Original

Intermittent administration of parathyroid hormone enhances primary stability of dental implants in a bone-reduced rabbit model

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(Received December 17, 2015; Accepted February 1, 2016)

Abstract: The aim of the study was to investigate the effect of parathyroid hormone (PTH) on primary stability of dental implants in a bone-reduced model. Ten female New Zealand white rabbits underwent ovariectomy and were administered glucocorticoid to induce osteoporosis. One group was administered PTH intermittently by subcutaneous injection for 4 weeks (PTH-group) and the other group was given injections of saline for 4 weeks (Osteoporosis; OP-group). After the administration period, implants were inserted into the distal femoral epiphyses of each animal. At implant placement, insertion torque (IT) and implant stability quotient (ISQ) were measured. Histological examination revealed newly formed trabecular bone around the implant socket in the PTH-group but not in the OP-group. The trabecular bone structures in the PTH-group appeared thicker than those in the OP-group. In the PTH-group, the mean IT value was significantly greater than that in the OP-group (29.8 ± 6.2 Ncm and 10.0 ± 2.1 Ncm, respectively; $P < 0.05$). The ISQ value in the PTH-group was significantly higher than that in the OP-group (74.7 ± 11.2 and 55.9 ± 13.5 , respectively; $P < 0.05$). Intermittent PTH administration could be an effective treatment for achieving favorable primary stability of dental

implants in patients with osteoporosis.
(J Oral Sci 58, 241-246, 2016)

Keywords: parathyroid hormone; primary stability; osteoporosis; rabbit.

Introduction

Successful implant treatment depends on the achievement of favorable primary stability, which in turn depends on sufficient bone quantity and quality (1). Osteoporosis is a skeletal disease that causes systematic loss of bone density and bone quantity (2). It has been reported that primary stability is decreased at sites of low bone density, for example those in patients with osteoporosis, and this may be a risk factor for implant failure (3). Thus, bone density at the implant placement site appears to be a crucial factor that is correlated with failure rate and primary stability (4,5). In fact, osteoporosis patients who undergo implant treatment have a less favorable outcome than patients with healthy bone (6). The most common secondary form of osteoporosis is that induced by glucocorticoid treatment for a great variety of inflammatory and allergic diseases. Glucocorticoid affects bone quality mainly by decreasing bone formation through a reduction of osteoblastogenesis and an increase of osteoblast and osteocyte apoptosis. Osteoporosis and osteoporotic fracture may be caused by cortical and trabecular bone loss (7). For these reasons, glucocorticoid-induced osteoporosis might be disadvantageous for achieving favorable primary stability.

Currently, intermittent administration of parathyroid

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doi.org/10.2334/josnusd.15-0717

DN/JST.JSTAGE/josnusd/15-0717

Fig. 1 Design of the animal experiment.

hormone (PTH) is clinically approved for enhancing bone formation and improving bone quantity as an anabolic pharmacological agent for patients with osteoporosis. PTH exerts clear anabolic effects on cancellous bone remodeling by increasing the number and activity of osteoblasts and decreasing their apoptosis (8,9). Moreover, PTH increases bone thickness not only in trabecular but also cortical bone (10,11). PTH therapy is applied to severe osteoporosis cases such as that induced by glucocorticoid, and it is expected to improve low bone density at implant placement sites and achieve favorable primary stability in such cases. Implant stability can be measured by clinical assessments such as insertion torque (IT) and resonance frequency analysis (RFA). Intermittent administration of PTH may improve primary stability evaluated by IT and RFA at sites of low bone density. However, studies of PTH have been limited mainly to those employing small animals such as mice or rats, for which application of dental implants is difficult. Therefore, few studies have evaluated the primary stability of implants using IT and RFA in osteoporosis models.

Recently, an osteoporosis model using rabbits was established by combining the effects of both ovariectomy and glucocorticoid administration (12,13). This model is easy to induce and highly reproducible. The bone-reduced rabbit model allows investigation of implant stability by evaluation of IT and RFA. The aim of the present study was to investigate the effect of PTH on the primary stability of implants in a rabbit bone-reduced model.

Materials and Methods

Ethics

The animal research protocol we employed was in accordance with the current version of the Japan Law on the Protection of Animals. This study was approved by the Research Facilities Committee for Laboratory Animal Science at Hiroshima University School of Medicine, Hiroshima, Japan (A-11-5-5). All surgery was performed under general anesthesia, and all efforts were made to minimize suffering during the experimental period.

Study design and animals

The study design is shown in Fig. 1. Ten female New Zealand White rabbits (age, 17 weeks; body weight, 3.0-3.5 kg) were used. The animals underwent ovariectomy and, 2 weeks later, received intramuscular injections of methylprednisolone acetate (Depo-Medrol, 0.5 mg/kg/day) for 4 consecutive weeks to prepare the bone-reduced animal models (12). Seven weeks after ovariectomy, the animals were divided in two groups: five rabbits that were injected with saline vehicle solution (Osteoporosis; OP group), and another five that received subcutaneous injections of PTH [1-34] (40 µg/day, 5 days weekly, Forteo, Pfizer, New York, NY, USA) (PTH group) for 4 weeks.

