarettes penetrates the box generated an experimental ETS. (B) The histological observation of the lung tissue of the rats exposed to the ETS environment. The lung tissues of the S group (b, f) and LS group (d, h) presented a high level of inflammatory cells. Additionally, telangiectasia and congestion were observed to be severe in the alveolar septum after 8 and 12 weeks' treatments. Yellow arrows indicate inflammatory cells, and black arrows indicate telangiectasia and congestion. Microscopic original magnification at 20× [Colour figure can be viewed at wileyonlinelibrary.com]

2.3 | Evaluations of alveolar bone loss and bone morphometric changes

At 8 and 12 weeks after treatment, the animals were euthanized by femoral artery bleeding. The maxillae were removed and fixed in 10% neutral buffered formalin for 48 hr. Following that, as mentioned above, the maxillae were dissected into two equal parts. The left part was stained with 1% aqueous methylene blue for general observation by stereomicroscope (OLYMPUS SZX16, OLYMPUS). On the images, six sites including the mesial, central, and distal sites of the buccal and palatal of the first molar were selected. The distance from the cementoenamel junction to the alveolar crest was measured at the same time, and the heights of alveolar bone loss from the six sites were recorded for averaging (mm).

2.4 | Alveolar bone histological analysis

The right part of the maxilla was decalcified with 10% EDTA (Edetate disodium, Yongda Chemical Reagent Development Center) solution for 8 weeks. The continuous slices (4 μm thickness) of the paraffin-embedded periodontal tissue between the first and the second molars were prepared for hematoxylin and eosin staining and immunohistochemistry staining for further histological observation using an optical microscope (OLYMPUS AX80, OLYMPUS).

2.5 | Immunohistochemical staining

Immunohistochemical staining was performed using the streptavidin-biotin method. Specifically, the histological sections were deparaffinized and rehydrated. Antigen retrieval was performed with 0.05% trypsin for 15 min at 37℃ and washed with PBS. Then, the sections were treated for endogenous peroxidase blockage with 3% hydrogen peroxide for 10 min. The sections were incubated with primary rabbit polyclonal anti-COX-2 antibody (Boster Biotechnology Company) at a dilution of 1:50 and primary rabbit polyclonal anti-SHP-2 antibody (Biosynthesis Biotechnology Company) at a dilution of 1:100 at 37℃ for 2 hr, and then washed with PBS. The sections were then incubated with a biotinylated secondary antibody (PV9000 kit, Zsbio Biotechnology Company) for 20 min at 37℃ and washed with PBS. Next, the sections were incubated with horseradish enzyme-labeled streptomyces ovalbumin (PV9000 kit, Zsbio Biotechnology Company) for 30 min at 37℃ and washed with PBS. Then, the section incubation with DAB was carried out for 5 min. The sections were washed in running tap water and then in distilled water. Counterstaining was performed with hematoxylin, followed by dehydration in a series of concentrations of alcohol, diaphanized in xylene, and finally, the coverslip was placed. We performed the negative controls for COX-2 and SHP-2 stainings in every experiment: (a) omitting primary antibody only using PBS buffer, (b) using primary antibody-matched IgG (Santa Cruz Biotechnology Company) at the same dilution as the primary antibody. Because COX-2 and

