Alendronate Effect in RANKL Concentration at Ovariectomized Sprague Dawley Rat

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Abstract

In this study, we analyze the relationship between alendronate concentration and serum-RANKL concentration

24 Sprague Dawley female rats that had given birth, aged 5 months were performed bilateral ovariectomy. Rats were divided into four groups. Groups A, B, C were treated with subcutaneous injection 4 times per week with alendronate 0.08 ml, 0.29 ml, 0.58 ml. Group O were not given alendronate, and were categorized as control group. The RANKL concentration were measured quantitatively with Enzyme-linked Immunosorbent Assays (ELISA) via blood sampling that were taken at before ovariectomy (day 0), prior to implantation (day 15), and intracardially after euthanasia (day 70).

As the result from study above, high dosage of alendronate there was no decrease in RANKL concentration, but there was a significant change in RANKL concentration. thus the hypothesis that the higher the dose of alendronate the higher the decrease of RANKL cannot be accepted

There is a relationship between high dosage of alendronate with the change in RANKL concentrations. The greatest RANKL changes were at concentrations of 35 mg (0.29 ml) and 70 mg (0.58 ml).

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Introduction

Dental implant, as a substitute for missing teeth, has become a popular alternative for people all over the world. Implant users are generally middle-aged men or women with one or more missing teeth as well as edentulous jaws. There are several factors that affects the success of implant treatment, one of the most important factor is bone quality. Bone quality plays an important role in the success of osseointegration and alveolar bone retention in implants.[1-3] Osteoporosis is a common disease that is found in this middle-aged group. It is a slow growing disease and requires lengthy research to see the therapeutic response. Osteoporosis therapy varies, one of which is widely used is a group of bisphosphonates.

Bisphosphonate are synthetic compounds extensively used for the treatment of systemic bone loss due to estrogen depletion. However, this drug has the side effects of osteonecrosis due to bisphosphonate (BON).[3] More than 90% of BON is found in patients with cancer and cases of metastatic bone disease who receive intravenous bisphosphonates.[2] Although oral bisphosphonate capability is 100 fold lower than intravenous use, the long-term of oral bisphosphonate usage will increases the risk of BON.[4-8]

BON is usually associated with a nitrogen-containing bisphosphonate. Oral bisphosphonates such as alendronate is one of the most widely used drugs for osteoporosis therapy. Duration of use is a very important factor, evidenced by the presence of more than 50% of osteoporotic patients with BON although there are no additional risk factors such as chemotherapy, head neck irradiation, diabetes and joint treatment such as steroid use, smoking habits.[9,10]

The purpose of this study was to analyze the relationship between alendronate concentration and serum-RANKL concentration.

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Materials and methods

Research design

This research was conducted at the Animal Laboratory from the *Institut Pertanian Bogor,* Bogor and histological analysis conducted at the National Science and Technology Development Agency (NSTDA), Thailand. 24 Sprague Dawley female rats that had given birth, aged 5 months weighing 200 grams. Rats were quarantined for 1 week. Afterwards, bilateral ovariectomy is performed. Alendronate (Fosamax[TM]) dose is adjusted to human dose. Alendronate administration is given immediately after ovariectomy is performed. The study used four groups of ovariectomized rats consisting of: a). group O (not given alendronate); b). group A (subcutaneous injection) (SC) 4 times per week with alendronate 0.08 ml); c). group B (injected SC every week with 0.29 ml); d). group C (injected SC every week with 0.58 ml).

Drug administration dose in rats was adjusted to 4 times per week of subcutaneous dose for alendronate 10 mg (0.08 ml), and once per week for dose 35 mg (0.29 ml) and 70 mg (0.58 ml). Calculation of alendronate dose in mice was obtained by converting alendronate dose in humans based on Hau and Hoosier's theory.[11]

Three weeks after ovariectomy, the vaginal smear is checked for 5 days to see if the rat has lost estrogen. On the 15th day, the implant (Pin Biocom, Biom Westlake, Hangzhou, PRC) was placed at mesial of maxillary first molars, and on the 70th day, rats were euthanized.

RANKL concentration

Blood sampling during the study was done 3 times. Intravenous blood collection is performed on rat's tail, before ovariectomy (day 0), prior to implantation (day 15), and intracardially after euthanasia (day 70). RANKL concentrations were measured quantitatively with Enzyme-linked Immunosorbent Assays (ELISA) (USCN, Life Science Inc, USA), at the end of the study to see the difference in RANKL concentrations before ovariectomy and when the implants were placed. The RANKL concentration in the sample was determined by comparing the optical density of the sample with the standard sample curve and directly written quantitatively.

Results

RANKL concentration analysis

RANKL concentrations in serum were analyzed to see differences in groups and inter-groups. The statistical analysis used was one-way ANOVA test to see the difference in the group while for inter-groups analysis was used General Linear Measures (repeated measure ANOVA) with post hoc Bonferroni ($P<0.05$). RANKL serum examination in experimental animals was staged into three, before ovariectomy (day-0), implant placement (day-15), and at euthanasia (day-70).

In group O, there was a significant difference, seen at RANKL concentrations between pre-ovariectomy compared to the time of implant placement ($P=0.005$) and pre-ovariectomy up to euthanasia ($P=0.000$). Similarly, there was a significant difference in RANKL concentrations before implant placement at euthanasia ($P=0.00280$) (Table 1).

