THE RAT AND THE SHEEP, ANIMAL MODELS FOR THE STUDY OF PERIODONTITIS AND INDUCED PERIIMPLANTITIS OF BACTERIAL STRAINS SPECIFIC TO HUMAN ORAL MICROBIOTE

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Abstract

Periodontitis and periimplantitis are two diseases that have as a common element the progressive loss of alveolar bone, eventually leading to the loss of teeth and dental implants. The causes of the two diseases are multiple but the composition of the local bacterial biofilm is one of the important triggers. The aim of this review was to establish the main bacterial strains that can induce experimental periimplantitis and periodontitis as well as the techniques by which diseases can reproduce. The rat and the sheep are commonly used animal models in this branch of research because it reflects the main characteristics of human periodontitis or periimplantitis. The results obtained from the recent literature show that *Porfiromonas gingivalis, Aggregatibacter actinomicemcomitans, Streptococcus oralis* or *Fusobacterium nucleatum* (bacterial species commonly found in the human oral microbiota) are among the bacteria that can easily reproduce the two diseases of the oral cavity. Induction techniques include oral gavage, ligation technique, lipopolysaccharide injection, or the use of preinfected implant devices. The data accumulated in this review will be useful for research on the pathology of periodontal or periimplant diseases but also the approach of innovative therapies.

Key words: periodontitis, periimplantitis, rat, sheep, bacterial biofilm.

Periimplantitis is an osteolytic inflammatory disease (Koutouzis T. and colab., 2017) induced by a number of factors that result in orofacial implant failure (1-47%) (Sun J., 2014). This condition is a major topic of interest in the field of implantology because technical progress has been made, demand is growing, especially among the elderly population (Nickenig H.J, 2008, Passia N., 2017), and standardized therapeutic schemes for preventing and combating periimplant disease are still insufficient due to the uncertainty of the pathogenic mechanism involved. The pioneer of the dental implant is Per-Ingvar Branemark, who in 1978 presented the first dental devices in the form of titanium root (Brånemark P.I., 1986) thus demonstrating the possibility of osseointegration by bringing the implant into direct contact with the bone surface (Pătrașcu I., 2021). Over time, a wide variety of dental implants have been introduced to the market, each with the aim of better osseointegration and limiting rejection phenomena.

Periodontitis is a chronic immunoinflammatory disease of the periodontium that results in the progressive loss of gingival tissue, periodontal ligament and finally, alveolar bone (Pihlstrom B.L., 2005). This condition may be associated with host susceptibility (Schenkein H.A. 2006) but is primarily initiated by subgingival biofilms containing a gram-negative commensal microbiota and opportunistic pathogens, and the body responds by activating polymorphonuclear cells. They release destructive reactive oxygen (superoxide, proteinase) that destroys host tissue, eventually causing osteoclastic bone resorption (Chapple IL.C., 2002). In order to look for optimal treatment solutions, the implant must be differentiated from periodontitis, a condition with which it shares common characteristics (Mombelli A., 1995). Both describe an inflammation of the mucosa, increased depth of the gingival pocket, bone loss observable on radiographic examination and the presence of the bacterial biofilm (Lindhe J., 2008). As in periodontitis, the composition of the biofilm that develops in the pockets around the implant is dominated by gram-negative bacteria (Leonhardt A., 1999). Recent studies focus on the

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individualization of diseases and claim that there are significant differences in the composition of the biofilm (Dabdoub S.M., 2013) or on the body response, in the sense that inflammatory infiltrate of periimpantitis occurs around the implant, and periodontitis, plasma cells, macrophages and lymphocytes are found on the surface of periodontal teeth (Piattelli A., 1998, Lang N.P., 2011). Histopathological analysis of inflammatory infiltrate in periimplantitis shows that it crosses the bone barrier, migrating to the trabecular space (Lindhe J., 1992), as opposed to periodontitis, in which the inflammatory infiltrate is limited to soft tissues (Marinello, 1995, Ericsson, 1996, Persson, 1996, Gotfredsen, 2002).

