



## Effect of pamidronate and calcium on implant osseointegration in ovariectomized rats

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### ABSTRACT

**Introduction:** Supplementation with minerals like calcium has been shown to be effective in preventing bone loss and increasing bone density. Thus, it can be assumed that if calcium supplementation is paired with bisphosphonates, greater benefit can be achieved. In this context, we decided to perform this animal study on ovariectomized rats to assess whether bisphosphonate supplemented with calcium can improve implant osseointegration compared to bisphosphonate and calcium alone.

**Materials and Methods:** In this animal study, 40 ovariectomized rats were divided into 4 groups prior to implant insertion. The first group received 5 mg/kg intramuscular calcium gluconate 3 times per week for 4 weeks. The second group received 5 mg/kg intramuscular pamidronate 3 times per week for 4 weeks. The third group was administrated a combination of both intramuscular calcium gluconate and pamidronate. The control group received no medication. The rats were sacrificed and the contact surface ratio (CSR) was evaluated 8 weeks after implant insertion in tibial bone.

**Result:** The pamidronate receiving group had significantly better CSR than the Ca receiving and control groups ( $P < 0.001$ ). Intravenous calcium did not improve CSR compared to the control group ( $P = 0.459$ ).

**Conclusion:** Short-term preoperative intravenous pamidronate can significantly improve bone to implant contact in post-menopausal animal models, whereas calcium supplementation has no beneficial effect.

**Keywords:** Pamidronate, Calcium, Ovariectomy, Dental Implant

### Introduction

Osseointegrated implants are increasingly used in oral rehabilitation of edentulous patients to restore full oral function and health. It has been postulated that some systemic conditions like osteoporosis can affect dental implant success to varying degrees [1, 2]. Osteoporosis is an osteometabolic condition which mostly occurs in postmenopausal women due to lack of estrogen as a result of loss of ovarian function [3]. Estrogen deficiency enhances bone remodeling in which bone resorption exceeds bone formation. This imbalance causes trabecular bone to become compromised [4]. Multiple therapeutic approaches, like bisphosphonate therapy, have been sought to reduce bone resorption. Bisphosphonates are powerful inhibitors of bone

resorption and their potency varies according to their structure. Besides decreasing bone loss, they increase bone mineral density, and decrease bone turnover [5]. Their inhibitory effect is present both in normal individuals and in those afflicted with a series of conditions accompanied by increased bone resorption, such as osteoporosis [6]. It has been shown that they may have the potential to improve osseointegration of endosseous implants in osteopenic subjects [7].

Supplementation with minerals like calcium has also been shown to be effective in preventing bone loss and increasing bone density [8]. Thus, it can be assumed that if calcium supplementation is paired with bisphosphonates, greater benefit can be achieved. In this context, we decided to perform this animal study on ovariectomized rats to assess whether bisphosphonate supplemented with calcium can

improve implant osseointegration compared to bisphosphonate and calcium alone.

## Material and Methods

In this controlled study, we used 40 six-month-old Wistar rats (4 groups, 10 rats per group) weighing between 300 to 350 gr; according to Tehran University of Medical Sciences guidelines for the care of laboratory animals. Animals were kept in separate cages in climate controlled conditions for 2 weeks in order to get adapted to laboratory conditions. They were fed standard laboratory diet and tap water. After 2 weeks, all rats were ovariectomized and were kept 6 more weeks in the laboratory for the osteopenia to happen. During this period, one rat from each group died.

### Study design

The 36 remaining ovariectomized rats were divided into 4 groups:

1. Calcium group: They received calcium gluconate 5 mg/kg 3 times per week intramuscularly for 4 weeks.
2. Bisphosphonate group: They received 5 mg/kg pamidronate intramuscularly 3 times per week for 4 weeks.
3. Calcium-bisphosphonate group: The rats in this group received both calcium gluconate and pamidronate with the above mentioned doses.
4. Control group: They received no medications.

After 4 weeks of drug therapy, dental mini-implants  $2.4 \times 10$  mm (DIO implant system, Busan, South Korea) were placed in tibial bone of each rat using the manufacturer suggested surgical protocol. For antibiotic prophylaxis, each rat received one million units of penicillin G intravenously 30 minutes prior to surgery. A bone healing period of 8 weeks was allowed for the osseointegration of mini-implants. One rat from the calcium-bisphosphonate group died during this period. Then, the rats were all sacrificed.

The tibial bone of each rat was dissected and soaked in formic acid for 6 weeks for decalcification. Then, they were processed and embedded in paraffin blocks. Using a rotary microtome, 10 serial sections of  $0.5 \mu\text{m}$  thickness were cut. Sections were stained by routine hematoxylin and eosin staining, and examined with light microscope to evaluate bone to implant contact. Then, contact surface ratio (CSR) was calculated, the average of CSR of 10 sections was recorded.

Statistical analysis was carried out using two-way ANOVA test with the help of the Statistical Package for Social Sciences (version 16; SPSS Inc., Chicago, IL, USA).

