Effects of Local Administration of Growth Hormone in Peri-implant Bone: An Experimental Study with Implants in Rabbit Tibiae

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Purpose: *The objective of this study was to evaluate the qualitative and quantitative differences that could appear in newly formed peri-implant bone around Screw-Vent implants placed in rabbit tibiae when treated with local administration of growth hormone (GH).* ***Materials and Methods:*** *Eight New Zealand rabbits were randomly divided into 2 groups: the experimental group, which received 4 IU of GH in the form of lyophilized powder added to the ostectomy site before implant placement, and the control group, which did not receive GH before implant placement. Animals were sacrificed 2 weeks later, and histologic sections were obtained for histomorphometry and observation under light microscopy.* ***Results:*** *The sections in the GH-treated group presented enhanced growth of new trabeculae from the periosteal tissue, and the bone-to-implant contact in the experimental group was significantly greater ($P < .05$).* ***Discussion:*** *Local administration of GH stimulated a more dramatic effect than that seen previously with systemic GH administration, prompting growth from both the periosteum and endosteum.* ***Conclusions:*** *Local administration of GH at the time of implant placement could enhance peri-implant bone reaction.* INT J ORAL MAXILLOFAC IMPLANTS 2003;18:807–811

Key words: *animal studies, bone remodeling, dental implants, growth hormone*

Growth hormone (GH) is a peptide with 191 amino acids, secreted by the anterior pituitary gland, that stimulates the growth process, acting as a metabolic and mitogenic regulator. Its effects are mediated primarily by insulin-like growth factor I (IGF-I), a peptide of 70 amino acids that is synthesized in almost all tissues, but fundamentally in the liver and in chondral tissue[1] under GH stimulation.[2]

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GH is one of the substances that regulate bone growth and bone remodeling in vivo,[3] but it has only recently been accepted that GH may also act as a locally produced growth factor that can be secreted by various types of cells[4] and may exert both endocrine as well as paracrine and autocrine effects.

GH is able to stimulate bone growth by direct stimulation of the epiphyseal chondrocytes[5] and osteoblasts.[6] GH also increases synthesis of IGF-I and IGF-II,[7] which stimulates the proliferation and differentiation of osteoblasts.[8] In addition, GH is able to stimulate bone protein synthesis and mineralization[9] and increase bone turnover.[10]

Systemic GH has been used for stimulating experimental bone fracture repair in both young and old rats, showing an increase of up to 400% in biomechanical properties when compared with an untreated control group.[11–14] Recent studies have shown that GH can also have a local effect. Guicheux and coworkers[15] observed that local administration of GH, released from a calcium phosphate–type biomaterial carrier, was able to improve the substitution process of biomaterials by bone through an acceleration of the bone remodeling process.

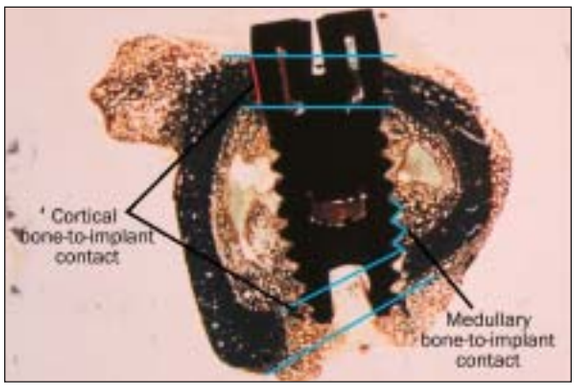

Fig 1 BIC contact at the cortical and medullary levels.

However, GH has rarely been applied locally during surgery to modify the osseointegration process.[16] The authors' working hypothesis has been that local administration of GH as a single dose at the time of implant surgery could accelerate the osseointegration process, inducing histologic differences compared to untreated control samples.

The objectives of the present study were the following:

1. To assess whether histologic differences appear in the peri-implant bone with the local administration of GH during surgery.
2. To evaluate quantitatively the peri-implant bone response with a morphometric analysis.