Research on peri-implantitis has highlighted a number of factors involved including: shape, implant location, occlusal overload, time allotted to osseointegration, implant-abutment connection, release of metal particles (Zandim-Barcelos D.L., 2019) and last but not least pathogenic bacterial accumulation on implant surface. Since the early 1990s, experiments have sought to mimic periimplantitis based on the idea that bacteria are directly responsible for producing the phenomenon resulting in progressive bone loss (Lindhe J., 1992, Lindhe J., 2008), especially through the biofilms they develop on both the surface of teeth and implants. Biofilms can appear early, being dominated by species of streptococci and species of actinomyces (Kumar P.S., 2012, Quirynen M., 2002) which represent the substrate for additional bacterial attachment (Rosan B., 2000), and *Fusobacterium nucleatum*, the most common bacterium in dental plaque is the bridge with late biofilms characterized of bacteria such as *Treponema denticola or Porphyromonas gingivalis* (Kolenbrander P.E., 2010).

As the implantology industry is booming, it is necessary that the devices be evaluated preclinically both in vitro but especially in vivo. For this, it is crucial to find an animal model that helps to understand the triggering mechanisms of periimplantitis and that mimics the condition encountered among human patients. Various animal species such as rabbit, mouse, rat, guinea pig, dog, pig (Wancket L.M., 2014), sheep, goat or nonhuman primate have been included in biocompatibility studies or in experimental induction of periimplantitis. Ethical reasons, maneuverability, accommodation conditions, feeding conditions or clinical follow-up are essential elements in choosing the animal model, and today small animals such as mice or rats are preferred because they are genetically similar to humans in a 90%, and biologically and economically, is the best option. Of these two species, it seems that the rat is preferable because the mouse used in the research of dental implants did not provide sufficient clinical data to assess osseointegration or periimplantitis, in this animal model the oral cavity is poor in spongy bone (Yue G., 2020).

Sheep have many practical advantages over other animal models. Although sheep have become more widely used as experimental animals, there are not enough studies on their use for intraoral experiments. Instead, sheep are a popular animal model in bone research in recent years.

The aim of this review was to establish the main bacterial strains that can induce experimental periimplantitis and periodontitis in rats and sheep, but also the techniques by which the disease can reproduce.

MATERIAL AND METHOD

Search strategies

An electronic search for English language publications was conducted in June-July 2021, in PubMed / Medline, Web of Science, Google Scholar and Science Direct databases, in the search strategy using terms such as dental implant, periimplantitis, periodontitis, animal model, experimental periimplantitis, bacterial plaque, biofilm, sheep, rat. Inclusion criteria included experimentally induced periimplantitis, experimental periodontitis, rat / sheep as a model of periimplantitis or periodontitis, and all techniques and methods used to induce the two diseases. Studies aimed at inducing mucositis, animal models or induction sites other than the oral cavity were excluded from the search. For this review, 187 articles were analyzed, of which 42 (published after 2010 and until now) contributed to the collection of information of interest.

RESULTS AND DISCUSSIONS

The rat as a candidate model for periimplantitis

Rodents are the most commonly used animal models in biomedical research, and in the field of periodontology they have been widely used due to the many similarities with humans in terms of periodontal and histopathological anatomy (Sun J., 2020). Rats have the advantage of profitability, the ease with which they are manipulated and allow the standardization of experimental conditions in genetically similar individuals and human-like molar structure. They are suitable for the study of diseases related to the destruction and regeneration of tissues even if in terms of periimplantitis has the disadvantage of small animal size and continuous growth of dentition. Another disadvantage is that the microbiota of the rat is different from that of humans, their size is small and therefore the

amount of tissue analyzed is small, resulting in the need for a large number of animals (Helieh S., 2011).

The dental formula of the rat is I 1/1, C 0/0, Pm 0/0, M 3/3, and the incisor has no roots. This animal model is often used in experimental periodontitis, due to the periodontal anatomy of the molar region, which is very similar to humans (*Table 1*) (Yamasaki A., 1979). For example, the marsh rice rat (*Oryzomys palustris*) can develop periodontal disease from the age of 2 weeks (Helieh S., 2011), characterized by gingival inflammation, pocket formation, ulceration, alveolar bone resorption and tooth mobility, especially on the mandibular molars. Periodontal disease has been shown to be dependent on dietary factors, so soft, high-carbohydrate foods promote the disease among young animals, and a diet rich in protein and fat has reduced the severity of the disease (Helieh S., 2011, Shaw J.H., 1969).