## Results

Of the 40 rats studied, 5 died during the study process. In the calcium receiving group and control group, the average

amount of CSR was 25% and 26%, respectively, while the bisphosphonate and bisphosphonate-calcium groups showed much higher ratios of 56% and 57%, respectively. The difference between pamidronate receiving groups (pamidronate-calcium and pamidronate alone) and non-pamidronate receiving groups (calcium and control groups) was statistically significant ( $P < 0.001$ ). However, no significant difference was observed between the calcium receiving group and control group ( $P = 0.459$ ) (Table 1).

*Table 1.* Comparison of contact surface ratio (CSI) among groups

	SD	P value
Ca-Pam	6.50	> 0.001
Ca-control	0.51	0.459
Ca-Pam-Control	6.49	> 0.001
PAM-Ca/Pam	3.29	0.992
Ca-CaPam	6.56	> 0.001

## Discussion

As people live longer, senile and postmenopausal osteoporosis are becoming more prevalent. Osteoporosis is a systemic skeletal disease mostly characterized by low bone mineral density and decreased trabecular bone volume [9]. Ovariectomized rats are widely accepted animal models for postmenopausal osteoporosis [10]. Multiple studies have been performed on ovariectomized animals to evaluate the effect of several agents like systemic and local bisphosphonates, parathormon (PTH), calcitonin, estrogen, and calcitriol on fixation of endosteal implants [11-15]. Qi et al. showed that 3 months after ovariectomy, osteoporotic changes were evident on femur of rabbits both on histopathology and bone mineral density (BMD) examinations [11]. In their study, both systemic and local treatment of zoledronate increased mineralized bone and implant bone contact, and the best result regarding maximal removal torque of implant was achieved in those animals who received a combination of both. Another study by Cardemil et al. showed that a systemic single dose of zoledronic acid resulted in lower level of bone remodeling marker in serum [16]. They also showed that the effect of systemic bisphosphonate is site-specific, since its administration improved bone-to-implant contact in the tibia, while the opposite was observed in the mandible. This could be due to diverse tissue responses to the drug in the tibia and mandible. In many other studies, it has been shown that administration of bisphosphonates, including alendronate and zoledronate, in combination with estrogen replacement therapy in ovariectomized animals could prevent negative effects of estrogen deficiency on bone microstructure and thus implant fixation [14, 17, 18]. Similar positive effects regarding bone mineral density and implant osseointegration have been achieved following the use of subcutaneous ibandronate in ovariectomized rats. Interestingly, the improvement in osseointegration was only seen in HA-coated implants compared to titanium-only implants [7].

In order to evaluate the effect of other hormone supplementation therapies on bone volume density (BVD), BMD, and implant fixation in ovariectomized animals and to compare its effect with alendronate, Skripitz et al. performed a study on ovariectomized Wistar rats. They concluded that PTH therapy increased BVD more effectively than alendronate, and that both PTH and alendronate improved implant bone contact significantly [12]. In another study in 2011, Chen et al. compared the effect of alendronate and calcitonin on ovariectomized rats concerning BMD and implant fixation. They found that both alendronate and calcitonin are able to effectively enhance the volume of bone mass surrounding the hydroxyapatite implants and also significantly lift up osseointegration rate. These effects were more pronounced with alendronate than with calcitonin. The effect of vitamin D supplementation on implant fixation in osteopenic animals has also been evaluated [13]. Nakamura et al. administered calcitriol, alendronate, or the combination of both to ovariectomized rats 8 weeks before the insertion of femoral implants and 4 weeks after the implantation. They found that applying the combination of alendronate and calcitriol to estrogen deficient animals, starting in the preoperative period, can improve cancellous bone mineral density and the stability of hydroxyapatite-coated implants [15].

In the present study, we mostly focused on the comparison of a specific systemic bisphosphonate (pamidronate) with intravenous calcium gluconate on early implant osseointegration by means of histomorphometry. The rationale behind administering calcium was that it has been shown that oral calcium supplementation in middle aged women can effectively reduce bone mineral content (BMC) loss in multiple bones of the human body [8]. However, in our study, no additional benefit was gained through the addition of calcium. We showed that short term preoperative intravenous pamidronate not only did not interfere with implant success, but also improved implant to bone contact surface area significantly. However, adding calcium did not affect the results significantly. In the current study, we found no adverse effect regarding the use of intravenous pamidronate including osteonecrosis. This could be attributed to the short term administration of the drug in the studied animals. Moreover, it can be attributed to the site-specific nature of osteonecrosis; since to the best of our knowledge this phenomenon is isolated to the jaw and has not yet been reported in other bones such as the tibia and femur [19]. In our study, all implants were placed in the tibia.

## Conclusion

It can be assumed that short term preoperative intravenous pamidronate can significantly improve bone to implant contact in post-menopausal animal models; whereas, calcium supplementation has no beneficial effect. The clinical application of these data on human subjects is yet to be studied.

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Conflict of Interest: 'None declared'.

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