Histologic, Morphometric, and Densitometric Study of Peri-implant Bone in Rabbits with Local Administration of Growth Hormone

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Purpose: *The objective of this study was to evaluate whether the local administration of growth hormone (GH) would influence the formation of peri-implant bone around titanium sheets placed in the tibiae of young rabbits.* ***Materials and Methods:*** *Thirty-two New Zealand rabbits were randomly placed in 1 of 2 groups: the experimental group, in which 4 IU (1.2 mg) of lyophilized powder (GH) was added to a surgically created defect at implant placement, or the control group, in which an implant sheet was placed without hormonal treatment. Animals were sacrificed at 1, 2, 3, and 6 weeks after surgery, and histologic sections were stained with Masson, Alcian blue, picrosirius, and hematoxylin-eosin and observed under light microscopy. The sections were analyzed morphometrically and densitometrically to calculate the amount of newly formed bone.* ***Results:*** *At week 2, GH-group sections showed enhanced growth of the trabeculae from the periosteal tissue, with thicker and more irregular trabeculae than those observed in control group specimens. A tendency toward greater bone area and lesser density was observed in the GH group, although the groups did not differ significantly. Nevertheless, bone-to-implant contact in weeks 2 and 6 was significantly greater in the GH group (*$P < .05$*).* ***Discussion:*** *An increase in the cortical response from periosteal and endosteal reactions was observed with the high local administration of GH, in disagreement with most authors. In the first phases of bone repair, the osteons were more disorganized; they were more organized by the sixth week.* ***Conclusion:*** *Local administration of GH could stimulate the first phases of the bone remodeling process in this experimental model.* Int J Oral Maxillofac Implants 2005;20:193–202

Key words: *bone remodeling, growth hormone, implants, rabbits*

Growth hormone (GH) is one of the most important regulatory substances in bone growth and bone remodeling in vivo.[1] It is actually considered a local growth factor, since it may have not only endocrine but also paracrine or autocrine effects.[2] GH is able to stimulate bone growth in a dose-dependent way by direct stimulation of the epiphyseal chondrocytes.[3] It stimulates the proliferation and differentiation of chondroprogenitor cells.[4] GH acts directly on the osteoblasts[5]; it also affects them indirectly by increasing insulin-like growth factor I (IGF-I) synthesis in their vicinity.[6] GH and IGF-I stimulate osteoblastic proliferation and differentiation.[7] GH stimulates bone turnover,[8,9] since it increases protein synthesis and mineralization[10] and, more specifically, bone matrix proteins.[11]

GH is able to enhance bone fracture repair in both young and old animals; its parenteral administration can increase the biomechanical properties of fractured bone up to 400%.[12–15] Recent studies have demonstrated that GH not only produces results after systemic administration but can also have a local effect. Guicheux and coworkers[16] have shown

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that local administration of GH, released from a carrier, a calcium phosphate–type biomaterial, produces an increase in bone growth by acceleration of the bone remodeling process.

However, GH has rarely been applied locally to enhance the osseointegration process.[17–19] Thus, the authors' working hypothesis has been that GH administered locally in a single dose at the time of the surgery could accelerate the peri-implant bone response.

The objective of the present study was to determine whether the local administration of 1 large dose of GH at the time of surgery would induce histologic, morphometric, and densitometric differences in the newly formed peri-implant bone of young rabbit tibiae at different times.

MATERIALS AND METHODS

The implantation material consisted of rectangular titanium sheets (Laboratorios Aragoneses, Madrid, Spain), 5 mm wide, 10 mm long, and 0.5 mm thick. The composition and surface topography of the sheets were analyzed in the Metallurgic and Metallotechnical Department of the High Technical School of Mining Engineering in Oviedo (Spain) and in the Coal Institute of Oviedo (Spain), Upper Council of Scientific Investigations, respectively. The titanium sheets were analyzed by atomic absorption spectrophotometry. Their surface was observed at 500× and 1,000× magnifications using a scanning electronic microscope. The degree of roughness was measured using a rugosimeter. The metallurgic analysis of the titanium sheets revealed that they were 98.5% ± 0.5% titanium. The surfaces of the sheets showed irregularities, with a maximum distance from peak to trough of 12 μm.

