

EFFECT OF SYSTEMIC INTERMITTENT ADMINISTRATION OF HUMAN PARATHYROID HORMONE (rhPTH[1-34]) ON THE RESISTANCE TO REVERSE TORQUE IN RABBIT TIBIAE

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The aim of this study was to evaluate the effect of intermittent administration of human parathyroid hormone [rhPTH (1–34)] on the removal torque of implants placed in rabbit tibiae. Twenty male New Zealand rabbits were submitted to implant surgery. Each animal received one machined screw-type implant (3.75 mm diameter × 8 mm length) in the proximal metaphysis of the right tibia. The rabbits were then divided into 2 groups: the test group (n = 10) received 6 µg/kg of rhPTH (1–34) subcutaneously in the dorsal region 3 days a week, and the control group (n = 10) received placebo. Removal torque was performed at 28 and 56 days after implant placement for both groups. The mean removal torque values at 28 days were 37.0 ± 4.36 Ncm and 47.4 ± 6.77 Ncm for control and test groups respectively ($P < .05$). At 56 days the reverse torque was 45.8 ± 3.96 Ncm for the control group and 55.8 ± 2.86 Ncm for the test group, indicating that the removal torque was significantly higher in the test groups ($P < .05$). These results demonstrated that intermittent treatment with rhPTH (1–34) enhanced the removal torque of implants in rabbit tibiae.

Key Words: teriparatide, removal torque, dental implants

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INTRODUCTION

The use of dental implants has become widely accepted as reliable treatment modality for anchoring dental restorations in the jaws. However, it was clinically observed that implants placed in the posterior maxilla had a low success rate because of the thin layer of cortical bone and low medullary density.¹⁻⁴

Several studies⁵⁻⁷ have evaluated the effects of some drugs on increase in bone quality, which could also act favorably to improve osseointegration. The most studied drugs are those indicated for the treatment of osteoporosis, such as alendronate,

raloxifene, and calcitonin, among others, and more recently, the recombinant human parathyroid hormone [rh-PTH(1–34)] teriparatide.

Teriparatide is a synthetic polypeptide obtained by the recombinant DNA technique, which contains the first 34 amino acids of the complete 84 amino acid molecule of endogenous human parathyroid hormone. Unlike other available drugs used in the prevention and treatment of osteoporosis, which act as antiresorptives drugs, teriparatide is the first of a class of agents that acts by stimulating bone formation through the activation and formation of osteoblasts; it promotes an increase in bone strength forming new bone tissue.^{7,8}

Previous clinical studies in animals, using intermittent administration of teriparatide, showed a significant increase in trabecular bone mass in rats,⁶ monkeys,⁹ and rabbits.¹⁰ Neer et al¹¹ performed a randomized, double-blind, placebo-controlled clinical study to evaluate the action of teriparatide in the management of severe osteoporosis in postmenopausal women. The authors showed a significant increase in bone mass and bone mineral density as well as a significant reduction in the risk of vertebral and nonvertebral fractures.

Therefore, it is believed that studying the effect of systemic and intermittent administration of the recombinant human parathyroid hormone in bone tissues around implants could help researchers understand the mechanisms and perhaps improve the success rate of implants inserted in areas of poor bone quality. Thus, the aim of this study was to evaluate the effect of systemic and intermittent administration of rhPTH(1–34) on the removal torque of implants placed in the tibiae of rabbits.

MATERIALS AND METHODS

Animals

Twenty New Zealand male rabbits, weighing 3.3 to 3.5 kg and ranging in age between 6 and 8 months, were used in this study. Each animal was placed in a 40 × 60 cm metal cage under controlled air and temperature (22 ± 2°C) conditions. During the experimental period, the animals received a solid diet and water ad libitum. The University of Santo Amaro Animal Research Committee approved this study, and all experimentation was conducted in accordance with the International Council of Laboratory Animal Science (ICLAS).

