Atatürk Üniv Diş Hek Fak Derg (*J Dent Fac Atatürk Uni*)
Cilt:31, Sayı:2, Yıl: 2021, Sayfa: 167-74

Araştırma/ Research Article

COMPARISON OF THE EFFECTS OF OMEGA 3 AND PROBIOTICS ON ALVEOLAR BONE LOSS IN RATS: AN IMMUNOHISTOCHEMICAL STUDY≠

OMEGA 3 VE PROBİYOTİKLERİN RATLARDA ALVEOLER KEMİK KAYBINA ETKİLERİNİN KARŞILAŞTIRILMASI: İMMÜNOHİSTOKİMYASAL BİR ÇALIŞMA≠

Dr. Öğr. Üyesi Burak DOĞAN* **Dr. Öğr. Üyesi Esra Sinem KEMER DOĞAN***
Prof. Dr. Özlem ÖZMEN**

Makale Kodu/Article code: 4487
Makale Gönderilme tarihi: 06.07.2020
Kabul Tarihi: 13.01.2021
DOI : *10.17567/ataunidfd.860173*

Burak Doğan : *ORCID ID:* 0000-0001-9011-1377
Esra Sinem Kemer Doğan: *ORCID ID:*0000-0003-3411-5925
Özlem Özmen : *ORCID ID:* 0000-0002-1835-1082

ABSTRACT
Aim: Omega 3 and probiotics have been demonstrated to have beneficial effects in periodontal disease pathogenesis. Inducible nitric oxide synthase is an important mediator regulating immune response. The objective of this study was to compare the impacts of omega 3 and probiotic supplementation on alveolar bone loss and gingival inducible nitric oxide synthase levels in an experimental periodontitis model.
Material and Methods: Thirty-two rats were separated into 4 equal groups as control, periodontitis, omega 3, and probiotic. Omega 3 or probiotic was supplemented by oral gavage and experimental periodontitis was induced to the rats in periodontitis, omega 3, and probiotic groups by ligature. Alveolar bone loss was measured histometrically. The number of neutrophils was counted and gingival inducible nitric oxide synthase levels were analysed immunohistochemically.
Results: Alveolar bone loss significantly increased in periodontitis induced groups, compared to control group and decreased in omega 3 and probiotic groups, compared to the periodontitis group ($p < 0.05$). Probiotic group had further decrease in alveolar bone loss than the omega 3 group ($p < 0.05$). The number of neutrophils and gingival inducible nitric oxide synthase levels were higher in the periodontitis, omega 3, and probiotic groups than those in the control group, and decreased in omega 3 and probiotic groups, compared to the periodontitis group ($p < 0.05$).
Conclusion: We suggest that omega 3 and probiotic administrations could reduce alveolar bone loss by decreasing gingival inducible nitric oxide synthase. Probiotic support seems to have more beneficial reducing alveolar bone loss, compared to omega 3.
Key words: Fatty Acids, Probiotics, Alveolar Bone Loss, Nitric Oxide Synthase Type II, Periodontitis

ÖZ
Amaç: Omega 3 ve probiyotiklerin periodontal hastalık patogenezinde yararlı etkilerinin olduğu gösterilmiştir. İndüklenebilir nitrik oksit sentaz immün yanıtı düzenleyen önemli bir mediyatördür. Bu çalışmanın amacı, deneysel periodontitis modelinde omega 3 ve probiyotik desteğinin alveoler kemik kaybı ve dişeti indüklenebilir nitrik oksit sentaz seviyeleri üzerine olan etkilerini karşılaştırmaktır.
Gereç ve Yöntem: Otuz iki sıçan kontrol, periodontitis, omega 3 ve probiyotik olarak 4 eşit gruba ayrıldı. Omega 3 ve probiyotik oral gavajla uygulandı ve periodontitis, omega 3 ve probiyotik gruplarındaki sıçanlara ligatür kullanılarak deneysel periodontitis oluşturuldu. Alveoler kemik kaybı histometrik olarak ölçüldü. Nötrofil sayısı hesaplandı ve dişeti indüklenebilir nitrik oksit sentaz seviyeleri immünohistokimyasal olarak değerlendirildi.
Bulgular: Alveoler kemik kaybı periodontitis indüklenen gruplarda kontrol grubuna göre anlamlı derecede arttı ve periodontitis grubuna kıyasla omega 3 ve probiyotik gruplarında azaldı ($p < 0.05$). Alveoler kemik kaybı, probiyotik grubunda omega 3 grubuna göre daha fazla azaldı ($p < 0.05$). Nötrofil sayıları ve dişeti indüklenebilir nitrik oksit sentaz seviyeleri kontrol grubuna göre periodontitis, omega 3 ve probiyotik gruplarında daha yüksekti ve periodontitis grubuyla kıyaslandığında omega 3 ve probiyotik gruplarında azaldı ($p < 0.05$).
Sonuç: Omega 3 ve probiyotik desteğinin dişeti indüklenebilir nitrik oksit sentaz seviyelerini azaltarak alveoler kemik kaybını düşürebildiğini öne sürmekteyiz. Alveoler kemik kaybını azaltmada probiyotik desteği, omega 3 ile karşılaştırıldığında daha yararlı görünmektedir.
Anahtar Kelimeler: Yağ Asidleri, Probiyotikler, Alveoler Kemik Kaybı, Nitrik Oksit Sentaz Tip II, Periodontit

