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ORIGINAL ARTICLE

The effect of systemically administrated zoledronic acid on the osseointegration of dental implants

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OBJECTIVE: The aim of conducting this study was to evaluate the effect of zoledronic acid (ZA) on the new bone formation (NBF) after the insertion of a titanium dental implant, which is very popular treatment in dentistry.

STUDY DESIGN: Twelve New Zealand white rabbits were used in this study. The rabbits were divided in two groups. ZA was systemically administered to the study group. Titanium implants were placed to the left and right tibias of the rabbits.

RESULTS: The data from the ZA group revealed a statistically significant increase in the bone mineral content and the bone mineral density. A non-decalcified histomorphometric examination conducted on the study group revealed a significant increase of NBF and bone-implant contact (BIC) at 2 and 4 weeks.

CONCLUSION: A single dose of systemic ZA administration increases the rate of NBF and augments the quality of the bone.

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Keywords: dental implant; zoledronic acid; osseointegration; non-decalcified histology

Introduction

Titanium is an excellent material for dental implants thanks to its biocompatibility, augmented resistance to corrosion, non-toxic effect on macrophages and fibroblasts, and reduced inflammatory response in peri-implant tissues (Ellerbe and Frodel, 1995). The success of endosseous implants is dependent on good osseointegration between the implant and the bone. Direct bone apposition on the implant surface with nearly no interposition of soft tissue leads to bone-implant fixation (Mavrogenis *et al*, 2009).

The most important problems of dental implants are the length of time between application and loading, and the low rate of clinical success in case of poor quality of bone (Lavos–Valereto *et al*, 2001). Despite of poor bone quality, different approaches have been used to enhance osseointegration and peri-implant-bone formation for rapid bone healing and predictable good improvement. For instance, recombinant growth factors (De Ranieri *et al*, 2005; Sachse *et al*, 2005) and bisphosphonates (BPs, Viera-Negrón *et al*, 2008) show that they improve the osseointegration of titanium alloy implants. In the recent studies, it was demonstrated that there was a positive effect of BPs, which were used in the treatment of osteoporosis and malignant hypercalcaemia, in bone recovery procedures (Wellington and Goa, 2003; Tekin *et al*, 2008; Viera-Negrón *et al*, 2008).

The most potent BPs compound is the clinically used zoledronic acid (ZA) (Body *et al*, 1999). Single dose of ZA, administered intraoperatively, demonstrated the positive effects on different models of bone healing (Little *et al*, 2003; Pampu *et al*, 2006; Yildiz *et al*, 2010), and thus, there are studies showing a positive effect on the osseointegration of orthopaedic implants. (Bobyn *et al*, 2005; von Knoch *et al*, 2005; Peter *et al*, 2005; Wise *et al*, 2005). However, no studies demonstrate a possible ZA effect on the osseointegration quality of dental implants.

Bisphosphonates have a well-documented profile of possible side effects. An initial influenza-like illness has been documented with the first infusion of BPs (Body *et al*, 2004). Renal failure has been noted in patients with cancer after repetitive high-dose infusions (Body *et al*, 2004). Recently, an association between BPs and osteonecrosis of the jaw was reported after tooth extraction (Marx, 2003). Most of these complications have occurred in patients with cancer who have often received monthly high-dose BP infusions.

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The aim of this study was to evaluate the effect of systemically administered ZA on osseointegration of titanium dental implants in rabbit's tibia.

Materials and methods

The approval for the present study was obtained from Selcuk University Experimental Medical Research and Practicing Centre Ethics Council, Konya, Turkey. The animals used for testing were also supplied by the same centre. The recommendations of the Helsinki Declaration, related to the protection of laboratory test animals, were strictly obeyed.

Two study groups were created – test animals and control animals, comprising six random adult male New Zealand white rabbits ($n = 12$; weight, 3.4–3.9 kg) in each group. The mean weights of animals in the control and test groups were similar ($P > 0.05$).

Implant preparation

The implants were produced from pure commercial titanium, and application sets produced specifically for the implant were utilized in this study. The implants were in screw form, measuring 3 mm in diameter and 5 mm in length. These were used in their natural form without exposing them to any process that would roughen them up. The implants were cleaned according to the decontamination procedures and were sterilized (Piattelli *et al*, 2003).

