POCKET MEASUREMENT METHODS IN WISTAR RATS PERIODONTITIS INDUCED BY BACTERIA AND THE INSTALLATION OF SILK LIGATURE: AN EXPERIMENTAL STUDIES

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ABSTRACT

Objective: The purpose of this study was to develop an experimental model of induction of periodontal disease in Wistar rats, using a combination of bacterial induction and binding of silk ligature with respect to pocket periodontal depth.

Methods: Experiments with a pre-posttest group design was applied. Five adult male Wistar rats from the Udayana University's Analytical Laboratory were included in the study. Measuring pocket depth in experimental animals using dental probes was previously administered. Then performed the installation of silk ligature and bacterial induction *Porphyromonas gingivalis*, on the mandibular anterior teeth. Release of silk ligature on day 7, without the action of debridement of plaque or calculus on rat tooth. Observation of the development of the testing animals on the 3d, 7d, and 11d. On the 11d, re-measurement of pocket depth was conducted.

Results: Periodontal tissue abnormalities with silk ligature placement and bacterial infiltration *Porphyromonas gingivalis* cause periodontal inflammation with periodontal pocket formation, with a mean depth of 3.32 mm, which was analyzed using *Wilcoxon p*<0.05.

Conclusion: In this study combining bacterial induction and the installation of silk ligature can shorten the induction of periodontal tissue disease characterized by the formation of pocket periodontal.

Keywords: Wistar rat periodontitis, Periodontal pocket, Periodontal probe

INTRODUCTION

Periodontal disease is one of the oral and dental diseases that is most commonly found in humans caused by several factors, one of which is due to the accumulation of bacterial plaque [1]. The WHO report (2003) suggests that diseases have high prevalence rates worldwide. Periodontitis is damage caused by the host defence. Clinically periodontitis is characterized by plaque accumulation, calculus and pocket formation, periodontal tissue inflammation and alveolar bone loss, gum bleeding, accompanied by pus with untreated halitosis resulting in tooth loss. Pathogenic bacteria are suspected of causing an inflammatory response, gingival and periodontal damage [2]. To study the phenomenon of periodontal inflammation and the effects of periodontal treatment, several animal models have been used as animal studies of periodontitis [3].

Periodontal disease can be divided into different phases and each can be studied separately depending on the animal model. This phase involves the colonization of biofilm-bacteria, invasion of bacterial products from epithelial tissue to connective tissue, destructive induction of host response to connective tissue and bone resorption. Improvement processes that follow tissue damage by selecting appropriate animal models in each of these phases can be analyzed individually, whereas in human research it is difficult to isolate specific steps and *in vitro* studies are less complex to examine specific phases [4]. Rats in particular, are models relevant for experimental periodontal studies [5]. The structure of the rat tooth gingiva is similar to human gingival sulcus, superficial and the presence of junctional epithelium on the tooth surface. Junctional epithelium is a pathway for the entry of foreign bodies and bacterial endotoxins, thereby causing the onset of inflammation [3].

The incidence of periodontal disease in rats is certainly rare so it needs to be induced to cause periodontitis. Experimental results indicate a horizontal bone loss in rats infected with *Actinomycetemcomitans aggregatibacter (Actinobacillus)* or *P.*

Gingivalis. Periodontitis is induced in mice by placing a silk or cotton ligature, which results in retention of bacterial plaque on the gingival sulcus around the molar teeth [6]. The rat induction model was performed by Ionel *et al.* by attaching a ligature to the mandibular anterior mandibular tooth. After 14d of ligature insertion, histopathologic results show signs of inflammation with neutrophil infiltration and alveolar osteolysis [3]. The accumulation of plaque bacteria produces toxins that will irritate the gingiva, the toxin stimulates a chronic inflammatory response in which the body will react by itself then the tissues and bones supporting the tooth will be damaged. The gingiva will separate from the tooth, forming the infected pocket (the space between the tooth and gingiva). As the pocket progresses deeper and broader it damages the gingiva and alveolar bone. In some experiments, mice periodontitis is performed by injecting oral bacterial bacteria such as *Porphyromonas gingivalis* to induce periodontal abnormalities [6].

