Effect of Irrigation Solution *Nannochloropsis Oculata* on Markers of Bone Repair in Chronic Periodontitis

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Abstract

Periodontitis caused by *Porphyromonas gingivalis* is an inflammatory disease that occurs worldwide. *Nannochloropsis oculata* contains ingredients that are antibacterial so that they can play a role in bone remodeling. The purpose of this study was to determine the effect of *Nannochloropsis oculata* on the amount of RANKL and OPG in periodontitis.

30 rats were divided into 5 groups, namely control group (K1), periodontitis group (K2), *Nannochloropsis oculata* therapy group (K3, K4, K5) given 0.25%, 1.25% and 2.5%. The number of RANKL decreases and the number of OPG increases. *Nannochloropsis oculata* is able to balance the amount of RANKL and OPG in chronic periodontitis.

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Introduction

Periodontitis is an inflammatory condition of the periodontal connective tissue caused by the accumulation of bacterial plaque on the tooth surface adjacent to the gingival tissue which damages the alveolar bone[1]. Periodontal diseases are prevalent both in developed and developing countries and affect about 20-50% of global population[2].

Microbial activity is increasing in periodontitis disease[3]. *Porphyromonas gingivalis* is the most common pathogenic microbe in chronic periodontitis[4]. The innate immune system, which includes leukocytes and other cells like those in the gingival epithelium and periodontal ligament, recognizes biofilm components as harmful signals[5]. Through the TLR-4 (Toll Like Receptor 4) receptor, cells detect the presence of Lipopolysaccharide (LPS) in *Porphyromonas gingivalis* bacteria[6]. The first line of phagocytes, which includes dendritic cells, macrophages, and neutrophils, express Toll-Like Receptors[6,7].

By encouraging osteoclastic activity, which leads to bone resorption, the increase in microbes has the potential to upset the equilibrium of the host response in periodontitis, the inflammatory response causes an imbalance between the resorption of bone and the formation of new bone, resulting in bone resorption[8]. The osteoclastogenesis-activator system of Receptor Activator of NF-kB (RANK)/Receptor Activator of NF-kB ligand (RANKL)/osteprotegerin (OPG) and the cytokine system of tumor necrosis factor ligands and the receptor superfamily interact to control bone resorption[9]. RANKL activation can be inhibited by OPG[10].

Antibacterial therapy for periodontitis is an option[11]. Antibacterial properties exist in algae.The green microalga *Nannochloropsis oculata* is one of them[12]. The marine-water microalga *Nannochloropsis oculata* is thought to be a good source of omega-3 fatty acids, specifically eicosapentaenoic acid (EPA)[13], flavonoids, vitamin E, carotenoid[14], terpenoids, flavonoids and alkaloids[15,16].

Nannochloropsis oculata used in in vitro studies on stem cell fibroblasts showed concentrations above 2.5% indicating toxicity[15], therefore in this study using concentrations of 0.625%, 1.25% and 2.5 %. The purpose of this study was to determine markers of alveolar bone healing

Materials and methods

This study used 30 white Rattus norvegicus wistar rats. There were 5 groups consisting of normal rats (K1), periodontitis rats (K2), groups 4,5 and 6 were periodontitis rats

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treated with *Nannochloropsis oculata* solution with concentrations of 0.625%, 1.25% and 2.5, respectively. %.

In a four-day experiment, drinking water of rats was given 20 mg of ampicillin and 20 mg of kanamycin, smeared with 0.1% chlorhexidine gluconate on the rat's gingiva. A cotton swab was used to apply a bacterial suspension of Porphyromonas gingivalis ATCC 33277 containing 1x109 CFU/mL in PBS topically to the buccal-lingual portion of the right-to-left molar region and anus. 1.5 mL of bacteria was given to the stomach of Wistar rats with a cannula syringe. This treatment was carried out three times for four days. In the third week after initial administration, periodontitis occurs[17,18].