Surgical procedures

General anaesthesia was induced before surgery through intramuscular injection of 35 mg kg^{-1} ketamine (Ketalar; Eczacıbaşı, Istanbul, Turkey) and 5 mg kg^{-1} xylazine (Rompun; Bayer, Leverkusen, Germany). The rear legs of all the rabbits were shaved and washed with an iodine solution, following which two per cent lidocaine was administered in the tibial area. Using sterile surgical techniques, an incision was made on the skin to expose the metaphysical area of the tibia. (Godoy *et al*, 2011; Park *et al*, 2011; Qi *et al*, 2012). The muscles were then dissected to allow the elevation of the periosteum. The flat surface on the lateral part of the proximal tibia was selected for implant. Holes were drilled into the tibia using a low-speed rotary instrument under constant irrigation with sterile saline following which a screw-type, pure titanium implant was placed. Two implants were inserted in each of the tibia of the rabbits' forelegs. Four implants were inserted in each animal, which means that a total of 48 implants were inserted (Figure 1). The muscle and fascia layers were closed with Vicryl resorbable sutures, while the skin was sutured using black silk for primary closure.

Zoledronic acid (Zometa; Novartis, Basel, Switzerland) was given to the experimental group by a single infusion of 0.1 mg kg^{-1}, diluted with 15 ml of normal saline in a 15-min perfusion with an infusion pump during the surgical procedure. Control group animals were administered only saline infusion for 15 min at the time of surgery (Little *et al*, 2003; Pampu *et al*, 2006; Yildiz *et al*, 2010; Tatli *et al*, 2011).

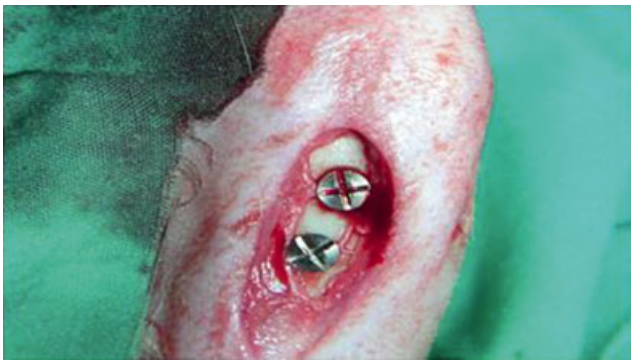

Figure 1 Two implants were placed in each rabbit's tibia

Animals from both the groups were placed in single cages. Post-operatively, antibiotic penicillin G procaine (40 000 IU kg^{-1}, once a day) and an analgesic (2 mg kg^{-1} tramadol hydrochloric, once a day) were applied for 5 days.

Randomly selected three rabbits from the test group and three rabbits from the control group (six rabbits in total) were sacrificed on 14th day, while the remaining others were sacrificed on 28th day. After the animals were euthanized, the implants and the surrounding bones were harvested en bloc and fixed in 10% neutral buffered formalin, for 14 days. The specimens were then prepared for non-decalcified histology.

Densitometric evaluation

The bone mineral density (BMD) and bone mineral content (BMC) were measured with a dual-energy X-ray absorptiometry (DEXA) and a densitometer (QDR 4500C; Hologic, Waltham, MA, USA) in a high-resolution mode, using the 'small animals' software supplied by the equipment manufacturer. The gap between the two implants was used for the assessments (Figure 2).

Preparation of the specimens and histomorphologic evaluation

The implants and the surrounding bones were harvested en bloc and fixed in neutral buffered formalin, dehydrated in 70%, 90%, 95% and 100% alcohol and embedded in a light-curing resin (Technovit 7200 VLC; Kulzer, Wehrheim, Germany). An Exakt sawing machine along with some grinding equipment (Exakt Apparatebau, Norderstedt, Germany) were used to cut and grind approximately 50-μm-thick sections that were then stained with 1% toluidine blue, prior to its evaluation under a light microscope. All the animals underwent histologic examination with the aid of an Olympus BX microscope (Olympus BX50; Olympus Optical Co. Ltd., Tokyo, Japan) that was connected to a computer. The osseointegration was observed via a light microscopy as the direct bone (toluidine blue stained) deposition on the implant surface (Park *et al*, 2011).