The time required for the occurrence of chronic periodontitis in mice is generally 3 w. Inflammation begins at week 1 (acute inflammatory state), week 2 (severe inflammatory state), and week 3 (Chronic inflammatory state). The period is almost equal to the time it takes for humans to produce the same disorder [6]. Research of induction of periodontitis by using ligature and bacterial induction has been done. Utama *et al.* induced periodontitis with a combination of ligature and bacterial injection 3x weekly for 3 w [8]. Noting the length of time and repetition of injections in rats, the purpose of this study to simplify the procedure and to shorten the time required for the induction of periodontitis in rats as well as obtained a method to measure the depth of pocket which is one of the clinical symptoms in periodontitis.

MATERIALS AND METHODS

Material

The research is experimental research with pre and post control group design. The study was conducted at Udayana University's

Analytical Laboratory, Bali, Indonesia with five Wistar rats. Research has got ethical clearance from the Faculty of Veterinary Medicine of Udayana University, Bali, Indonesia.

Wistar rats

Preparing male Wistar rats weighing between 250-300g weighed in digital scales. Animals try to be adapted for 1 w at the research site to adapt to the environment. The male Wistar rat was placed in a clean cage with good ventilation with a length of 50 cm, a width of 40 cm, a height of 40 cm with a temperature of 25-27 °C, moisture of 7-75% and light 12h of light and 12h of darkness. Bedding in experimental animals is by the husk. Bedding is replaced every three days is and rats are fed standard AD II pellets and ad libitum (unlimited) drinking water. Environmental health and animal monitoring is done daily.

Inclusion criteria

Male rats Wistar strain, rats ages 8-10 w, weight 250–300g.

Exclusion criteria

A hyperactive rat who bites his friend. Drop out criteria when the Wistar rat is sick or dead during research determined by the veterinarian.

Phorphyromonas gingivalis bacteria

Preparing bacterial colonies to be induced into Wistar rats is *Porphyromonas gingivalis* ATCC 33277. Made per litre culture media: 5g yeast extract, 5g peptone, 200 ml goat blood fill up to 1l. Next in an autoclave for 15 min. After the cold medium was inoculated 1 ose bacterium *P gingivalis* and incubated at 35 °C, with a population density of 2.59×10^8 CFU/ml.

Anaesthesia and induction of periodontitis

a. Anaesthesia in mice was performed using HCl Ketamine as much as 80 mg/kg or equivalent about 0.22 ml intramuscular in the thigh muscle.

b. Approximately 10-15 min later, the rats began to look limp and their movements slowed, searching for gingival sulcus gaps and measurements, then mounting the silk ligature on the lower anterior teeth. Silk ligature is strongly bonded in the cervical area of the tooth by wrapping it around the anterior teeth so it is not easily removed. Silk ligature is inserted and pushed into the gingival sulcus with the help of dental explorer.

Induction of bacteria *P gingivalis*

Induction of bacteria *P gingivalis* with intramuscular injection using 1cc needle syringe at the buccal mandible of the mandibular anterior of 0.25 ml. Performed only one injection at the beginning of the experiment.

Observation stage

Observing changes in rat, rat's movements, conditions, abilities and appetite on the 3rd, 7th and 11th d. On a daily basis, the life conditions of the rats remain monitored. Release of silk ligature on day 7, without the action of debridement of plaque or calculus on rat tooth.

Pocket depth measurement

Before the pocket depth measurement is done, the rats are inserted into plastic bottles of the size corresponding to the rat to make it easier to hold. The mouth of the rat is retained with a tool that can help the opening of the mouth so that the hand is not bitten during pocket measurement. Measurements using the Osung dental probe PCP 12, which is thin and flattened edges making it easy to fit into the pocket. This dental probe with the size of each strip is marked with size 3-6-9-12, on the probe mounted rubber stopper for border measurement of pocket depth. Pocket measurements by moving the probe begin from the distobuccal-buccal-mesiobuccal, distolingual, lingual, mesiolingual surface. At the time of measurement, it turns out the most accessible area and has the deepest pocket is in mesiobuccal. Then the pocket depth measurement results are measured with a line starting from the tip of the probe to the rubber stopper limit. The entry of the dental probe can be seen from the buccal mucosal epithelial because it is so thin that it is clearly visible.