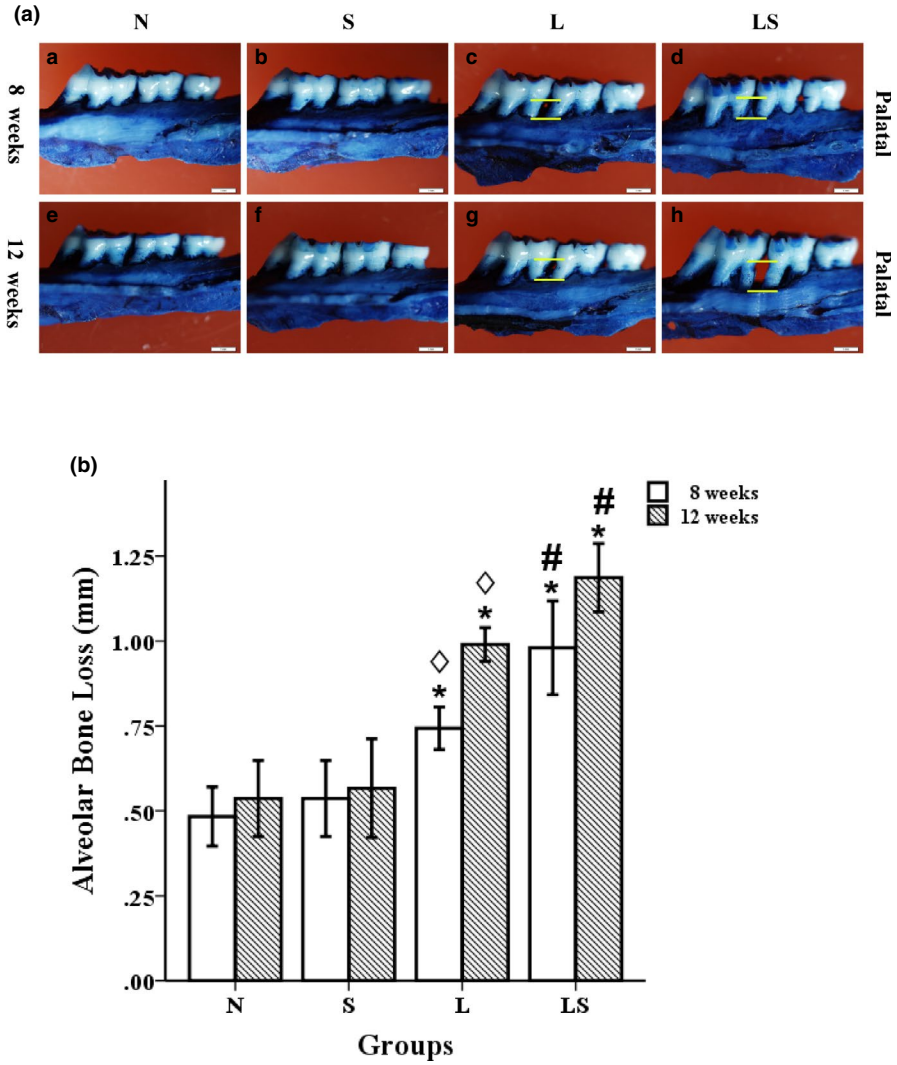


FIGURE 2 (A) Illustrative photographs of the periodontal alveolar bone loss in the maxilla. The distance between the yellow lines indicates the height of alveolar bone resorption. Original magnification at 16×. (B). Comparison of the alveolar bone loss among N, S, L, and LS groups. Data represent the mean ± *SEM* of alveolar bone loss (mm) in each group at 8 and 12 weeks after treatments. * indicates $p < .05$ compared to N group; ♢ indicates $p < .05$ compared to S group; # indicates $p < .05$ compared to L group [Colour figure can be viewed at wileyonlinelibrary.com]

SHP-2 primary antibodies are both rabbit polyclonal IgG, the negative controls for COX-2 and SHP-2 are the same. The positive immunohistochemical staining of the antigen in all sections exhibited brown staining in the cytoplasm. These areas were evaluated by ImageJ software (Motic Medical 6.0, Motic) as previously described by Cheng, Dong, Lin, Sun, and Chen (2012). In addition, the average optical density value was measured to determine the levels of COX-2 and SHP-2 expression in the periodontal tissues (Pei, Lin, Dai, & Yin, 2007).

2.6 | Statistical analysis

The data are presented as mean ± standard error of the mean (*SEM*). Differences among the experimental groups were analyzed by two-way ANOVA followed by Bonferroni's multiple comparison test. *p*-Values <.05 were considered significant. All statistical analyses were performed using SPSS 21.0 software (Statistical Product and Service Solutions 21.0, IBM).

3 | RESULTS

3.1 | Evaluation of experimental simulated ETS model in rats

After 8 weeks of exposure to the ETS environment, the color of the lungs of most experimental rats in the S group and LS group turned pale, and brownish-black spots on the surface of lungs were occasionally observed. These changes tended to be more obvious in the lung tissue of rats exposed to ETS at 12 weeks after treatments in S and LS groups. Under an optical microscope, at 8 weeks, some inflammatory cells were observed in the interstitial lung and pulmonary bronchial wall, and severe telangiectasia and congestion were present in the alveolar septum in S and LS group (Figure 1B,b,d). At 12 weeks, the numbers of inflammatory cells were appreciably increased, and more telangiectasia and congestion were observed (Figure 1,B,f,h). However, none of the changes described above were found in lung tissues of rats in the N and L groups at 8 or 12 weeks (Figure 1B,a,e,c,g).