Digital images were obtained from 96 specimens prepared for non-decalcified histology. All the measurements were calculated under 40× magnification through IMAGEJ 1.4 (image processing and analysis in java

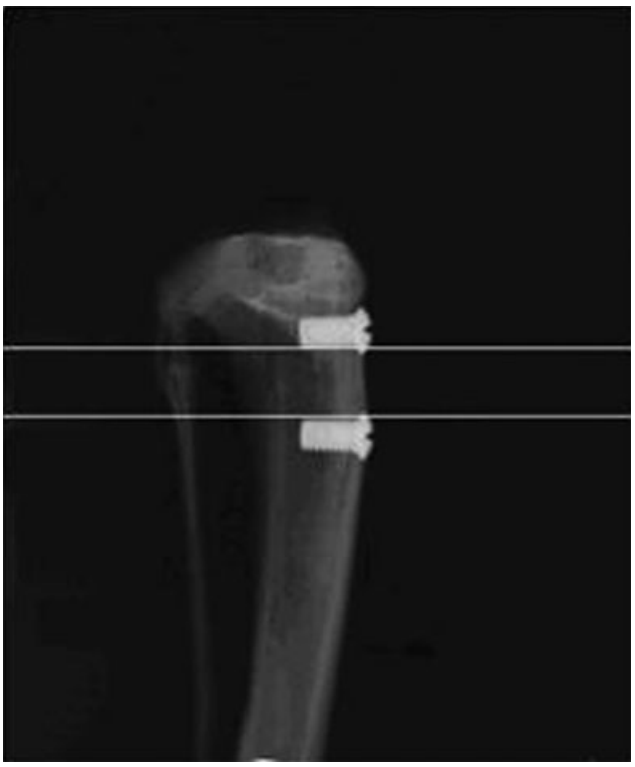

Figure 2 The gap between the two implants was used for the densitometry assessment

freeware; National Institute of Mental Health, Bethesda, MD, USA) software. The distance from 0.5 mm from top point of threats was determined as total area (TA) (Figure 3). The percentage of the new bone formation (NBF), with toluidine blue stain on the implant surface, was calculated as the proportion from the NBF area to the total area (% of NBF = NBF/TA × 100). The ratio of BIC was calculated as the proportion from the all the osseointegrated spaces around the implant (OSI) to total perimeter of the implant (TPI) (% of BIC = OSI/TPI × 100).

Statistical analysis

The densitometry and histomorphometry values were analysed using the Mann–Whitney *U*-test, while the SPSS (version 16.0) statistical program for Windows XP (SPSS, Chicago, IL, USA) was used for statistical analysis. The statistical data are expressed as mean (s.d.), and $P < 0.05$ was accepted as statistically significant.

Results

There was no gain in body weight in either of the groups. We observed no gastrointestinal side effects such as vomiting or diarrhoea from the ZA application. Mortality, infection or wound dehiscence were not recorded in this protocol.

The results of the densitometric and histomorphometric analyses of the NBF in the control and ZA groups are shown in Table 1.

