Influence of Alendronate Administration Regimen on the Final Outcome of Implant Osseointegration in an Osteoporotic Model

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Abstract

This study aimed to evaluate the bone response around titanium implants in osteoporotic rabbits receiving oral alendronate (ALN) for one month either simultaneously with implant placement (IP) or stopped immediately before surgery. Six weeks before IP, 34 adult female New Zealand white rabbits were submitted to intramuscular injections of dexamethasone (3 mg/kg twice a week) to induce osteoporosis-like conditions. Two animals were sacrificed and histological examinations of their leg bones were performed to confirm the osteoporosis-like condition, as compared to a healthy animal. The remaining 32 rabbits were then divided into four equal groups. Group I represented osteoporosis-like animals receiving implants. For Groups II, III and IV, osteoporosis-like animals received calcium phosphate cement before IP. Animals in Group II received implants without any systemic treatment for osteoporosis. In Group III, ALN was simultaneously started with IP. In Group IV, ALN was stopped immediately before surgery. At 12 weeks post-surgery all animals were sacrificed. Both bone-implant interface and mineralized bone area percentage (MBA%) were microscopically evaluated. Group IV showed a significant gain in MBA % ($p < 0.05$), along with the most favorable implant integration outcome. Our results suggest that finishing an ALN course before implant placement could enhance the healing capacity around implants.

Key words: Dental implants, alendronate sodium, histological analysis, osteoporosis, osseointegration

Introduction

Osteoporosis is a progressive bone disease that is characterized by a decrease in bone mass and density, leading to an increased risk of fracture (Alldredge *et al.,* 2009). Osteopenia is a condition where bone mineral density (BMD) is lower than normal. It is considered by many physicians to be a precursor to osteoporosis. However, not every person diagnosed with osteopenia will develop osteoporosis. More specifically, osteoporosis is diagnosed when BMD is less than or equal to 2.5 standard deviations below that of a young (30–40 years), healthy adult women reference population. This is translated as a T-score. However, osteopenia is defined as a BMD T-score between -1.0 and -2.5, as mentioned by the World Health Organization (WHO) report in 2007. Previous researchers have demonstrated that osteoporosis can impair the process of implant osseointegration (Cho *et al.,* 2004; Duarte *et al.,* 2005).

Bisphosphonates, such as alendronate (ALN), risedronate, ibandronate and clodronate, are an important group of drugs used for the treatment of metabolic and oncologic pathologies involving the skeletal system (Küçük *et al.,* 2011). Their nuclear structure consists of two phosphate groups joined by a single carbon atom. They are potent inhibitors of bone resorption and have structural similarity to inorganic pyrophosphate (Serra *et al.,* 2008). They have a strong affinity for mineralized bone and therefore exert their effects mainly by inhibiting osteoclast-mediated bone resorption and normalizing the high rate of bone turnover (Gao *et al.,* 2009).

After administration, bisphosphonates are rapidly cleared from the circulation and localized to hydroxyapatite (HA) bone mineral surfaces, especially at sites of increased osteoclast activation (Theriault and Hortobagyi, 2001; Zenios *et al.,* 2004). *In vitro* studies on the effects of bisphosphonates on the nuclear factor-kappaB (RANK)/ligand (RANKL)/osteoprotegerin

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(OPG) system have been controversial. Viereck *et al.* (2002) demonstrated an up-regulation of OPG mRNA in primary trabecular human osteoblasts during exposure to pamidronate and zoledronate. Pan *et al.* (2004) hypothesized that the effect of zoledronate on the recruitment and differentiation of osteoclast precursor cells is mediated indirectly by increasing OPG protein secretion and decreasing trans-membrane RANKL expression in osteoblasts. In contrast, it was found that pamidronate and ALN left OPG and RANKL expression unchanged in osteoblasts, while they decreased osteoclast formation in co-culture experiments (Kim *et al.,* 2002).

There have been only a few studies on the effects of bisphosphonates on the RANK/RANKL/OPG system *in vivo* so far. In patients with osteoporosis associated with beta-thalassaemia, repeated treatment with pamidronate was shown to decrease OPG serum levels over a period of 12 months. Treatment was associated with an increase in BMD and a decrease in markers of bone turnover, while RANKL levels remained unchanged during the study period (Voskaridou *et al.,* 2003).

The necessity for repairing and remodeling of bone increases when conducting any surgical intervention. Compounds of bisphosphonates have high affinity for bone tissue, especially in areas that are remodeling. Depending on the dose, route and time of drug administration such capabilities may be seriously undermined (Grant *et al.,* 2008; Montoya-Carralero *et al.,* 2010).

ALN (4-amino-1-hydroxybutylidene-1,1-bisphosphonate sodium) has been shown to be one of the most potent bisphosphonates in inhibiting bone resorption both *in vitro* and *in vivo*. Although the detailed mechanism of action of ALN is still unclear, it has been shown to inhibit osteoclasts' ruffled border formation, and decreases the osteoclastic resorption without destroying osteoclasts. ALN may also promote the deposition of bone matrix protein, osteocalcin, and collagen by osteoblasts (Mochida *et al.,* 2002). The ability of ALN to affect systemic bone remodeling raises natural questions about the drug's influence on dental implant osseointegration (Chacon *et al.,* 2006).

Osseointegration of an implant is a wound healing process that depends upon host bone quality and quantity, its healing capacity, and various other systemic conditions. Osseointegration is based on intimate bone–implant contact achieved during healing. Thus, any condition affecting bone quality or quantity, or micro-architecture resulting in reduction in cancellous bone volume and bone–implant contact (which results in reduced bone tissue available around the implant) could theoretically have a negative impact on the success rate of an endosseous implant (Qi *et al.,* 2004; Ruggiero, 2011).

