Parathyroid hormone 1–34 enhances titanium implant anchorage in low-density trabecular bone: A correlative micro-computed tomographic and biomechanical analysis

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Abstract

The use of osseous titanium implants is the standard of care in dentistry and orthopaedic surgery. Nevertheless, implantation in low-density bone has a poor prognosis and experimental studies show delayed implant anchorage following gonadectomy-induced bone loss. Intermittently administered human parathyroid hormone 1–34 [1αhPTH(1–34)] is the leading bone anabolic therapy. Hence, this study assessed whether 1αhPTH (1–34) enhances titanium implant integration in low-density bone. Threaded titanium implants, 0.9 mm in diameter, were inserted horizontally into the proximal tibial metaphysis of 5-month-old rats, 7 weeks postorchiectomy (ORX). Subcutaneous administration of 1αhPTH(1–34), at 5, 25 and 75 μg/kg/day commenced immediately thereafter and lasted for 8 weeks. Quantitative micro-computed tomography (μCT) at the implantation site was carried out at 15 μm resolution using high energy and long integration time to minimize artifacts resulting from the high implant radiopacity. Osseointegration (OI) was calculated as percent implant surface in contact with bone (%OI) quantified as the ratio of “bone”-to-total voxels in contact with the implant. Additionally, the trabecular bone volume density (BV/TV), trabecular thickness (Tb.Th), trabecular number (Tb.N) and connectivity density (Conn.D) were measured in the peri-implant bone. All μCT parameters were stimulated by 1αhPTH(1–34) dose-dependently; the percent maximal enhancement was %OI = 143, BV/TV = 257, Tb.Th = 150, Tb.N = 140 and Conn.D = 193. The maximal values of %OI, BV/TV and Tb.Th in 1αhPTH(1–34)-treated ORX rats exceeded significantly those measured in the implantation site of untreated sham-ORX controls. The same specimens were then subjected to pullout biomechanical testing. The biomechanical parameters were also enhanced by 1αhPTH(1–34) dose-dependently, exceeding the values recorded in the sham-ORX controls. The percent 1αhPTH(1–34)-induced maximal enhancement was: ultimate force = 315, stiffness = 270 and toughness = 395. Except for the BV/TV and Tb.Th, there was no significant difference between the effect of the 25 and 75 μg/kg/day doses. There was a highly significant correlation between the morphometric and biomechanical parameters suggesting the use of quantitative CT as predictive of the implant mechanical properties. These findings demonstrate that 1αhPTH(1–34) effectivly stimulates implant anchorage in low-density trabecular bone and thus the feasibility of administering 1αhPTH(1–34) to improve the clinical prognosis in low-density trabecular bone sites.

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Introduction

Endosseous implantation using uncemented titanium prostheses is commonly performed in trabecular bone sites such as the jaws, vertebrae and ileum. A prerequisite for successful implant anchorage is de novo bone formation that leads to
osseointegration (OI), namely, direct bone–implant contact. A second critical factor for mechanical fixation is the peri-implant trabecular bone (PIB), which connects the implant to the external cortical envelope [1,2]. The importance of an appropriate local trabecular bone mass is illustrated by the high failure rate of implants inserted into sites with low trabecular bone density [3,4]. In addition, a handful of studies report a vast delay in implant anchorage in experimental animals with gonadectomy-induced bone loss [5–8].

Although experimental studies on agents applied locally at trabecular implantation sites reported the stimulation of implant anchorage [9,10], clinically such local treatments have not been applied yet to low-density bone locations. Experimental systemic administration of drugs such as estrogen, bisphosphonates and calcitonin also suggests their clinical use to improve implant anchorage [11–13]. However, these agents are mainly anti-resorptive thus preventing bone loss. Therefore, they are primarily intended to inhibit implant-induced bone loss [11–13] rather than stimulate implant anchorage in sites with pre-existing low bone density.

