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Literature Review

Rat Models of Periodontitis: A Focused Review on Ligature, Bacterial Inoculation, and LPS-Induced Approaches

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Periodontitis, animal model, ligature, lipopolysaccharide, bacteria

ABSTRACT

Introduction: Periodontitis is a common chronic inflammatory disease that leads to progressive destruction of the gingiva, periodontal ligament, and alveolar bone. This is due to a dysbiotic oral microbiome that triggers an exaggerated host immune response. Preclinical rat models are indispensable in periodontal research, allowing for controlled investigations of disease mechanisms and therapeutic interventions.

Review: This review examines three widely used rat models of experimental periodontitis, including ligature-induced, oral bacterial inoculation, and lipopolysaccharide (LPS) injection. The ligature-induced model is favored for its rapid and reproducible induction of localized inflammation and bone loss, although mechanical trauma may confound the microbial effects. Oral inoculation with pathogens such as Porphyromonas gingivalis simulates natural infections and chronic progression, offering greater biological relevance. The LPS model provides a controlled inflammatory stimulus that facilitates the study of host immune pathways and cytokine expression; however, it lacks microbial complexity. Recent studies have explored the combination of ligature and LPS methods to synergistically intensify inflammation and bone loss, thereby improving model robustness for therapeutic evaluation.

Conclusion: Each animal model offers specific benefits for studying periodontitis development and treatment. Although these models have certain limitations, ongoing improvements in research methods will increase their relevance to human health. Advances in technology, imaging, and microbiological techniques are expected to improve the accuracy and usefulness of these models. These developments will enhance our understanding of the disease and contribute to the discovery of more effective and targeted treatment options for periodontitis.

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INTRODUCTION

Periodontitis is a chronic inflammatory disease characterized by the progressive destruction of the supporting structures of the teeth, including the gingiva, periodontal ligament, and alveolar bone.[1,2] The disease results from a dysbiotic shift in the oral microbiome, which elicits an aberrant host immune response leading to tissue breakdown and alveolar bone resorption.[1] If left untreated, periodontitis can result in tooth mobility, tooth loss, and systemic complications, significantly affecting patients' quality of life.[2]

Preclinical animal models are indispensable tools in periodontal research, offering controlled environments to study disease pathogenesis and evaluate novel therapeutic strategies.[3] These models help bridge the translational gap between in vitro findings and human clinical studies by enabling detailed mechanistic insights and reproducible intervention testing.[3] Among animal models, rodents, especially rats and mice, are widely used due to their manageable size, lower maintenance cost, well-characterized immune system, and compatibility with diverse experimental manipulations.[3,4]

Three commonly utilized rat models for inducing periodontitis include the ligature-induced model, oral bacterial inoculation, and lipopolysaccharide (LPS) injection.[4–6] The ligature model, which involves placing a silk or nylon suture around molars to promote plaque accumulation and inflammation, is appreciated for its rapid onset and reproducibility.[7,8] Oral inoculation with human or murine-derived periodontal pathogens such as *Porphyromonas gingivalis* mimics the natural route of infection, although colonization efficiency can be variable.[4] In contrast, the LPS injection model induces localized inflammation by directly stimulating Toll-like receptor pathways, providing a robust and quantifiable inflammatory response with minimal microbial interference.[9] Each model offers distinct advantages and is selected based on specific research aims, including host-pathogen interactions, immune modulation, and bone loss kinetics.

REVIEW

Ligature-induced periodontitis

The ligature-induced periodontitis (LIP) model is one of the most commonly used preclinical approaches for inducing periodontal disease in rats. This model involves the placement of a ligature, typically made from silk, nylon, or stainless steel wire, around the cervical region of the maxillary or mandibular molars to initiate localized plaque accumulation and gingival inflammation.[10,11] The ligatures serve both as mechanical irritants and as retention sites for bacterial biofilm, triggering a host inflammatory response that mimics key features of human periodontitis.[7]

Ligatures are generally applied around the first molars, and the duration of placement typically ranges from 7 to 21 days, depending on the study objectives and desired severity of bone loss.[3] During this period, rapid progression of periodontal inflammation is observed, leading to significant alveolar bone resorption as confirmed by histological and micro-computed tomography (micro-CT) analyses.[3,11] This characteristic makes the LIP model particularly useful for evaluating the acute phases of periodontal breakdown and host response.

Due to its simplicity, consistency, and rapid onset of disease, the LIP model has become a standard tool for evaluating anti-inflammatory agents, regenerative biomaterials, and surgical procedures aimed at reversing or mitigating periodontal destruction.[10,12] It enables reproducible experimental conditions and controlled induction of disease severity, facilitating clear comparisons between treatment groups.[3,13]

However, the LIP model has limitations. The act of placing ligatures introduces mechanical trauma to the gingival tissues, which may confound the inflammatory response by introducing non-microbial inflammation.[11] Additionally, the artificially rapid progression of disease may not accurately reflect the chronic, multifactorial pathogenesis of human periodontitis.[14,15] Despite these concerns, the LIP model remains indispensable for short-term mechanistic studies and preclinical screening of therapeutic interventions in periodontitis.

Bacterial oral inoculation and LPS-induced periodontitis

Oral inoculation with periodontal bacteria and direct lipopolysaccharide (LPS) injection are two alternative methods for inducing experimental periodontitis in rats, each offering distinct research advantages. These models complement the ligature-induced model by allowing researchers to focus on specific microbial or immune pathways in disease progression.