In a pathological condition such as osteoporosis, where large numbers of osteoclasts are continuously recruited, and each osteoclast takes a "test bite" of bone before becoming inactivated, the accumulated effect may be sufficient to cause unaffordable bone loss. Consistent with this notion, bisphosphonates impaired the function of osteoclasts in a model for disuse osteopenia in dogs, but as they were unable to overcome the strong stimulus for osteoclast recruitment, resorption was reduced only to a moderate extent (Yang *et al.,* 2005). Apparently the cells become inactivated by the bisphosphonates, but remain in the area for a long time (Aspenberg and Fahlgren, 2011).

Implant osseointegration may be adversely affected because of localized bone resorption. This might be due to the strong resorptive stimulus, which continuously recruits new osteoclasts. Therefore, we hypothesized that initial treatment targeting osteoclast recruitment would be more efficacious in implant integration, rather than a treatment reducing osteoclast activity.

No previous reports have discussed the impact of the interference time of bisphosphonates in osteoporosis treatment on the final outcome of implant osseointegration. In view of this, the purpose of the present study was to evaluate the bone response to titanium implants grafted with injectable calcium phosphate in osteoporotic rabbits when treated with oral ALN at two different intervals. It was administered for one month, either simultaneously with implant placement or immediately withdrawn before surgical intervention.

Materials and methods

In this prospective animal study, 34 adult female New Zealand white rabbits aged 8 months and weighing 4-5 kg were used. They were housed individually in stainless steel cages in a standard animal facility at the Faculty of Science, Al Azhar University for Boys. Access to food and tap water were available *ad libitum*. After a 10-day acclimatization period, all the animals were subjected to the osteoporosis induction protocol. Six weeks before implant placement, all animals were submitted to intramuscular injections of dexamethasone (3 mg/kg twice a week) to induce osteoporosis-like conditions (Zhang *et al.,* 2012).

At the end of the dexamethasone-injection period, two animals were randomly selected and sacrificed. Histologic examination of their leg bones was performed to confirm the osteoporosis-like condition, as compared to a healthy animal. The remaining 32 rabbits were randomly divided into four equal groups of eight rabbits each: Group I represented the induced osteoporosis model and acted as the negative control. Group II was the positive control group with induced osteoporosis and received calcium phosphate cement (CPC) [PD Vital Os cement® (injectable and resorbable

calcium phosphate bone grafting cement) CalciphOs Technology, CH-1800 Vevey Switzerland]. Group III included the OP-test group of animals, which received CPC+ALN-sodium tablets [(Osteomepha™-70), SIGMA Pharmaceutical Industries], simultaneously with implant placement. The animals received oral doses of 10 mg once a week via an oropharyngeal tube, which assured that all the medication was ingested. It was taken in the morning of the same day for one month on an empty stomach. Animals were only allowed to ingest water 2 hrs before and 2 hrs after each drug dosing (Chacon *et al.,* 2006). Group IV contained the OP-animals, which were given ALN-sodium for 1 month, which was stopped immediately before receiving the implants + CPC.

In this study the TUT implant system [(submerged implant type), TUT Dental implants, Egyptian Co. for Dental Implants (ECDI) Cairo, Egypt] was used. The implants are fabricated from commercially pure medical titanium grade 3. This implant is of a cylindrical form and threaded, with a tapered vented end. The surface of the implant is treated by sand blasting with aluminum oxide and acid-etched, which increases the implant surface roughness, thus increasing bone–implant contact and promoting osseointegration. In each rabbit, one implant of 3.4 mm diameter and 7 mm length was placed unilaterally into one of its femurs.

For each group, two rabbits were kept under the same living conditions to be sacrificed at the same time with their corresponding group to ensure persistence of the OP-condition during the time of the experiment.

Surgical protocol

The animals were fasted for 12 hrs before surgery. Ketamine (50 mg/kg) and xylazine (20 mg) were administered intramuscularly. Surgical procedures were approved by the ethical principles of the health research ethics committee adopted by the Faculty of Medicine for Girls, Al Azhar University, Cairo, Egypt. Titanium dental implants were placed using a standardized surgical protocol. Once general anaethesia was established, the femur region of each animal was shaved and washed with iodine; an approximately 3 cm long incision was made through the skin and the fascia to expose the femur shafts through blunt dissection.

Using a physio-dispenser, a round bur was used to mark the desired locations of implants. Bone was perforated using segmental drills at a low speed with saline cooling, and the bone holes were carefully threaded. The implants were gently screwed into place for Group I. For Groups II, III and IV, CPC was applied in the osteotomy sites *(Figure 1)*, and then the implants were gently screwed into place, followed by placing the healing covers *(Figure 2)*. The site of

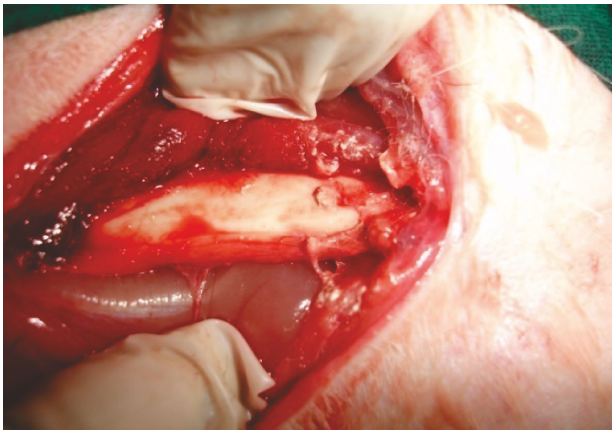

Figure 1. Calcium phosphate cement (CPC, black dotted ring) applied to the osteotomy site.