Intermittently administered human parathyroid hormone 1–34 [ihPTH(1–34)] is the only clinically approved bone anabolic therapy for osteoporotic patients [14–16]. This peptide has been demonstrated to stimulate implant fixation in normal bone [17–20] and reverse gonadectomy-induced trabecular bone loss in the jaw, appendicular and axial skeleton in experimental animals [21–23]. In addition, it has been recently demonstrated in ovariectomized (OVX) rats that preimplantation rescue of bone loss with iahPTH(1–34) results in improved osseointegration and PIB density [24].

Premedication with iahPTH(1–34) to enhance implant anchorage is often unattainable, particularly in orthopaedic surgery where implant surgery is frequently nonselective. Therefore, the purpose of the present study was to test whether initiation of iahPTH(1–34) treatment at the time of implantation improves prosthesis anchorage. Indeed, quantitative micro-computed tomographic (μCT) and biomechanical analyses in the proximal tibial metaphysis of rats with established orchiectomy (ORX)-induced bone loss show that commencing iahPTH(1–34) treatment at the time of titanium implant insertion markedly enhances the implant anchorage, thus demonstrating the feasibility for clinical use of such therapy.

Materials and methods

Animals

The experimental protocol was approved by the Institutional Animal Care and Use Committee of the Hebrew University-Hadassah Medical Center. Thirteen-week old male Sprague–Dawley rats were purchased from Harlan Laboratories (Jerusalem, Israel) and maintained at the animal research facility of the Hebrew University-Hadassah Medical Center. Animals were fed purina (Koffolk 19–520, Koffolk Ltd., Tel Aviv, Israel) and water ad libitum throughout the experiment. The study design is shown schematically in Fig. 1. The rats were divided randomly into four groups of bilateral ORX animals and one group of sham-ORX animals. Each group consisted of 10 rats. Seven weeks were then allowed to pass prior to implantation to permit significant bone loss to occur in the ORX animals. At this time, titanium implants were inserted into the proximal metaphysis of the right tibia in each animal. Human PTH(1–34) was chemically synthesized as previously reported and purified by HPLC [25]. Daily subcutaneous injections (5 days a week) of either 5, 25 or 75 μg/kg of human PTH(1–34) or vehicle (VEH) only (saline containing 0.001N HCl and 2% heat-inactivated rat serum) were then administered to the respective ORX groups. The animals were sacrificed 8 weeks after implant insertion and the implantation site was subjected to μCT and biomechanical analyses. Animals with postoperative swelling or ulceration and specimens with malpositioned implants or excessive bone formation around the extracortical part of the implant were excluded from the study, leaving a sample of 6–8 specimens per group (Fig. 1).

Fig. 1. Schematic representation of experimental design. Note that sham-ORX animals were given neither iahPTH(1–34) nor vehicle.

Fig. 2. Implant insertion site in secondary spongiosa in proximal tibial metaphysis of ORX rat. 2-D μCT frontal slice through implant mid-longitudinal axis; dotted lines delimit PIB volume of interest, extending 0.9 mm both proximal and distal to axis.
Implantation

We used turned (smooth) threaded titanium implants (Dentatus, Hägersten, Sweden). The implant shank measures 5 mm in length. Its largest diameter is 0.9 mm tapering to 0.55 mm near the tip. Implants were cleaned in denaturated 70% ethanol in an ultrasonic bath and gas-sterilized in 10% ethylene oxide. All surgical procedures were performed under aseptic conditions. The implant insertion path was prepared using a round, 0.8 mm in diameter, low-speed dental bur, from the antero-medial aspect of the right tibia towards the postero-lateral ridge. The cortical penetration hole was approximately 1.5 mm distal to the proximal growth plate which is visible as a line brighter than the bone. The implant shaft was then threaded into the path leaving 0.5 mm outside the tibia (Fig. 2) to enable its attachment to the biomechanical testing device.

