The effect of zinc supplementation on collagen of periodontitis rat

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ABSTRACT

Background: Zinc, co-factors of DNA- and RNA-polimerase, having great role in tissue healing. In periodontitis, collagen type I is the main fiber constructing periodontal structure is degenerated and be the main cause of loss of teeth among adult in Indonesia. Zinc deficiency is still a nutritional problem. The aim of the study is to obtain the influence of 500 and 200 μg zinc supplementation (Zn) per day for 7 days to periodontal collagen of periodontitis (PI) rat through histologic feature.

Method: In the experimental study with factorial design, 28 adult male Wistar rats were used. Subjects were grouped simply randomly into 6 i.e Healthy (H) at the start, H at the end, P + Te-tracycline (T), P + T + Zn, and P + T + Zn, Subject other than H-groups were induced periodontitis by Porphyromonas gingivalis bacteria. Zinc concentration was measured by AAS Flame. Periodontal tissue was stained with Mallory's PTA stain and ANOVA was used to analyze difference between mean of Zn concentration of groups studied.

Result: There were similarity in the feature of groups H at the start, H at the end, and P + T + Zn, in sense of irregularity, length, and solidity of collagen. P group had irregular, and short collagen. Groups of P + T with shorter collagen, had similarity to Health at the end, P + T + Zn, shorter collagen similar to P + T + Zn. There was significant difference in Zn concentration between H at the end P group. No significant differences among Zn concentrations of Subject groups. Great variety of Zn concentration found among groups probably were the cause of absence of differences, although means of the Zn concentration values depicted it.

Conclusion: Zinc supplementation dosage 500 μg/day given to periodontitis rat beside Teri-tracycline, gives better effect to collagen structure compared to 200 μg/day.

Key words: Zinc supplementation - collagen - periodontitis rat - histologic feature - Zn concentration

ABSTRAK

Praptiwi, Siti Fatimah Muoi, Soeharyo Hadisaputro, Suryono - Pengaruh suplemenatasi seng pada sertat kolesterol tikus periodontitis.


Tujuan: mengetahui pengaruh suplementasi Zn dosis 500 μg (Zn) dan 200 μg (Zn) per hari selama 7 hari pada kolagen jaringan periodontal tikus yang sakit (Periodontitis = P) melalui gambaran histologisnya.


Simpan: Suplementasi Zn dosis 500 μg pada tikus periodontitis dengan pengobatan Tetracyclin memberikan pengaruh lebih baik pada struktur kolagen dibanding 200 μg.
INTRODUCTION

Zinc (Zn) is an important mineral in protein synthesis for growth, recovery from injury, etc. Zinc deficiency would cause impaired chemoattractant function and neutrophil function, and also DNA damage. The impact is the pressure of the immune function; infection will frequently occurred and further, there will be disturbance of tissue repair. As cofactors of DNA- and RNA-polymerases, there will be decreased of collagen synthesis in Zn deficiency.

Periodontitis is the continuation of gingival inflammation as a response to odontopathic bacteria infection. Although the calculated incidence rate for periodontitis in Indonesia was 2.07%, with its rapid formation of periodontal pocket, 10% at 20 years old and 40% at fiftyies directing to loss of teeth, the disease is a vast health problem in community. Porphyromonas gingivalis (P. gingivalis) is the most aggressive odontopathic bacteria. Protease and toxin of the bacteria would cause collagen degradation, and according to Hoag and Pawlak (1996) would direct to fast formation of gingival pocket. This pocket makes periodontitis as the main cause of rapid loosening teeth among adult.

Effort to overwhelm bacterial infection could be done a.o by using Tetracycline. The broad spectrum antibiotic slowing the growth of bacteria by interfering with the production of proteins needed by the bacteria to grow. This action of the antibiotic gives time to body's defense mechanism to destroy the bacteria.

Inflammation as the body response to infection, proceeds in 3 basic stages, in forms of vasodilation and increased leakage from capillaries, migration of phagocytes to the site of infection, and tissue repair, respectively. The second stage resulted extravasation, that is exude of blood cells out of the vessel to enter surrounding tissue to conduct body defense functions. Tissue repair indicated by new cells that are produced by mitosis replace damaged fibers and other tissue structures.

Healing is homeostasis and integrated functioning. Many systems interact to protect the body from damage and maintain stable life-supporting functions. In the traumatized or septic tissue, the cytokine interleukin-1 (IL-1) is increasing, which enhance the expression of metalloprotein (MT). This change would increase Zn uptake through and transport to the gut. The increase of IL-1 would give proliferative effect on endothelial vascularization and fibroblast.

The function of fibroblast in periodontal ligament is to synthesize collagen. The fibrillar collagen is the main component of periodontal tissue, which provides the tensile strength. In each fibril there are tropocollagen molecules constructed longitudinally. The molecules are connected one to each other through intermolecular bond with increasing number according to advancing age. Older fibril collagen will become rigid and brittle.

Beside Zn, protein is nutrient extremely needed for body defense and healing. Zn requirement for rat is 0.0012% of its daily food consumed and 40μg/day for its growth. The need of protein for growth is 25 - 39% of daily food consumed. Rat in average consume food 5g/100g of body weight per day.