*Hatay Mustafa Kemal University Faculty of Dentistry, Department of Periodontology, Hatay.
**Burdur Mehmet Akif Ersoy University Faculty of Veterinary Medicine, Department of Pathology, Burdur.
≠This study was supported by the the Hatay Mustafa Kemal University Scientific Research Projects Commission (Project number: 18.M.090).

Kaynakça Bilgisi: Doğan B, Kemer Doğan ES, Özmen Ö. Omega 3 ve probiyotiklerin ratlarda alveoler kemik kaybına etkilerinin karşılaştırılması: immünohistokimyasal bir çalışma. Atatürk Üniv Diş Hek Fak Derg 2021; 31: 167-74.
Citation Information: *Dogan B, Kemer Dogan ES, Ozmen O. comparison of the effects of omega 3 and probiotics on alveolar bone loss in rats: an immunohistochemical study. J Dent Fac Atatürk Uni 2021; 31: 167-74.*

INTRODUCTION

Periodontitis is one of the most prevalent diseases worldwide and its prevalence increases gradually with age.[1] Dental plaque initiates an inflamematory response in the gingiva and inflammation leads dramatic consequences on periodontal tissues in susceptible patients.[2]

The effects of adjunctive treatments on periodontal disease pathogenesis have been investigated for a period of time. Omega 3 is an essential fatty acid and has beneficial effects in various inflammatory diseases via modulating inflammatory mediators, oxidetive response, lipids,[3] and pathogenic bacteria.[4] Similarly, probiotics, live microorganisms which are benefitcial on host health,[5] improve immune response[6] and regulate microbiota.[7] Omega 3[8-10] and probiotics[11-15] have been reported to improve clinical periodontal parameters in humans and reduce alveolar bone loss (ABL) in experimental studies,[16-21] although some researchers could not indicate a significant difference.[22-26]

Nitric oxide synthase (NOS) is an enzyme which takes part in the synthesis of nitric oxide (NO), an important molecule in several biological activities such as regulation of vascular tonus and immune response. NOS has 3 isoforms known as inducible NOS (iNOS), endothelial NOS (eNOS), and neuronal NOS (nNOS). NO is vital for inflammatory and immune response, however its excessive amounts could be toxic and associated with different diseases, thus dual activity of iNOS related NO is mostly affected by concentration.[27]

Proinflammatory cytokines and lipopolysaccharides (LPS) were shown to stimulate iNOS synthesis in periodontal ligament cells.[28] Increased gingival iNOS mRNA levels were demonstrated in inflamed tissue and it was suggested that iNOS may have a role in the inflammatory response of periodontitis.[29] Many reports indicated that omega 3[30-32] and probiotics[33,34] were able to reduce iNOS. Probiotic administration was also presented to decrease salivary NOS in patients with chronic periodontitis.[35]

It seems that both omega 3 and probiotics may have beneficial effects on host immune system via similar pathways and a possible synergistic mechanism between these adjuvants has recently been reported,[36] however none of the previous studies have compared their effects. The purpose of this study was to exhibit and compare the effects of omega 3 and probiotic supplementation on ABL and gingival iNOS levels in a ligature-induced periodontitis model.

MATERIALS AND METHODS

Ethics was provided by the Süleyman Demirel University Animal Experiments Local Ethics Committee (No: 05.05.2018, 13/06). Thirty-two adult Wistar albino rats (224.78±16.73 g) were separated into 4 equal groups (n=8) as control (C), periodontitis (P), omega 3 (O3), and probiotic (Pro). The study was conducted for a period of 44 days and rats were kept in standardized cages in a room at 21-23°C heat and 55–60% humidity, with 12 hours of light and 12 hours of darkness.

Experimental Period

During the induction of experimental periodontitis and sacrification, 80 mg/kg ketamine hydrochloride and 10 mg/kg xylazine combination was used.[20] After anaesthesia, sterile 3.0 silk sutures were ligated in a subgingival position around the cervix of maxillary 2nd molars of the rats in the P, O3, and Pro groups on the 30th day for two weeks.[37] In order to prevent the movement of the ligature, two knots were placed at the mesio-buccal side of the 2nd molars.