In group A, ovariectomized rats with alendronate 10 mg (0.08 ml) 4 times per week, no significant difference in RANKL concentrations between pre-ovariectomy compared with implant placement time and implant placement time compared with euthanasia. This indicates the absence of active RANKL-RANK process to form osteoclasts. RANKL changes in group A were lower than in group O, bone mass could be maintained with alendronate 10 mg (0.08 ml) compared with no alendronate (Table 1).

In-group B, RANKL concentrations changed significantly between implant placement time and euthanasia ($P=0.018$), whereas in group C, there was no significant difference between pre-ovariectomy compared with implant placement time. Results with significant differences were obtained before implantation with euthanasia ($P=0.040$) and before ovariectomy with euthanasia ($P=0.006$). RANKL concentrations change significantly, this means the use of RANKL and RANK in increased bone formation can occur, resulting in more osteoclasts in the bone and more bone loss occur.
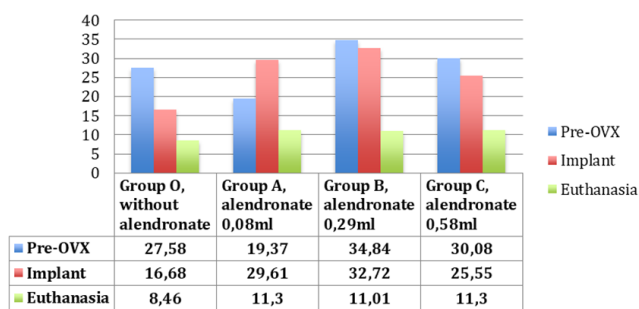
	Group O, without alendronate	Group A, alendronate 0,08ml	Group B, alendronate 0,29ml	Group C, alendronate 0,58ml
Pre-OVX	27,58	19,37	34,84	30,08
Implant	16,68	29,61	32,72	25,55
Euthanasia	8,46	11,3	11,01	11,3

Table 1. RANKL Concentration Diagrams in the O, A, B, C Groups In Pre-Ovariectomy (day 0), Implant Installation (day 15) and Euthanasia (day 70)

Discussion

The RANK/RANKL/OPG system is important in bone renewal. RANKL has been identified as a major mediator of osteoclast formation. Many factors can cause bone loss such as parathyroid hormone, TNF, IL-1 but all by stimulating RANKL expression by osteoblasts. RANKL is a ligand that interacts with RANK receptors expressed by osteoclasts. The interaction between RANKL and RANK leads to the maturation of osteoclasts into multinucleated osteoclasts and become activated osteoclasts. RANKL expressed by osteoblast derivatives is cell-bound RANKL whereas expressed by T lymphocytes is soluble RANKL.[12,13] The RANKL effect can be neutralized by the osteoprotegerin feed receptor (OPG), which plays an important inhibitory role in the regulation of osteoclastic bone resorption. In this study, RANKL was performed soluble serum from pre-ovariectomy (day 0), implant installation (day 15) and euthanasia (day 70).

The correlation between OPG/RANKL serum and the risk of osteoporotic fractures is still controversial.[14–16] In preclinical study data indicates high levels of RANKL and low OPG, are associated with high bone turnover and bone loss. but this data is not found in clinical evidence.[17] Low serum OPG is found in vertebral fractures in postmenopausal osteoporotic women.[15] Another study said the increased risk of hip and wrist fractures in women with high OPG serum.[14] A prospective population analysis showed that low serum RANKL levels were associated with a high risk of atraumatic fracture regardless of age, sex, menopausal status, OPG level, and lifestyle. Thus in this study will be an assessment of RANKL concentrations changes, compared to assessing the increasement or decreasement of RANKL concentrations that are still controversial.

In group O there was a significant change in RANKL concentrations between pre-ovariectomy and implantation and pre-ovariectomy with eutahanasia. According to previous research, the effect of estrogen deficiency caused by ovariectomy on mandibular distraction osteogenesis was unfavourable and slower than non-ovariectomy group.[18] RANKL will increase in number in osteoporosis patients, thus bone remodeling more towards the formation of bone cavity by osteoclasts rather than bone formation by osteoblasts.[19] It is not mentioned whether RANKL binds to a cell or a soluble that forms an osteoclast.

In groups A, B, and C at day 0 and at the time of implant installation (day 15) there was no significant difference. It is estimated that the bone condition is almost the same at the time of pre-ovariectomy and implant installation, the effect of the absence of estrogen covered directly by alendronate, the formation of osteoclasts were decreased, so that RANKL concentration is equal to before ovariectomy. In groups B and C there was a significantly decreased during implant and euthanasia (70th day). Here we see that RANKL concentration changes sharply, indicating reduced osteoclasts formed.[20] Groups O, B, and C have significant differences in RANKL serum pre-ovariectomy and euthanasia serum concentrations. In groups A, B, and C the actual anti-resorptive effect of alendronate.

From this research it is seen that alendronate effect will increase sharply with time. The longer the use of alendronate will change the RANKL concentration and will give a significant difference on the 70th day compared to pre-ovariectomy (day 0). Changes in pre-ovariectomy (day 0) and implant installation have started but the peak is on the 70th day. Dose and duration of alendronate use will have an effect on RANKL concentration.

Conclusions

This study shows that there is a relationship between high dosage of alendronate with the change in RANKL concentrations. The greatest RANKL changes were at concentrations of 35 mg (0.29 ml) and 70 mg (0.58 ml). Irritation

of the jawbone like implant placement in patients that taking long-term bisphosphonates and with high doses should be monitored for complications. Further research and dissemination of information regarding the side effects of bisphosphonates with high concentrations should be increased.

Declaration of Interest

The authors report no conflict of interest and the article is not funded or supported by any research grant.

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