Implant surgery

One screw-type machined implant (3.75 mm in diameter and 8 mm length; ACE, Surgical Supply,

Brockton, Mass) was surgically placed in the right proximal tibia of each animal. In each rabbit, anesthesia was induced with intramuscular injection of 0.25 mL/kg Xylazine (Virbaxyl 2%, Virbac do Brasil, Roseira, São Paulo, Brazil) and 0.5 mL/kg ketamine (Francotar, Virbac do Brasil, Roseira, São Paulo, Brazil). Anesthesia was maintained with an additional dose of 0.2 mL ketamine, and this was repeated if necessary. After anesthesia, each rabbit was submitted to a trichotomy of the lower right limb. Antisepsis was performed using sterile brushes drenched in a topical solution of iodopovidone, and sterile cotton fields were placed to maintain asepsis. Local infiltration of the anesthetic 2% lidocaine (Alphacaine, DFL, Rio de Janeiro, Brazil) 1.8 mL/rabbit was administered. After a digital location of the tuberosity of the proximal tibial metaphysis, a 3 cm long incision was made in the skin, and subcutaneous and muscular tissues were displaced until the bone surface was exposed. Special care was taken with regard to the location of implant fixation, which was standardized: implants were always fixed 5 mm from the most distal part of the tuberosity, in the core of the ventral surface of the exposed tibia. The implants were placed with a precision torque gauge instrument, and the insertion torque was standardized at 20 Ncm. The periosteum was replaced, and muscular tissue was sutured by continuous suturing with a reabsorbable suture (Vicryl 5.0, Johnson & Johnson, Langhorne, Penn). The skin was sutured with interrupted suture using mononylon suture (Ethicon 5.0, Johnson & Johnson).

Administration of the recombinant human parathyroid hormone and experimental protocol

The rabbits were randomly allocated into 2 groups of 10: control group (placebo) and test group rhPTH(1–34). The animals in group A received placebo and those in group B received the rhPTH(1–34); both were administered in systemic and intermittent mode.

A disposable injection pen was used to administer the rhPTH(1–34); it contained 3.3 mL of the hormone at a dose of 250 µg/mL (Forteo, Eli Lilly do Brasil Ltda, São Paulo, Brazil). Each pen releases 28 doses of 20 µg of a sterile, isotonic, colorless, and transparent solution, which must be administered by means of a subcutaneous injection. Hormone administration in the test group started on the same day the implants were placed. The 6 µg/kg dose of the hormone was injected in the dorsal subcutaneous area 3 times a week during the period between 8 and 10 AM, until all data were obtained. Administration of placebo in the control group started on the same day the implants were placed.

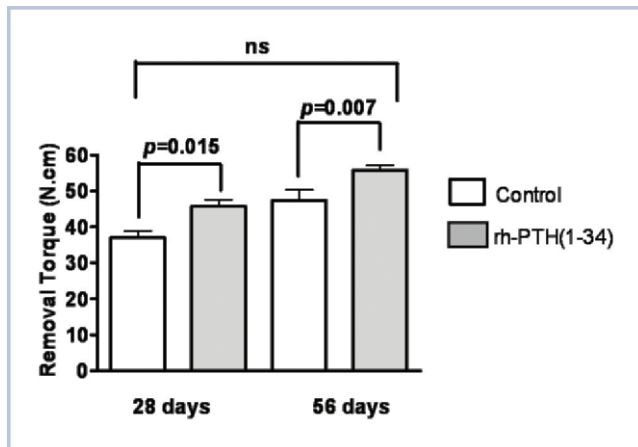


FIGURE 1. Mean + standard deviation of the removal torque values in each group for 28 and 56 days. Mann-Whitney test (difference between groups) $P < .05$; Wilcoxon test (intragroup differences) $P > .05$; ns = nonsignificant.

Removal torque

Evaluations of the removal torque were made on the 28th day and 56th day after the surgery. The animals were subset into 4 subgroups with 5 rabbits each: Control Group 1 (Control: 28th day); Control Group 2 (Control: 56th day); Test Group 1 (Test: 28th day) and Test Group 2 (Test: 56th day).

After 28 and 56 days, the animals with implants were anesthetized, and the tibial implant was fully unscrewed with a torque gauge manometer (BGT60CN Torque Gauge Manometer, Tohnichi, Tokyo, Japan). To connect the torque meter, bone or cartilage that had formed on the top of the implants was carefully removed using a scalpel and a fissure bur. When an implant was unscrewed, the peak torque value fell quickly when the rupture between bone and implant occurred. Up to this moment no macroscopic movement of the implant was evident. After rupture, the continued unscrewing required low torque.