Micro-computed tomography

At the time of sacrifice, tibiae with implants were separated, transferred for 48 h to phosphate-buffered formalin and then kept in 70% ethanol. For a detailed qualitative and quantitative 3-D evaluation, the proximal 15 mm of the tibia was examined by a μCT imaging system (μCT 40, Scanco Medical, Bassersdorf, Switzerland). For image acquisition, the specimens were mounted on a turntable shifted automatically in an axial direction parallel to the long axis of the implant. The X-ray tube voltage was set to 70 kV, in order to allow maximal X-ray transmission through the highly opaque titanium implant. To maximize signal-to-noise ratio, the system was operated at 114 mA (maximal current for the 70 kV setting) and the longest integration time, 300 ms. Micro-tomographic

![Fig. 3. Drawings of jig for pullout testing. Top, proxo-distal view of tibia; inset, enlarged part shown in right middle. Bottom, antero-posterior view of tibia; inset, enlarged part shown in left middle. Arrows, pull force vector; (1) ball joint; (2) plate for attachment to testing system; (3) tightening screw; (4) ring fixture for tibia; (5) tweezers fixture for implant; (6) proximal tibia; (7) implant.](image)

![Fig. 4. 2-D μCT images of cross-sectional plane through implant mid-longitudinal axis. Arrows, bone–implant contacts; C, cortical bone; PIB, peri-implant trabecular bone. Representative specimens with median BV/TV values.](image)
slices were acquired at 1000 projections and reconstructed at a spatial nominal resolution of 15 μm. These settings have been recently reported suitable for accurate analysis of titanium implants osseointegration [26]. A constrained 3-D Gaussian filter ($\sigma = 1.2$ and support = 1) was used to partly suppress the noise in the volumes.

The titanium and mineralized tissue were segmented from each other and from the bone marrow, including the immediate implant vicinity, by applying a multi-level thresholding procedure [27–29]. The %OI was calculated as the ratio between bone and total voxels in contact with the implant. The PIB volume of interest included the entire trabecular compartment between the cross sectional planes 0.9 mm proximally and 0.9 mm distally from the implant longitudinal axis (Fig. 2). The following morphometric parameters were calculated in the PIB: bone volume density (BV/TV), trabecular thickness (Tb.Th), trabecular number density (Tb.N) and connectivity density (Conn.D).

Biomechanical testing

Following the µCT analysis, the specimens were progressively rehydrated [30] and kept in phosphate-buffered saline for 48 h prior to the biomechanical testing. The implant head of each specimen was firmly connected to a commercial material testing system (Zwick 1456, Ulm, Germany). For near linear displacement, we used a jig designed specifically for the present study whereby the bony part of the specimen is loosely placed in an open ring (the ring slot is for the implant head) and ball joints connect the fixtures (Fig. 3). At pulling, the bone lies firmly against the inner aspect of the ring. The pullout testing was performed at a 1 mm/min displacement rate. Ultimate force, stiffness and toughness were determined from the resultant load-displacement curves.

Statistical analysis

The SigmaStat software (SPSS, Chicago, IL, USA) was used throughout. Differences in morphometric and biomechanical parameters were analyzed by analysis of variance (ANOVA). When significant differences were indicated by ANOVA, group means were compared using the Student–Newman–Keuls method for multiple comparisons. Pearson correlation coefficients were calculated to assess the relationship between biomechanical and quantitative µCT measurements.