All rats were fed *ad libitum* with standard pellet diet and water. In addition to the *ad libitum* diet, omega 3 or probiotics were supplemented to the rats in the O3 and Pro groups, respectively. Omega 3 fish oil including 60% EPA and 40% DHA (Voonka fish oil omega 3, Eczacıbaşı, İstanbul, Turkey) at 40 mg/kg dose[26] and *Lactobacillus* (L. casei, L. plantarum, L. acidophilus and L. delbrueckii subspecies bulgaricus), *Bifidobacterium* (B. longum, B. breve and B. infantis), and *Streptococcus salivarius subspecies thermophiles* strains containing probiotics (VSL#3, VSL Pharmaceuticals, Gaithersburg, Maryland, USA) at 13x10^{9}/kg dose[38] were applied in 1 ml saline via oral gavage along the experimental period.

Histopathologic and Histometric Examination

Harvested right maxilla samples were fixed in neutral formalin (10%) and decalcified with 0.1 M EDTA solution for 1 week. Samples were then routinely processed, embedded in paraffin and cut into 5 µm sections in the coronal plane of the tooth. Then the samples were stained with hematoxylin and eosin and evaluated by a light microscope under X10

objective (Olympus CX41, Olympus Corporation, Tokyo, Japan). ABL was measured histometrically in the intermolar area of the first and second molars by calculating the distance between cementoenamel junction and alveolar bone crest.[39] Additionally, count of neutrophils in the junctional epithelium (JE) and connective tissue (CT) was examined in an area of 0.05x .05 mm under a magnification of X400 (Olympus CX41, Olympus Corporation, Tokyo, Japan).[40] Analyses were performed using a digital programme (Database Manual Cell Sens Life Science Imaging Software System, Olympus Co., Tokyo, Japan).

Immunohistochemical Analysis

For immunohistochemistry, left maxillary halves were immunostained with iNOS antibody using a streptavidin biotin technique (Anti-iNOS antibody [ab15323], Abcam, Cambridge, UK). Primary antibody was used at a 1:100 dilution, incubated for a period of 60 min, and biotinylated secondary antibody and streptavidin-alkaline phosphatase conjugate was used for immunohistochemistry. A commercial kit (EXPOSE Mouse and Rabbit Specific HRP/DAB Detection IHC Kit [ab80436], Abcam, Cambridge, UK) was processed as a secondary antibody and 3,3'-diaminobenzidine as chromogen for 5 min. For negative controls, a primary antiserum step was omitted. Analyses were performed by a blinded examiner (Ö.Ö.). To evaluate the percentage of positive stained cells, 100 cells were counted in 10 different fields for each section under X40 objective (Olympus CX41, Olympus Corporation,Tokyo, Japan) using a commercial analysis software (BAB Bs200Pro Image Processing and Analysis Systems 4.0, Ankara, Turkey).

Statistics

The power of the study was >99% for ABL (effect size=4.03, α=0.05) (G*power, v.3.1.9.2 for Windows, University of Kiel, Kiel, Germany). The normality of distribution was evaluated by the Kolmogorov-Smirnov test, and Levene's test of homogeneity of variance was applied. None of the evaluated parameters was normally distributed. Kruskal-Wallis test followed by Mann Whitney U test was used to compare histopathological, histometric, and immunohistochemical scores between the groups. Calculations were made using a statistics programme (SPSS 15.0, SPSS Inc., Chicago, IL, USA). Variables were presented as mean±standard deviation. $p < 0.05$ was regarded as the significance.

RESULTS

ABL was higher in the periodontitis groups (P, O3, and Pro), than the C group, and lower in the O3 and Pro groups, compared to the P group ($p < 0.05$) (Figure 1). Pro group demonstrated a significant decrease in ABL, compared to the O3 group ($p < 0.05$) (Figure 2). The number of neutrophils in JE and CT was significantly increased in the P, O3, and Pro groups, than those in the C group, and reduced in the O3 and Pro groups, with no difference between them ($p > 0.05$), in comparison with the P group ($p < 0.05$) (Figure 3). Immunohistochemical images are shown in Figure 4. Gingival iNOS was lower in the C group than the other groups ($p < 0.05$). Compared to the P group, decreased numbers were obtained in the O3 and Pro groups ($p < 0.05$), although O3 and Pro groups had similar levels ($p > 0.05$) (Figure 5).

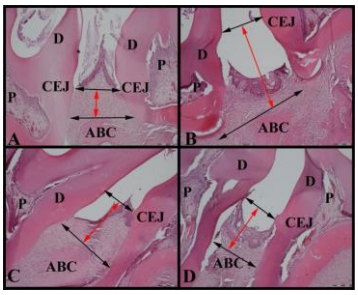

Figure 1. Histometric measurement of alveolar bone loss. A: control group, B: periodontitis group, C: omega 3 group, D: probiotic group. Red arrows indicate the distance between the cementoenamel junction (CEJ) and alveolar bone crest (ABC). D: dentin, P: pulp, Bars=200 µm

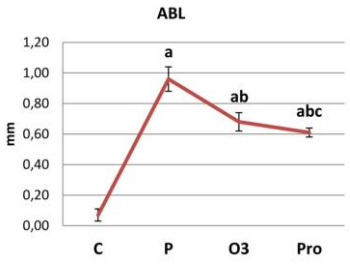

Figure 2. Alveolar bone loss (ABL) levels among the groups. C: control group, P: periodontitis group, O3: omega 3 group, Pro: probiotic group. [a]Significant difference compared to C group ($p < 0.05$), [b]Significant difference compared to P group ($p < 0.05$), [c]Significant difference compared to O3 group ($p < 0.05$).