Thirty-two 3-month-old female New Zealand rabbits, weighing 2.5 kg each, were used as experimental animals. Two titanium sheets were placed in each rabbit, 1 on the medial side of each tibia. The methodology followed was that of Tresguerres and colleagues.[17] 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 to expose the internal side of the tibia in the diaphysis-proximal metaphysis. After cutaneous-periosteal detachment, a longitudinal ostectomy along the longitudinal axis 1 mm wide and 10 mm long was performed in each tibia. In the experimental group (n = 16), the ostectomies were treated locally with 4 IU (1.2 mg) of recombinant human GH (rhGH) (Saizen, Serono Laboratories, Madrid, Spain) in the form of lyophilized powder. In the control group (n = 16), the titanium sheet was simply impacted into the medullar canal without prior GH treatment. The sheets were maintained in position by the periosteal flap sutured with Dexon (Davies and Geck, Wayne, NJ). The skin was sutured with silk. Oxytetracycline was administered orally to both groups to prevent postsurgical infection.

Four animals from each group were sacrificed at 1, 2, 3, and 6 weeks after surgery. The tibiae were dissected from soft tissues and fixed in 10% buffered pH 7 formaldehyde for posterior embedment in 2-hydroxyethylmethacrylate resin, according to method of Donath and Breuner,[20] so as to cut undecalcified bone and titanium simultaneously with the Exakt microtome (Exakt Apparatebau, Norderstedt, Germany). Blocks of 20-mm thickness, including the sheet, were obtained, and transversal cuts of 200-μm thickness were made with the Exakt cutting band to obtain the definitive histologic cut of the bone and titanium. Each section obtained was then ground with the Exakt grinder until a final thickness of 50 to 80 μm was obtained for the study under a light microscope (Leica, Wetzlar, Germany). Morphometric and densitometric studies were subsequently performed to quantify the bone response around the titanium sheets. This histologic study was performed in the Department of Morphological Sciences and Surgery in the Medical School in the University of Alcalá de Henares, Madrid.

Light Microscopy

The histologic analysis was made using the following stains[21]:

- Masson stain, to differentiate the calcified bone tissue (in green color) from the uncalcified osteoid (in orange-red)
- Alcian blue, which indicated the presence of sulfated acid proteoglycans (which stained blue and formed bands) coinciding with zones of significant bone resorption
- Picrosirius stain, which, when observed under polarized light, allows the observation of birefringence in collagen fibers
- Hematoxylin-eosin, which allowed the differentiation of individual cells (hematoxylin stained the nuclei and eosin stained the cytoplasm)

Morphometry

A morphometric study to quantify the bone area was performed. This was carried out with the MIP-4 imaging analyzer (a computerized system that performs area and volume measurements; Digital Image System, Barcelona, Spain). The morphometric parame-

ters calculated were bone area and bone-to-implant contact (BIC). Bone area was defined as the ratio between the area occupied by bone and the total area, including bone and marrow tissue areas (Fig 1), and provides information about the quantity of the newly formed bone.[17] BIC is defined by calculating the length of bone surface border in direct contact with the implant perimeter.[22] These measurements were made with a 10× objective in all fields of each specimen, by counting the number of intersections of bone and titanium over the implant surface. Finally, the results were expressed as a percentage of the implant surface covered by bone. For each sample, various sections were obtained and one of them was randomly chosen for the statistical evaluation.

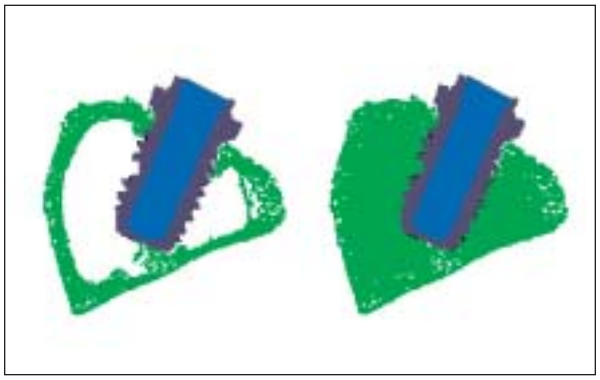

Fig 1 Bone area was the ratio between the area occupied by bone *(left)* and the total area *(right)*.

Densitometry

A histologic densitometric study was made to measure the bone mineral density (BMD) at 0.5 cm above and 0.5 cm below the sheet using dual energy x-ray absorptiometry. The XR Norland densitometer (Norland Medical Systems, Fort Atkinson, WI) was used to obtain the BMD expressed in g/cm^2).

Statistical Analysis

The mean values ± SEM for bone area, BIC, and BMD were calculated for each group. The groups were compared using the Student *t* test. The results and the statistical analysis were elaborated with the SPSS 11.0 computer system (SPSS, Chicago, IL).