Nannochloropsis oculata solution therapy was applied topically to the gingival sulcus for ten days following periodontitis in groups 3, 4, and 5. *Nannochloropsis oculata* solution therapy was administered to group K1 at a concentration of 0.625 percent, to group K2 at a concentration of 1.25 percent, and to group K3 at a concentration of 2.5 percent. *Nannochloropsis oculata* was applied to the mandibular gingival sulcus in the molar region of *Wistar* rats with the help of a syringe given for 15 days.

The mandibles of rats were taken and examined histopathologically for the examination of RANKL and OPG expressed by osteoblasts. The examination was carried out using a microscope with a magnification of 400x .

Results

Table 1 shows the mean and standard deviation of the low RANKL and OPG expressions in the K1 group, in the K2 group, the periodontitis group showed an increase while the *Nannochloropsis oculata* (K3 - K5) group showed a balanced amount of RANKL and OPG expression.

Table 2 shows that there was a significant difference between the normal group, the treatment group and the periodontitis group. There was no significant difference between the normal group and the treatment group, namely groups 1, 2, 3 with the administration of *Nannochloropsis oculata* solution with concentrations of 0.25%, 1.25% and 2.5%

Kelompok	RANKL (Mean ± SD)	OPG (Mean ± SD)
K 1	4.33 ± 1.21	6.17 ± 1.33
K 2	16.83 ± 3.76	7.17 ± 1.722
K 3	9.00 ± 1.41	9.50 ± 1.87
K 4	11.50 ± 3.56	11.33 ± 1.37
K 5	11.00 ± 2.37	8.81 ± 1.33

Table 1. the mean value of the RANKL and OPG expression in each group.

	K 1	K 2	K 3	K 4	K 5
K 1		0.000*	0.006*	0.000*	0.000*
K 2			0.000*	0.002*	0.001*
K 3				0.119	0.208
K 4					0.749
K 5					

Table 2. RANKL Expression LSD test results.

	K 1	K 2	K 3	K 4	K 5
K 1		0.272	0.001*	0.000*	0.006*
K 2			0.015	0.000*	0.073
K 3				0.050	0.461
K 4					0.009
K 5					

Table 3. RANKL Expression LSD test results.

Table 2 shows that there was a significant difference between the K1 group in normal rats and the K2, K3, K4, K5 groups, namely the group of rats given *Porphyromonas gingivalis* bacteria. The periodontitis group (K2) and the group treated with *Nannochloropsis oculta* showed significant differences. There was no significant difference between the groups given *Nannochloropsis oculata* with concentrations of 0.25%, 1.25% and 2.5%.

Table 3 shows that there is no significant difference between the K1 group in normal rats and the K2 group, namely periodontitis rats, but there is a significant difference between the K1 group and K3, K4, K5 groups, namely the group of rats given *Porphyromonas gingivalis* bacteria and *Nannochloropsis occulta* therapy. The periodontitis group (K2) and the group treated with *Nannochloropsis oculta* showed no significant difference. There was no significant difference between the groups given *Nannochloropsis oculata* with concentrations of 0.25%, 1.25% and 2.5%.

Discussion

The normal group (K1) had lower levels of RANKL and OPG expression, the periodontitis group had higher levels, the therapy group (K3-5) had lower levels of RANKL expression, and the OPG expression group had higher levels that were equivalent to RANKL. expression. The high amount of expression of RANKL, which is involved in osteoclastogenesis, leads to a higher amount of expression of OPG, which is involved in antiosteoclastogenesis. A cytokine called receptor activator of nuclear factor-B ligand (RANKL) is the one that causes bone loss[19]. Table 1 shows an increase in the RANKL, OPG in the periodontitis group in osteoblasts. Human periapical lesion RANKL mRNA expression is significantly higher than that of healthy periapical tissue[20]. Table 1 also shows the increase in RANKL is also accompanied by an increase in OPG but the amount is not balanced. At various parts of the human skeleton, bone resorption can be triggered by either increased RANKL or decreased OPG local expression[10].