The oral inoculation model aims to mimic natural disease progression by introducing pathogenic bacteria such as *Porphyromonas gingivalis* into the oral cavity of rats.[16] Repeated application of bacterial suspensions over several weeks allows for microbial colonization, gingival inflammation, and bone loss that better represent the chronic nature of human periodontitis.[4,16] Although this method offers high biological relevance, colonization efficiency can vary and can co-aggregate with competing commensal bacteria.[4,17–19]

The LPS injection model relies on the direct administration of purified bacterial LPS, commonly derived from *P. gingivalis*, into the gingival tissues.[9,20] This approach induces a strong localized immune response characterized by elevated pro-inflammatory cytokines such as IL-1β and TNF-α, leading to osteoclastogenesis and alveolar bone loss.[20] Studies have shown that repeated LPS injections can result in neuroinflammation and systemic effects, suggesting a link between periodontitis and cognitive dysfunction.[21] The LPS model is particularly valuable for studying molecular inflammatory pathways and testing targeted anti-inflammatory therapies.[22]

To enhance the severity and consistency of the disease, some studies have employed a combination of ligature placement and LPS injection. This dual induction model intensifies both microbial and immunologic components of periodontitis, leading to more pronounced alveolar bone resorption and connective tissue breakdown compared to either method alone.[20] It has been validated as a reproducible and efficient model to evaluate both preventive and regenerative periodontal therapies *in vivo*.[20]

These models are commonly used to investigate pathophysiological mechanisms and test the efficacy of novel interventions such as anti-inflammatory drugs, biomaterials, and host-modulating therapies.[3,23] However, their artificial nature and rapid progression may limit their translational value in humans. Bacterial oral inoculation requires technical expertise and has variability in pathogen colonization, while LPS injections do not fully replicate the polymicrobial complexity of periodontitis.[20,21]

Comparison of rat periodontitis models

Experimental periodontitis models in rats have significantly contributed to our understanding of the mechanisms of periodontal disease and facilitated preclinical testing of therapeutic interventions. Among the models discussed, the LIP model remains the most widely used because of its straightforward methodology, reproducibility, and rapid disease induction. By placing ligatures around the molars, plaque accumulation is mechanically promoted, leading to localized inflammation and alveolar bone loss typically within 1 to 3 weeks.[3,10] This controlled environment enables consistent evaluation of bone resorption and tissue destruction, making it ideal for studying acute responses and the efficacy of regenerative therapies.[8,13] However, the model's reliance on mechanical trauma introduces a confounding factor that may amplify inflammatory responses beyond those triggered by natural bacterial colonization.[11] Moreover, the accelerated progression seen in LIP may not fully replicate the chronic and multifactorial nature of human periodontitis.[3,21]

The bacterial oral inoculation model attempts to mimic natural infection by repeatedly applying periodontal pathogens, notably *Porphyromonas gingivalis*, to the oral cavity.[3] This method more closely represents the polymicrobial etiology and chronic progression of periodontitis, allowing detailed exploration of host-pathogen interactions and immune regulation over an extended timeframe.[20] Nevertheless, achieving consistent and stable colonization remains challenging due to the complexity of the oral microbiome and the need for antibiotic pre-treatment to reduce competing flora, which may introduce additional variability.[20] This limits its reproducibility compared with the ligature model.

LPS-induced periodontitis, through direct gingival injection of bacterial endotoxins, offers precise control of the inflammatory stimulus and facilitates mechanistic studies of host immune responses and

osteoclastogenesis.[10,21] This model rapidly elicits elevated cytokine expression and alveolar bone resorption, providing a platform for examining inflammatory signaling pathways and systemic effects, such as neuroinflammation associated with periodontitis.[20,21] However, the absence of live bacteria and biofilm formation in LPS models restricts its capacity to simulate the complex microbial environment crucial for disease pathogenesis.[21]

The combination of ligature and LPS injection has emerged as a strategy to synergistically harness mechanical and endotoxin-driven inflammation, resulting in exacerbated bone loss and inflammatory markers beyond those induced by single methods.[20] This dual approach enhances disease severity and consistency, offering a robust platform for evaluating novel therapeutic agents targeting both microbial and host-derived factors.[20]

Strengths and limitations

Each model exhibits distinct strengths that are suited to specific research goals. The rapid and reproducible induction of the ligature model makes it the gold standard for therapeutic screening. In contrast, the oral inoculation model mimics natural infection dynamics and provides invaluable insights into microbial pathogenesis. The ability of the LPS model to isolate inflammatory mechanisms is critical for understanding host responses and systemic implications. Nonetheless, limitations such as artificial induction, mechanical trauma, variability in bacterial colonization, and lack of microbial complexity necessitate careful consideration when extrapolating findings to human disease.[10,11,20,21]

Future perspectives

Advances in molecular biology, imaging, and genetic engineering promise to refine these models and enhance their translational relevance. Incorporating omics technologies and in vivo imaging could offer unprecedented insights into disease progression and host-microbe interactions.[3] The development of genetically modified rats with altered immune or bone metabolism pathways may provide more accurate disease models that reflect patient heterogeneity. Moreover, integrating microbiome transplantation or synthetic biofilms could address the limitations of bacterial complexity in the current models. Finally, the evolution of combined or hybrid models integrating multiple induction methods and systemic conditions, such as diabetes or aging, will better simulate the multifactorial etiology of periodontitis. Such innovations will accelerate the discovery of targeted, personalized therapeutics to mitigate the significant public health burden imposed by periodontal disease.

CONCLUSION

Rat models of periodontitis, including ligature-induced, oral inoculation, and LPS-induced methods, provide valuable platforms for investigating the mechanisms of periodontitis and testing interventions. Each model has specific advantages and limitations related to disease induction, progression, and microbial complexity. Careful consideration of these factors is essential to align the model selection with the research objectives. Continued refinement and integration with advanced methodologies are necessary to improve translational relevance and to support the development of effective periodontal therapies.

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