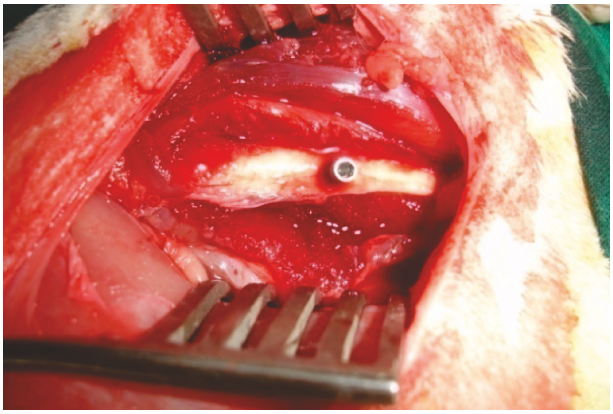

Figure 2. The implant, after being screwed into place, is followed by placing the healing covers.

implantation was then irrigated profusely with normal saline, the soft tissue was repositioned and approximated, and the fascia and skin were sutured with black silk suture *(Figure 3a, 3b)*.

The area was then washed with a mixture of iodine and 70% (v/v) ethanol. Each animal received a preoperative intra-muscular injection of garamycin (gentamicin) at a dose of 15 mg/kg/day. The antibiotic regimen continued for three successive days postoperatively to prevent infection after surgery. The animals were allowed full-weight bearing without any mobility restrictions immediately post-operatively.

At the end of the 12th week after implant placement, all rabbits were sacrificed by an overdose of

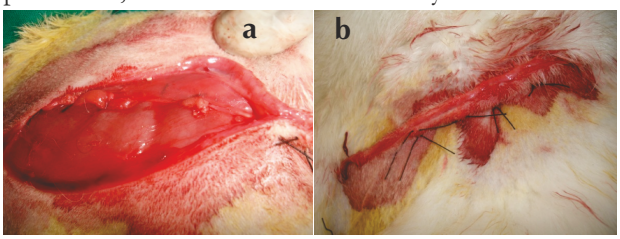

Figure 3. The soft tissue was then repositioned and approximated: (a) the fascia is sutured first using a bioresorbable suture; (b) the skin is closed using black silk suture.

intravenous pentobarbital. At the time of sacrifice, there was no indication of any inflammation around the implants.

The implanted femurs were removed, and the bones containing the implants were divided into two equal groups: one was used for examination by scanning electron microscope (they were immediately immersed in 2.5% gluteraldehyde in phosphate buffer, pH 7.4 for 6 hrs, then dehydrated in a graded series of ethanol for 10 minutes at each gradation). The other group was used for histological examination by light microscopy using toluidine blue stain, where they were immediately immersed in 10% (v/v) buffered formalin solution for fixation of the specimens for 48 hrs, then dehydrated in a graded alcohol series for 10 hrs. The specimens were embedded in methyl methacrylate without decalcification. After polymerization, sections were made through the longitudinal access of the implants and through the surrounding non-decalcified bone. The embedded tissue was cut into 150 µm thick sections with a low speed diamond wheel using tap water lubrication.

Scanning electron microscope (SEM) examination

The sections were sanded with abrasive paper under tap water in order to obtain a uniform surface finish. Specimens were gold sputtered and examined using an SEM (Philips, XL30, 5600 MD; Eindhoven, Holland). The bone–implant interface was examined and the gap distances between bone and implants were measured throughout the length of the implant body in all the study groups.

Light microscopic (LM) examination

Specimens were further ground and submitted for longitudinal sections, then 100 µ thick sections were mounted on glass slides. Sections were stained with toluidine blue for 2 minutes, then cover-slipped. Toluidine blue staining was utilized for optimal demonstration of mineralized bone and osteoid seams, osteoblasts and osteoclasts, in order to analyze the different stages of bone formation and remodeling patterns. It allows visualization of newly formed bone and distinguishes between mature and immature bone, as mineralized bone stains light purple and non-mineralized osteoid is colorless to pale blue.

The histological sections were examined by LM to detect the bone architecture around the implant and the mineralized bone area percentage (MBA%) using an image analyzer computer system (Leica Qwin 500 Software, Germany). It comprises LM supplied with a digital camera together with a computer, which is capable of performing high-speed digital image processing for the purpose of measurements. The image analyzer was calibrated automatically to convert the measurement units (pixels) produced by the image analyzer program into actual micrometer units.

Statistical analysis

Data are presented as mean and standard deviation (SD) values. A one-way ANOVA test was used to compare among the four groups. Tukey's test was used for pair-wise comparisons between the mean values when the ANOVA test was significant. The significance level was set at $p \leqslant 0.05$. Statistical analysis was performed with IBM® SPSS® Statistics Version 20 for Windows.

Results

Histological results

Evaluation of the histological sections of Group I showed such typical osteoporotic features as reduction in bone volume, represented by multiple relatively wide areas of bone marrow spaces and thinning of bone trabeculae. Besides the presence of poorly mineralized bone, osteocytes were haphazardly arranged with loss