3.2 | Measurement of alveolar bone loss

In general observation, periodontitis in rats in L and LS groups, which were ligatured surgically with a stainless wire around the cervical of the maxillary first molars, was generated successfully as characterized by the periodontitis main clinical features such as alveolar bone loss and furcation exposure. The most severe alveolar bone resorption was observed in the LS group, which was exposed to both wire ligature and ETS, and the extent of alveolar bone resorption exhibited a time-dependent tendency: Bone loss was greater in the group exposed to 12 weeks of treatment than in the group exposed to 8 weeks (Figure 2A,B). The height of the alveolar bone loss was determined on the images of the gross specimens by stereomicroscope. The L group displayed more alveolar bone loss than the N and S groups (both $p < .05$, Figure 2B). The LS group exhibited obvious alveolar bone loss that was significantly different from the N group and L group ($p < .05$, Figure 2B).

3.3 | Alveolar bone histological analysis

Histopathological analysis of the adjacent region between the first and second molars of the N group indicated that few inflammatory cells infiltrated into the periodontal tissues and that the shape of the alveolar crest was regular with normal feeding patterns at 8 and 12 weeks (Figure 3,a,e). In the same region of the S group, mild inflammatory cell infiltration and mild resorption of alveolar bone were observed at 8 and 12 weeks (Figure 3,b,f). Conversely, the L and LS groups at 8 and 12 weeks presented a higher level of inflammatory cell infiltration, greater attachment loss, and alveolar bone resorption with the destruction of both the cementum and alveolar processes. And the periodontal damage exhibited a time-dependent tendency coupled with the ligature or ligature with ETS subjected animals for 8 and 12 weeks (Figure 3c,g,d,h). The destruction of periodontal tissues of the LS group emerged earlier and more severely than in the S and L groups (Figure 3d,h).

3.4 | Immunohistochemical expression of COX-2 in periodontal tissue

The expression of COX-2 was mainly located in the cytoplasm of fibroblasts (Figure 4A,B; red arrow) and osteoclasts (Figure 4A,B; black arrow) in periodontal tissue. At 8 and 12 weeks, there were few positive expressions of COX-2 in the N group (Figure 4A,a,e and Figure 4B,a,e) and a mild COX-2 positive expression in the S group (Figure 4A,b,f and Figure 4B,b,f). Increasing positive expression of COX-2 was detected in the L group (Figure 4A,c,g, and Figure 4B,c,g). Furthermore, a stronger and more positive expression of COX-2 was observed in the LS group (Figure 4A,d,h, and Figure 4B,d,h). There were no positive stainings among the COX-2 negative controls and the representative images from the N and 12 weeks LS groups' slices are shown in Figure S1. Figure 4C shows the analysis of COX-2 expression among the four groups. The expression of COX-2 in the L group was significantly increased compared to that in the N and S groups (both $p < .05$, Figure 4C).

