Research progress of ligature-induced periodontitis model in mice

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Abstract: *Periodontitis is a chronic inflammatory disease caused by bacterial pathogens, characterized by inflammatory infiltration and progressive alveolar bone loss. This paper provides an overview of the mechanism, methods and treatment of model establishment and provides relevant information for future studies using the ligature-induced periodontitis model in mice.*

Keywords: *ligature, Periodontitis model, mice, treatment*

1. Introduction

Periodontitis is a biomembrane mediated chronic inflammatory disease of the supporting tissues of the teeth, which destroys the gingiva and alveolar bone and ultimately leads to tooth loss[1], affecting approximately 3.5 billion people worldwide[2].The prevalence of periodontitis is quite high, ranging from 17.6% to 90% reported in different regions of the world[3-4]. The Fourth National Oral Health Epidemiology Survey reported that less than 10% of adults in China are periodontally healthy and the detection rate of periodontal pockets of 4 mm and above is over 50% [5]. In addition, there are numerous epidemiological and experimental studies showing that periodontitis is closely associated with various diseases such as cardiovascular disease [6], diabetes [7] and Alzheimer's disease [8], posing a serious threat to human health. Therefore, the search for the etiology and treatment of this disease has been a hot topic of research for scholars at home and abroad.

Because clinical studies of periodontitis involve interactions between genes, behavior and plaque, and because human studies face challenges, such as ethical issues and differences in individual propensity to progress with periodontitis, animal models are used to assess the pathogenesis of periodontitis and to identify new drugs or therapeutic interventions prior to clinical trials in periodontal studies [9]. Mice are convenient and versatile animal models [10] that have been used in periodontal studies. The main focus is on microbiology and immunology. The mouse model has the advantages of a wide range of genetically engineered strains, relatively low cost, short study times and a molar gingival zone similar to that of humans. In addition, a large amount of data has been collected on the immune system, allowing a more in-depth understanding of the pathogenesis of periodontitis [11-12].

Over the last few decades, various models of periodontitis in mice have been established [13]. Ligation-induced periodontitis models offer several advantages over other models, including rapid disease induction, predictable bone loss and the ability to study periodontal tissue and alveolar bone regeneration[10]. Ligation removal can play a large role in the study of inflammatory regression and healing response. It has also been shown that ligation does not cause intense inflammation and alveolar bone loss in germ-free mice, suggesting that the persistent inflammation and bone loss in the ligation model is dependent on the accumulation of bacteria during the ligation process and is not due to mechanical trauma[14]. Due to the complexity of the pathogenesis and clinical manifestations of periodontitis, the establishment of stable and reliable animal models that can mimic the clinical features of human periodontitis is one of the key points of research. This paper reviews the research progress of ligature-induced periodontitis model in mice.

2. Ligature-Induced Periodontitis in Mice

The pure silk thread ligation is a simple and effective method to establish periodontitis animal model. The operation method is mainly to ligate fine silk, nylon wire, orthodontic stainless-steel wire, etc. in the cervical area or gingival sulcus of the mice's teeth, so that the continuous accumulation of plaque at the ligation site can cause the destruction of periodontal support tissues and the resorption of alveolar bone in the corresponding area to achieve the purpose of establishing periodontitis model in a short period of time. Currently, the silk thread ligation method is widely used because it is simple and less time-consuming to perform, but ligatures can become loose or lost, so it is important to regularly check for dislodgement of the ligature material during modelling or replace it as needed [15-16].

Lin[17] used 5-0 silk thread tied around the second molar of mice during ligation according to the protocol of Abe et al. and found that the ligation-induced periodontitis model showed acute alveolar bone loss and soft tissue inflammation in the early stages (within 7 days). Yoon[11] also demonstrated this disease progression in a human ligature-induced periodontitis model, with no significant differences in the first 3 days and substantial inflammatory infiltration and alveolar bone loss on days 4-8.

