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Research Report

The role of probiotic on alveolar bone resorption

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ABSTRACT

Background*: Probiotics are microbes derived from the group of lactic acid bacteria that work to maintain the health of hosts. Probiotics can also be used to improve oral health. Periodontal disease is usually marked with gingival inflammation and alveolar bone resorption. Gram negative anaerobic bacteria that play important role in human periodontal disease are Porphyromonas gingivalis. (P. gingivalis). P. gingivalis is a virulent bacteria in vivo or in vitro, and mostly found in subgingival plaque of periodontitis patients.* ***Purpose****: This study is aimed to know the role of probiotics to inhibit the resorption of alveolar bone induced with P. gingivalis.* ***Methods****: This study used male wistar rats divided into 4 groups. Group I was control group (without treatment); group II was induced with P. gingivalis ATCC 33277 for 5 days; group III was induced with P. gingivalis ATCC 33277 and also injected with probiotics (Lactobacillus casei ATCC 4224) for 5 days simultaneously; and group IV was induced with P. gingivalis ATCC 33277 for 5 days and also injected by probiotics (Lactobacillus casei ATCC 4224) in the next 5 days. After that, the samples were decapitated, taken their alveolar bone, and then were examined by immunohistochemistry to observe osteoclast activity in alveolar bone resorption by using tartrate-resistant acid phosphatase (TRAP) expression. All data were then analyzed statistically.* ***Results****: It is known that there were significant differences of TRAP expression among all those treatment groups ($p < 0.05$).* ***Conclusion****: It then can be concluded that probiotics can decrease osteoclast activity in periodontal tissue of wistar rats, so it can inhibit alveolar bone resorption.*

Key words*: Probiotics, Porphyromonas gingivalis, Lactobacillus casei, tartrate-resistant acid phosphatase, osteoclast*

ABSTRAK

Latar belakang*: Probiotik adalah mikroba dari golongan bakteri asam laktat yang bekerja mempertahankan kesehatan host dan probiotik dapat digunakan untuk meningkatkan kesehatan rongga mulut. Penyakit periodontal ditandai dengan adanya keradangan pada gingiva dan resobsi tulang alveolar. Bakteri Gram negatif anaerob yang sangat berperan dengan penyakit periodontal pada manusia adalah Porphyromonas gingivalis (P. gingivalis). P. gingivalis merupakan bakteri yang virulen, baik diuji secara in vivo maupun in vitro, dan banyak ditemukan pada plak subgingiva penderita periodontitis.* ***Tujuan****: Penelitian ini bertujuan untuk mengetahui peran probiotik dalam menghambat resorbsi tulang alveolar yang diinduksi P.gingivalis.* ***Metode****: Penelitian ini memakai tikus jenis wistar jantan sebagai sampel dan dibagi menjadi 4 kelompok: Kelompok I yaitu kontrol tanpa perlakuan; kelompok II di induksi P.gingivalis ATCC 33277 selama 5 hari; Kelompok III di induksi P.gingivalis ATCC 33277 ditambah suntikan probiotik (Lactobacillus casei ATCC 4224) selama 5 hari secara bersamaan; dan Kelompok IV di induksi P.gingivalis ATCC 33277 selama 5 hari ditambah suntikan probiotik (Lactobacillus casei ATCC 4224) 5 hari selanjutnya. Setelah itu sampel didekaputasi, diambil tulang alveolar dilakukan pemeriksaan imunohistokimia untuk melihat aktivitas osteoklas dalam resopsi tulang alveolar dengan mendeteksi tartrate-resistant acid phosphatase (TRAP). Data yang dikumpulkan dianalisis secara statistik.* ***Hasil****: Terdapat perbedaan yang bermakna antar kelompok untuk ekspresi TRAP ($p < 0.05$).* ***Kesimpulan****: Dapat disimpulkan bahwa probiotik dapat menurunkan aktivitas osteoklas pada wistar tikus yang mengalami periodontitis sehingga dapat mencegah resorbsi tulang alveol.*

Kata kunci*: Probiotik, Porphyromonas gingivalis, Lactobacillus casei, tartrate-resistant acid phosphatase, osteoklas*

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INTRODUCTION

Nowadays, dental treatment of oral periodontal disease is highly needed although the success rate of the treatment is still low. During the active phase of this disease there will be gingival bleeding and exudation which can cause quick damage, such as alveolar bone loss within weeks or months then causing tooth loss and tooth-movement.[1]

Periodontal diseases caused by Lypopolysaccharide (LPS), can cause the occurrence of alveolar bone destruction. If the periodontium disease occurred during the eruption, it can cause the increasing of osteoclasts in alveolar bones which then causes premature eruption.