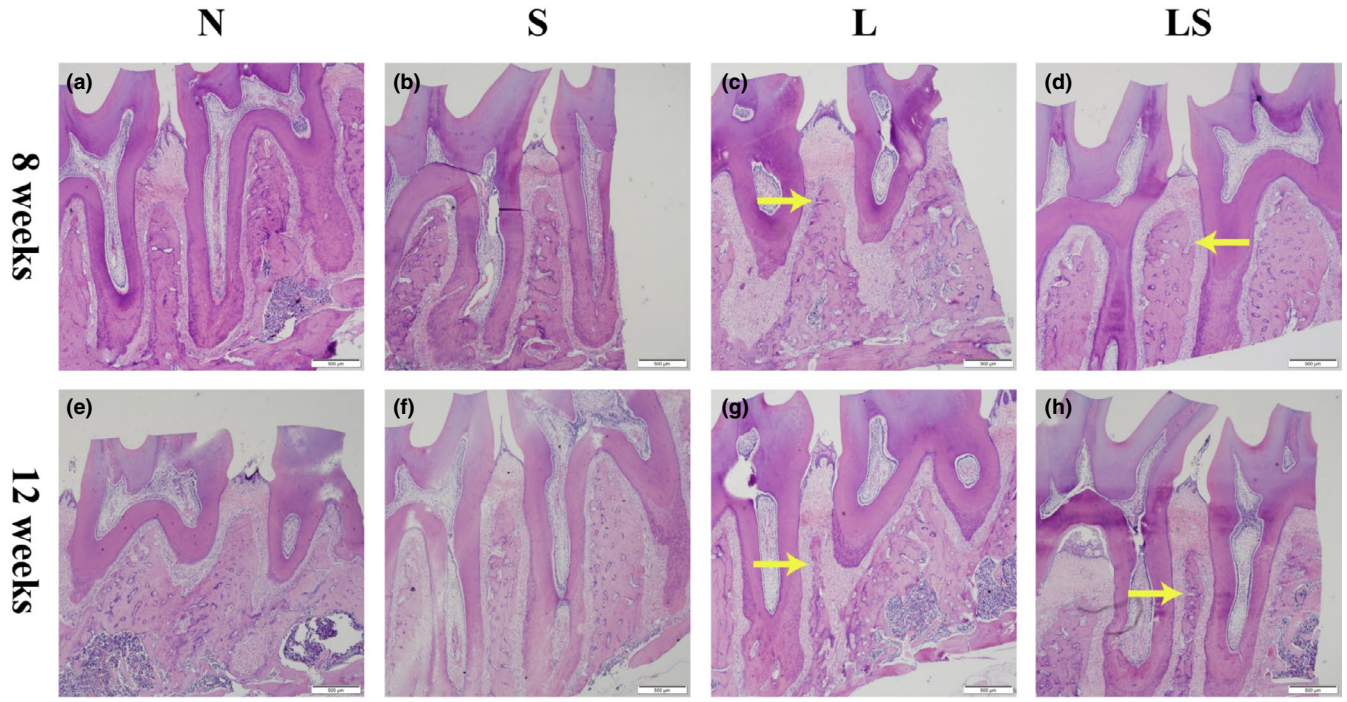


FIGURE 3 Histological analysis of alveolar bone. The L group (c, g) displayed intense cellular infiltration and destruction in the cementum and alveolar process, and the LS group (d, h) presented the most severe destruction, while the N group (a, e) showed minimal alveolar bone resorption and S group (b, f) represented mild destruction. Yellow arrows indicate alveolar bone resorption. Microscopic original magnification at 40× [Colour figure can be viewed at wileyonlinelibrary.com]