Decaputation is performed for histopathologic examination by HE (Hematoxilin Eosin) staining and 400x objective used to know microscopic fig. of fibroblast cell activity, presence of inflammatory cells such as lymphocytes, osteoclast cells.

Instruments used

To find out that the method of induction of periodontitis is through the use of silk ligature and the induction of *Porphyromonas gingivalis* bacteria had a significant relationship with periodontal pocket, the data was analyzed by *Wilcoxon Test*; p-value = 0.041 (significant $p<0.05$), with an average pocket depth of 3.32 mm.

RESULTS AND DISCUSSION

From an observation of rats after induction on the third day, the rats looked restless, in pain and did not want to eat. Around the anterior tooth, gingiva appears swollen and reddish.

Fig. 1: Rats before and after installation of silk ligature on day 7

Fig. 1, observation on the 7th d the rat still appeared restless but could eat mushy food (food dipped into the water first). Gingiva looked reddish and when touched with a bleeding probe, tooth decay occurs. Silk ligature began to be released without disturbing the debridement at the surface of the anterior teeth. The hope was that the accumulation of plaque and inflammatory calculus will continue until the 11th d. Observation of the changes that occurred in rat gingiva on

the 3rd and 7th d showed a change in clinical symptoms leading to periodontitis. Periodontitis begins with the presence of gingival inflammation (gingivitis) characterized by swelling and gingival bleeding, then deepening of the gingival sulcus causing pocket gingiva [9]. The occurrence of periodontitis begins with the entry of bacterial products in the form of *Lipopolysaccharide* by bacterial induction on the 1st d, resulting in a response by the host, on the 3rd d, gingivitis is

characterized by swelling and redness around the anterior gingiva teeth. Observation on the 7th d showed inflammation in on-going nerve

support tissue, which is characterized by bleeding on the probe and the presence of tooth mobility.

Fig. 2: Measurement of pocket depth with periodontal probe

As shown in fig. 2, the rat movements began to calm and the diet remained the same, still choosing soft food on the 11th d. The gingiva appeared bluish and the stability of the tooth weakened with degeneration of degree 2. On the 11th d, there was no longer bleeding but there was wobble of teeth and rats did not want hard food, this condition leads to the occurrence of periodontitis. The results of this observation are almost identical to those observed in rats performed by Siregar *et al.* [10]. In accordance with the opinion of Jacob and Nath, immunological reaction of the host response to agents is almost the same as humans. Since the 1st d of the induction of LPS,
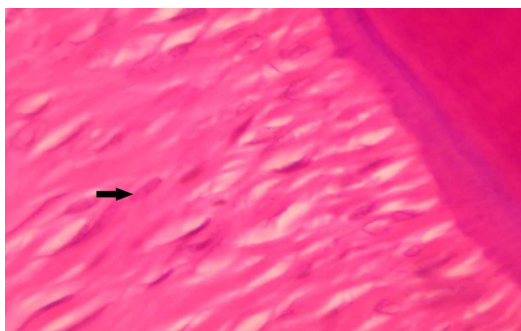
proinflammatory cytokines have begun to appear (TNFα and IL-1), on the 2nd d, TNFα expression, macrophages, and fibroblasts in the junctional epithelium are on the rise, there is an increase in osteoclast and pro-osteoclast expression in the alveolar bone margin that lasts for three days. On the 7th d, there was a decrease and the amount was almost the same as the *Lipopolysaccharide*-induced control [11]. *Porphyromonas gingivalis* infection in periodontal tissue increased the expression of TNFα and IL-10. Pathologic condition induced by *P gingivalis* can be inhibited by the expression of IL-10 [12].

Table 1: Results of pocket depth measurements in rat induced periodontitis
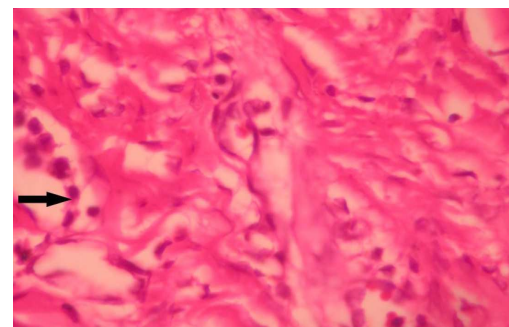
Pocket	N	*Mean*	SD	*P*
Pre-induction	5	0.000	0.000	0.041
Post-induction	5	3.320	0.334	