The loss of bone substance is actually caused by the excessive activity of osteoclasts. Organic acids secreted by osteoclasts can dissolve bone mineral density resulting in degradation of collagen. Unfortunately, the giving of bone graft is only used for repairing bone damage that already exists, but can not be used for prevention. The procedure of bone graft therapy takes a very long time and complex.[2]

In recent years, many of probiotic have continually developed to be an actual research topic since the potential of probiotics in the future is enormous. The study of Krasse *et al*.,[3] showed the decreasing of gingival bleeding and the reducing level of moderate and severe gingivitis after therapy with probiotics on days 0 and 14 compared to that with placebo. Nevertheless, the damage of the alveolar bone is still not proven. It is because probiotic bacteria can stimulate the immune systems, such as improving the function of macrophage phagocytosis, natural killer cells, monocytes and neutrophils, and can also stimulate the secretion of IgM and increase the production of IgA with the final result that can increase the production of immunoglobulin.[4]

Oral cavity is the first part of gastrointestinal tract so that some actions of probiotics also play a role in the ecosystem of the oral cavity. Lactobacillus in the oral cavity derived from healthy and unhealthy periodontal tissues showed antimicrobial activity against bacteria that causes periodontitis such as *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis (P.gingivalis)*, and *Prevotella intermedia*. *Lactobacillus* can also produce antimicrobial components, such as organic acids, hydrogen peroxide, carbon peroxide, diacetil, antimicrobial substances which have low molecular weight and bacteriocin, as well as low pH.[5]

Osteoclasts produce tartrate resistant acid phosphatase (TRAP) enzyme in large numbers which is an enzyme produced in osteoclast precursorsionly, called as a marker of osteoclasts. Mature osteoclasts then express receptors for calcitonin and prostaglandins which are inhibitors of hormones or growth factors although not directly.[6] Therefore, the purpose of this study is to see the expression of TRAP in experimental animals, induced with *P. gingivalis* after the administration of probiotics.

MATERIALS AND METHODS

The research was conducted on male wistar rats in the age of three months with 170–200 grams of body weight. Those rats were fed with the same food. Next, the acclimatization was conducted for a week before they were subjected to adaptation to location and food.

The samples were devided into 4 groups, 10 samples each: group I is a control group without any treatment; group II is a treatment group induced with *P. gingivalis*; group III is a treatment group induced with *P. gingivalis* and at the same time also injected with probiotic bacteria; group IV is a treatment group induced with *P. gingivalis* for 5 days, and then followed with the injection of probiotic bacteria after 5 days.

Lactobacillus casei (*L. casei*) (ATCC 4224) was conducted first on media with procedures in accordance with protocols from the factory. Then, the infection of periodontal tissues by using *P. gingivalis* (ATCC 33277) injected at the junctional epithelium at the gingival sulcus in the first incisive teeth of the right mandibular labial part, with 2×10^8 cfu dose, once a day for 5 days. Next, the administration of probiotic bacteria was conducted by injecting the same area at the time of *P.gingivalis* induction. The dose used was 2×10^8 cfu/ml given once a day for 5 days. The administration of probiotic bacteria was then conducted in 2 ways, group III given simultaneously and group IV administered after the induction of *P.gingivalis* for 5 days.

The experimental animals either in the control groups or in the treatment groups, would be decapitated for their alveolar bone and gingival region of their first insisive of their right mandibular labial part. Decalcified samples was conducted in order to release inorganic material in bone without damaging the protein by giving 5% citric acid for 5 days. Fixation (paraffin embedding) was conducted followed with cutting process by using microtome with transverse direction (mesio-distal direction), where the thickness of the cuts was related with the needs. Meanwhile, to see resorption caused by osteoclast activity as a result of periodontal infection, TRAP detection was conducted in order to be used as a cytochemical marker. For analyzing data, Kruskal-Wallis test was conducted, if there is difference, the test would be followed by Mann-Whitney test.