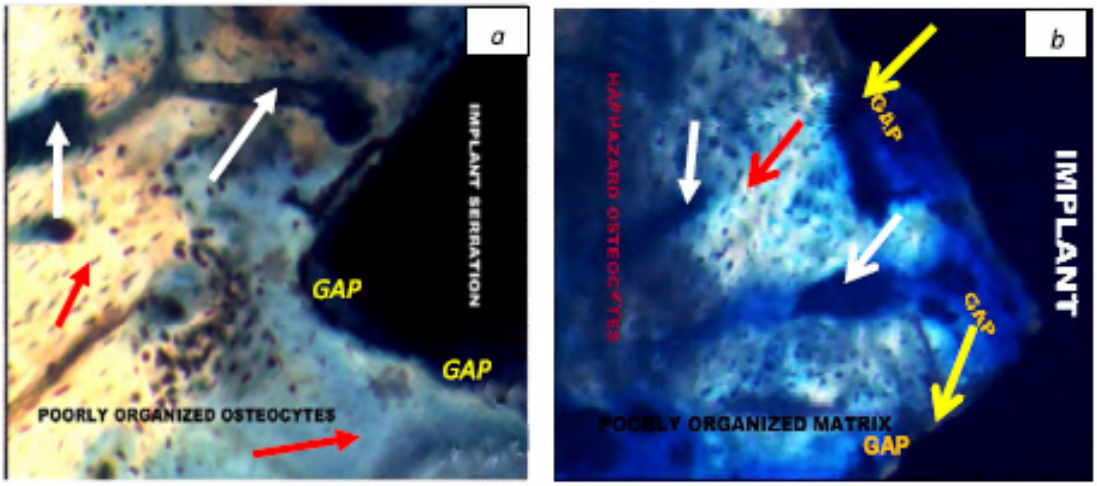

Figure 4. Histological micrographs of osteoporotic bone showing widening of bone marrow spaces (white arrows). Osteocytes are poorly formed and sporadically detected with loss of their architecture; poorly mineralized bone is shown (light blue and colorless, as indicated by red arrows). Wide gap areas between bone and implant are seen (yellow arrows). Toluidine blue stain, x200.

of their normal shape, size and architecture, which is proof of osteoporosis and the validity of the applied protocol in this experiment. Wide gaps were also noticed between bone and implant, indicating improper osseointegration due to the induced osteoporosis *(Figure 4a, 4b)*. Group II showed poorly organized osteocytes. Osteoblast-like cells lining the active new bone formation were observed around the marrow spaces (remodeling sites). Newly formed osteoid was also seen in direct contact with the cement layer, and CPC became islands incorporated in the new bone. A smaller gap space was also seen in contact with matrix and osteocytes of the newly formed bone adjacent to the cement, which may be due to the osteoconductive action of the cement *(Figure 5)*.

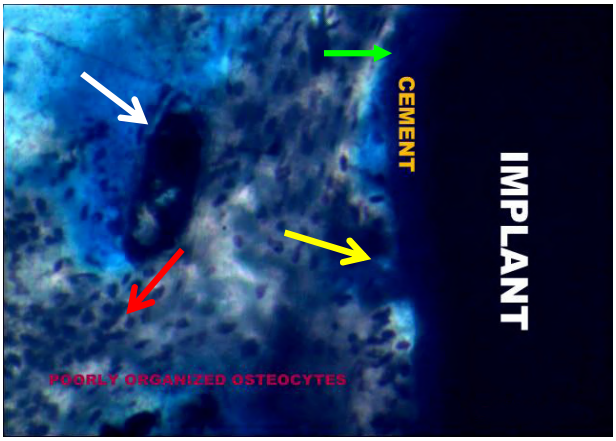

Figure 5. Group II showed an obvious cement layer intervening between the bone and implant, with haphazardly arranged osteocytes (red arrows). Wide bone marrow spaces are seen lined with osteoblast-like cells (white arrow). Newly formed osteoid is seen in direct contact with the cement layer (light blue), with incorporated islands of cement (yellow arrow). A minimal gap can be also seen in contact with the matrix (green arrow). Toluidine blue stain, x200.

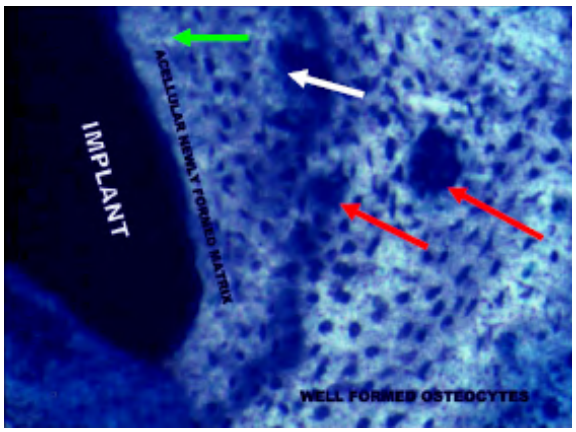

Figure 6. Group III showed well formed and arranged osteocytes around Haversian canals (red arrows). Acellular osteoid matrix can be seen at the bone–implant interface (green arrow). The line of demarcation is lined with osteoclasts between old and newly formed bone (white arrow). A small gap can be seen on the surface of the implant. Toluidine blue stain, x200.

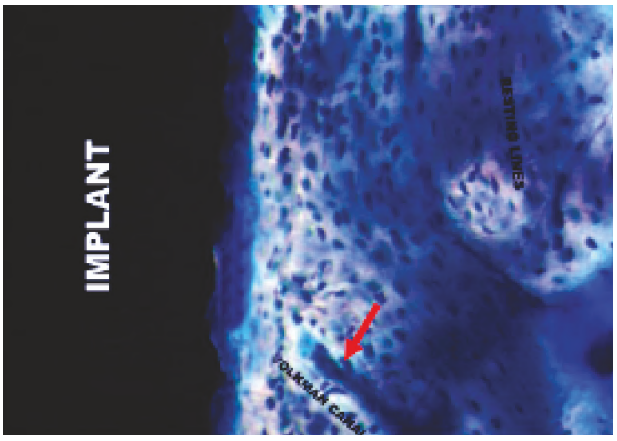

Figure 7. Group IV showed an appropriately organized and formed Haversian system with well-formed osteocytes and Volkman's canal (red arrow). A resting line is seen demarcating the bone turnover activity. Toluidine blue, x200.

Group III showed well-formed osteocytes and Haversian system. The newly formed bone in direct contact with the implant was nearly acellular; minimal gap space was also noticed. A line of demarcation between the old bone and the newly formed bone was clearly seen and it was lined by large multinucleated osteoclasts *(Figure 6)*. In Group IV, almost regular bone structure was seen, i.e. a well organized and well formed Haversian system was noted with well formed osteocytes. Newly formed bone in direct contact with the implant was nearly acellular. A resting line was seen indicating normal bone turnover activity *(Figure 7)*.

