Peer Reviewed, Open Access, Free
Published Quarterly
Mangalore, South India
ISSN 0972-5997
Volume 7, Issue 4; Oct-Dec 2008

Short Report

Effect of fish oil on lipopolysaccharide-induced hydroxyapatite loss in rat alveolar bone: A Preliminary Study

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Citation

Indahyani DE, Santoso ALS, Utoro T, Soesatyo MHNE, Sosroseno W. Effect of fish oil on lipopolysaccharide-induced hydroxyapatite loss in rat alveolar bone: A Preliminary Study *Online J Health Allied Scs.* 2008;7(4):7

URL

http://www.ojhas.org/issue28/2008-4-7.htm

Submitted: Nov 24, 2008; Accepted: Jan 15, 2009 Published: Feb 25, 2009

Abstract:

Dietary fish oil has been shown to inhibit bone resorption and, therefore, the aim of the present study was to test the hypothesis that fish oil alters lipopolysaccharide (LPS)-induced hydroxyapatite loss in rat alveolar bone. Rats were divided into four groups. The animals injected with saline or *Escherichia coli*-derived LPS into the maxillary alveolar mucosa on the buccoapical site of the molar region daily for 8 days were served as a negative or positive control, respectively. Other groups of animals were injected with LPS and orally treated with fish oil at the same day with or after LPS injection. The results of the present study showed that the hydroxyapatite contents of alveolar bone in rats treated with fish oil at the same day with or before LPS injection were significantly higher than those in rats injected with LPS alone, but still lower than those in untreated animals. Therefore, the present study suggests that oral treatment with fish oil may reduce LPS-induced hydroxyapatite loss in rat alveolar bone

Key Words: Alveolar, Bone, Fish Oil, Hydroxyapatite, Rat

Introduction:

Dietary fish oil, which is rich in n-3 fatty acid, to improve bone remodelling has been a focus attention mainly due to the fact that it inhibits bone resorption but enhances bone formation. (1) Indeed, previous reports demonstrating that fish oil inhibits alveolar bone loss induced by bacterial infection (2,3) or orthodontic appliances (4) in an animal model may reflect the inhibitory properties of fish oil on osteoclast activities.

Bone resorption mediated by osteoclasts is characterized by hydroxyapatite and type I collagen loss at the site of resorption pit formation.(5) Lipopolysaccharide (LPS) derived from enteric or oral bacteria has been shown to induce alveolar bone resorption in an animal model, (6,7) suggesting that this bacterial constituent may stimulate hydroxyapatite loss at the site of the alveolar bone resorptive area. Therefore, the aim of the present study was to test a hypothesis that fish oil may reduce LPS-induced hydroxyapatite loss in the rat alveolar bone.

Materials and Methods:

Male five days old Wistar rats were divided into 4 groups, each consisted of 20 animals and assigned to AIN 93 diet as previously described. (2) Group I and group II were injected with 50 µl of PBS and 5 µg of LPS derived from *Escherichia coli* (Sigma Chemical Co., St. Louis) dissolved in 50 µl of PBS, respectively, using syringe (27GX½") into the maxillary alveolar mucosa on the buccoapical site of the molar region daily for 8 days as described previously. (6) Group III was injected with LPS and orally treated with 1 ml of fish oil (Sigma) on the same day until the day of the sacrifice. (2) Group IV was orally treated with fish oil for 5 days and then injected with LPS. Animals were sacrificed by cervical dislocation on day 9, 13, 17 and 21, except that group IV was sacrificed on day 13, 17 and 21. Maxillary alveolar bones were isolated, heated at 100°C for 30 minutes and then minced. The levels of hydroxyapatite content were assessed by using X-ray diffractometer (Shimadzu, Kyoto, Japan). The experimental design was approved by the ethical committee of Faculty of Medicine, Gadjah Mada University. Data was statistically analyzed by a repeat measurement test using a statistical package (SysStat Software Inc., San Jose, CA, USA)..

