The Effect of Alendronate to Osseointegration of Dental Implant at Ovariectomized Sprague Dawley Rat

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Abstract

This study aimed to analyze the effect of alendronate concentration on osseointegration of dental implants based on the bone area on area of interest and necrotic bone

24 Sprague Dawley female mice that have given birth, weighing 200 grams aged 5 months. All rats performed bilateral ovariectomy. All rats were divided into four groups; group O (not given alendronate); group A (subcutaneous injection) (SC) (4 times per week with alendronate 0.08 mL); group B (injected SC every week with 0.29 mL); group C (injected SC every week with 0.58 mL). The implant was placed on the fifteenth day when mice had hypoestrogen condition after 2 weeks post-ovariectomy. Histomorphometric examination was performed and stained with Masson Trichrome technique.

The highest percentage of bones formation around the implants were achieved in group B, which shows the best osseointegration of the implant. The lowest percentage of bones formation was group O, the ovariectomy without alendronate group. There was a significant difference in group B (alendronate 0.29 mL) when compared with O (control) and A (alendronate 0.08 mL), and C (alendronate 0.58 mL).

This study shows that there is a correlation between alendronate concentration and bone percentage around the implant in the area of interest on histomorphometry examination. The highest bone increase in the group with alendronate 35mg (0.29 mL) per week. Whereas in the alendronate group of 70mg (0.58mL) per week, the resulting bone formation was less in the area of interest with the worst percentage of necrotic bone from the four groups.

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Introduction

Nowadays, dental implants are one of the alternatives in replacing tooth loss in the jaw. Successful integration of bone implants involves several factors such as shape, topography, implant's surface, and surgical techniques, implant loading and also the quality and quantity of bone implant recipients. Bone quality is the most important factor in the success of osseointegration and alveolar bone retention. In patients with osteoporosis, bone mass and bone density become low. Several studies have reported the effect of osteoporosis on the success of implant in jaw bone.[1–3] Osteoporosis occurs in postmenopausal women because of lack of estrogen. Osteoporosis 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.

Bisphosphonates are pyrophosphate analogs that are effective in inhibiting bone resorption and have been used extensively for systemic bone loss therapy caused by estrogen deficiency. This drug is also used for malignant lesion therapy such as breast cancer, prostate cancer, and multiple myeloma and bone disorder therapy such as Paget's disease. [4] However, this

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drug has the side effects of bisphosphonate osteonecrosis (BON). Generally, BON is associated with long-term therapy of bisphosphonates. Therefore osteonecrosis is suspected to be a disease process that depends on time and dose. [5, 6] The alendronate effect that can cause osteonecrosis in osteoporosis, the implant users in this group should be wary, especially for the patients with osteoporosis and dental implant users who use alendronate therapy or patients with osteoporosis and alendronate users who then use dental implants.

The use of alendronate to avoid bone loss around tooth implants has been studied. [7, 8, 9, 10] But the alendronate molecular mechanisms of osteoclasts and osseointegration of dental implant still not studied well. On the other hand, there is a contradiction between the effectiveness of alendronate therapy in suppressing bone resorption and the adverse side effects of jaw osteonecrosis that are difficult to cure completely. Based on the background, the purpose of this study was to analyze the effect of alendronate concentration on osseointegration of dental implants based on the bone area on area of interest and necrotic bone.

Materials and methods

Research Design

24 Sprague Dawley female mice that have given birth, weighing 200 grams aged 5 months. All rats performed bilateral ovariectomy. Alendronate dose for human are converted to mice dose based on Hau and Hoosier's theory. [11]. All rats were divided into four groups; group O (not given alendronate); group A (subcutaneous injection) (SC) (4 times per week with alendronate 0.08 mL); group B (injected SC every week with 0.29 mL); group C (injected SC every week with 0.58 mL).

The vaginal smear is checked at 3 weeks post-ovariectomy for 5 days, to see if the mouse in hypoestrogen condition. On day 15th, the mesial implants were implanted from the maxillary first molars and the 70th day in mice in euthanasia. Then histomorphometry examination were performed.

Installation of Implants

Subcutaneous alendronate administration was given immediately after ovariectomy in mice. Six ovariectomized rats were not given alendronate. The implant was placed on the fifteenth day when mice had hypoestrogen condition after 2 weeks post-ovariectomy. Fifteen days post-ovariectomy, under general anesthesia and skin cleansing with iodine soap, an implant was placed on the mesial of the maxillary first molar, in areas where there is no contact with the antagonist teeth. The implant is placed at 1500 rpm with saline solution. A titanium screw implant with a length of 4 mm and a diameter of 1 mm is placed until the thread reaches the cortical portion of the bone. After implantation, amoxicillin 100mg/kg of body weight was administrating subcutaneous and oral paracetamol 300mg/kg of body weight was given.