Scanning electron microscopy results

On examination of the specimens, Group I showed a markedly irregular, rough porous surface of bone surrounding the implant body. There were multiple variably sized holes and cavities, which reflects the increase in number and size of bone marrow spaces. Clear wide gap spaces intervening between the implant surface and bone were also seen and accurately measured *(Figure 8a, 8b)*. Group II showed no enhancement in the porous appearance of the bone surrounding the implant, but there was a noticeable decrease in the measured gap spaces between the implant and bone *(Figure 9a, 9b)*.

Examination of Group III revealed a smoother appearance of the bone surrounding the implant, while there was a marked reduction in the size of pores and cavities. The newly formed bone around the implant was clearly seen as an electron dense area just adjacent to the implant surface, and a clear reduction of gap spaces was noticed between the implant and bone

Histomorphometric analysis

Group IV showed the statistically significant highest MBA%. In Group III, a statistically significant lower MBA% was shown, followed by Group II. However, Group I showed the statistically significant lowest mean MBA% *(Table 1)*.

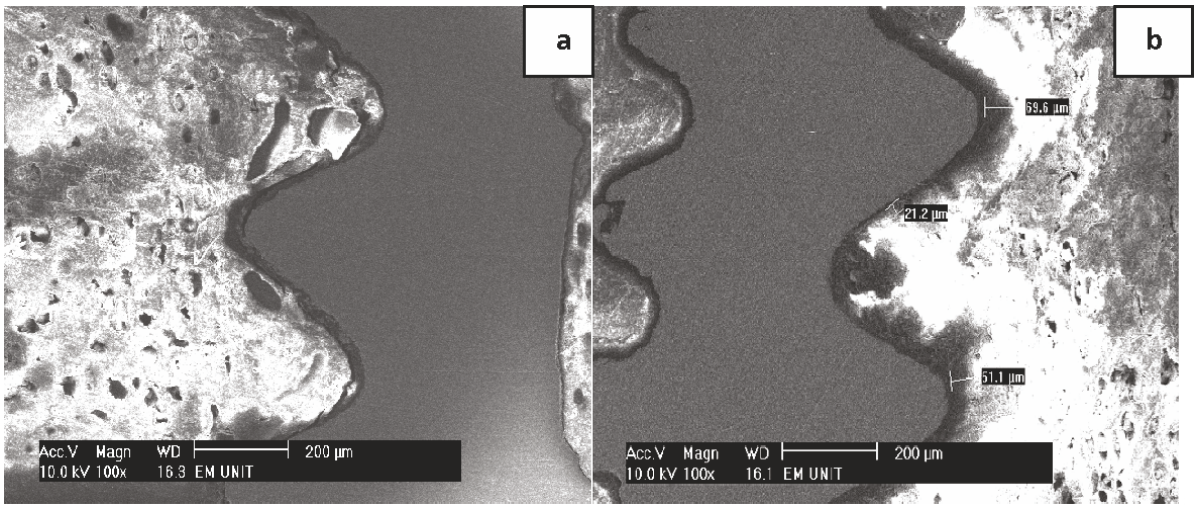

Figure 8. SEM of Group I showed: (a) rough porous bone with multiple variable sized holes and pores, with the larger holes near the implant surface; (b): measurements of the gap areas at the bone–implant interface, (x200).

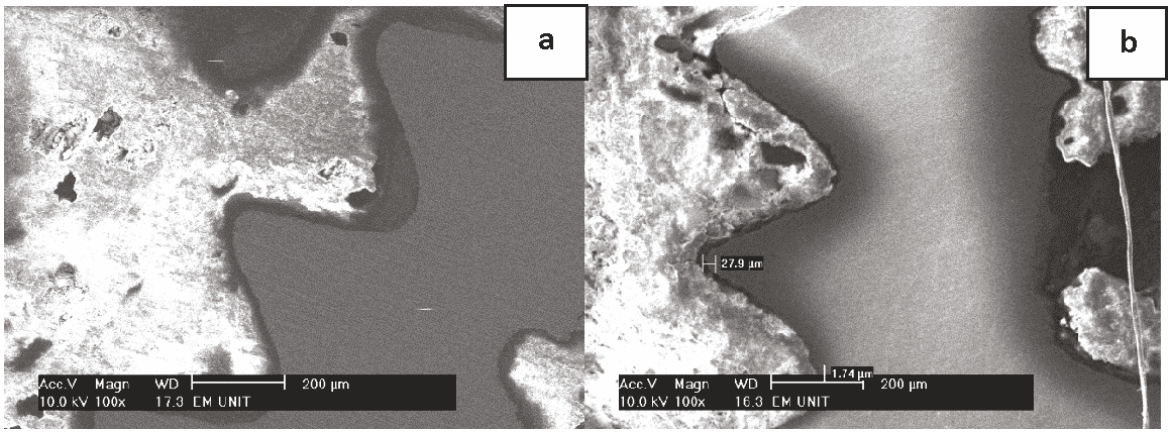

Figure 9. Group II demonstrated (a) multiple areas of cavitations and holes; (b) a decrease in the measured gap size at the implant–bone interface. SEM, x200.

With regard to gap distance, Group I showed the highest mean, while Group II showed a statistically significant lower mean gap distance. Although the lowest gap distance was found in Group IV, a non-meaningful difference ($p > 0.05$) was revealed when compared to Group III *(Table 2)*.

There was a statistically significant inverse (negative) correlation between gap distance and MBA% ($r = -0.893$; $p < 0.001$). An increase in gap distance was associated with a decrease in MBA%, as shown in *Figure 12*.