During the operation we found that the small size of the mice's mouths and teeth created technical difficulties in the placement of the ligature wires. Marchesan[10] set up a 3D printing and ligature holder to place a double-knotted 2.5 mm silk ligature between two mouse molars to improve modelling speed. However, the application of this method is somewhat limited by the fact that 3D printing technology is not yet widespread. Li[18] provided a new and effective method of ligating between mandibular molars in mice by means of C+NiTi root canal files and orthodontic wires (0.20 mm in width and 3-5 mm in length). The results showed an abrupt increase in bone loss in the periodontitis group on days 3-6 and a relatively smooth progression on days 6-10. In addition, the value of the distance from the enamel bone boundary to the top of the alveolar bone crest in the 1-day group was similar to that of the unligated control group, indicating that the progressive bone loss observed reduces the likelihood of injury triggered by the placement of orthodontic wires.

Ai[12] optimised the ligature model by bending the orthodontic filament into a small loop to both reduce the rate of ligature loss and mitigate mucosal tissue damage. It was found that a significant amount of alveolar bone loss occurred on day 6 rather than day 3, with progressive bone resorption occurring between days 6-12. This suggests that there can be variation in the timing of bone loss due to the diversity of ligature models.

Ligation-induced experimental periodontitis allows analysis of many aspects of periodontitis, including bacterial interactions and ecological imbalances, periodontal inflammatory response and osteobiology, and is therefore considered a suitable model for studying oral microbial and host interactions[15]. The destruction of periodontal tissue by periodontitis in humans is caused by microorganisms. The host immune response to bacteria is thought to play a very important role in the progression of periodontal tissue destruction. In contrast, the ligation-induced periodontitis model in mice facilitates the observation of the host response[19].

3. Ligation-induced periodontitis model with bacteria in mice

Porphyromonas gingivalis (Pg) plays an important role in periodontitis by releasing virulence factors such as Lipopolysaccharide (LPS),which induces host immune response and periodontal tissue damage[16].LPS,a component present in the cell wall of Gram-negative bacteria, has been shown to induce the secretion of a variety of inflammatory factors[20], including prostaglandins and cytokines,and these mediators cause periodontal tissue inflammation through the activation of multiple pathways[21].Pg-LPS can continuously stimulate host immune cells, leading to the release of a large number of bioactive substances, such as cytokines, nitric oxide and reactive oxygen species,causing cell damage and apoptosis, and ultimately inflammation[22], and can also induce cognitive impairment in mice [23].

The periodontal inoculation model consists of local microinjections of bacteria or some isolated bacterial component, such as LPS, into the proximal palatal gingiva between the molars[24].This approach promotes periodontal inflammation as evidenced by increased pro-inflammatory cytokines, migration of junctional epithelial cells to the root tip and osteoclastogenesis, which in turn causes persistent alveolar bone resorption [25]. Studies have shown that the 7 th and 15 th days of ligation model have a great influence on bone loss, but the intensity of bone loss decreases on the 30 th day, while the sustained bone loss began from the 15 th day in the gingival injection and gastric perfusion model

constructed by Pg [26]. A faster rate of alveolar bone loss, higher levels of inflammatory factor expression, periodontal immune cell infiltration and osteoclast activation have also been reported in ligated models compared to Pg and Pg-LPS models[12]. The role of Pg has now been confirmed by 16s rRNA gene sequencing[27], so we can also add bacteria to ligation to induce a mouse model of periodontitis.

Kim[28] established artificial periodontal pockets by placing ligature wires around the maxillary second molars in mice. The ligatures were removed after 1 week of placement and PBS or Pg was applied to the gingival pockets for 5 weeks (3 times a week). When measuring alveolar bone loss from the enamel bone boundary to the top of the crest of the alveolar bone, bone loss was found to occur only at the location where Pg was applied. This indicates that the Pg-induced bone loss is specific. Akkaoui[29] found significantly higher concentrations of TNF-α, IL-6 and IL-1β in gingival tissue in ligation + Pg-LPS injection compared to ligation alone.