Tables 2 and 3 show significant differences between group 1 and the group of rats with periodontitis. Bacteria-reactive immune response may induce RANKL-expressing T-cells in the mouse periapical bone loss lesion[20]. RANKL is produced as a membrane-bound or secreted ligand by osteoblasts, fibroblasts, or activated T- and B-cells. An osteoclast-stimulating cell membrane-bound factor known as receptor activator of NF-B ligand (RANKL), a member of the Tumor Necrosis Factor (TNF) ligand superfamily, has been identified[10].

In order to control inflammation, a healthy periodontium maintains a sophisticated dynamic equilibrium of inflammatory cytokines. When this equilibrium shifts in favor of proinflammatory cytokines, periodontal tissue is destroyed[21]. Periodontal bone destruction has been linked to a variety of mediators, including nitric oxide, cytokines, and arachidonic acid metabolites[3]. Neutrophils are major layers in periodontitis. Th17 and exFoxp3Th17 activation is dependent on IL-6 production from stromal cells in the periodontal tissue, such as periodontal ligament cells (PDL cells). Periodontitis-related inflammatory cytokines, such as tumor necrosis factor (TNF) and interleukin-1 (IL-1) produced from host cells exposed to the dental biofilm, produce a synergistic effect by increasing IL-6 synthesis[21]. In synovial inflammation, proinflammatory cytokines like IL-1, IL-6, TNFa, and IL-17 strongly promote RANKL production by activating the NF-kB pathway in synovial cells and T cells, which activates osteoclasts[22].

Tables 2 and 3 of the periodontitis therapy group showed a significant difference with group 1 and group 2, but when viewed from table 1 for RANKL there was a decrease in the treatment group compared to the periodontitis group. The OPG markers showed a significant reduction with the normal group but seen in table 1 the average number of OPGs was able to offset the number of RANKL, meaning that the alveolar bone had improved compared to the periodontitis group. *Nannochloropsis oculata* contains alkaloids[15]. Through RANKL-dependent pathways like TRAF6, NFB, MAPK, and NFATc1 signaling pathways, alkaloids reduce osteoclastogenesis[23].

Decreased RANKL or increased OPG expression could result in enhanced bone formation, leading to osteopetrotic conditions. The involvement of the RANKL-OPG system is well established in the pathogenesis of diseases of bone and mineral metabolism, such as rheumatoid arthritis, postmenopausal osteoporosis, Paget's disease and bone malignancies, such as multiple myeloma[10]. Bone mass maintenance is made possible by high levels of osteoprotegerin (OPG), which competes with RANKL to prevent the formation of ligand-receptor complexes[19].

Polyunsaturated fatty acids (PUFAs) are present in *Nannochloropsis Sp*[24]. Osteoclast formation was significantly inhibited by PUFAs in response to RANKL[25]. The production of -3 PUFA will inhibit pro-inflammatory cytokines like IL-1, IL-6, and TNF-, preventing bone resorption[26]. Through regulation of RANKL expression induced by proinflammatory cytokines, flavonoids can inhibit osteoclastogenesis[27].

OPG prevents all of the downstream molecular events that result in osteoclast differentiation and bone resorption by binding to RANKL and preventing its subsequent interaction with RANK. OPG prevents all of the downstream molecular events that result in osteoclast differentiation and bone resorption by binding to RANKL and preventing its subsequent interaction with RANK. Bone mass maintenance is made possible by high levels of osteoprotegerin (OPG), which competes with RANKL to prevent the

formation of ligand-receptor complexes. Osteoprotegerin is a deceptive protein that prevents bone resorption by capturing and binding to it. Bones are protected from resorption by high OPG levels[10,28].

Conclusions

Nannochloropsis oculata is able to balance the amount of RANKL and OPG in chronic periodontitis.

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Declaration of Interest

The authors report no conflict of interest.

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