MATERIALS AND METHODS

A total of eight 3-month-old New Zealand rabbits, weighing 2.5 kg each, were used as experimental animals. Rabbits were randomly divided into 2 groups. Rabbits in both the control and experimental groups had one 3.3×8-mm Screw-Vent implant (Paragon Implant Company, Encino, CA) placed in the internal side of each tibia. In addition, the experimental animals received 4 IU of recombinant human GH (Saizen; Serono Laboratories, Madrid, Spain) in the form of lyophilized powder placed in the ostectomy site before placement of the implant.

After intramuscular anesthesia with ketamine (Imalgene 1000, 0.75 mg/kg; Merial, Lyon, France) and xylazine (Rompun, 0.25 mg/kg; Bayer, Leverkusen, Germany), an incision was made on the internal side of the tibia, at the union of the diaphysis/proximal metaphysis. After detachment of the cutaneous-periosteal tissues, the bone bed was prepared for implant treatment following instructions of the implant manufacturer, using internal/external cooling Paragon drills. The implants were placed and achieved primary stability; then, the periosteal flap was sutured with Dexon sutures (Davis & Geck, Wayne, NJ) and the skin with silk sutures. Oxytetracycline was administered orally to prevent postsurgical infection in both groups.

The animals were sacrificed 2 weeks after surgery. Both tibiae were dissected from their soft tissues, fixed in 10% buffered pH 7 formaldehyde, and embedded in 2-hydroxyethylmethacrylate resin, according to the Donath and Breuner method,[17] so as to cut undecalcified bone and titanium simultaneously with the Exakt microtome (Exakt Apparatebau, Norderstedt, Germany). The histologic analysis was conducted under a light microscope (Leica, Wetzlar, Germany) with sections stained with Masson stain and picrosirius.[18] These procedures were performed in the Department of Morphological Sciences and Surgery at the Medical School in the University of Alcalá de Henares, Madrid, Spain.

A morphometric study to quantify the newly formed bone around the implants was performed later with a MIP-4 imaging analyzer (a computerized system that performs area and volume measurements; Digital Image System, Barcelona, Spain). The parameter calculated was bone-to-implant contact (BIC), which is defined as the length of bone surface border in direct contact with the implant perimeter (×100%).[19] The BIC was measured at the cortical zone in contact with the implant (cortical level) and at the medullary zone in contact with the implant (medullary level) (Fig 1). These measurements were made with a 10× objective in all fields of each specimen by counting the number of intersections over the implant surface. Finally, the results were expressed as a percentage of the implant surface covered by bone at the cortical and medullary levels. For each sample, various sections were obtained and one of them was randomly used for the statistical evaluation.

The BIC mean values ± standard error of the mean (SEM) of each group were calculated. The groups were tested by the Student *t* test. The results and the statistical analysis were elaborated with the SPSS 11.0 computer system (SPSS, Chicago, IL).

RESULTS

At 2 weeks after implant placement and GH treatment, the experimental group sections demonstrated a greater periosteal and endosteal response than the control group sections. More newly formed trabeculae could be seen in the sections with GH. These trabeculae were thicker and more irregular than the control group sections (Figs 2 and 3).

Fig 2 Section obtained from a specimen without GH, showing poor periosteal and endosteal response (Masson; original magnification ×10).

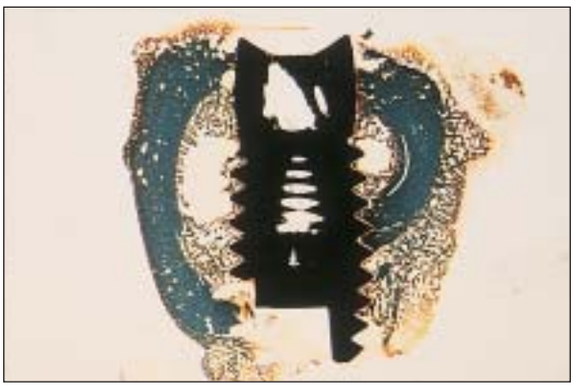

Fig 3a Section from a specimen with GH showing a greater periosteal and endosteal response. In this case the new trabeculae were mostly mineralized *(green)* and were perpendicular to the old cortex (Masson; original magnification ×10).