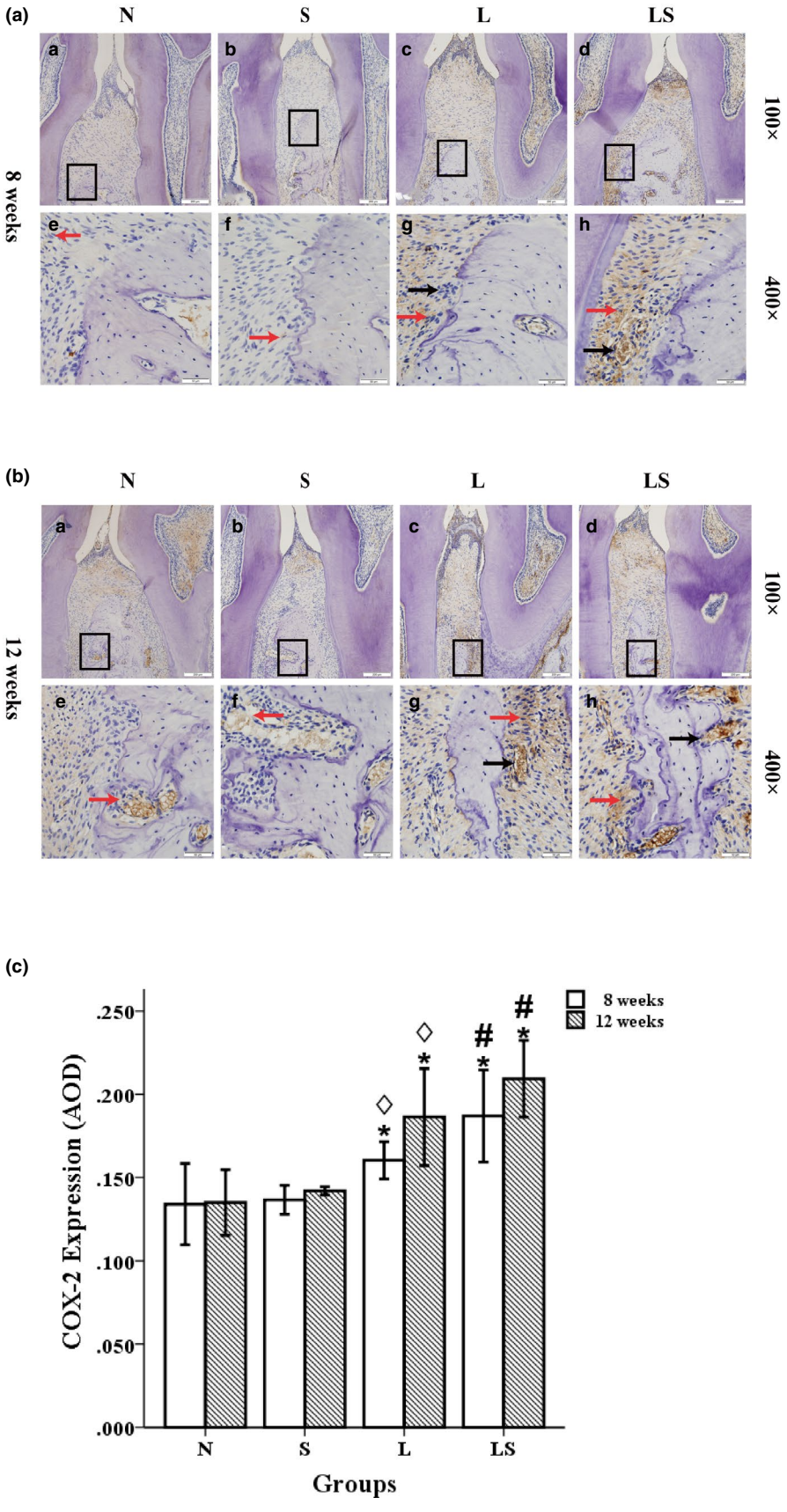
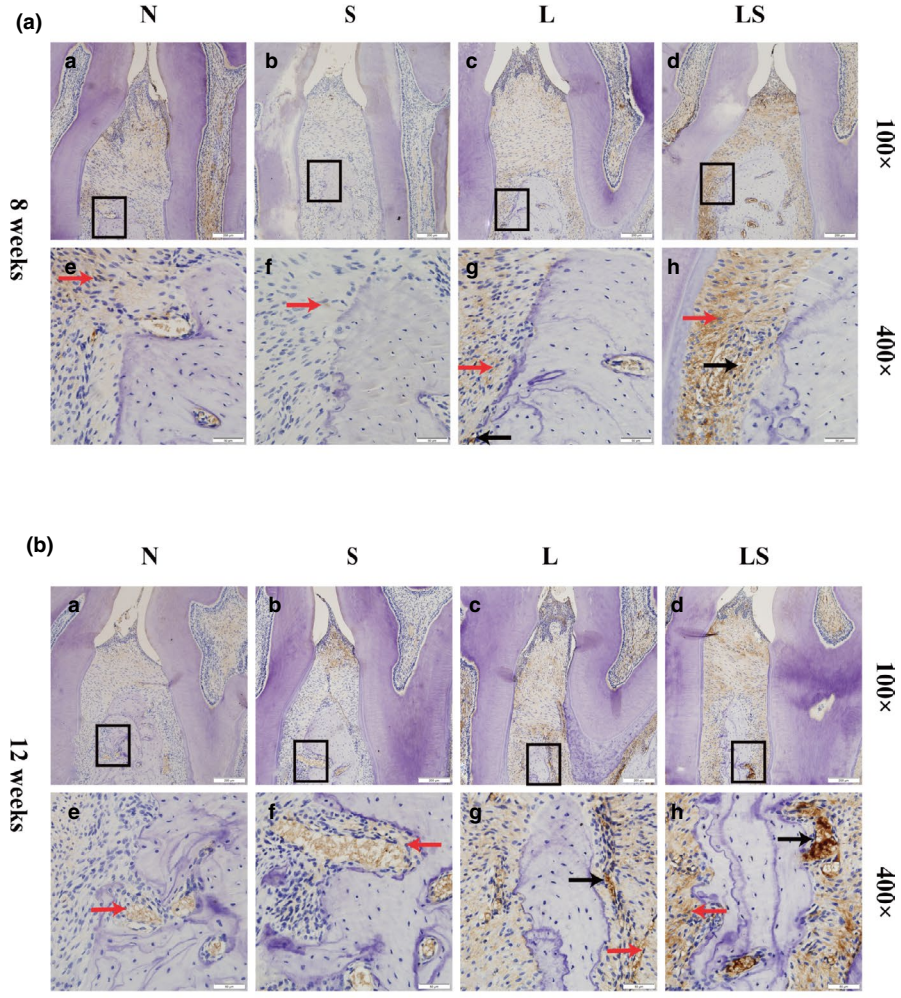