Discussion

The indication for implant use should sometimes be reconsidered in the presence of possibly interfering systemic or local factors (Alsaadi *et al.,* 2007). It is assumed that compromised bone metabolism, as in osteoporosis, would negatively affect the healing process in the bone tissue surrounding the implants (Eastell *et al.,* 2011; Cardemil *et al.,* 2013).

The number of patients suffering from or at risk for osteoporosis and seeking implant therapy is increasing considerably. Evidence-based management of these patients becomes increasingly difficult when bisphosphonate therapy is coupled with the process of osseointegration. A clear understanding of bone metabolism, implant integration, and BP pharmacology is needed to make educated treatment decisions. The objective of this project was to evaluate the effect of a one-month oral ALN course on bone–implant integration when given either simultaneously with or immediately stopped before implant insertion.

Experimental models of osteoporosis in rabbits are useful to investigate anabolic agents because they have an active Haversian remodeling system and can achieve skeletal maturity quickly (Baofeng *et al.,* 2010). Animal sacrifice was carried out 12 weeks after implant insertion because this time frame was suitable for completion of the osteoconductive phase for implants in the osteoporotic rabbit model (Chacon *et al.,* 2006).

Because the osteopenic condition probably inhibits new bone formation (Nikolaou *et al.,* 2009), CPC was selected as a standard line of treatment in this study. Accordingly, this may explain the significantly

Table 1. Mean and standard deviation (SD) values and results of one-way ANOVA and Tukey's tests for comparing mean area percentage among the four groups.

Group I		Group II		Group III		Group IV		*p*-value
Mean	SD	Mean	SD	Mean	SD	Mean	SD	
14.2 [d]	2.1	19.7 [c]	2.2	34.1 [b]	2.8	45.1 [a]	3.6	<0.001*

*Significant at $p \leqslant 0.05$. Different letters indicate statistically significantly differences.

Table 2. Mean and standard deviation (SD) values and results of one-way ANOVA and Tukey's tests for comparing gap distances among the four groups.

Group I		Group II		Group III		Group IV		*p*-value
Mean	SD	Mean	SD	Mean	SD	Mean	SD	
49.4 [a]	7.1	24.4 [b]	4.6	6.2 [c]	1.5	2.6 [c]	0.8	<0.001*

*Significant at $p \leqslant 0.05$. Different letters indicate statistically significantly differences.

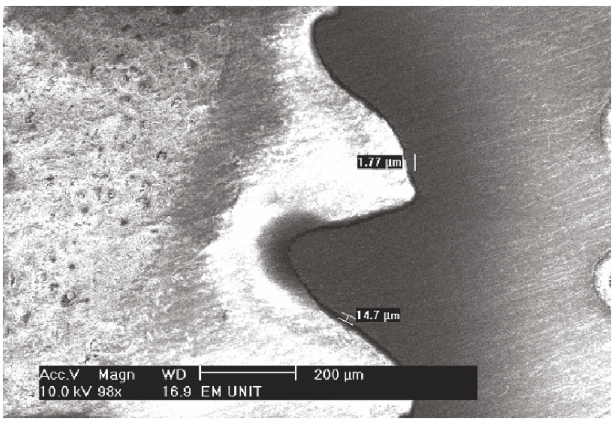

Figure 10. Group IV showed the smoothest, most regular appearance of bone, as the cavities and pores were almost not visible. Also, this group showed the minimal measured gap distance

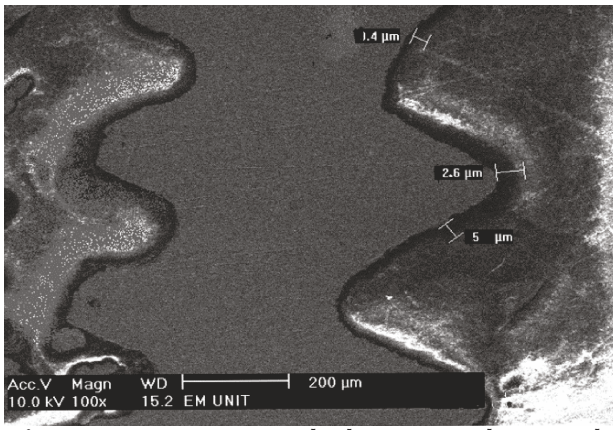

Figure 11. Group IV had a normal, smooth appearance of the bone surrounding the implant. Minimal gap distances at the bone–implant interface were also detected. SEM, x200.

better outcome that was revealed in Group II as compared to Group I (the non-grafted osteoporotic animals). CPC was identified as an excellent alloplastic material for osseous augmentation because of the unique combination of osteoconductivity, biocompatibility and moldability, in addition to its bone-like apatite final setting product, bioactivity, self-setting characteristics, and low setting temperature (Shih *et al.,* 2013).

However, even though CPC is stable *in vivo*, the resorption rate of this bone cement is very slow, which poses difficulty for clinical use in normal conditions. This was of negligible significance in this study as CPC was used in an osteoporotic model, where accelerated degradation occurs (Van de Watering *et al.,* 2013).

Bone is a highly metabolically active tissue in which the processes of osteoblastic bone formation (anabolic activity) and osteoclastic resorption (catabolic activity) are continuous throughout life. Therefore, the capacity of bone tissue to respond to injuries such as fracture or implant placement is associated with several mechanisms and may be affected by different conditions (Ennis, 2010).

Recently it was concluded that osteoporosis has definitive negative effects on healing of autologous bone grafts, decreasing osseointegration and fixation of adjacent implants, whereas treatment with bisphosphonates can efficiently invert the negative effect of osteoporosis and promote osseointegration and fixation of implants with autologous bone grafts (Qi *et al.,* 2012). The authors also suggested that administration of these drugs, used systemically, locally, or combined, may be an effective strategy for improvement of osseointegration and fixation of implants, especially in sites with poor bone quality, e.g., implants in cases with osteopenia or osteoporosis.