Results:

The results of the present study showed that injection with LPS (group II) resulted in significant hydroxyapatite loss in rat alveolar bone as compared with group I ($p<0.05$). Interestingly, the levels of hydroxyapatite contents in the animals treated with fish oil at the same day with (group III) or before LPS injection (group IV) were higher than those in group II, but were still lower than those in group I ($p<0.05$). Furthermore, the levels of hydroxyapatite contents in group IV were not significantly difference than those in group III at day 13 and 21 ($p>0.05$), but were higher than those in group III at day 17 ($p<0.05$).

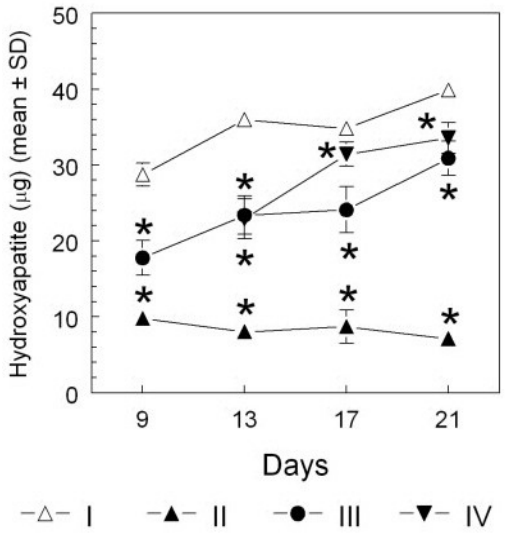

Fig. 1. Effect of oral treatment with fish oil on lipopolysaccharide-induced hydroxyapatite loss in rat alveolar bone. See text for detail of the groups. (*) significant difference at $P < 0.05$. SD = standard deviation.

Discussion:

The present study showed that oral treatment with fish oil at the same day with or before LPS injection at the rat alveolar mucosa increases the levels of alveolar bone hydroxyapatite formation, suggesting that fish oil treatment may reduce LPS-induced hydroxyapatite loss in rat alveolar bone. Since alveolar bone resorption is characterized by hydroxyapatite loss, (5) the results of the present study are in accordance with the previous reports showing that fish oil inhibits bacterially induced alveolar bone resorption in rats

(2,3) but not by another previous study.(7) The reason(s) to explain the discrepancy between the present and previous study (7) is unclear, but it could be differences in the experimental design. Yet, the exact mechanism(s) by which fish oil reduced LPS-induced hydroxyapatite loss in rat alveolar bone as seen in the present study is not well understood. One possibility is that fish oil might inhibit the synthesis of osteoclast-activating cytokines, such as PGE_2, but enhance the alkaline phosphatase activity, thereby decreasing osteoclast activities but up-regulating osteoblast functions and, hence, bone hydroxyapatite formation.(8) Our previous study showing that treatment with fish oil reduces the number of periapical osteoblasts in dental pulp-exposed rats (2) may support this contention. Alternatively, it has been demonstrated that osteoclast-mediated bone hydroxyapatite loss occurs in extracellular pH lower than 7.4.(5) Experiments are now underway to assess whether or not fish oil might suppress osteoclast-activating cytokines and/or increase extracellular pH and subsequently, decrease hydroxyapatite loss at the LPS-injected alveolar bone site in rats as seen in the present study.

The extrapolation of the present study in humans is speculative. Prophylactic benefits of fish oil in the reduction of rat alveolar bone loss due to periodontopathic bacterial infection (3) or dental pulp exposure (2) have been documented. The present study clearly showed that oral treatment with fish oil reduces LPS-induced hydroxyapatite loss in rat alveolar bone. Yet, whether or not fish oil may be beneficial as a complimentary treatment in bacterially induced alveolar bone loss in humans remains to be investigated further.

In conclusion, the results of the present study showed that hydroxyapatite contents of LPS-injected alveolar bone in fish oil-treated rats were significantly higher than that in untreated animals, suggesting that fish oil treatment may reduce LPS-induced hydroxyapatite loss in rat alveolar bone.

Acknowledgements:

This work was supported by the Postgraduate Research Fund from the Ministry of National Education, the Indonesian government. The authors gratefully thank to Dr. Endang Tri Wahyuni (Department of Chemistry, Faculty of Mathematic and Natural Sciences, Gadjah Mada University) for her excellent technical guidance.

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