Zn deficiency, also high dose of Zn 1000μg/g food consumed/day for 2 weeks, would lengthen recovery time by the change of inflammatory response. The rate of wound closure was significantly slower in mice fed Zn 1000μg/g compared with mice fed the 500μg/g, despite the fact that there was no significant difference in skin and serum Zn levels between the 2 groups. High Zn intake may decrease Copper (Cu) absorption, leading to Cu deficiency and anemia, and may play an important role in the immunodeficiency. For human, 50 mg Zn/day per oral in the form of ZnSO₄ was reported to fasten wound closure, as for rat described by Lawrence and Bacharach (1964) it would be 50 x 0.018 mg or 900μg.

Recovery process consists of 3 phases, i.e. inflammation, proliferation, and remodeling.

Proliferation phase is characterized by fast growth of cell surrounding wounded tissue, and new vascularization is produced to repair the decay.

To emphasize the existence of collagen, Mallory's triple stain was employed. Stained with Mallory's, the collagen fibers are identified by the blue color, and red blood cells inside the blood vessels appear red.
MATERIAL AND METHODS

In this experimental study with factorial design, the subject were 29 adult male Wistar rat of the 5th generation of the strain, namely LPPT 5. Subjects were acclimated for 4 days in the individual cage, made of stainless steel, with placed Zn at the bottom to let the urine and feces go down. The subjects then grouped simple randomly into 6, i.e Healthy (H) at the start, H at the end, Periodontitis (P), P+Tetracycline (T), P+T+Zn, and P+T+Zn. Five rats from group Healthy at the start sacrificed to obtain normal value of periodontal Zn concentration. All four subject of other healthy group were kept healthy until the end of the study; they were Healthy at the end. Four other groups were induced periodontitis through Porphyromonas gingivalis (P. gingivalis) ATCC 53977 standard strain bacteria inoculation, 3 times during 4 days. Approximately 10^6 CFU of live bacteria in 100µl of Phosphate Buffered Saline (PBS) were directly introduced to the stomach and colorectal region, using canulated syringe. The bacteria was also inoculated to gingival ridge of molars region, upper, lower, right and left, using cotton bud. Each week after inoculation, a subject was sacrificed. The periodontal tissue separated, then soaked in buffer formalin solution to prepare for histologic examination to find sign of periodontitis. After the finding, a week was needed to make chronic condition of periodontitis. Further, 7 days treatment were given to each group according to the groups' name as follows: no treatment given to Periodontitis (P), group for group of P+Tetracycline (T), 18 mg/day of the Tetracycline powder, diluted in sterile water was given; and groups of P+T+Z, and P+T+Z, received T 18 mg/day + Zn 500 µg/day and T 18 mg/day + Zn 200 µg/day respectively. The Tetracycline made by Ningxia Qiyuan Pharmaceuticals Co. Ltd. Zn used is Zn sulphate (ZnSO₄)·7 hydrate. After those 7 days treatment, all subjects sacrificed, and the periodontal tissue of molar regions were separated from maxillar and mandibular bone for histologic examination and measurement of Zn concentration. Zn concentration was measured using Atomic Absorption Spectrometer (AAS) Flame. To prepare the periodontal tissue for histologic examination, the tissue specimen were soaked in formalin buffer and undergo the Mallory staining procedure. Light microscope with 40x magnification was used in histologic examination, and 80x for group P. Other halves of the specimen were kept in small Eppendorf tube and kept in cold storage before measurement of Zn concentration.

RESULT AND DISCUSSION

The study was approved by 'Komisi Etik Penelitian Keselihatan Fakultas Kedokteran Universitas Diponegoro dan RS dr. Kariadi Semarang'. Twenty nine adult male Wistar rats were acclimated in the individual cages for 4 days, with sufficient lighting. Each subject was fed 20g/day Rat Bio 22 containing 73.53 ppm Zn, with free access to tap water containing 9.50 ppm Zn. The rats were 8 weeks old at the start of the study. Food consumed for rat, according to McCoy (1971) in average is 5g/100 g body weight/day. Mean body weight of rat studied was 364.34g, which would consume 5 x 3,643 g = 18,215 g food/day. The amount of protein in Rat Bio 22 is 22% minimum. In this study, the rat was sufficiently fed, involving Zn and protein.

Further, the subject were grouped into 6, simple randomly. Unless 2 Healthy groups, 4 groups were induced periodontitis using P. gingivalis bacteria 3 times during 4 days.

After 3 weeks, there was sign of periodontitis, indicated by presence of extravasation. To make a chronic periodontitis, a week is needed after the finding of periodontitis. Then, as being planned, no other treatment given to Periodontitis (P). For group of P+Tetracycline (T), 18 mg/day of Tetracycline powder diluted in sterile water was given. Groups of P+T+Z, and P+T+Z, received T 18 mg/day + Zn 500 µg/day and T 18 mg/day + Zn 200 µg/day respectively.