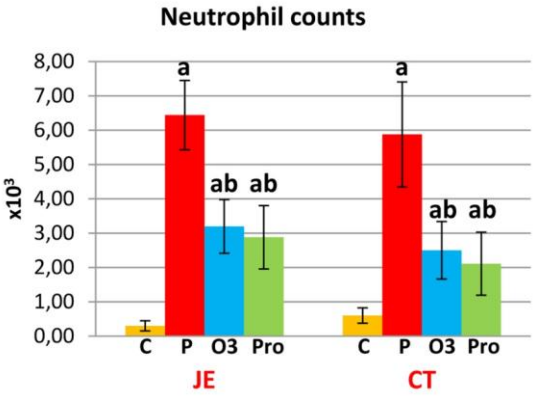

Figure 3. The number of neutrophils in junctional epithelium (JE) and connective tissue (CT). C: control group, P: periodontitis group, O3: omega 3 group, Pro: probiotic group. [a]Significant difference compared to C group ($p < 0.05$), [b]Significant difference compared to P group ($p < 0.05$).

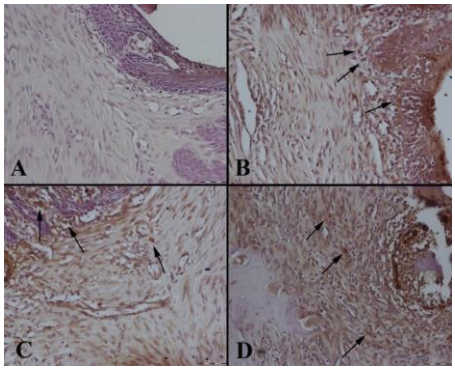

Figure 4. Inducible nitric oxide synthase expirations in gingiva. A: Negative expiration in intact gingival mucosa in control group, B: Marked expiration in numerous cells in ulcerated gingival mucosa in periodontitis group, C: Decreased expiration in some epithelial and mesenchymal cells in omega 3 group, D: Moderate expiration in some submucosal cells in probiotic group. Black arrow: Immunohistochemically-positive stained cells. Bars=50 µm.

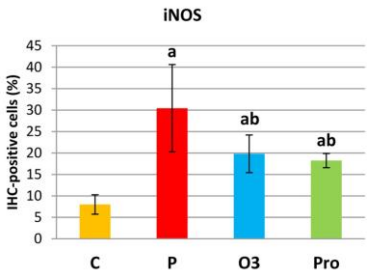

Figure 5. Immunohistochemically (IHC), the number of inducible nitric oxide synthase (iNOS)-positive cells in gingiva. C: control group, P: periodontitis group, O3: omega 3 group, Pro: probiotic group. [a]Significant difference compared to C group ($p < 0.05$), [b]Significant difference compared to P group ($p < 0.05$).

DISCUSSION

There has been no study comparing the effects of omega 3 and probiotics on ABL. We revealed that both omega 3 and probiotic supplementation decreased ABL. Additionally; probiotic administration had higher reduction in ABL, compared to omega 3. Furthermore, omega 3 and probiotic administrations significantly suppressed gingival iNOS, which was increased by periodontitis induction.

Omega 3[3] and probiotics[7] could be beneficial on host immune system by regulating various inflammatory mechanisms. Moreover, they are able to inhibit pathological microbiota.[4,7] A number of studies have been performed investigating the effects of omega 3 and probiotics on periodontal disease.[8-15] In humans, dietary supplementation with omega 3 in addition to scaling and root planning (SRP) had higher decreases in gingival index (GI), probing depth (PD), and clinical attachment level (CAL), than SRP in patients with chronic periodontitis and it was suggested that omega 3 could be used as a modulator in the treatment of periodontitis.[9] In postmenopausal women, omega 3 administration combined with SRP indicated greater improvements in PD and CAL, than SRP.[10] In a recent study, Castro Dos Santos et al.[8] presented increased clinical attachment gain in pockets with PD ≥5 mm by omega 3 and aspirin supplementation in addition to SRP in chronic patients with type 2 diabetes. On contrary, Martinez et al.[23] did not demonstrate a significant effect of 1-year omega 3 administration with SRP on the clinical results in chronic periodontitis patients. Keskiner et al.[22] also could not exhibit an effect of omega 3 on clinical parameters of chronic periodontitis patients in a randomized controlled clinical study with 6 months follow-up.