Nitrogen-containing bisphosphonates such as ALN, pamidronate, risedronate, ibandronate, and zoledronate exert their effects by inhibiting components of the intracellular mevalonate pathway, resulting in impaired membrane localization of small

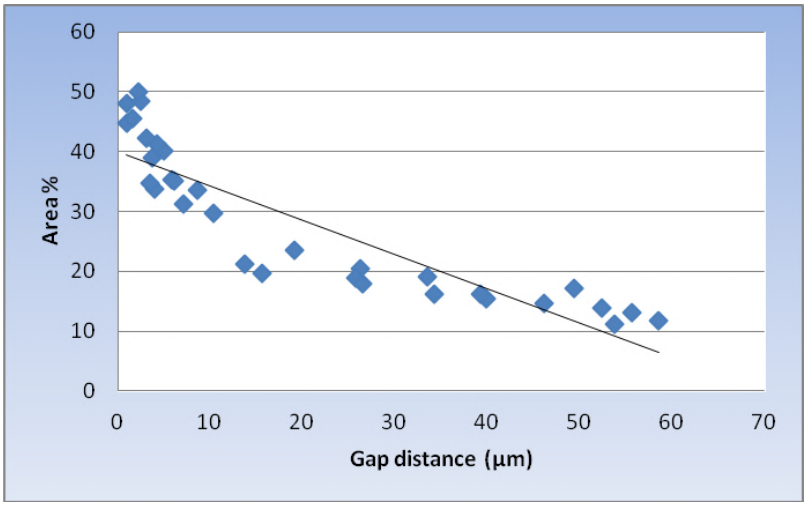

Figure 12. A scatter diagram shows the negative correlation between gap distance and mineralized bone area percentage.

guanosine triphosphatases, an important signaling molecule involved with maintaining osteoclast cell morphology, integrin signaling, membrane protein trafficking and cell survival (Morris and Einhorn, 2005; Einhorn, 2010). They accumulate in maximum concentration in the osseous matrix and in osteoclasts, mainly during the first 24-48 hr of medication, and the concentration remains high over long periods of time (Montoya-Carralero *et al.,* 2010).

ALN was the selected bisphosphonate in this study. It is a widely used and powerful drug in the treatment of osteoporosis and is considered as an alternative to estrogens, which, among other side effects, might be involved in the development of endometrial cancer (Rossouw *et al.,* 2002).

In treatment of osteoporosis, oral bisphosphonates (in most cases) or intravenous pharmacological agents are the drugs of choice, because as a result of their mechanism of action they are effective in increasing BMD and reduce the risk of fractures (Pazianas *et al.,* 2007). Although the oral route of bisphosphonate administration is much safer than the intravenous one, recommendations published by the American Dental Association in 2006 warn that the placement of dental implants or guided bone regeneration involves an increased risk of jaw osteonecrosis in these patients. On the other hand, it was reported that patients who take oral bisphosphonates are no more at risk of implant or bone graft failure than other patients (Bell and Bell, 2008).

Consistent with this observation, Cartsos *et al.* claimed that the mode of bisphosphonate use could result in different risk profiles. They reported an increased risk of inflammatory conditions with intravenous use of bisphosphonates, whereas they did not find this risk with oral administration (Cartsos *et al.,* 2008). The oral route was preferred in our experiment.

In this study, the histological analysis for Group I was in accordance with findings by Dalle *et al.* in 2005, who reported that glucocorticoid-induced osteoporosis resulted in marked thinning of bone trabeculae. Group II did not show improvement in the general condition of the osteoporotic bone, but the new bone formation adjacent to the cement was clear, with the residual cement layer becoming incorporated into the newly formed bone in the form of islands. The same findings were reported by Tsai *et al.* in 2008.

In Groups III and IV, ALN treatment resulted in increased bone trabecular thickness with restoration of normal lamellar architecture. Additionally, an increase in the number of normal osteocytes, with well organized bone matrix and newly formed bone around osseous defects and endosseous implants were noticed. No interposition of fibrous tissue was noticed at the perimeter of the implant, which confirms the osseointegration observed under LM (Bitto *et al.,* 2009).

Consistent with that, Jakobsen *et al.* (2007) demonstrated that ALN treatment increased fixation of implants inserted in cancellous bone after 4 and 12 weeks in a canine model by increasing bone volume and density.

In this study, the histomorphometric analysis of MBA% showed statistically significantly higher values in ALN-treated groups (III and IV) than the untreated ones. This could be attributed to the positive effect of ALN on bone mineralization. Interestingly, the effects of ALN on the function of osteoblasts were recently analyzed (Li *et al.,* 2012). Co-incubation of the MG63 cell line with ALN at different concentrations was investigated. They found that the calcium deposition by MG63 was increased at the lower concentration, with consequent benefits in bone formation.

Furthermore, the effects of PTH and ALN (alone and in combination) on both the bone architecture and mineralization in ovariectomized rats were examined. ALN treatment increased the peak of mineral content

above the other treatment groups at both trabecular and cortical surfaces (Campbell *et al.,* 2011).

In contrast, Denissen *et al.* (2000) found no histological differences in bone quality or the amount of bone mineralization when comparing implants with or without ALN; they also found no significant differences in bone repair. Additionally, Chacon *et al.* reported in their study that the effect of systemically administered ALN has no distinct effect on implant integration of femur and tibia of osteoporotic female rabbits. They found that bisphosphonates most likely induce a state of compromised bone metabolism, and thus implant integration and maintenance may be a problem (Chacon *et al.,* 2006).

On the other hand, it was reported by some researchers that, although ALN might have negative effects on the derived material properties (structural properties normalized by bone geometry and fractional bone volume), these negative effects were counteracted by an increase in bone volume such that there was no deterioration of the biomechanical properties at the structural level (Allen and Burr, 2007).