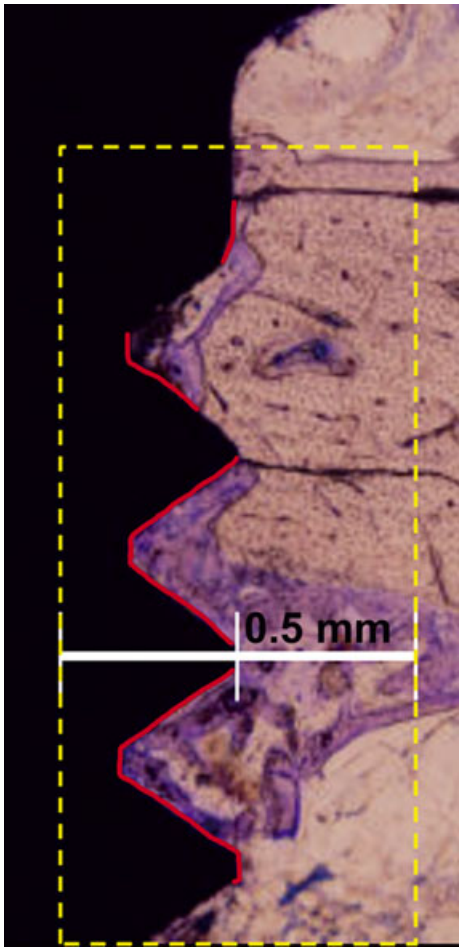

Figure 3 It shows that the total area is distance from 0.5 mm from top point of threats (red line: the bone-implant contact surface)

Table 1 The results of the densitometric and histomorphometric analyses of the NBF in the control and ZA groups

	Control	*ZA*	*Increase (%)*
2 weeks			
BMC	0.16 ± 0.05	0. 22 ± 0.01[a]	33
BMD	0.40 ± 0.05	0.50 ± 0.030[b]	24
NBF	13.75 ± 0.86	16.91 ± 0.66[c]	23
BIC	19.61 ± 3.42	23.00 ± 6.22[d]	17
4 weeks			
BMC	0.17 ± 0.03	0.31 ± 0.07[e]	73
BMD	0.45 ± 0.02	0.61 ± 0.030[f]	36
NBF	16.91 ± 0.66	21.50 ± 0.52[g]	27
BIC	23.19 ± 4.95	27.38 ± 2.22[h]	18

BIC, bone-implant contact; BMC, bone mineral content; BMD, bone mineral density; NBF, new bone formation; ZA, zoledronic acid.
For 2 week: [a]$P < 0.05$ compare with BMC control group, [b]$P < 0.05$ compare with BMD control group, [c]$P < 0.05$ compare with NBF control group, [d]$P < 0.05$ compare with BIC control group.
For 4 week: [e]$P < 0.05$ compare with BMC control group, [f]$P < 0.05$ compare with BMD control group, [g]$P < 0.05$ compare with NBF control group, [h]$P < 0.05$ compare with BIC control group.

Bone densitometry analysis

After 2 and 4 weeks of healing periods, the BMD and BMC values in ZA groups were significantly high in

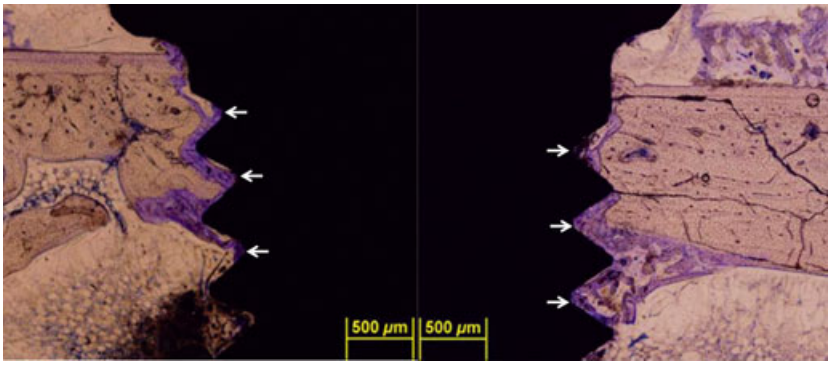

Figure 4 Histomorphometric analysis after 4 weeks in the control group

comparison with the control groups. There was no significant difference between the 2- and 4-weeks control animals in terms of the BMD and BMC values, while these values were significantly different in the ZA groups.

Histomorphometric analysis

The histomorphometric analysis after 2 and 4 weeks revealed that the percentage of NBF around the implant in the ZA group was significantly greater than the control group. After 2 and 4 weeks, the BIC values in the ZA group were also significantly higher than in the control group (Figures 4 and 5).

At the end of 4 weeks, sign of NBF stained toluidine blue around implant was very small, and unhealed bone areas appeared in the control groups (Figure 4), whereas abundant stained toluidine blue and complete healing around implant were observed in the ZA group (Figure 5).