Implant procedure and evaluation of primary stability

All procedures were performed under anesthesia with sodium pentobarbital (10 mg/kg, i.v.; Somnopentyl, Kyoritsu Seiyaku Corporation, Tokyo, Japan). All implant sockets in the distal epiphysis of the knee joint of both femurs were prepared according to the Brånemark protocol in the manufacturer's instructions. Briefly after the knee joint was exposed, an implant surgical system (Fig. 2a) (iChiropro, Bien-air, Bienne, Switzerland) with a rotary speed not exceeding 800 rpm was used for consecutive application of a 2.0-mm round drill, 2.0-mm twist drill, 3.0-mm pilot drill, 3.0-mm twist drill, and countersink drill. After implant socket preparation, the implants (3.75-mm diameter, 7.0-mm length, Brånemark System MKIII, Nobel Biocare, Gothenburg, Sweden) was inserted until the color indicator was level with the bone ridge and the maximum IT during the insertion was recorded (Fig. 2b). After implant insertion, RFA was performed using an Osstell (Osstell AB, Gothenburg, Sweden) to measure the implant stability quotient (ISQ) (Fig. 3a). Measurements were performed three times from two different directions, and the values obtained for each implant were averaged. The ISQ measurements were carried out in accordance with a previous study (Fig. 3b) (14-16).

Fig. 2 (a) Implant surgical system. (b) Insertion torque was recorded automatically during implant insertion.

Fig. 4 The IT value for the PTH group was higher than that for the OP group ($P < 0.05$).

Histological observation and histomorphometric analyses

After the rabbits had been sacrificed, the femurs were harvested and further fixed in 10% neutral formalin for 2 weeks. After implant removal, tissue blocks were cut and decalcified with hydrochloride solution (KC-X, FALMA, Tokyo, Japan) for 5 days, dehydrated through a graded ethanol series, cleared with xylene, and embedded in paraffin. Sections 5 μ m thick were obtained from each block and stained with hematoxylin and eosin. Histological observation was performed by light microscopy (BZ-9000, Keyence, Osaka, Japan).

Histological images were digitized and histomorphometrically analyzed using NIH ImageJ (National Institutes of Health, Bethesda, MD, USA), and bone formation area was measured as the total ratio of cortical to trabecular bone area. The regions of interest for calculation of the ratio of bone formation area were those in the area surrounding the implant socket 1.5 mm lateral to it and 3.0 mm vertical from the top of the cortical bone. The measurement area was limited to the cortical bone section.

Fig. 3 (a) Resonance frequency analysis device. (b) Measurement of the implant stability quotient was performed using an Osstell. Measurement was performed three times along both the short and long axes to obtain average values for the placed implant.

Fig. 5 The ISQ value for the PTH group was higher than that for the OP group ($P < 0.05$).

Statistical analysis

The data obtained, i.e. IT, ISQ and bone formation area, were presented as mean $\pm$ standard deviation. Statistical analysis of the data was performed using the Mann-Whitney U test. Statistically significant differences were defined as $P < 0.05$.

Results

Primary stability evaluation

Figure 4 shows the IT results obtained using the implant surgical system as an automatic recording assessment tool. The IT value was significantly higher in the PTH group than in the OP group (29.8 ± 6.2 Ncm and 10.0 ± 2.1 Ncm, respectively; $P < 0.05$).

Figure 5 shows the ISQ results obtained using the Osstell system for RFA. The ISQ value was significantly higher in the PTH group than in the OP group (74.7 ± 11.2 and 55.9 ± 13.5 , respectively; $P < 0.05$).

Histological observation and histomorphometric analyses

Trabecular bone formation around the implant socket was detected in the PTH group but not in the OP group. The trabecular bone structures in the PTH group appeared to

Fig. 6 (a) Histological specimen from the PTH group. (b) Thick trabecular bone structures are evident around the implant socket.

Table 1 Ratio of bone formation area

	% (SD)
PTH	48.3 (3.6)*
OP	36.1 (9.5)*

SD; standard deviation

* $P = 0.0275$

be thicker than those in the OP group (Fig. 6ab). Increased trabecular and cortical thickness was also observed in the PTH specimens. In the OP specimens, marrow fibrosis and tunneling resorption were seen (Fig. 7ab). Improved trabecular connectivity as well as increased cortical thickness after PTH treatment was detected in both sections.

The ratio of bone formation was significantly higher in the PTH group ($48.3 \pm 3.6\%$) than in the OP group ($36.1 \pm 9.5\%$) ($P < 0.05$) (Table 1).