FIGURE 4 A, B, Immunohistochemical expression of COX-2 in the periodontal tissue at 8 (A) and 12 weeks (B) after treatments. Stronger and more positive expression of COX-2 was observed in the L and LS groups compared to the N and L groups, respectively. The rectangle indicates the areas (100×) observed under the microscope at higher (400×) magnification. Red arrows indicate fibroblasts, and black arrows indicate osteoclasts. Microscopic original magnification at 100× and 400×, respectively. (C) The comparison of COX-2 expression in the periodontal tissue among the N, S, L, and LS groups. Data represent the mean ± *SEM* of COX-2 expression as the average optical density (AOD) in each group. * indicates $p < .05$ compared to the N group; ♢ indicates $p < .05$ compared to the S group; # indicates $p < .05$ compared to the L group [Colour figure can be viewed at wileyonlinelibrary.com]



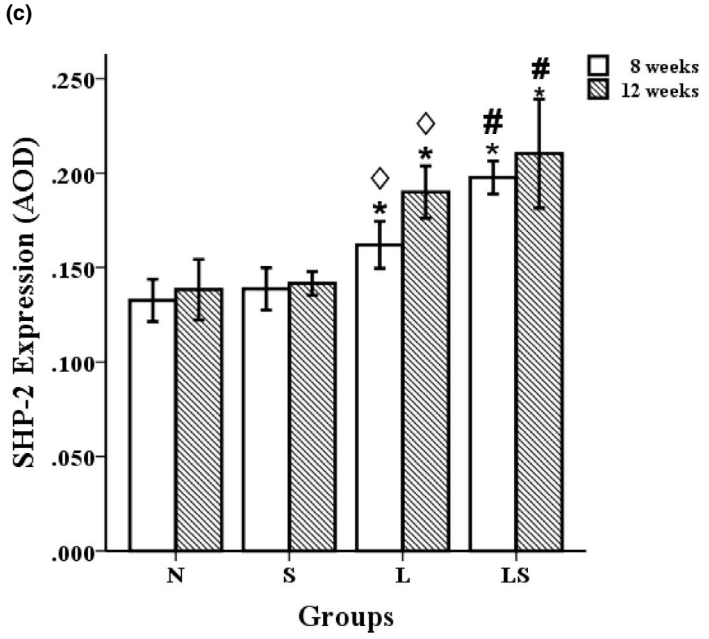


FIGURE 5 A, B, Immunohistochemical expression of SHP-2 in the periodontal tissue at 8 (A) and 12 weeks (B) after treatments. Stronger and more positive expression of SHP-2 was observed in the L and LS groups compared to the N and L groups, respectively. The rectangle indicates the areas (100×) were observed under the microscope at higher (400×) magnification. Red arrows indicate fibroblasts, and black arrows indicate osteoclasts. Microscopic original magnification at 100× and 400×, respectively. (C) The comparison of SHP-2 expression in the periodontal tissue among N, S, L, and LS groups. Data represent the mean ± *SEM* of SHP-2 expression as the average optical density (AOD) in each group. * indicates $p < .05$ compared to the N group; ♢ indicates $p < .05$ compared to the S group; # indicates $p < .05$ compared to the L group [Colour figure can be viewed at wileyonlinelibrary.com]

There was also a significant increase in COX-2 expression in the LS group in comparison with the N and L groups at 8 and 12 weeks ($p < .05$, Figure 4C), which exhibited a time-dependent tendency as well.