Discussion

A large number of studies involving dental implants have been conducted to shorten the bone healing time and for improving the quality of osseointegration in cases where the bone is poor. Materials like platelet rich plasma (Fontana *et al*, 2004), melatonin with fibroblast growth factor-2 (Takechi *et al*, 2008) and bone morphonogenic protein-2 (Becker *et al*, 2006) were used by some researches. In accordance with these findings, ZA, which is one of the BPs, has positive effect upon osseointegration of orthopaedic implants (Bobyn *et al*, 2005; von Knoch *et al*, 2005; Peter *et al*, 2005; Wise *et al*, 2005). However, there is no study concerning the effect of systemic ZA application on the osseointegration quality of dental implants.

Many different animal models have been reported in dental implant studies including sheep, dogs, pigs, rats and rabbits (Pearce *et al*, 2007). Rabbit model is a special preference because of its ease of use and proper bone size. In terms of bone mineral densities and long bone fracture rigidity, human and rabbit bones have been reported to be similar (Wang *et al*, 1998; Pearce *et al*, 2007). Therefore, this study was performed with a well-established rabbit model for investigating the process of dental implant osseointegration (Piattelli *et al*, 2003; Yildiz *et al*, 2010; Godoy *et al*, 2011; Park *et al*, 2011; Qi *et al*, 2012).

Systematic application of BPs have been widely used in the clinical treatment of various systemic metabolic bone diseases including Paget's disease (Walsh *et al*, 2004), hypercalcemia of malignancy (Wellington and Goa, 2003) and post-menopausal osteoporosis (Bone *et al*, 2004). It is clear that all active BPs inhibit bone resorption, bone turnover and, therefore, bone loss at the tissue level. (Rodan and Fleisch, 1996).

In the present study, ZA was administered as a single dose of 0.1 mg kg^{-1}. (Little *et al*, 2003; Pampu *et al*,

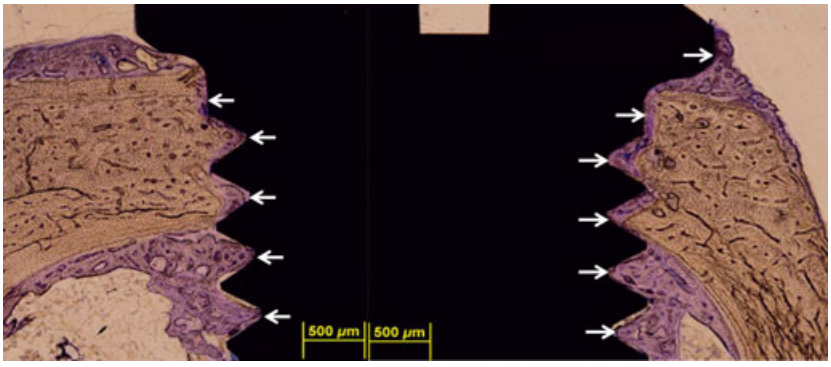

Figure 5 Histomorphometric analysis after 4 weeks in the zoledronic acid group

2006; Yildiz *et al*, 2010; Tatli *et al*, 2011). It has been proved that the plasma concentration of the drug gradually declines within 28 days (Chen *et al*, 2002). Thus, a repeat dose of ZA could be administered 28 days after the initial single dose, if required. Therefore, the administration of intra-operative single dose of 0.1 mg kg^{-1} ZA was thought to be sufficient throughout the osseointegration period of implants in the present study.

Oral surgical procedures including dental implants are not recommended for cancer patients being administered intravenous Bps because of the risk of Bps-related osteonecrosis of the jaws (Scully *et al*, 2006). However, some authors claim that there is not a risk of Bps-related osteonecrosis of the jaws developing as a result of implant surgery in patients taking Bps with the doses used for the treatment of osteoporosis (Fugazzotto *et al*, 2007; Grant *et al*, 2008).