Discussion

The results of this study indicate that PTH can increase the primary stability of dental implants as determined by IT and ISQ values. Primary stability is dependent on bone quality and quantity in the implant placement site. At sites with poor bone density, such as in osteoporosis, the primary stability is decreased. The development of low bone density animal models has been essential in studies of osteoporosis (17). The most common method for inducing osteoporosis in rats or mice is ovariectomy. However, commercialized dental implants cannot be applied in such small animals. In contrast, mature rabbits are sufficiently large for insertion of dental implants. Therefore, they have been used to evaluate osseointegration or stability of dental implants (18). However, rabbits are remarkably resistant to conventional strategies for inducing bone loss, and thus ovariectomy alone is seldom successful (12,13). In the present study, an osteoporosis

Fig. 7 (a) Histological specimen from the OP group. (b) Few trabecular bone structures are evident around the implant, and tunneling resorption can also be seen, whereas the marrow is mostly present in the outer portion of the implant socket.

model using rabbits was established by combining the effect of both ovariectomy and glucocorticoid administration. Glucocorticoid affects bone quality by decreasing bone formation through a reduction of osteoblastogenesis and an increase of osteoblast and osteocyte apoptosis. Glucocorticoid reduces runt-related transcription factor 2 (Runx2), which promotes osteoblast differentiation (19). Also, it controls the Wnt signaling pathway in correlation with bone formation, and reduces the proliferation of osteoblasts and accelerated apoptosis (20-23). Through these mechanisms, bone formation is suppressed and bone density is reduced.

Promotion of osteoclast resorption and suppression of bone formation in cortical and trabecular bones due to glucocorticoid administration have been demonstrated in the same experimental osteoporosis rabbit model that was used in the present study (12). Dual-energy X-ray analysis has shown that bone mineral density was significantly decreased in a combined ovariectomy and glucocorticoid administration model in comparison with that in an untreated model (12). Furthermore, in terms of mechanical strength analysis, our previous study showed that the maximum mechanical bone strength in the bone-reduced rabbit model was lower than that in a normal rabbit model. Therefore, the glucocorticoid-induced model showed a greater reduction of bone density and mechanical strength of femoral cortical bone (16). Intermittent PTH administration affects bone quality by promoting preosteoblastic proliferation and osteoblast bone formation. In addition, PTH inhibits the degradation of Runx2 (24,25) and apoptosis of osteoblasts (26). These findings suggest that PTH has an antagonistic

effect on the action of glucocorticoids. Almagro et al. reported that implant stability could be improved by PTH administration in osteoporosis models; in that study, PTH administration was performed after implant placement, and implant stability was evaluated in terms of osseointegration (27). However, implant stability as primary stability was not clarified, because in this study it appears that implant stability after placement and the bone condition at implant placement seem to be similar. Our previous study of primary stability demonstrated that the IT and ISQ values were significantly lower in the osteoporosis rabbit model than in the untreated rabbit model. Furthermore, a previous evaluation of tibial bone mechanical strength by the 3-point bending test indicated that bone strength in animals with osteoporosis was significantly lower than that in normal animals (16).

The results of the present study reflected osteoporosis due to a decrease of bone density. Histological assessment demonstrated a thicker and more trabecular bone structure in PTH specimens than in OP specimens. IT and ISQ measurements were used for evaluation of primary stability; however, the techniques used for measuring these parameters differ substantially. IT can be used for evaluation of bone quality and primary stability at the time of implant insertion (28). Normally, IT is strongly correlated with the mechanical strength of cortical bone (29). Therefore, reduced bone density caused by osteoporosis affects the IT value, as was evident in the OP group. It is considered that reduced bone density in the area surrounding the implant socket caused by ovariectomy and glucocorticoid was ameliorated by PTH administration. Therefore, the IT value was significantly higher in the PTH group than in the OP group because of the promotion of bone metabolism. RFA has been performed as an effective and non-invasive method for measuring the stability of implants (30,31). We used the Osstell® system to assess RFA. The resonance frequency measured from the response signal obtained by Smart-Peg was then calculated as the ISQ, which ranged from 1 to 100. The ISQ values of successfully stabilized implants are reported to range from 57 to 82, reflecting the condition of the bone. The ISQ indicates whether an effective amount of bone is surrounding the implant, and the rigidity of the bone-implant interface in cortical and cancellous bone (32,33). We found that the mean ISQ value was over 70 in the PTH group and approximately 56 in the OP group, indicating a more favorable bone condition or primary stability in the PTH group. It is considered that the reduced density of cortical and cancellous bone was ameliorated by PTH. Data in the literature regarding the appropriate dosage of PTH

for achieving such an effect are lacking. A low PTH dose of less than 10 µg/kg three times a week is reportedly effective for enhancing bone metabolism (34). High PTH dosages have also been reported to promote metabolism and thus bone formation (35). In this study, the PTH dose rate was set at approximately 15-20 µg/kg 5 times a week, which was considered to be within the range used in numerous *in vivo* studies (usually 15-60 µg/kg 5 times a week) (27). Intermittent PTH administration improves primary implant stability at sites of low bone density. We plan to conduct further studies for evaluation of osseointegration or under-loading in order to confirm the utility of PTH treatment for osteoporosis.

Acknowledgments

This study was supported by a Scientific Research Grant (No. 15K11160) from the Japan Society for the Promotion of Science.

Conflict of interest

The authors declare no competing financial interests.

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