Results

Using settings of the µCT system that result in enhanced X-ray transmission through the titanium implant, we were able to generate high resolution tomographic images that clearly depict the bone–implant interface, thus identifying bone in contact with the implant and quantifying the %OI. These images demonstrate that the higher iahPTH(1–34) doses markedly enhanced both OI and PIB density (Fig. 4). Quantitatively, the comparison between ORX/VEH and sham-ORX rats showed that ORX resulted in 14%, statistically insignificant decrease in the %OI (Fig. 5A). Only the ORX/PTH75 animals showed a statistically significant increase in the %OI over both the sham-ORX animals (26%) and ORX/VEH controls (43%). Still, the
The overall response pattern of this parameter was that of a dose-response relationship (Fig. 5A). In the PIB, the ORX resulted in a significant 34% reduction in BV/TV (ORX/VEH vs. sham-ORX, Fig. 5B), comparable to the ORX-induced bone loss in implant-free metaphyseal trabecular bone [22]. The iahPTH(1–34) treatment induced a significant dose-response stimulation of the PIB parameters. The magnitude of this stimulation was similar to that reported for implant-free trabecular bone in the same post-ORX rat model [22]. The BV/TV showed a maximal increase of 258% (in the 75 μg/kg/day group) as compared to the ORX/VEH group. Furthermore, at the 25 and 75 μg/kg/day doses, the treatment resulted in respective 14% and 43% higher BV/TV compared to sham-ORX controls (Fig. 5B). The increase in the PIB BV/TV apparently resulted from a maximal 150% stimulation of the Tb.Th (Fig. 5C) and 140% enhancement of the Tb.N as compared to the vehicle treated ORX controls (Fig. 5D). ORX did not result in reduced Tb.Th values. However, in the ORX/PTH25 and ORX/PTH75 animals, Tb.Th was 15% and 43% higher, respectively, than in the sham-ORX controls (Fig. 5C). The Tb.N showed a different pattern with a 22% ORX-induced decrease and full rescue by all three iahPTH (1–34) doses. However, the Tb.N values did not significantly exceed the level in sham-ORX rats, even at the maximal dose (Fig. 5D). The morphometric parameter mostly affected by ORX was Conn.D, which assesses the structural integrity of the trabecular network. Its level in ORX/VEH rats was only 43% compared to the sham-ORX controls. The iahPTH(1–34) induced only partial, 40% rescue of this decrease at the lowest and highest doses, with maximal 70% rescue at the medium dose. However, the difference between 25 and 75 μg/kg/day doses was statistically insignificant (Fig. 5E).

Table 1: Correlation coefficients ($r^2$ values) between structural and biomechanical parameters of the bone–implant complex

<table>
<thead>
<tr>
<th>Ultimate force</th>
<th>Stiffness</th>
<th>Toughness</th>
</tr>
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<tbody>
<tr>
<td>%OI</td>
<td>0.516</td>
<td>0.240</td>
</tr>
<tr>
<td>BV/TV</td>
<td>0.719</td>
<td>0.433</td>
</tr>
<tr>
<td>Tb.Th</td>
<td>0.598</td>
<td>0.314</td>
</tr>
<tr>
<td>Tb.N</td>
<td>0.473</td>
<td>0.318</td>
</tr>
<tr>
<td>Conn.D</td>
<td>0.371</td>
<td>0.277</td>
</tr>
</tbody>
</table>

All coefficients are significant at $P < 0.008$.

To assess the biomechanical effects of iahPTH(1–34), we used the pullout test because most of the loading applied to functioning implants has a major axial vector. The ORX resulted in small, statistically insignificant decreases in all three biomechanical parameters (Fig. 6). As in the case of the structural analysis, the iahPTH(1–34) induced a dose-dependent stimulation of these parameters. Exceptionally marked increases, which exceeded the values obtained in sham-ORX controls, were observed at the 25 and 75 μg/kg/day doses. The maximal increases (obtained with either 25 or 75 μg/kg/day) were 315, 270 and 395% over the ORX/VEH rats in ultimate force, stiffness and toughness, respectively. No significant differences were noted between animals treated with the 25 and 75 μg/kg/day doses induced small, statistically insignificant enhancement.

All pairs of structural and biomechanical parameters demonstrated significant correlation coefficients. In general, the strongest relationship of the structural parameters was with the ultimate force and the weakest with stiffness (Table 1). These data indicate that at least circumstantially the iahPTH(1–34)-induced stimulation of OI and PIB properties is closely linked to the enhancement of bone biomechanical properties of the bone–implant complex.

**Discussion**

Using high-resolution μCT-based morphometry and biomechanical testing, we demonstrate that administration of iahPTH (1–34), commencing at the time of implantation, markedly enhances the anchorage of titanium implants in low-density trabecular bone. Furthermore, the quality of the iahPTH(1–34)-enhanced anchorage exceeds implant integration in normal trabecular bone of the sham-ORX controls.