Although proliferation of both cell types (oral fibroblasts and oral epithelial cells) was inhibited in a ZA concentration-dependent manner (Ravosa *et al*, 2011), this is distinct from a recent study by Scheper *et al* (2009), who reported enhanced susceptibility of HaCat keratinocytes to ZA-induced apoptosis relative to gingival fibroblasts when treated with ZA. Moreover, another study showed that zoledronate did not affect angiogenic markers in the bone marrow or soft tissue wound healing in the oral cavity (Yamashita *et al*, 2011). In this study, no side effect such as osteonecrosis of the jaw and impaired soft tissue healing were seen.

The bones of the oral cavity differ from those of the appendicular skeleton in a number of ways. The jaw bone is derived from neural crest mesoderm, forms primarily via intramembranous ossification, contains mostly fatty marrow and is chronically exposed to the outside environment and micro-organisms (McCauley and Li, 2007). Another apparent unique quality of the bones of the oral cavity is their susceptibility to osteonecrosis of the jaw (ONJ) in association with BP treatment (Ruggiero *et al*, 2004; Ruggiero, 2009). Although this is a relatively rare side effect of BP treatment (approximately 5% of patients treated with BPs for cancer), the severity of the condition as well as the absence of any known pathophysiology or effective treatment makes it a significant issue in the dental community (Allen and Burr, 2009; Allen, 2011). To our knowledge, no data are available concerning the relationship between single-dose administration and the possible side effects of BPs.

In a long-term study associated with BPs, there was no osteonecrosis development and carried no risk in terms of dental implant applications and graft procedures (Bell and Bell, 2008). Yildiz *et al* (2010) evaluated whether ZA influences bone healing around titanium implants inserted in ovariectomized rabbits. They concluded that ZA may prevent the negative influence of oestrogen deficiency on bone healing around titanium implants inserted in ovariectomized rabbits.

ZA was reported to increase tibia resistance and mineralization in the healing region of distraction osteogenesis (Little *et al*, 2003). It was also reported that the ZA leads to a significant increase in the BMC and BMD in areas of recovery and around the pins that provide bonny fixation of distraction tools in distraction osteogenesis (Pampu *et al*, 2006). Likewise, similar findings from the areas between two implants were observed in this study.

In a dog model, the effect of ZA on bone was examined, in which an enhancement of bone growth into porous tantalum implants was found. (Bobyn *et al*, 2005). Moreover, it demonstrated that increasing the mechanical fixation of an orthopaedic implant and peri-implant bone density were zoledronate dose-dependent (Peter *et al*, 2005). Wise *et al* (2005) noticed that zoledronate affects some of the material properties of the cortical bone and allows the newly formed subperiosteal bone to remain and therefore affect the overall quality of the bone in total hip arthroplasty. In addition to this, bone resorption was markedly decreased by a single dose of ZA in orthopaedic joint replacement (von Knoch *et al*, 2005). An annual infusion of ZA after the repair of a low-trauma hip fracture was associated with a reduction in the rate of new clinical fractures and improved survival (Lyles *et al*, 2007).

We can infer from the present study that intra-operative that is, involving application of ZA, which was previously documented to be effective in 0.1 mg kg^{-1} dose, promotes osseointegration of dental implants. Additionally, ZA did not affect the angiogenesis in bone around implant. This might be explained by inhibitory effects on osteoclasts, and improving effects on osteoblast proliferation, maturation and differentiation. Densitometry data from the bone tissue in the near vicinity of the implant and values related to BIC were significantly increased by this application in both early as well as late phases. Moreover, it would be provided data with cell culture study to indicate improvement of osseointegration on the implant surface with ZA.

In conclusion, while the results of previously conducted studies and the present study indicate positive effects, new studies on optimum dosage that can possibly affect the osseointegration and on mode of application have yet to be conducted. Additional studies are necessary to evaluate the effects of re-dosing on the osseointegration of the dental implant compared with the application of a single dose.

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Conflict of interest

We certify that there is no conflict of interest between authors and with any financial organization regarding the material discussed in the manuscript.

Author contributions

Dr. Mustafa Ayan designed the study and did the surgery. Dr. Doğan Dolanmaz designed the study and did the surgery.

Dr. Ahmet Mihmanlı designed the study and wrote the paper. Dr. Aslı Ayan did the densitometric evaluations. Dr. Mehmet Kürkçü did the non-decalcified histological evaluations.

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