RESULTS

It is known that the lowest expression of TRAP occurred in group 4 induced with *P. gingivalis* for 5 days, and then induced also with probiotic *L. casei* (Table 1). Based on the mean data of TRAP expression, normality and homogeneity tests are then conducted by using Kolmogorov Smirnov and Levene test. From the test results, it is known that the

Table 1. The mean results of TRAP expression

No.	Groups	n	Mean	Standard Deviation
1	I	10	4.54	1.34
2	II	10	2.75	1.83
3	III	10	1.85	1.08
4	IV	10	1.15	0.58

Note: Group I : Control; Group II: Induced with *P. gingivalis*; Group III: Induced with *P. gingivalis* and *L. casei* at the same time; Group IV: Induced with *P. gingivalis* and followed with *L. casei.*

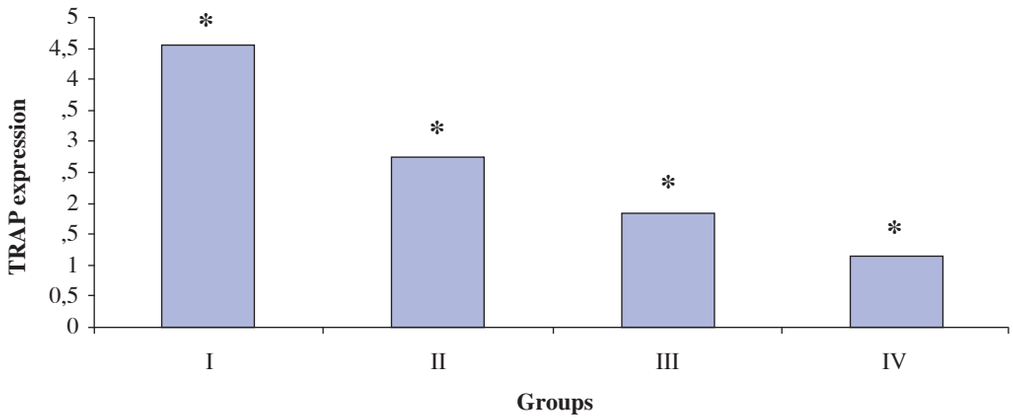

Figure 1. The graph of *Mann-Whitney test* result of the mean of TRAP expression.
*: Significant difference ($p < 0.05$)
Group I: Control, Group II: Induced with probiotic bacteria, Group III: Induced with *P. gingivalis* and *L. casei* at the same time, Group IV: Induced with *P. gingivalis* and followed with *L. casei*.

mean data is not normal. Therefore, non-parametric test is then performed. Non-parametric test conducted is Kruskal-Wallis test. Kruskal-Wallis test results shows a significant difference ($p < 0.005$). It indicates that the administration of probiotics affects the expression of TRAP. Further testing is then conducted, namely Man-Whitney test. The results of Mann-Whitney test also shows significant differences in all of those treatment groups ($p < 0.005$) (Figure 1). The results of TRAP staining expression by using immunohistochemical techniques in the region of the dental alveolar bone tissue of the first insisive of the right mandibular labial part of wistar rats can be seen in figure 2.

DISCUSSION

The results of this study demonstrated the influence of probiotics on the expression of TRAP-induced *P. gingivalis*. TRAP is used as marker of osteoclasts and macrophages. The results showed that the decreasing of osteoclast formation in the surrounding of alveolar bone was characterized by the reducing of TRAP expression. The results of this study also indicated that the lowest mean of the expression of TRAP was showed in group IV, the treatment group of male wistar rats induced with LPS of *P.gingivalis*, for five days, and then also injected by probiotic bacteria, *Lactobacillus casei,* for next five days.