Table 1

Similarities and histological differences of the oral cavity of the rat and human. (Listgarten M.A., 1975, Page R., 1982)

Similarities	Differences
superficial gingival bone and attachment of the junctional epithelium to the surface of the teeth	keratinization of the crevicular epithelium in rats
junctional epithelium appears to be a pathway for foreign substances, bacterial endotoxins and inflammatory cell exudates	the relationship between the gingival and junctional epithelium with desmosomal contact between the most superficial cells of the gingival epithelium and the non-keratinized cells of the junctional epithelium
	progressive change of the position of the molars in the three-dimensional space, resulting in the global movement in an occlusal-distal-buccal direction compared to the occlusal-mesial drift observed in humans
	the rat is resistant to periodontal disease
	weak inflammatory response in rats (neutrophils, few lymphocytes and an absence of plasma cells in the gingival tissues)

Sheep as a model of periodontitis and periimplantitis.

Their use has been reported in studies of critically sized bone defect models (Griffon, 2001), periodontal studies (Duncan, 2003), and techniques for augmenting facial bone / maxillary sinus (Haas, 2003). Their popularity is most likely related to their nature as higher-level vertebrates and their nonpet status. They are easily available, cheap to buy and maintain and respond well to surgical procedures (Salmon R, Duncan W, 1997). Disadvantages include difficulty in handling, requirement for large housing and lack of research information compared to other animal models. (An Y., Friedman R., 1999).

The dental anatomy of sheep differs significantly from that of humans. An edentulous area of 3 to 5 cm separates the mandibular incisors from the teeth of the cheek. The small and fragile mandibular premolars have a long and prominent hypsodont crown compared to the small mesial and distal root. Molar, premolar, periodontium and metabolic rate in sheep is similar to that in humans, as well as bone loss that occurs in sheep, on the age of aging (Vlaminck et al., 2008). The use of a sheep model in orthopedic infection studies was first reported in 1973, and since then they have been developed as models in chronic osteomyelitis studies. Orthopedic models using sheep and goats are well accepted, as their larger bone and spinal canal sizes allow the assessment of fasteners that would otherwise need to be modified for use in smaller animals, such as rabbits or rats (Stewart, 2012).

Sheep have a predisposition to periodontitis, a condition called “broken mouth“which according to microbiological research involves both local and disseminated bacteria in the rumen. The clinical manifestation is an acute one, most often associated with nutritional deficiencies. Diet has an important local effect on bacterial plaque formation and the development of periodontal inflammation. Vitamin deficiency, such as vitamin C, B12 and D, increases the prevalence and progression of periodontal disease, as well as reduced intake of magnesium, calcium, iron and zinc (Dommisch et al., 2018).

There is strong evidence to suggest that periodontitis is one of the factors leading to implant loss through the development of periimplantitis, and patients with periodontitis have a greater loss of dental implant and alveolar bone. The relationship between an oral biofilm-specific pathogen, the host response, and tissue destruction is a necessary first step toward the future goal of mechanistic studies under biofilm-mediated conditions, such as periodontitis or peri-implantitis (Lee D.W., 2014).

Numerous studies have attempted to identify pathogens associated with peri-implant infections. The methods were based on anaerobic cultures, microscopy, polymerase chain reaction, in situ hybridization of fluorescence or DNA-DNA hybridization, resulting in the detection of gram-negative, mobile cocci comprising *Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola* (red complex) also other species from *Treponema I to III* groups and *Synergistetes cluster A.* (Belibasakis G.N., 2016). In addition to these bacterial species, common in periimplantitis and periodontitis, *Peptostreptococcus spp.* or *Staphylococcus epidermidis* and *Staphylococcus aureus* were also identified, but only at the level of the implanted device. Comparative studies regarding the specific microbiota in periimplantitis have shown the presence of several genera such as: *Butyrivibrio, Campylobacter, Eubacterium, Prevotella (Prevotella nigrescens), Selenomonas, Streptococcus (Streptococcus nonmutan, Streptococcus mutans), Actinomyces, Leptotrichia, Propionibacterium, Peptococcus, Eubacterium spp., Lactococcus* and *Treponema*. Periimplant crevicular fluid analysis detected species of *Acinetobacter, Micrococcus and Moraxella* (Freire M.O, 2011, Belibasakis G.N, 2021).