Cheat[30] found that the number of osteoclasts on the alveolar bone surface of WT mice ligated alone was reduced by about 50% and that the expression level of NLRP3 inflammatory vesicles was increased by 68% in Pg-treated mice. This suggests that the presence of Pg induces a stronger immune response and promotes the production of cytokines in periodontal tissue and peripheral blood.

The ligation and Pg-LPS models are representative models of human periodontitis, however, it remains controversial as to the most appropriate method of periodontitis formation in ligation-induced periodontitis models.

4. Microbiology

Periodontitis is a microbially driven disease and dental plaque is a complex microbial community of which bacteria are the most abundant component [31]. Ai [12] used 16S rDNA whole genome sequencing in an orthodontic wire ligation mouse model and found that the dominant mouse oral phylum was consistent with that of humans. In addition, principal component and principal coordinate analysis showed that the phylum Aspergillus and Clostridium were most abundant in the ligation model, suggesting that they were associated with periodontitis, and the thick-walled phylum and the actinomycete phylum were most abundant in the healthy control group, indicating that the oral microbial community shifted from symbiotic to non-symbiotic. These results suggest that ligation leads to a dysbiosis of the oral microbial community.

In an ecologically dysregulated environment, some microorganisms interfere with the physiological functions of the immune system, causing alterations in the oral microbiota and neutrophil recruitment, which are associated with alveolar bone loss [32]. The massive accumulation of neutrophils in periodontal pockets is a hallmark of periodontitis and is a contribution to tissue destruction through the release of matrix metalloproteinases and reactive oxygen species. Histology and immunohistochemistry can be used as an aid to measure the level of inflammation, tissue degradation and immune response generated in each experimental group [33].

5. Immunology

In periodontitis, inflammatory damage by microorganisms, abnormal host-microbiota action and inappropriate and sustained activation of the immune system induce a pro-inflammatory immune response, leading to inflammatory bone loss. Pro-inflammatory immune responses are initiated by toll-like receptors and other pattern recognition receptors that play a key role in acute inflammatory responses, cell signaling and apoptosis. The tlr9-mediated immune response can induce inflammatory alveolar bone loss [34-35]. In addition, ST2 receptors are expressed on many immune and non-immune cells and activate the NF-κB and MAPK pathways, leading to the production of inflammatory cytokines [36].

PI3Ks are a family of enzymes involved in a variety of cellular functions and pathological conditions, of which the gamma and delta types are involved in the control of the immune system and inflammation, and PI3Ks control the IL-17 signalling pathway, which induces RANKL expression in osteoblasts/osteoclasts. Treatment of mice with ligamentous periodontitis using PI3Kγ inhibitors showed reduced alveolar bone resorption and downregulation of IL-17A and RANKL gene expression in gingival tissue[37]. It has been found that platelets can not only interact directly with other inflammatory cells in the immune inflammatory response, but also regulate other inflammatory cells indirectly by secreting immune mediators. On day 7 of ligation in mice, a diffuse infiltration of platelets and leukocytes was observed in the gingival tissue and the proportion of their aggregates was significantly increased[38].

This suggests that platelet-leukocyte interactions are critical for the progression of inflammation.

6. Treatment

Periodontitis is a common type of inflammatory bone loss and a risk factor for systemic disease. In addition to basic maintenance and surgical treatment, effective medication can be used to control the periodontal inflammatory response and inhibit alveolar bone resorption, such as the application of peptides, proteins and plant extracts. The pathogenesis of periodontitis involves a dysregulation of inflammation, which is the target of periodontitis treatment.

Sema4D, a member of the Semaphorin family of secreted membrane-bound proteins, induces alveolar bone resorption with elevated local soluble Sema4D (sSema4D), TNF-α and RANKL when ligatures are attached to maxillary molars in mice for 7 days. Removal of the ligature and administration of the drug significantly promoted bone regeneration. The data suggest that sSema4D released by osteoclasts may perform a dual function, reducing bone formation while upregulating bone resorption [39]. The natural animal form of vitamin D is cholecalciferol (also known as vitamin D3). Vitamin D3 requires two hydroxylations to become 1, 25-dihydroxyvitamin D3 (1,25(OH)$_2$D3) before it can be activated, and exogenous 1,25(OH)$_2$D3 supplementation reduces alveolar bone loss and gingival inflammation due to ligating periodontitis in mice [40].