Fig 3b Section from another specimen with GH, with more endosteal and periosteal reaction than the section shown in Fig 3a. In this specimen, the new trabeculae were more disorganized and more irregular, and less mineralization had occurred than in the previous specimen (Fig 3a) (Masson; original magnification ×10).

Fig 3c Section from another specimen with GH, with an exaggerated reaction from the endosteum and fundamentally from the periosteum. The new periosteal trabeculae can be seen; some of them are perpendicular and mineralized. In another area, the new periosteal trabeculae were irregular, without zones of mineralization (Masson; original magnification ×10).

Birefringent neoformed collagen fibers were seen with the picrosirius stain, when polarized. More birefringent collagen fibers were seen in the sections with GH than in the sections without GH (Figs 4a and 4b).

The quantitative morphometric analysis obtained with the MIP-4 analyzer showed more BIC in the GH group, with statistical significance (Table 1; Figs 5 and 6). Mean BIC ± SEM at the cortical level was 66.67% ± 4.9% in the experimental group and 28.78% ± 2.6% in the control group, which was statistically significant ($P < .05$). Mean BIC ± SEM at the medullary level was 51.49% ± 6.9% in the GH group and 18.34% ± 2.5% in the control group, which was also statistically significant ($P < .05$).

DISCUSSION

In recent years, several substances have been used to improve peri-implant bone response: bone morphogenetic proteins,[20,21] growth factors,[22] and more recently hormones, such as GH.[16,23] Systemic administration of GH has been used to increase bone mass[14] and to improve the fracture repair processes.[11,12] Other authors have studied the stimulating effects of local GH on bone formation in rat mandibles[24] or the enhancing effects of local GH on formation of new bone and bone resorption using a phosphate/calcite matrix GH-releasing system.[15] The authors' previous data have also shown an increase in the peri-implant bone response with the local administration of GH in an osteoporotic rabbit model.[16]

The present histologic results showed an increase in newly formed bone trabeculae in the GH-treated group, derived from the periosteum and eventually from the endosteum. These findings are partially in disagreement with the results of most authors conducting research in this area, who propose that GH can stimulate the periosteal reaction without affecting the endosteum. Andreassen and coworkers,[14,25]

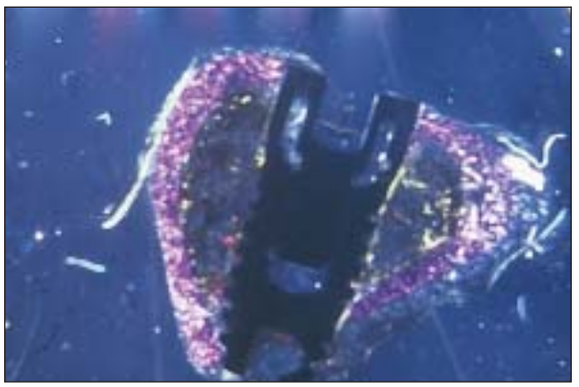

Fig 4a Section from a specimen without GH seen under polarized light, showing lesser birefringent neoformed collagen fibers (picrosirius; original magnification ×10).

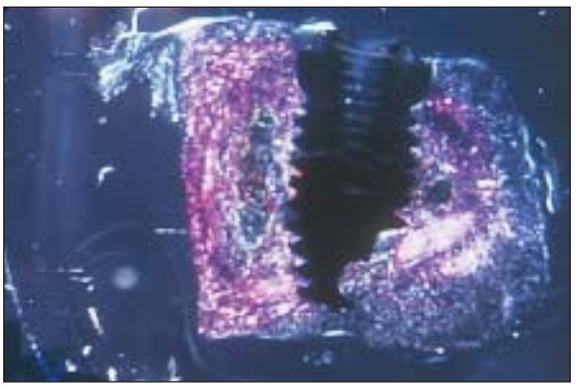

Fig 4b Section from a specimen with GH seen under polarized light, showing more birefringent neoformed collagen fibers (picrosirius; original magnification ×10).