Moreover, LPS of *P. gingivalis* induced in the anterior alveolar bone where there is a seed of rat incisors in order to obtain the condition of periodontitis. *P. gingivalis* had several virulence factors which are LPS, fimbriae, toxic metabolism results, and proteases. These virulence factors could lead to diseases either directly or indirectly by activating host cells to release mediators of LPS inflamatory.[7] *P. gingivalis* is actually an important etiological factor in periodontitis that can induce inflammation and cause periodontal tissue damage. LPS of *P.gingivalis* can even stimulate inflammatory cytokine expression in monocytes and gingival fibroblasts, and also induce bone resorption activity.[8] Periodontal disease caused by LPS showed the occurrence of alveolar bone destruction. If periodontal disease occurred during the eruption, it can cause the increasing of osteoclasts in alveolar bone which can cause premature eruption.[9]

LPS is an endotoxin which can bind surface receptors, cluster of differentiation-14 (CD14), on macrophages and monocytes. Toll-like receptor-4 (TLR4) of macrophages and monocytes that bind with bacteria by the presence of CD14 then would induce the secretion of cytokines and other

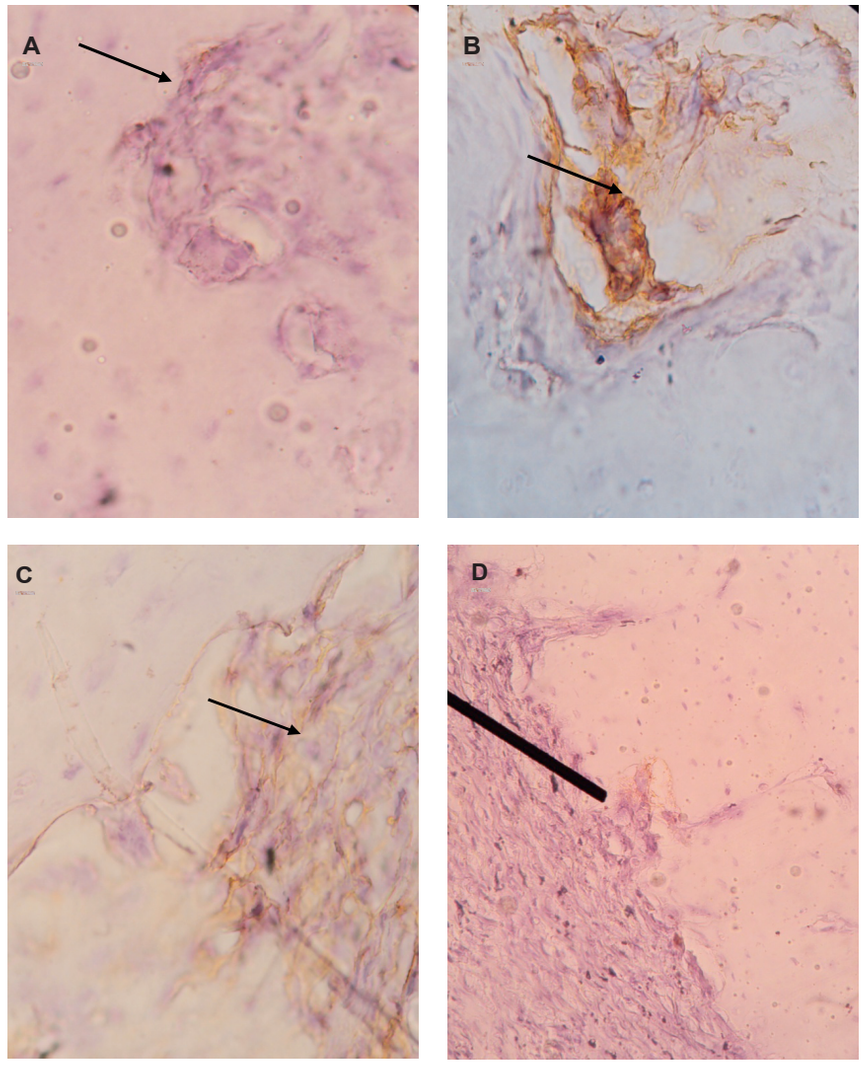

Figure 2. Immunohistochemistry figure of the alveolar bone of the first incisive of the right mandibular (labial part) of wistar rats. TRAP expression shows brown spots around the the alveolar bone (shown with arrows). A) Group I: Control; B) Group II**:** Induced with *P. gingivalis;* C) Group III: Induced with *P. gingivalis* and *L. casei* at the same time; D) Group IV: Induced with *P. gingivalis* and followed with *L. casei.*