Resveratrol, a natural polyphenol found in different plants, was administered intraperitoneally at 10 mg/kg of resveratrol monomer or dimer on the 15th day after induction in a mouse model of experimental periodontitis to activate the Nrf2 pathway, promote bone healing and reduce local oxidative damage [41]. PPAR is a member of the nuclear hormone receptor and acts as a transcription factor regulating gene expression in metabolism and inflammation. After anti-inflammatory treatment with PPARα agonists, the ligated group showed a significant reduction in TNF-α, IL-1β, IL-6 mRNA levels and RANKL/OPG ratio, and a significant reduction in TRAP-positive cells[42]. Erythropoietin (EPO) promotes the differentiation of mesenchymal stem cells into osteoblasts and is involved in the interaction between osteoblasts and osteoclasts, and has also been shown to regulate excessive inflammation by antagonising and modulating pro-inflammatory cytokines, such as TNF-α [43]. Ginseng is a traditional Chinese herb with a long history of medicinal use. Ginsenoside Rd (Rd) is a bioactive component extracted from ginseng and has been shown to have antibacterial and anti-inflammatory activity. Rd treatment of periodontitis in mice resulted in reduced levels of inflammatory factors (IL-1b and IL-6) in periodontal tissue and a significant reduction in resorption and destruction of alveolar bone [44].

Pyrroloquinoline quinone (PQQ) is a new coenzyme that is widely found in foods. Ligation-induced periodontitis mice supplemented with a PQQ diet for 2 weeks and found significantly lower alveolar bone loss, osteoclast numbers, cellular senescence-associated cells and cytokine expression compared to untreated periodontitis mice. It may be that periodontal damage is alleviated by regulating redox homeostasis and cellular senescence [45].

Drugs currently used to treat diabetes may have a direct beneficial effect on periodontitis by reducing inflammation, bone loss and enhancing bone formation [46]. Patients with both diabetes and periodontitis have higher levels of systemic inflammatory markers than those with periodontitis alone, and treatment of periodontitis has been shown to result in a substantial reduction in systemic inflammatory markers in diabetic patients compared to those with periodontitis in general health [47]. Bisphosphonates such as zoledronic acid are currently the first-line treatment for degenerative bone diseases such as osteoporosis. Evaluation of 3- and 10-week ligation-induced periodontitis mice treated with zoledronic acid showed increased inflammatory infiltration and bone loss at the ligation site in the 10-week group, along with a 61% increase in the number of osteoclasts [48]. Periodontitis induced by prolonged ligation around the second molar in ApoE -/- mice revealed a significant increase in the levels of pro-inflammatory cytokines such as IL-1β, IL-6 and TNF-α [49].

The ligature model was used to study the effects of various treatments and therapeutic agents on periodontitis, including reduction of bacteria and their causative factors, anti-inflammation, and prevention of alveolar bone loss. It helps us to find suitable therapeutic agents and to understand the underlying mechanisms.

7. Conclusions

The ligature-induced periodontitis model in mice provides useful information on the pathogenesis,

microbial response and treatment of periodontitis. Although the model does not reflect all aspects of the mechanisms or causes of periodontitis in humans, the triggering of significant bone loss is a key feature of the model. This model is therefore an ideal technique for studying the mechanisms of periodontitis-induced alveolar bone resorption and inhibiting alveolar bone loss. Ligation-induced periodontitis appears to be a model of acute periodontitis, and although the model may not reflect the long-term bone loss and inflammatory infiltration of chronic periodontitis, the ligation-induced experimental periodontitis model in mice is an effective way to explore the molecular mechanisms of periodontitis. A practical and reproducible model that truly mimics the natural pathogenesis of periodontitis in humans needs to be further explored and developed.

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