Note: *Wilcoxon Test*: significant *p*<0.05

Table 1 summarizes the pocket depth measurement results obtained data between 3.00 mm to 3.80 mm, with the average pocket depth of 3.320 mm. *Wilcoxon test* analysis obtained a significant relationship *p*-value = 0.041 (significant *p*<0.05), to the depth of pocket after the induction of periodontitis. Pocket periodontal is a deepening of the gingival sulcus which is one of the clinical symptoms of periodontitis. Pocket formation is due to the migration from epithelial crevicular towards the apical. Dental plaque results in the accumulation of bacterial products in the gingival sulcus and deeper penetration, thus stimulating the occurrence of inflammation. Inflammation destroys soft tissue, proliferation and migration of the apical junctional epithelium, decreases the gingival fibers so that the sulcus deepens and, if continued, the breakdown of the periodontal ligament and the destruction of alveolar bone and tooth mobility [13].

Fig. 4: There was an increase in the number of fibroblast cells and lymphocyte cells in mice treated on day 11 (HE, 400x)

Fig. 3: Periodontal tissue in control rats visible fibroblast cells no visible lymphocyte cells and osteoclast cells (HE, 400x)
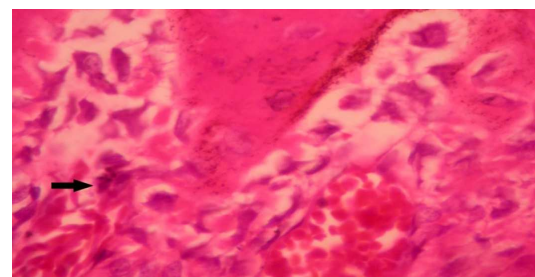
Fig. 5: Overview of osteoclast cell in rat treatment on day 11(HE, 400x)

Fig. 3-5 demonstrate the histopathologic examination after treatment on the 11th d. We found an increase in inflammatory cells, such as neutrophils and lymphocytes, as well as an increase in the number of fibroblast cells and osteoclast cells, as shown below. Histopathological examination results show the presence of inflammatory cells such as neutrophils, lymphocytes, fibroblast cells, and osteoclasts. The host response to bacterial metabolism products triggers epithelial junctional cells to produce cytokines and stimulates neutrons to produce neuropeptides that cause local vascular vasodilation. Neutrophils leave the blood vessels and migrate to the site of inflammation in response to chemokines. Early lesions follow, with an increasing number of neutrophils in connective tissue and the emergence of macrophages, lymphocytes, plasma cell, and mast cells [14]. *Porphyromonas gingivalis* stimulates dendritic cells, macrophages, and T cells to activate TNFα and IL-1β that activate fibroblasts to stimulate the release of matrix metalloproteinase as an enzyme that degrades the molecular matrix in collagen and damages the periodontal ligament [15]. TNFα is a proinflammatory cytokine that promotes osteoclastogenic processes that stimulate RANKL expression. *Porphyromonas gingivalis* increases osteoclastogenesis production by activating TNFα and IL-1β that stimulate B-lymphocyte and T-lymphocyte cells such as Th1 and Th17, which then correlate to RANKL expression. RANKL results in the maturation of preosteoclasts into adult osteoclasts and results in alveolar bone resorption in periodontitis [16]. In the opinion of Cekiki *et al.* in the presence of an increase in the number of lymphocyte cells, the destruction of fibroblast cells and the increase in the number of osteoclast cells in tissue histopathology results in a sign of periodontitis [14]. The 11th d after induction, rats showed histopathologic features of fibroblast activity, lymphocyte and osteoclast cells, this indicates that the anterior teeth of rats had periodontitis.

CONCLUSION

In this study, the combination of ways through bacterial induction and the installation of silk ligature can shorten the induction of periodontal tissue disease characterized by the formation of pocket periodontal.

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AUTHOR CONTRIBUTIONS

All authors have contributed to provide suggestions and thoughts in this research in accordance with their respective disciplines so that this research can run smoothly and successfully obtain results in accordance with research objectives.

CONFLICT OF INTERESTS

All the authors hereby declare that there is no conflict of interest

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