Models of induction of periodontitis and periimplantitis

Techniques for inducing periodontitis or periimplantitis in rats involve inoculating specific pathogenic bacteria by oral gavage, intraoral injection or by placing ligatures around teeth or implants. The difficulty of reproducing the diseases occurs when the bacteria are applied in a growth phase incompatible with the formation of biofilm, especially when the indigenous flora also intervenes.

The "ligature-induced" defective pattern is commonly used to initiate periodontitis and periimplantitis in rats. Thus, a silk thread impregnated or not with pathogens is placed around the implant or tooth. Placement of a ligature leads to the accumulation of dental plaque and microulceration of the sulcular epithelium which, in turn, facilitates the invasion of periodontal pathogens into the connective tissue. Loss of periodontal attachment and resorption of alveolar bone occurs predictably over a 7-day period in rats (Nowotny A., 1983, Bezerra M.M., 2000, Bezerra M.M, 2002, Lohinai Z., 1998, Xie R., 2011). The role of bacteria in this model is supported by the findings that osteoclastogenesis and alveolar bone resorption are improved by the application of gram-negative bacteria (Lohinai Z., 1998). However, ligament-induced traumatic injury is limited only to study the pathological mechanisms of human peri-implantitis (Klausen B., 1991).

The lipopolysaccharide (LPS) application model was used to examine innate immune hosts using either LPS injection into the gingival tissue or LPS into the gingival scroll. The lipopolysaccharide component (LPS) of the cell wall of gram-negative bacteria is a significant inflammatory stimulus that triggers an innate immune response. The commonly used injection site is the palatal appearance of the first upper molars, but some studies have also performed injections on the interdental papilla between the first and second lower molars (Dumitrescu A.L., 2004, Nishida E., 2001). In rodents this pattern causes severe inflammatory responses of the peri-implant tissue and significant bone loss. Despite the sensitivity and accuracy in inflammatory induction, the use of LPS in the induction of periodontitis or periimplantitis is not similar to human disease due to the lack of bacterial colonization.

The rodent model that uses pre-infected implants, which investigates the host's responses against titanium implants on the surface of which bacteria form biofilm (Freire et al., 2011).

Infection model with *Aggregatibacter actinomycetmcomitans* (*AA*)

Rodents, although preferred as a model for inducing periodontal disease or periimplantitis, have the disadvantage that bacteria used to induce the disease process only temporarily infect the oral cavity, as rodents are not natural hosts for many human bacteria. A well-documented exception to this general principle is infection of the rat with *AA* which naturally colonizes the oral cavity. It has been hypothesized that *AA* may form a biofilm on titanium implants, which in turn can be used as a colonizing substrate for other bacterial species. Aa is common to periodontitis and periimplantitis, easily forms a biofilm on implants, and in rats, they lead to clinical reproduction of the disease, from tissue destruction to osteolysis.

The wid type of *AA* adheres to rat mouth epithelial cells, is frequently found in rice rats, while Sprague Dawley rat is difficult to detect, although it can also colonize it (Fine D.H., 2005).

The *AA* model was also used to examine periodontal bone resorption and the host's systemic response to infection. Li et al (Li Y., 2010) examined the role of T, B and CD4 + cells in adaptive immunity resulting in increased lymphocyte counts in regional lymph nodes as well as high levels of IL-2, IL-1, TNF, CD40 ligand, FasL, RANKL and osteoprotegerin.

Oral gavage model

The introduction of human bacterial strains by oral gavage and the subsequent impact on the periodontium has been studied in different rodent models (48). Various bacterial strains associated with periodontitis in humans have been used in this model, including *Porphyromonas gingivalis*, AA, *Tannerella forsythia*, and *Treponema denticola* (Garlet G.P., 2006, Sharma A., 2005, Lee S.F., 2009, Kesavalu L., 2007, Okada Y., 2010). Rats are usually given a known number of bacteria in a viscous suspension (2% carboxymethylcellulose) administered orally. Although the infection is transient, 45% of rats exposed to *Porphyromonas gingivalis* and 80% exposed to *Treponema denticola* or *Treponema forsythia* were found to harbor these bacteria after 4-6 weeks. Significant bone loss can be measured histologically, by macroscopic analysis or by computed tomography. Alveolar bone resorption is usually assessed around the maxillary molars, because the induction of bone loss in the lower molars is slower due to the thicker cortical alveolar bone and wider bucolic dimensions (Polak J., 2005).