Invernici et al.[11] revealed that supplementation of *Bifidobacterium animalis subspecies lactis* (B. lactis) containing lozenges in addition to SRP had further reductions in PD and CAL in chronic periodontitis. Similarly, supplementation with yogurt containing B. lactis for 28 days decreased levels of gingival inflammatory markers including plaque index (PI), GI, and bleeding on probing (BOP).[13] Meenakshi et al.[12] reported improved clinical periodontal parameters with adjunctive administration of L. casei Shirota to SRP. *Lactobacillus reuteri* (L. reuteri) supplementation to initial periodontal treatment was suggested to be

useful in moderately deep pockets.[14] Tekce et al.[15] also showed that SRP plus L. reuteri significantly decreased PI, GI, BOP, and PD, compared to SRP alone. On contrary, Pelekos et al.[25] did not indicate a significant effect of L. reuteri supplementation on clinical outcomes of patients with chronic periodontitis. Montero et al.[24] also reported that multi probiotic administration did not lead to a change in GI, although improved microbiological outcomes were observed in patients with gingivitis.

In addition to the human studies, the role of omega 3 and probiotics were researched in experimental studies. Decreased ABL levels with omega 3 supplementation were demonstrated previously.[16-18] Recently, omega 3 and/or aspirin was exhibited to be effective reducing ABL by modulating osteoclastogenesis.[31] Furthermore, fish oil diet was shown to decrease ABL in hypercholesterolemic rats with ligature-induced periodontitis.[41] B. lactis administration significantly decreased CAL and ABL in an experimental periodontitis study.[19] Garcia et al.[21] presented that local application of *Saccharomyces cerevisiae* in combination with SRP significantly decreased ABL in the furcation area of the mandibular first molars of rats. They suggested that probiotic supplementation alone or in addition to SRP were effective in the management of periodontitis. Foureaux Rde et al.[20] also stated that probiotics could reduce tissue destruction caused by periodontal disease. On the other hand Vardar-Sengul et al.[26] could not indicate a significant effect of therapeutic and/or prophylactic omega 3 administration on ABL, although inflammatory markers were improved.

We presented that both omega 3 and probiotic supplementation decreased ABL, and probiotic supplementation reduced ABL much more than omega 3. Many adjunctive supports have been investigated and researchers have been studying to explore the optimum adjuvant in periodontal disease pathogenesis. Recent studies have shown the probable synergistic mechanisms between omega 3 and probiotics[36,42] but there has been no study whether these adjuvants have equal roles on host or one of them is superior to other, thus the rationale of this study was to compare the effects of these adjuvants. The effects of omega 3 and probiotics on periodontal pathogenesis may depend on the dose, application method, and model used. Also lack of microbiological outcomes, which were possibly affected by omega 3 and probiotic administration, limited to comment on the results. Keskiner et al.[22] revealed that the impact of omega 3 may be dose dependent. Vardar-Sengul et al.[26] asserted that 14 days of administration was not long enough to demonstrate an effect on ABL.[26] Similarly to Vardar-Sengul et al.[26] we used omega 3 at 40 mg/kg dose but contrary them, supplementation continued 44 days. Our results are in accordance with the literature exhibiting omega 3 or probiotics could reduce ABL. Furthermore, probiotics were shown to be superior to omega 3 regarding reducing ABL, which needs to be explored further.

Omega 3 and probiotic supplementation was shown to reduce gingival iNOS together with a decrease in the count of neutrophils in both JE and CT, compared to the P group. iNOS and NO are regulatory molecules of host response in nature, and high amounts of NO depending abnormal iNOS activity plays a role in various diseases, thus iNOS has both useful and harmful outcomes.[27] It has been manifested that iNOS is synthesized by a stimulation of proinflammatory mediators and LPS,[28] and iNOS derived NO is able to stimulate osteoclast differentiation.[43] In ligature-induced periodontitis models, elevated gingival iNOS[44] and iNOS mRNA levels[39] were observed in the periodontitis groups, compared to the controls. iNOS inhibition was also improved clinical periodontal parameters and reduced experimental gingivitis in dogs,[45] and decreased ABL in an experimental periodontitis model.[43] In our study, increased gingival iNOS levels in the P group, compared to the C group supports previous studies.

Omega 3 administration was indicated to decrease iNOS and NO in LPS-stimulated macrophages.[30] α-linolenic acid, one of the fatty acids in omega 3,[46] was presented to downregulate iNOS gene expression by impeding nuclear factor kappa B and mitogen-activated protein kinase signaling.[32] In a recent study, omega 3 and/or aspirin decreased iNOS protein synthesis in LPS-stimulated cells.[31] Similarly, *Lactobacillus brevis* (L. brevis) inhibited iNOS activity which was induced by LPS.[33] Treatment with *Weissella cibaria* also reduced NO and iNOS expressions together with mRNA levels of iNOS in LPS-induced cells, indicating that probiotic administration was able to inhibit NO by suppressing iNOS.[34] In patients with chronic periodontitis, supplementation of L. brevis decreased salivary NOS activity.[35] Authors suggested that anti-inflammatory effects of L. brevis may be depended on decreased iNOS activity and, therefore

NO dependent prostaglandin E2 expression and matrix metalloproteinase activation.[35] Decreased iNOS levels in the O3 and Pro groups, compared to the P group confirm earlier findings.