Histomorphometry Examination

Histomorphometric examination was performed at the National Science and Technology Development Agency (NSTDA), Thailand. The specimen was cleaned, cut into 3-5 mm by using EXAKT 300, and fixated using formalin for 48 hours. The specimens were dehydrated, and infiltrated with glycolmethacrylate, submergence in the mold with Technovit 7200 VLC for 16 hours, and then the specimen were mounted, cut with EXAKT 300 and smoothed with EXAKT 400 CS. Histological staining with Masson Trichrome technique.

Results

Analysis of Bone Percentage of Implants in AOI (Histomorphometry Examination)

The results of the bone acquisition around the implant data in the area of interest were analyzed by one-way ANOVA with post hoc LSD. The specimen was stained using Masson Trichrome technique. Area of interest was marked with green rectangular shape (Figure 1). In group O, some implants are unstable in the bone. No osseointegration of implant appears in histomorphometry examination. Which indicate the bone is not formed in sufficient quantities. There was a significant difference between B and O ($p = 0.021$), A with B ($p = 0.062$), and B with C ($p = 0.059$). In group B, percentage of bone formed around the implant was higher than in group O. Poor osseointegration around implant also happened in group C. This may be the result of an infection process when implant was planted, or the particle of necrotic bone around the implant that break apart when tissue preparation due to chronic infection after implant placement.

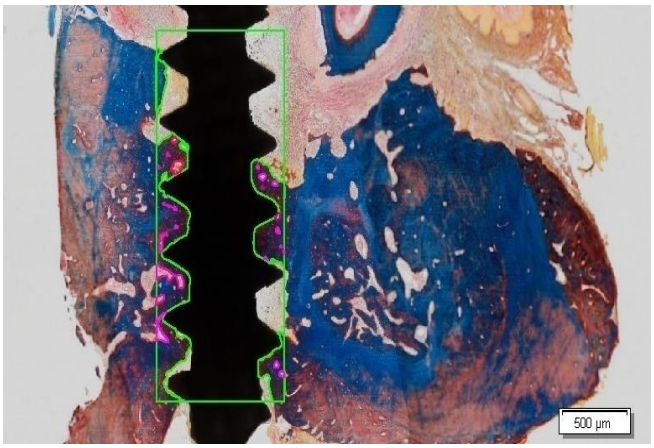

Figure 1. Masson Trichrome staining and bone (blue), the area of interest (green)

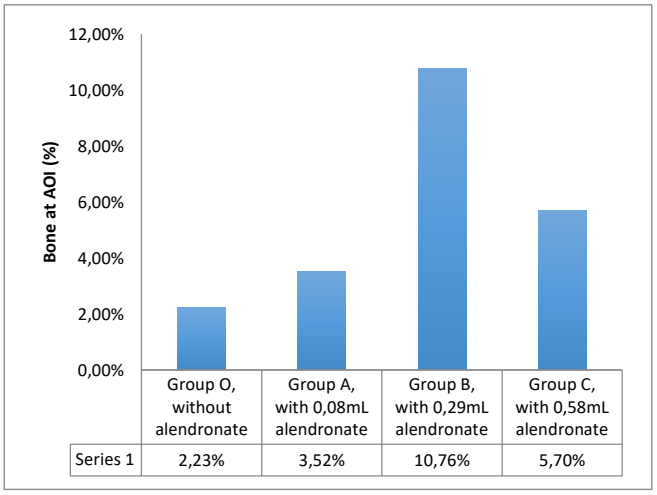

Figure 2. Percentage of bone histomorphometry in the AOI (area of interest) of each group

The lowest percentage from histomorphometric examination was group O, the ovariectomy without alendronate group (Figure 2). There is pure osteoporosis happened in this group. Therefore, the initial healing of the implants is poor and the bone in the area of interest is little. There was a significant difference in group B when compared with O and A, and C ($p = 0.021$; $p = 0.062$, $p = 0.059$), the highest percentage of bones formation around the implants were achieved in this group, which shows the best osseointegration of the implant. This was observed until the 70th day, during euthanasia.

Analysis of Alendronate Concentration and Osteointegration of Necrotic Implants and Bones

From chart (Table 1), necrotic bone (5%) was observed in group C, the group with the highest alendronate dose, 70mg (0.58 mL), although not statistically significant. When viewed as a whole that osseointegration of implants in this group is also poor (5.7%), seen from the least bone formed in the area of interest (Figure 2).