inflammatory mediators, proinflammatory cytokines and prostaglandin E-2 ($PGE_{2)}$. Those mediators then stimulated osteoclast formation derived from stromal/osteoblast cells through cell-to-cell binding which is *receptor activator for NF-κ B ligand* (RANKL) in osteoblasts by *receptor activator for NF-κ B* (RANK) on osteoclast progenitor. LPS would stimulate the increasing of osteoblast RANKL that functions are for the formation of osteoclasts, so osteoblasts in having differentiation and proliferation for multiplying themselves could not be impaired. Mice injected with LPS *E. coli* in their first maxillary molar mucosa region would have the increasing number and size of osteoclasts in each additional doses of LPS, and later would cause alveolar bone resorption. Bone resorption is actually caused by the degradation of the crystal structure of hydroxyapatite (HA) and the organic structure of collagen due to the low pH, from ±3.0 to 4.5, caused by the activity of osteoclasts.[9]

In the treatment group given LPS of *P. gingivalis*, the expression of TRAP in alveolar bone was increased due to the level of LPS on gingival crevicular fluid associated with the increasing of gingivitis severity. Fine *et al. cit.* Kusumawadini[10] showed that LPS levels were correlated with the percentage of Gram-negative bacteria in healthy periodontal tissue and periodontitis. It also suggested that LPS has biological activity contributing to the pathogenesis of periodontal diseases.

The production of lactic acid by *Lactobacillus* can make pH low and also inhibit the growth of pathogenic bacteria. *Lactobacillus* is a probiotic bacterium that can prevent the growth of black-pigmented anaerobic bacteria considered to play a role in periodontal diseases in subgingival area.[11]

One of the factors triggering periodontal diseases is Gram-negative bacteria on tooth root surface, a biofilm. LPS and other compounds can improve access to the

gingival tissues, initiate and lead immunoinflammation causing the production of pro-inflammatory cytokines in high levels, which can induce the production of metalloproteinase matrix resulting in the destructions of tissue, periodontal ligament, and alveolar bone resorption.[12] *L. casei* of probiotic bacteria, can regulate the balance of local and systemic immune responses against infection by releasing proinflammatory cytokines and activating natural killer (NK) cells in order to eliminate pathogenic bacteria.[13] Probiotic bacteria can also form biofilms as oral mucosal defense layer against oral diseases. This biofilm layer can prevent pathogenic bacteria invading the tissue by filling the empty cavities of the tissue that can be entered by pathogenic bacteria. In addition, biofilms may prevent the growth of cariogenic bacteria and other bacteria causing periodontal diseases.[14]

Lactobacilli, produce several antimicrobial compounds including organic acids (lactic acid, acetic acid, succinic acid), hydrogen peroxide, and bacteriosin as well as adhesive inhibitors that may affect oral bacteria. Inflammation of the gingiva is one of the effects of food spoilage caused by pathogenic bacteria. Probiotics control the growth of pathogenic bacteria in order to prevent gingivitis. Probiotics have low pH so that bacteria can not form dental plaque and calculus plaque causing periodontal diseases.[15]

Probiotics also produce antioxidants which can prevent the stain and plaque formations by neutralizing free electrons required for the formation of minerals or calculus. Besides that, probiotics can also damage *putrescence odors* by fixating toxic gas and turning it into gas needed for metabolism.[16]

L. casei can be used for the treatment of vascular endothelial cells in mice with coronary arteritis.[17] *L. casei* is indicated to be able to stimulate PMN cells on endothelial cells, and also able to increase intercellular adhesion molecule-1 (ICAM-1). The increasing of these materials can reduce inflammation in coronary arteries of studied animals. Probiotics, can also control the growth of pathogenic bacteria in order to prevent gingivitis. Probiotics actually have low pH so that bacteria can not form dental plaque and calculus plaque causing periodontal diseases.

It can be concluded that probiotics can influence the decreasing of osteoclast activity in periodontal tissue of wistar rats, so it can inhibit alveolar bone resorption.

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