Higher levels of gingival iNOS were reported in patients with periodontal disease, compared to the controls, and neutrophils were shown to express substantial amounts of iNOS, which could be an important pathway in periodontal disease pathogenesis.[47] When decreased neutrophil counts in the O3 and Pro groups were considered, we suggest that decreased inflammatory response may prevent neutrophil recruitment in JE and CT which finally leads to reduced iNOS production in the supplementation groups, compared to the P group.

CONCLUSIONS

Within the limitations, we conclude that omega 3 and probiotics are able to reduce ABL by decreasing gingival iNOS. Although probiotics likely to have better contribution on reducing ABL than omega 3, underlying biological mechanisms are needed to be investigated.

Acknowledgements
The authors declare that there were no other contributors involved in this work.
Conflicts of interest statement
The authors declare no conflict of interest

REFERENCES

1. Kassebaum NJ, Bernabe E, Dahiya M, Bhandari B, Murray CJ, Marcenes W. Global burden of severe periodontitis in 1990-2010: a systematic review and meta-regression. J Dent Res 2014;93:1045-53.
2. Kononen E, Gursoy M, Gursoy UK. Periodontitis: A multifaceted disease of tooth-supporting tissues. J Clin Med 2019;8:1135.
3. Connor WE. Importance of n-3 fatty acids in health and disease. Am J Clin Nutr 2000;71:171S-5S.
4. Chanda W, Joseph TP, Guo XF, Wang WD, Liu M, Vuai MS, Padhiar AA, Zhong MT. Effectiveness of omega-3 polyunsaturated fatty acids against microbial pathogens. J Zhejiang Univ Sci B 2018;19:253-62.
5. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, Morelli L, Canani RB, Flint HJ, Salminen S, Calder PC, Sanders ME. Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol 2014;11:506-14.
6. Arat Maden E, Altun C. Probiyotikler ve ağız sağlığı. Atatürk Üniv Diş Hek Fak Derg 2012;22:334-9.
7. Sanders ME, Merenstein DJ, Reid G, Gibson GR, Rastall RA. Probiotics and prebiotics in intestinal health and disease: from biology to the clinic. Nat Rev Gastroenterol Hepatol 2019;16:605-16.
8. Castro Dos Santos NC, Andere NM, Araujo CF, de Marco AC, Kantarci A, Van Dyke TE, Santamaria MP. Omega-3 PUFA and aspirin as adjuncts to periodontal debridement in patients with periodontitis and type 2 diabetes mellitus. Randomized clinical trial. J Periodontol 2020.
9. Deore GD, Gurav AN, Patil R, Shete AR, Naiktari RS, Inamdar SP. Omega 3 fatty acids as a host modulator in chronic periodontitis patients: a randomised, double-blind, palcebo-controlled, clinical trial. J Periodontal Implant Sci 2014;44:25-32.
10. Elgendy EA, Kazem HH. Effect of omega-3 fatty acids on chronic periodontitis patients in postmenopausal women: a randomised controlled clinical study. Oral Health Prev Dent 2018;16:327-32.
11. Invernici MM, Salvador SL, Silva PHF, Soares MSM, Casarin R, Palioto DB, Souza SLS, Taba M, Jr., Novaes AB, Jr., Furlaneto FAC, Messora MR. Effects of bifidobacterium probiotic on the treatment of chronic periodontitis: A randomized clinical trial. J Clin Periodontol 2018;45:1198-210.
12. Meenakshi SS, Varghese S. Adjunctive effect of probiotic (lactobacillus casei shirota) to scaling and root planing in the management of chronic periodontitis. Drug Invention Today 2018;10:1381-6.
13. Kuru BE, Laleman I, Yalnizoglu T, Kuru L, Teughels W. The influence of a bifidobacterium animalis probiotic on gingival health: A randomized controlled clinical trial. J Periodontol 2017; 88: 1115-23.
14. Ince G, Gursoy H, Ipci SD, Cakar G, Emekli-Alturfan E, Yilmaz S. Clinical and biochemical evaluation of lozenges containing lactobacillus reuteri as an adjunct to non-surgical periodontal therapy in chronic periodontitis. J Periodontol 2015;86:746-54.