After 7 days treatment, all subjects were sacrificed. Periodontal tissues of each group divided into 2. Half of each specimen were soaked in formalin buffer, then undergo Mallory staining as part of histologic examination. The other halves were kept in Eppendorf small tube and ready for AAS procedure to measure Zn concentration.
a. Histologic feature

FIGURE 1 and 2 demonstrate difference between collagen of the two. Collagen in FIGURE 1 of Healthy tissue at the start of 3 weeks old subject was thinner than collagen in FIGURE 2 of 23 weeks subject. The thicker collagen seen from older subject may be due to the increasing number of tropocollagen. intermolecular bond inside the collagen by advancing age.11

FIGURE 3 denoting chronic inflammation in periodontitis. It depicts the degradation of collagen by bacteria,10 at the same time with new collagen to repair.15 In traumatized tissue, II-1 is increasing and gives proliferative effect on vascularization and fibroblast.15 Inflammation as the body response to infection, showed extravasation indicated by the exude of blood cells to surrounding tissue.16

FIGURE 4. Periodontitis treated with Tetracycline (P+T). The antibiotic slowing the growth of bacteria. By doing so, it permits body defense mechanism to destroy the bacteria. Tissue in this chronic periodontitis had more healthy collagen fiber compared to that of Figure 3, but fewer blood vessels compared to a normal one15 seen in FIGURE 2.
FIGURE 5. Periodontitis treated with Tetracycline and Zn of 500μg showed the benefit of Tetracycline in the same way as group (P+T). Accumulated Zn in the area of inflammation as the effect of the increasing II-1 enhanced protein synthesis including fibroblast. Fibroblast produced sufficient collagen as part of tissue repair.

FIGURE 6. Periodontitis treated with Tetracycline and 200μg Zn of P+T+Zn group of 23 weeks old rat. Thin collagen were constructed regular, with smaller blood vessels compared to that in FIGURE 5.

b. Zinc concentration

Data of periodontal Zn concentration using AAS Flame is presented in TABLE 1.

Mean value of Zn concentration between groups of H at the start i.e. 52.732 ± 49.60 ppm and H at the end i.e. 26.752 ± 8.40 ppm analyzed with t-test gave no significant difference (p=0.341), although there was difference in the mean value seen between the two. Great variation of Zn concentration at start group might be the cause of no significant difference. The variation might be due to 'gnawing-cage' habit of some subjects in group H at the start which gave impact to higher Zn concentration in periodontal tissue. Those habit could be seen by defective plaited Zn at the bottom of some cages.

Using t-test to analyze difference between Zn concentration of groups H at the end i.e. 26.752 ± 8.40 ppm and P i.e. 10.560 ± 9.31 ppm, there was significant difference (p=0.042) between the two. Zn is used abundantly in defense mechanism in periodontitis, so that the concentration in periodontal tissue was decreased. This situation was improved by giving Tetracycline to fight against the bacteria in P+T group, while Zn gave great contribution in the recovery from injury. The synergic effect of Tetracycline and Zn gave the mean value of Zn concentration increasing to 21.877±9.93 ppm.

Zn concentration of groups P+T+Zn, i.e. 23.742±23.31 ppm and P+T+Zn, i.e. 17.233±22.61 ppm analyzed by t-test gave no significant difference (p=0.711), although there was different seen by their mean value. Great variety of Zn concentration value of some subjects in both two groups which could be seen by the SDs might be the cause of the non significance.
Mean value of Zn concentration of P+T+Zn₁ group i.e 23.742 ± 23.31 ppm was the nearest to Health at the end group i.e 26.752 ± 8.40 ppm compared to others. There was no significant difference (p=0.813) of Zn concentration between the two. Great variety of Zn concentration value of some subjects in P+T+Zn₁ group might be the cause of the non significance.

Tetracycline slowing the growth of bacteria by interfering with the production of proteins needed by the bacteria to grow. Slowing the bacteria’s growth allows the body defense mechanisms to destroy them.¹ Sufficient amount of Zn gave contribution on strengthening body defense mechanism¹ and all at once accelerate recovery from injury ¹ by its proliferative effect on fibroblast ¹ as seen in group P+T+ Zn₁.

In general, in Periodontitis groups i.e P; P+T; P+T+Zn₁; P+T+Zn₂ ANOVA test gave no significant difference of Zn concentration among groups (p=0.731), although mean values showed the difference.

Great variety of Zn concentration value of some subjects in the groups seen by the SDs might be the cause of the non significance.

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**CONCLUSION**

In periodontitis rat treated with Tetracycline, dosage of Zn supplementation 500 µg / day for 7 days gave better effect to structure of collagen compared to 200 µg. There were no significant difference of Zn concentration among Periodontitis groups, although the mean values showed it. It is recommended to prevent the subject from gnawing object containing Zn in this case part of the cage, in order not to confuse the result of the study. Cage
made completely of stainless steel would be a good choice.

ACKNOWLEDGEMENT

The author wish to thank Experts, Staff and technicians in Microbiology Department and Pathology Anatomy Department Faculty of Medicine Gadjah Mada University, for the service and patient guidance. The author is grateful for the generosity of Phapros Pharmaceutical Industry to offer the Tetracycline used in this study.

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