Our results contrast with another rabbit femoral condyle study where ALN had no positive effect when it was incorporated into bone cement and inserted into the defect (Bodde *et al.,* 2008), which could be because ALN appeared to evoke a toxic response that may have contributed to the absence of a positive bone healing response. This suggests that the ALN effect on osteoblast activity may be more important for increasing bone formation during healing of bone defects than ALN effects on osteoclast activity (Cottrell *et al.,* 2010).

It was found that under steady-state conditions with normal mineralization, the proportion of bone that remains unmineralized is directly proportional to the rate of bone turnover (Chavassieux *et al.,* 1997). ALN decreases the rate of bone turnover without inhibition of mineralization in long-term clinical use (Fuchs *et al.,* 2011). The decrease in bone remodeling activity was found to prolong the lifetime and increase the number of bone structural units. Hence, ALN increases the duration of secondary mineralization, leading to an increase in the degree of bone mineralization, which is a major determinant of bone strength (Bala *et al.,* 2011).

With regard to Group IV, although the ALN course was stopped before surgical interference, our results revealed that this group had a statistically significantly higher MBA% than Group III. This could be related to the role of ALN in improving the bone quality before implant placement. Consequently, this will enhance the bone healing capacity around implants, being one of the most important determinants in the success of the osseointegration process (Qi *et al.,* 2012).

Moreover, Duarte *et al.* (2003) found that ovariectomized rats maintained normal bone turnover after ALN treatment withdrawal. Similar studies also revealed an accelerated bone loss following withdrawal of estrogen therapy, but not after withdrawal of ALN or combination therapy (Duarte *et al.,* 2005). The prolonged residual skeletal effects of bisphosphonates are probably a consequence of their strong affinity to HA crystals. Bisphosphonates bound to bone mineral are released during bone resorption by osteoclasts, which could lead to a localized accumulation of the drug in newly formed bone. Depending on the duration of treatment and bisphosphonate-specific requirements, these compounds can remain for years, a fact that raises natural questions about the possibility of stopping treatment with oral bisphosphonates before surgical implant intervention, considering the benefit/risk ratio for discontinuation of the drug (Mellado-Valero *et al.,* 2010).

The SEM results confirmed the histological results and revealed a notable effect on bone morphology, as manifested by roughness, multiple porosities and cavitations in Group I, indicating osteoporotic changes. In addition, they revealed the presence of a wide gap between the implant and bone, denoting improper osseointegration. For Group II, there was less enhancement of general bone morphology and roughness, but there was a decrease in the gap distance between the implant surface and the bone. This decrease was due to the presence of CPC, which induces the formation of new bone around the implant, enhancing the osseointegration process (Shih *et al.,* 2013).

Both Groups III and IV revealed a more uniform and smoother appearance of bone, with Group IV being the best. Using the measurements of the gap distance for all groups, Groups III and IV showed significantly lower means compared to Groups I and II. In accordance with our findings, Duarte *et al.* (2003) found significant differences between the study and control groups, with lower values in the spongy region of the group with induced osteoporosis. Kim *et al.* (2011) found no differences in new bone formation in extraction sockets, bone area around the implant site, or bone–implant contact in the bisphosphonate group compared to the control group.

Recently, comparing groups with and without ALN therapy revealed increases of 14.9% and 29.6% in BMD, as detected radiographically and by dual energy x-ray absorptiometry, respectively, in favor of ALN (Lucisano *et al.*, 2013).

The results of the present study revealed a non-significant difference ($p > 0.05$) between Groups III (6.2 ± 1.5 μm) and IV (2.6 ± 0.8 μm). Although the difference was not significant, gap distances in Group IV were still lower than those in Group III, indicating that ALN administration before implant placement may have a beneficial effect on enhancing the osseointegration process. Additionally, our results showed that the significant gain in MBA% was

correlated with the most favorable implant integration outcome, as demonstrated in Group IV. In accordance, it was reported that dental implant fixation depends upon both bone–implant contact and bone formation around the implant (Gao *et al.,* 2009).

Bisphosphonates are capable of both inhibiting particle-induced osteoclastic bone resorption and stimulating osteoblastic bone formation, resulting in a net gain of peri-implant cortical and cancellous bone. It was also suggested that bisphosphonates might promote gene expression of key osteogenic transcription factors, including BMP-2 and core binding factor alpha subunit 1 (cbfa-1), by acting as successive differentiation triggers, which secondarily result in a pronounced recruitment, proliferation and anabolic activation of osteoblasts (von Knoch *et al.,* 2007). More recently, it was reported that ALN seemed to decrease bone resorption but not bone formation (Kim *et al.,* 2011).

However, bisphosphonates may be insufficient to inhibit ongoing bone resorption around the prostheses, while prophylaxis is another matter: bisphosphonates appear to be efficacious for improvement of early postoperative healing, and might thereby reduce the risk of failure (Hilding and Aspenberg, 2006).

Ultimately, the prophylactic protocol utilized in Group IV could be advisable because of its possible role in improving the bone quality before implant placement, which is mandatory during the early periods of healing. In osteoporotic patients, this regimen might be also considered a safer tool to reduce the inflammatory risk of bisphosphonates in human jaws, as it could avoid its prolonged skeletal effects.

Conclusion

According to our results, osteoporosis might be considered as a risk factor in implant therapy, but this risk could be avoided with regular treatment. However, finishing an ALN treatment course prior to implant placement might be highly advisable. This prophylactic regimen was found to enhance the bone healing capacity, resulting in a more favorable clinical outcome of implant osseointegration. Further studies in humans to understand more about proper implant use and survival in the complex bone environment under osteoporotic-like conditions are encouraged.

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