15. Tekce M, Ince G, Gursoy H, Dirikan Ipci S, Cakar G, Kadir T, Yilmaz S. Clinical and microbiological effects of probiotic lozenges in the treatment of chronic periodontitis: a 1-year follow-up study. J Clin Periodontol 2015;42:363-72.
16. Kesavalu L, Vasudevan B, Raghu B, Browning E, Dawson D, Novak JM, Correll MC, Steffen MJ, Bhattacharya A, Fernandes G, Ebersole JL. Omega-3 fatty acid effect on alveolar bone loss in rats. J Dent Res 2006;85:648-52.
17. Bendyk A, Marino V, Zilm PS, Howe P, Bartold PM. Effect of dietary omega-3 polyunsaturated fatty acids on experimental periodontitis in the mouse. J Periodontal Res 2009;44:211-6.
18. Kesavalu L, Bakthavatchalu V, Rahman M, Su J, Raghu B, Dawson D, Fernandes G, Ebersole J. Omega-3 fatty acid regulates inflammatory cytokine/mediator messenger RNA expression in Porphyromonas gingivalis-induced experimental periodontal disease. Oral Microbiol Immunoll 2007; 22:232-9.
19. Oliveira LF, Salvador SL, Silva PH, Furlaneto FA, Figueiredo L, Casarin R, Ervolino E, Palioto DB, Souza SL, Taba M, Jr., Novaes AB, Jr., Messora MR. Benefits of Bifidobacterium animalis subsp. lactis Probiotic in Experimental Periodontitis. J Periodontol 2017;88:197-208.
20. Foureaux Rde C, Messora MR, de Oliveira LF, Napimoga MH, Pereira AN, Ferreira MS, Pereira LJ. Effects of probiotic therapy on metabolic and inflammatory parameters of rats with ligature-induced periodontitis associated with restraint stress. J Periodontol 2014;85:975-83.
21. Garcia VG, Knoll LR, Longo M, Novaes VC, Assem NZ, Ervolino E, de Toledo BE, Theodoro LH. Effect of the probiotic Saccharomyces cerevisiae on ligature-induced periodontitis in rats. J Periodontal Res 2016;51:26-37.
22. Keskiner I, Saygun I, Bal V, Serdar M, Kantarci A. Dietary supplementation with low-dose omega-3 fatty acids reduces salivary tumor necrosis factor-alpha levels in patients with chronic periodontitis: a randomized controlled clinical study. J Periodontal Res 2017;52:695-703.
23. Martinez GL, Koury JC, Martins MA, Nogueira F, Fischer RG, Gustafsson A, Figueredo CM. Serum level changes of long chain-polyunsaturated fatty acids in patients undergoing periodontal therapy combined with one year of omega-3 supplementation: a pilot randomized clinical trial. J Periodontal Implant Sci 2014;44:169-77.
24. Montero E, Iniesta M, Rodrigo M, Marin MJ, Figuero E, Herrera D, Sanz M. Clinical and microbiological effects of the adjunctive use of probiotics in the treatment of gingivitis: A randomized controlled clinical trial. J Clin Periodontol 2017;44:708-16.
25. Pelekos G, Ho SN, Acharya A, Leung WK, McGrath C. A double-blind, paralleled-arm, placebo-controlled and randomized clinical trial of the effectiveness of probiotics as an adjunct in periodontal care. J Clin Periodontol 2019;46:1217-27.
26. Vardar-Sengul S, Buduneli E, Turkoglu O, Buduneli N, Atilla G, Wahlgren J, Sorsa T, Baylas H. The effects of selective COX-2 inhibitor/celecoxib and omega-3 fatty acid on matrix metalloproteinases, TIMP-1, and laminin-5gamma2-chain immunolo-calization in experimental periodontitis. J Periodontol 2008;79:1934-41.
27. Cinelli MA, Do HT, Miley GP, Silverman RB. Inducible nitric oxide synthase: Regulation, structure, and inhibition. Med Res Rev 2020;40:158-89.
28. Kim YS, Pi SH, Lee YM, Lee SI, Kim EC. The anti-inflammatory role of heme oxygenase-1 in lipopolysaccharide and cytokine-stimulated inducible nitric oxide synthase and nitric oxide production in human periodontal ligament cells. J Periodontol 2009;80:2045-55.
29. Hirose M, Ishihara K, Saito A, Nakagawa T, Yamada S, Okuda K. Expression of cytokines and inducible nitric oxide synthase in inflamed gingival tissue. J Periodontol 2001;72:590-7.
30. Aldridge C, Razzak A, Babcock TA, Helton WS, Espat NJ. Lipopolysaccharide-stimulated RAW 264.7 macrophage inducible nitric oxide synthase and nitric oxide production is decreased by an omega-3 fatty acid lipid emulsion. J Surg Res 2008;149:296-302.
31. Yang M, Li L, Soh Y, Heo SM. Effects of omega-3 fatty acids and aspirin on Porphyromonas gingivalis-induced periodontitis in rats. J Periodontol 2019;90:1307-19.
32. Ren J, Chung SH. Anti-inflammatory effect of alpha-linolenic acid and its mode of action through