Oral infection by topical administration of bacteria was also performed in rats. Many of these studies examined the Sprague Dawley strain (Lazar V., 2017).

One aspect that has been discussed in the oral infection model is the use of a single bacterial species versus the use of two or more microorganisms associated with periodontal disease. The complexity of bacterial stimulation is supported by the findings that the persistence of *Porphyromonas gingivalis* in the oral cavity of rats at 4 weeks after the initial challenge is significantly increased from 45 to 80-100% when this bacterium is co-infected with *Treponema forsythia* and *Treponema denticola*. Alveolar bone loss is significantly higher in animals caused by a polymicrobial oral infection than by monoinfection (Lazar V., 2017) but careful analysis of recent studies shows that periodontitis is induced by *Porphyromonas gingivalis*, compared to periimplantitis in which the polymicrobial biofilm is preferred.

If in terms of rat as a model of periimplantitis and periodontitis there are numerous studies, in the literature for sheep, the results are very poor, despite their similarity to humans, in terms of bone. Very few studies have used sheep as a model of periodontitis using the ligation technique mainly (Alexandru el al., 2019). Bacteria with implication in the study of periodontitis experimental sheep are represented by species of *Prevotela* (*Prevotella buccae, Prevotella intermedia, Prevotella loescheii, Prevotella melaninogenica*) and *Porphyromonas* (*Porphyromonas asaccharolytica, Porphyromonas endodontalis, Porphyromonas gingivalis, Porphyromonas gula*) (Borsanelli et al, 2017). The oral microbiota of ovine periodontitis is compatible with that found in human periodontitis. A study conducted by Silva et al., 2019, showed that the most common microorganisms in sheep with severe periodontitis were *Tannerella forsythia, Treponema denticola , Fusobacterium nucleatum and Porphyromonasgingivalis* while *AA , Enterococcus* gum were detected in none of the samples analyzed.

As in the case of the rat, the induction of periodontal disease is most easily achieved with the help of the *Porphyromonas gingivalis* strain that caused epithelial infiltration, collagen decomposition and bone resorption similar to the degenerative processes specific to human periodontitis. In addition, significantly higher levels of IgG antibodies against *Porphyromonas gingivalis* antigens have been observed in sheep with periodontitis, levels similar to those in humans (Genco, 1998).

In current research, based on keywords, no studies of experimental periimplantitis in sheep have been found, with bacterial implication which means that this animal model is still unexplored in this field.

CONCLUSIONS

Porphyromonas gingivalis is one of the most important periodontal pathogens, which has the ability to adhere to and invade the epithelial tissue of the oral cavity in both rats and sheep. *Aggregatibacter actinomycetemcomitans* can easily colonize the rat's oral cavity and titanium implants. *Fusobacterium nucleatum* is an important periodontal agent, especially in forms of rapid and progressive periodontal disease. *Prevotella intermedia* is pigmented in black, while *Bacteroides forsythus* is a non-pigmented gram-negative bacterium. These bacteria produce pro-inflammatory lipopolysaccharides and extracellular proteases that could destroy IgA immunoglobulins. *Microseptopreptococcus spp* has been positively associated with dental implant failure. Spirochetes (*Treponema vincentii* and *Treponema denticola*) have been observed to a greater extent in patients with periodontal disease than in healthy individuals and are capable of producing pro-inflammatory lipopolysaccharides and unusual metabolic products such as indole, hydrogen sulphide and ammonia, which are potentially toxic to host cells.

Therefore, used alone or in combination these bacteria can reproduce both periodontitis and periimplantitis in rats. This model reflected the

main characteristics of the two human diseases and may be a useful tool for future research into the relevant pathological pathways of peri-implant diseases, as well as for new therapeutic approaches.

Due to the similarity with humans in terms of bone structure, size, common oral microbiota and susceptibility to periodontitis, sheep can be an animal model for the study of periimplantation.

ACKNOWLEGMENTS

This review is part of the bibliographic study of the doctoral thesis entitled: *Evaluation of osseointegration of dental implants using anti-rejection medication in a rat model* (PhD student Ancuța Diana - Larisa) and part of the of EuroNanoMed III Joint Translational Call – ANNAFIB research project.

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