3.5 | Immunohistochemical expression of SHP-2 in periodontal tissue

The expression of SHP-2 was mainly located in the cytoplasm of fibroblasts (Figure 5A,B; red arrow) and osteoclasts (Figure 5A,B; black arrow). At 8 and 12 weeks, there were weak and few SHP-2 expressions in the N (Figure 5A,a,e, and Figure 5B,a,e) and S groups (Figure 5A,b,f, and Figure 5B,b,f). A significant positive existence of the expression of SHP-2 was detected in the L group (Figure 5A,c,g, and Figure 5B,c,g). Stronger and more positive expression of SHP-2 was observed in the LS group (Figure 5A,d,h, and Figure 5B,d,h). There were no positive stainings among the SHP-2 negative controls. Figure 5C shows the analysis of SHP-2 expression in the four groups. The expression of SHP-2 in the L group was significantly increased compared to the N and S groups (both $p < .05$, Figure 5C). And there was a significant increase in SHP-2 expression in the LS group in comparison with the N and L groups at 8 and 12 weeks of treatments ($p < .05$, Figure 5C), which also exhibited a time-dependent tendency.

4 | DISCUSSION

In the present study, we investigated the effects of ETS on periodontal tissue in a rat model of periodontitis and the molecular factors potentially associated with inflammation and bone loss. We examined alveolar bone loss and the expression of COX-2 and SHP-2 in periodontal tissue in rats under an ETS condition at different time periods. Our results demonstrated that ETS increased alveolar bone resorption in periodontitis model rats. Further, ETS enhanced the expression of COX-2 and SHP-2 in periodontal tissue, indicating that ETS is associated with the process of periodontal inflammation and alveolar bone resorption in periodontitis.

Environmental tobacco smoke remains a major public concern around the world. Based on the health consequences of smoking in the United States, the economic and healthcare effects of ETS remain substantial (Makadia, Roper, Andrews, & Tingen, 2017). Some studies have concluded that many compounds in ETS such as nicotine, ammonia, nitrogen, and sulfur oxides have harmful effects on periodontal health (Akinkugbe et al., 2016). Periodontitis is an inflammatory condition of the periodontal tissue (Yucel-Lindberg & Båge, 2013). However, the inflammatory factors involved in aggravating the destruction of periodontal tissues under ETS require further exploration.

Alveolar bone loss is the most remarkable feature of periodontitis. PGE2, which can be derived from COX-2, is associated with periodontal tissue damage in periodontitis, as it is a potent stimulator of bone resorption (Kim et al., 2012; Sundar, Javed, Romanos, & Rahman, 2016). The most well-known cytokines involved in periodontitis are TNF-α and IL-1β, which may influence and amplify the inflammatory response, causing tissue destruction and bone loss. The release of TNF-α and IL-1β during periodontitis activates osteoclasts and osteoblasts to produce COX-2-mediated PGE2, triggering bone resorption (Guimarães et al., 2016). Furthermore, it has been documented that COX inhibitors (COX-2 selective or non-selective) can effectively reduce bone destruction in periodontitis (Jagadish & Mehta, 2014; Queiroz-Junior et al., 2009). Recent research has demonstrated that treating periodontitis rats with COX-2 inhibitors results in significantly reduced alveolar bone loss as well as decreased PGE2 expression compared to the untreated periodontitis group (Moro et al., 2019). Therefore, it can be concluded that COX-2 is a sensitive marker of inflammation and destruction of periodontal tissue in periodontitis. In the present study, the L group treated with ligature displayed a significant increase in damage to the periodontal supporting tissues. In addition, we demonstrated that ligature-induced periodontitis is associated with increased expression of COX-2 in both fibroblasts and osteoclasts. These results are in concordance with the previous literature which suggests that levels of PGE2 or COX-2 were elevated in progressing periodontal lesions (Taxman et al., 2012). According to our results for the levels of alveolar bone loss and expressions of COX-2 and SHP-2 (Figure 2, 4, and 5), the influence of ligature on the periodontal tissues was more important than that of smoking factors during the progress of periodontitis. However, when rats with periodontitis in the LS group were exposed to ETS, the alveolar bone loss, as well as COX-2 expression, in periodontal tissue was significantly increased compared to those in the L group. Therefore, our results suggest that ETS can accelerate alveolar bone resorption and aggravate an inflammatory reaction in periodontitis in rats.