Statistical analysis

The differences in the removal torque between the groups and between the different experimental periods (28 and 56 days) were assessed using Mann-Whitney and Wilcoxon tests respectively ($P < .05$).

RESULTS

The mean removal torques for the groups are presented in the Figure. The test group presented the highest means in both experimental periods, compared with the control group ($P < .05$). However,

there was no intragroup difference between 28 and 56 days ($P = .065$).

DISCUSSION

This study was first planned when the authors considered the advantages of studying the influence of rhPTH(1-34) on the process of bone modulation around implants, and therefore on the removal torque, supposing that its action on the osteoblasts could produce any degree of improvement in osseointegration.

In the literature it was seen that the rabbit is commonly used as a model to evaluate the healing of endosseous implants, as well as in removal torque evaluations.¹²⁻¹⁷ Concerning the choice of this model, another important point was the time required for the dynamics of bone remodeling to take place after the implants were placed. In rabbits it occurs in about 6 weeks. On the other hand, rabbits exhibit Haversian remodeling quite similar to people,¹⁸ and as a result they would be more suitable for studying the bone remodeling process. In addition, the present research protocol evaluated 10 animals ($n = 10$) per group and in the subgroup there were 5 ($n = 5$), which was considered satisfactory for the study and statistical evaluations. The same number of animals was used by other authors, as found in the literature.^{12,16,19,20}

The concern about performing the implant removal torque tests soon after the animals were killed was to prevent dehydration, which could change and interfere in the physiologic and mechanical properties of the evaluated system, as previously reported by Peng et al.²¹

In our study, the removal torque evaluations were performed at 28 and 56 days, because a period of 42 days would be the time necessary for obtaining osseointegration in rabbits, as has been reported previously.¹² The evaluation at 28 days was useful for observing whether rhPTH(1-34) accelerated the bone remodeling process, as the values obtained in the test group were statistically higher than those obtained in the control group. These findings are in agreement with several studies that evaluated the effects of the intermittent administration of rhPTH(1-34).²²⁻²⁴ Studies showed increases in bone formation on both periosteal and endocortical surfaces, resulting in increased bone area and cortical area in rabbits^{11,24} and an increase in bone density around titanium dental implants placed in the tibia of ovariectomized rats,²⁵ which could explain the better removal torque values in groups that received the hormone.

The improvement in the removal torque observed

at 28 and 56 days in the test group was useful to demonstrate that intermittent administration of rhPTH(1–34) did not act unfavorably on the process of osseointegration. However, no improvement in the removal torque could be observed in the statistical analysis between the intragroup analyses for the 2 groups. This statement could certainly be confirmed if histologic and histomorphometric analyses of the areas were performed. This result differs from those of Sennerby et al,¹³ who obtained the same mean removal torque values (35 Ncm) in evaluations at 42, 84, and 168 days, but showed a high correlation with the results of Johanson and Albrektsson,¹² who verified a progressive increase in removal torques at 21, 28, 84, 168, and 365 days. However, it must be pointed out that the aforementioned studies did not evaluate hormones, only the effect of time on osseointegration of the dental implant.

Analysis of the results demonstrated that the mean values obtained in test groups were statistically higher than those obtained in the control groups; this can be explained as a positive action of the hormone on the osseointegration process, considering that higher scores of removal torque must be related to a larger quantity of bone in contact with the implants. These results were also related by Skripitz et al,²⁶ who observed a greater effect on new bone formation inside a titanium chamber inserted in the proximal tibia of rats.

Although the mean removal torques between the test and control groups showed no significant differences ($P > .05$), the increased mean in the test group represented a positive result and suggests an improvement in osseointegration in the presence of the hormone.

These findings suggest that one way to increase success in rehabilitation with implants, mainly in areas of poor bone quality, would be to look for treatments that can improve bone modulation, perhaps by thickening the cortical and density of the medullary bone. Further animal (with histologic evidence) and potentially clinical studies are needed to support the use of rhPTH(1–34) as a valid treatment modality. In conclusion, the present study demonstrated an increase in the removal torque of implants placed in rabbit tibiae with intermittent administration of rhPTH(1–34).

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