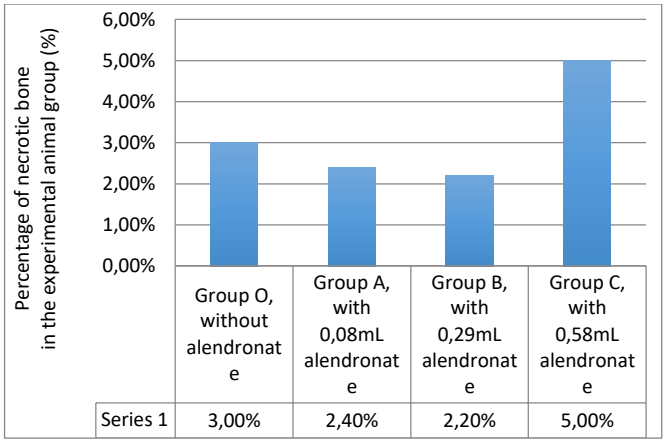

Table 1. Percentage of necrotic bone in the experimental animal group

Discussion

Research on implant osseointegration in experimental animal studies and human studies has been done with varying results. One study reported that implant osseointegration in experimental animals give a better results by applying local alendronate to the implant. [12] Whereas some other studies suggest systemic (subcutaneous) administration are better. [13, 9, 10] Furthermore, research on the absence of bisphosphonate effects on implant placement in experimental animal studies and human studies were proposed by several researchers. [14, 15, 16] Studies said that bisphosphonate use on human has an adverse effect in implant osseointegration were proposed by Marx and Starck. [17, 18] They reported radiolucent lesion, open bone, odor, pain, infection, candidiasis, fistulas and secondary osteomyelitis. While some other researchers reported periimplantitis occurred around the implant in patients with bisphosphonate users.[16, 19]

In recent years there has been much thought to improve osseointegration of bone in implants. In the literature, some medicinal substances to condition the surface of the implant in order to increase or maintain bone mass and quality, some studies were conducted using bisphosphonates to interfere with bone resorption mechanisms. Alendronate attached to the implant endosteal to evaluate the effect of bone regeneration, the result suggests that the percentage of bone in the implant is significantly increased.[12] In addition, the use of topical bisphosphonates in the form of clodronate solution may be given to the patient before implant placement. Topical bisphosphonate on implants has more contact with new bone than the control group.[20]

Group O is a group that is only ovariectomized but not given alendronate, some implants have no osseointegration. More than 20% of implants fail and come off, starting in the second week. This finding is in line with studies stating that with the discontinuation of estrogen hormone there is an increased bone loss.[21] Studies said that mandibular distraction osteogenesis was unfavourable and slower in ovariectomized rats than non-ovariectomy group, suggesting the influence of estrogen in bone repair in the histological and histomorphometric analysis.[22]

In group A, the experimental animals were ovariectomized and administered alendronate 10mg (0.08 mL) 4 times daily per week, the skin in the injecting area underwent hyperemia and hair loss. Subcutaneous injection should not have significant effect or pain in animal experiments, but in this research alendronate may have reddish and hair loss effects because intravenous injection is sufficiently intensive to cause an inflammatory reaction and feather loss. Group A differed from the other groups, usually, subcutaneous injection had no such complications provided that the fluid inserted did not exceed 1 mL in the adult rat.[23]

In group C, the experimental animals were ovariectomized and given the highest alendronate dose of 70mg (0.58 mL), some mice having an infection characterized by swelling more than 5 days and followed by pus formation and the infection occurs on the implant installation area in almost all experimental animals. It happens because alendronate will break down the amount and activity of osteoclasts and show a decrease in bone turnover to none at all. When an invasion occurs such as an implant-mounting trauma, the bone has a tendency to super-infection. Alendronate suppresses bone remodeling processes where micro fractures caused by implant placement are not regenerated by osteoclasts, while osteoblasts do not form new bones that contact to the implant. This leads to implant failure in patients treated with osteoclast inhibitors.[5,24-28] In the histologic examination of group C, the most common necrotic bone was observed, as did the histomorphometric examination of bone formation was low around the implant in AOI. In group A, B C the bone percentage in AOI is higher than that of group O. This may be due to alendronate ability to strongly affinity with hydroxyapatite crystals. Bisphosphonates are bound to bone mineral and released during bone resorption by osteoclasts. This will cause the accumulation of these drugs locally and interfere with osteoclasts directly.[29]

Conclusions

This study shows that there is a correlation between alendronate concentration and bone percentage around the implant in the area of interest on histomorphometry examination. The highest bone increase in the group with alendronate 35mg (0.29 mL) per week. Whereas in the alendronate group of 70mg (0.58mL) per week, the resulting bone formation was less in the area of interest with the worst percentage of necrotic bone from the four groups. The use of bisphosphonates increases the incidence of osteonecrosis, so further research is required at higher species levels such as *Macaca sp* to see the long-term side effects of the drug as well as the pharmacokinetics of bisphosphonates. Practitioners should be more careful and explain the complications that can occur in patients with osteoporosis bisphosphonate users, especially in patients that will undergo implant placement.

Declaration of Interest

None declared.

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