In this study, we also detected the involvement of SHP-2 in periodontitis. SHP-2, a ubiquitously expressed protein tyrosine phosphatase, is required for many signal transductions (Chong & Maiese, 2007). It is believed that SHP-2 is highly correlated with multiple cell signaling processes, such as growth, survival, proliferation, differentiation, and apoptosis, and is particularly important in chronic inflammation (Coulombe & Rivard, 2016).

We observed that the expression of SHP-2 in the L and LS groups was significantly higher than that in the N and S groups after interference factors were inflicted upon the experimental rats and that the expression of SHP-2 was located in fibroblasts and osteoclasts of periodontal tissues. These results suggest that SHP-2 is involved in the pathogenesis of periodontitis. Fibroblasts are major cells in periodontal tissue. MacGillivray et al. (2003) observed that SHP-2 plays pivotal roles in IL-1β-induced maturation of focal adhesions in fibroblasts and consequently in modulating the signaling pathways leading to ERK activation. SHP-2 can regulate fibroblast activation and fibrosis by controlling TGFβ-induced STAT3 signaling. Inactivation of SHP2 prevents TGFβ-induced JAK2/STAT3 signaling, reduces fibroblast activation,

and ameliorates experimental fibrosis (Zehender et al., 2018). Osteoclasts, balanced with osteoblasts, are responsible for the resorption of mineralized matrix and are pivotal in the process of bone remodeling. In the case of periodontitis, the dynamic reconstruction balance of alveolar bone is destroyed via inflammatory factors and cytokines; the activity of osteoclast differentiation is responsible for the alveolar bone resorption (Schenkein, Barbour, & Tew, 2007). SHP-2 is one of the key factors in the process of osteoclast formation and plays an important role in bone resorption (Shalev & Elson, 2019). SHP-2 regulates osteoclastogenesis by promoting preosteoclast fusion and is involved in the modulation of bone formation (Zhou et al., 2015). Macrophage colony-stimulating factor (M-CSF) and receptor activator of nuclear factor kappa B ligand (RANKL) are two essential factors in the process of osteoclast differentiation and formation. A study revealed that osteoclastogenesis stimulated by M-CSF and RANKL was defective in SHP-2-deficient mice. Osteoclasts were absent from the bones of SHP-2-deficient mice (Bauler et al., 2011). Obvious expressions of SHP-2 in the L group here suggest that SHP-2 is involved in the process of periodontal tissue destruction in periodontitis. The release of SHP-2 during periodontitis may regulate the fibroblast activation and/or activate osteoclasts to induce bone resorption. A significantly increased expression of SHP-2 in the LS group compared with the L group suggests that ETS could influence SHP-2 expression significantly and accelerate alveolar bone resorption in periodontitis conditions and SHP-2 is involved in the process of ETS interference through the impact on fibroblasts and osteoclasts.

5 | CONCLUSIONS

Our findings indicate that ETS increases alveolar bone loss in periodontitis with enhanced expression of COX-2 and SHP-2 in periodontal tissues. Further investigation is needed to explore the role of COX-2 and SHP-2 in ETS-associated periodontitis.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTION

Xiangjun Li: Investigation; Writing-original draft; Writing-review & editing. **Xiangyang Liang:** Data curation; Formal analysis. **Shujuan Li:** Methodology; Project administration. **Xia Qi:** Supervision; Visualization. **Ning Du:** Software; Validation. **Dongru Yang:** Conceptualization; Funding acquisition; Resources.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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