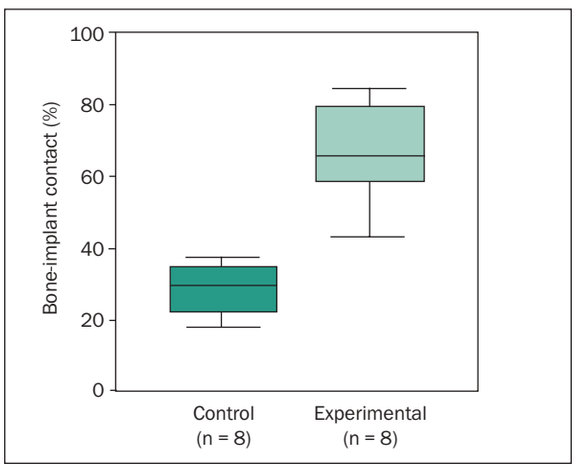

Fig 5 Cortical bone-to-implant contact. In the experimental group, mean BIC (± SEM) was 66.67% ± 4.9%, and in the control group, mean BIC was 28.78% ± 2.6%. These differences were statistically significant ($P < .05$).

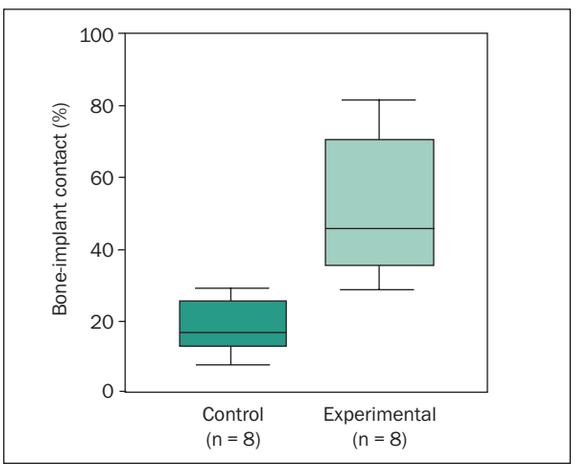

Fig 6 Medullary bone-to-implant contact. In the experimental group, mean BIC (± SEM) was 51.49% ± 6.9%, and in the control group, mean BIC was 18.34% ± 2.5%. These differences were statistically significant ($P < .05$).

Mosekilde and associates,[26] and Martínez and colleagues[27] showed that systemic GH was able to increase the cortical mass exclusively from the periosteum. In the present results, the endosteal reaction could have been induced by the high local levels of GH. The newly formed trabeculae were more irregular in the GH group. However, Jorgensen and coworkers[28] observed newly formed bone after the systemic administration of GH in growing rats and concluded that it had the characteristics of normal bone, with concentric lamellae forming osteons. The present results suggest that locally administered GH in a single dose of 4 IU (1.2 mg) could exert an "impulse effect" in the first hours of the process of osseointegration,[16] recruiting more preosteoblasts and thus leading to an acceleration of the process.

The morphometric data revealed that there was significantly greater BIC in the GH-treated group than in the control group. These results obtained with the local application of GH are similar to those of Lynch and associates,[22] who observed that local administration of platelet-derived growth factor and IGF-I were capable of stimulating the regeneration of bone around titanium dental implants in the early phases of healing. These data are in agreement with Cochran and colleagues,[20] who found more BIC in implants that were treated with local recombinant human bone morphogenetic protein-2.

CONCLUSION

The local administration of GH was able to enhance the peri-implant bone response around Screw-Vent implants placed in young rabbit tibiae at a statistically significant level.

Table 1 Bone-Implant Contact in Examined Species

Implant sample	Cortical BIC (%)	Medullary BIC (%)
Control group (no GH)		
1	18.11	16.03
2	33.94	29.22
3	35.96	16.21
4	26.82	18.06
5	27.71	22.68
6	38.03	10.72
7	31.70	25.88
8	18.00	7.98
Experimental group (with GH)		
1	52.48	47.91
2	43.03	35.32
3	64.31	43.11
4	65.09	35.31
5	66.02	28.62
6	80.75	68.61
7	77.31	71.37
8	84.44	81.67

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