It was previously reported in osteoporotic humans and other experimental models of low-density bone that the iahPTH(1–34) bone anabolic treatment is effective over a relatively narrow therapeutic window ranging between 10 and 80 μg/kg/day.
Here too, the iahPTH(1–34) stimulated both the morphometric and biomechanical parameters in a dose-dependent manner between 5 and 75 μg/kg/day. The 5 μg/kg/day (low) dose had a minimal, not always statistically significant effect, while the 25 (medium) and 75 (high) μg/kg/day doses had similar effects on all parameters except for BV/TV and Tb.Th, for which the high dose induced a stronger effect than the medium. These considerations indicate that the effective anabolic dose range in this model is approximately one log, a rather narrow window considering the clinical scenario where excessive doses result in increased frequency of adverse effects [15].

The duration and time of onset of the iahPTH(1–34) administration are also of major importance when coming to balance the efficacy against potential side effects and cost [14–16]. It is therefore meaningful that the present stimulation of prosthetic anchorage was accomplished with the iahPTH(1–34) treatment commencing at the time of implantation. It was at least as effective as iahPTH(1–34) rescue of gonadectomy-induced bone loss prior to implantation [24]. This comparison suggests that premedication with iahPTH(1–34) is unnecessary, rendering iahPTH(1–34) therapy useful in both elective and nonselective instances of implant insertion in low-density trabecular bone sites.

The few studies that analyzed the relationship between morphometric and biomechanical parameters of implant anchorage reported only a weak correlation between structural and functional properties [26]. The present rather high correlation coefficients between the morphometric and biomechanical results are attributable to (i) standardization of the implant insertion; (ii) the high precision of the 3-D μCT imaging and quantitative evaluation; and (iii) the jig used for biomechanical testing, which permitted the application of an axial vector. Jointly, these measures resulted in a high signal-to-noise ratio. Although all the present correlation coefficients are statistically significant, the coefficients with the biomechanical parameters tend to be higher for the PIB BV/TV than for the %OI. In addition, the present ORX and iahPTH(1–34) administration as well as previously reported OVX and estrogen treatment [5,11] had a substantially stronger effect on BV/TV than on the %OI, suggesting that systemic hormonal manipulations target mainly the PIB whereas OI may be more effectively enhanced by implant surface modifications [9,38–40]. It is also important that the weakest morphometric–biomechanical relationship is with Conn.D and that iahPTH(1–34) induced only partial rescue of the PIB connectivity, but more than doubling of the biomechanical parameters. Together, these findings suggest that the new bone induced by iahPTH(1–34) is structurally different from normal bone but still fully compatible mechanically.

As reported recently in ORX rats [22], the ORX-induced PIB loss was characterized mainly by reduced Tb.N, whereas its rescue by iahPTH(1–34) increased primarily the Tb.Th, significantly above the normal level. This is apparently because changes in bone resorption, which mediate the gonadectomy-induced trabecular bone loss, affect mostly Tb.N [41], whereas changes in bone formation target Tb.Th for the most part [42], Likewise, it has been recently reported that bone loss primarily affects the connectivity and overall number of trabeculae, whereas increased strength results from overall trabecular thickening even in the absence of improved connectivity [43]. Although our results show full reversal of the decrease in Tb.N and almost full rescue of the ORX-induced loss of trabecular connectivity, further modeling is required to determine whether Tb.Th is indeed the most important factor in this system.

While generally endosseous implantation is a highly successful procedure in orthopaedics and dentistry, low-density skeletal sites, focally formed or consequent to systemic bone loss, present a substantially poorer prognosis of the implant bone complex. The present enhancement of implant anchorage in low-density bone offers a promising approach to improving the anchorage of endosseous implant in these sites by iahPTH (1–34) therapy. The anticipated duration of such treatment is relatively short, thus limiting the undesired impact of subcutaneous injections and potential adverse effects. In addition, the correlation between μCT and biomechanical parameters suggests high-resolution quantitative CT for noninvasive clinical prediction of the implantation prognosis.

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References