the inhibition of nitric oxide production and inducible nitric oxide synthase gene expression via NF-kappaB and mitogen-activated protein kinase pathways. J Agric Food Chem 2007;55:5073-80.
33. Liu Y-W, Ong W-K, Su Y-W, Hsu C-C, Cheng T-H, Tsai Y-C. Anti-inflammatory effects of Lactobacillus brevis K65 on RAW 264.7 cells and in mice with dextran sulphate sodium-induced ulcerative colitis. Beneficial microbes 2016;7:387-96.
34. Yu H-S, Lee N-K, Choi A-J, Choe J-S, Bae CH, Paik H-D. Anti-inflammatory potential of probiotic strain Weissella cibaria JW15 isolated from Kimchi through regulation of NF-κB and MAPKs pathways in LPS-induced RAW 264. 7 Cells. J Microbiol Biotechn 2019;29:1022-32.
35. Riccia DN, Bizzini F, Perilli MG, Polimeni A, Trinchieri V, Amicosante G, Cifone MG. Anti-inflammatory effects of Lactobacillus brevis (CD2) on periodontal disease. Oral Dis 2007;13:376-85.
36. Eratte D, McKnight S, Gengenbach TR, Dowling K, Barrow CJ, Adhikari BP. Co-encapsulation and characterisation of omega-3 fatty acids and probiotic bacteria in whey protein isolate–gum Arabic complex coacervates. J Func Foods 2015; 19: 882-92.
37. Kirzioglu FY, Tozum Bulut M, Dogan B, Fentoglu O, Ozmen O, Carsancakli SA, Ergun AG, Ozdem M, Orhan H. Anti-inflammatory effect of rosuvastatin decreases alveolar bone loss in experimental periodontitis. J Oral Sci 2017;59:247-55.
38. Esposito E, Iacono A, Bianco G, Autore G, Cuzzocrea S, Vajro P, Canani RB, Calignano A, Raso GM, Meli R. Probiotics reduce the inflammatory response induced by a high-fat diet in the liver of young rats. J Nutr 2009;139:905-11.
39. Kuo PJ, Fu E, Lin CY, Ku CT, Chiang CY, Fu MM, Fu MW, Tu HP, Chiu HC. Ameliorative effect of hesperidin on ligation-induced periodontitis in rats. J Periodontol 2019;90:271-80.
40. Yoshinaga Y, Ukai T, Nakatsu S, Kuramoto A, Nagano F, Yoshinaga M, Montenegro JL, Shiraishi C, Hara Y. Green tea extract inhibits the onset of periodontal destruction in rat experimental periodontitis. J Periodontal Res 2014;49:652-9.
41. Antona ME, Ramos C, Stranges A, Monteiro AF, Gonzales Chaves MM, Mandalunis P, Zago V, Friedman SM, Macri EV. Fish oil diet effects on alveolar bone loss, in hypercholesterolemic rats. Arch Oral Biol 2020;109:104553.
42. Kobyliak N, Falalyeyeva T, Bodnar P, Beregova T. Probiotics supplemented with omega-3 fatty acids are more effective for hepatic steatosis reduction in an animal model of obesity. Probiot Antimicrob Proteins 2017;9:123-30.
43. Herrera BS, Martins-Porto R, Maia-Dantas A, Campi P, Spolidorio LC, Costa SK, Van Dyke TE, Gyurko R, Muscara MN. iNOS-derived nitric oxide stimulates osteoclast activity and alveolar bone loss in ligature-induced periodontitis in rats. J Periodontol 2011;82:1608-15.
44. Kirzioglu FY, Ozmen O, Dogan B, Bulut MT, Fentoglu O, Ozdem M. Effects of rosuvastatin on inducible nitric oxide synthase in rats with hyperlipidaemia and periodontitis. J Periodontal Res 2018;53:258-66.
45. Paquette DW, Rosenberg A, Lohinai Z, Southan GJ, Williams RC, Offenbacher S, Szabo C. Inhibition of experimental gingivitis in beagle dogs with topical mercaptoalkylguanidines. J Periodontol 2006; 77: 385-91.
46. Doughman SD, Krupanidhi S, Sanjeevi CB. Omega-3 fatty acids for nutrition and medicine: considering microalgae oil as a vegetarian source of EPA and DHA. Curr Diabetes Rev 2007;3:198-203.
47. Batista AC, Silva TA, Chun JH, Lara VS. Nitric oxide synthesis and severity of human periodontal disease. Oral Dis 2002;8:254-60.

Sorumlu Yazarın Yazışma Adresi
Burak Doğan
Hatay Mustafa Kemal University,
Faculty of Dentistry Department of Periodontology
31001 Hatay/Turkey
Phone: +90 326 245 60 60- 17171
Fax: +90 326 245 50 60
Email: burakdogann@gmail.com