RESULTS

Macroscopic Analysis

In some of the GH-treated tibiae obtained in week 2, macroscopic differences were observed between the experimental and control groups (Fig 2). These tibiae showed a higher growth of newly formed tissue. In the tibiae obtained at weeks 1, 3, and 6, no macroscopic differences could be seen.

Light Microscopy

The histologic results showed more newly formed trabeculae, from the periosteum and, to a lesser degree, from the endosteum, in weeks 1 and 2 in the GH experimental group sections than in the control group sections (Fig 3).

Thicker and more irregular trabeculae were observed in the experimental group than in the control group, in which a higher degree of parallelism with the sheet was seen (Fig 4). This periosteal response was more irregular next to the implant and was more marked in the GH-treated animals sacrificed in the first and second weeks. In sections from week 1, bone resorption was greater in the experimental group. More cavities were seen in the experimental group sections (Fig 5) and more blue bands (highlighted by Alcian blue stain) were seen around the resorption cavities, coinciding with zones in which proteoglycans were released from the matrix by osteoclastic resorption. At weeks 2 and 3, no differences in resorption were observed between the 2 groups. At week 6, bone resorption was found to be greater in the control group than in the experimental group (Fig 6). The osteons in the cortex in the sixth week were more organized in the GH group than in the control group (Fig 6). In the samples obtained in the first and second weeks after surgery, chondrocyte nests were observed inside the cortex in the GH-treated group but not in the control group (Figs 7 and 8).

Morphometric Results

Mean values of bone area ± SEM were calculated. In week 2, the morphometric data revealed more bone area in the experimental group than in the control group (0.64 ± 0.02 vs 0.6 ± 0.02); however, this difference was not statistically significant. Three and 6 weeks postsurgery, the mean bone area was slightly greater in the control group than in the experimental group (0.63 ± 0.03 at 3 weeks and 0.62 ± 0.01 at 6 weeks versus 0.60 ± 0.02 at 3 weeks and 0.58 ± 0.01 at 6 weeks) (Fig 9), but again, the difference was not significant. No statistically significant differences were found among the groups in regard to mean bone area.

BIC means ± SEM were calculated for samples obtained 2 and 6 weeks postsurgery. BIC was significantly greater in the GH-treated group at both 2 weeks (23% ± 2% vs 15% ± 2%) and 6 weeks (34% ± 2% vs 26% ± 2%) ($P < .05$) (Fig 10).

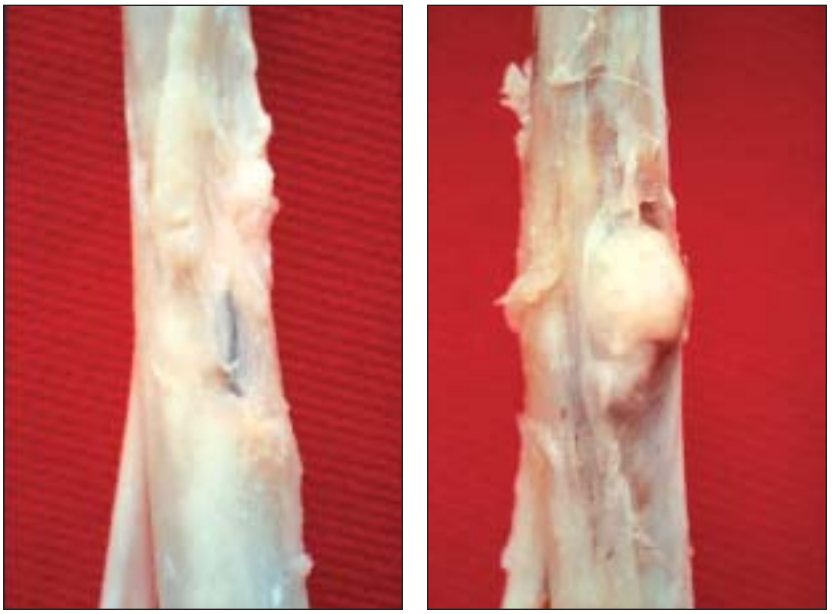

Fig 2 Macroscopic view of 2 tibiae obtained from rabbits sacrificed at 2 weeks. The tibia without GH *(left)* showed a lesser degree of growth in the peri-implant tissues. The tibia with GH *(right)* showed great growth in the newly formed peri-implant tissues.

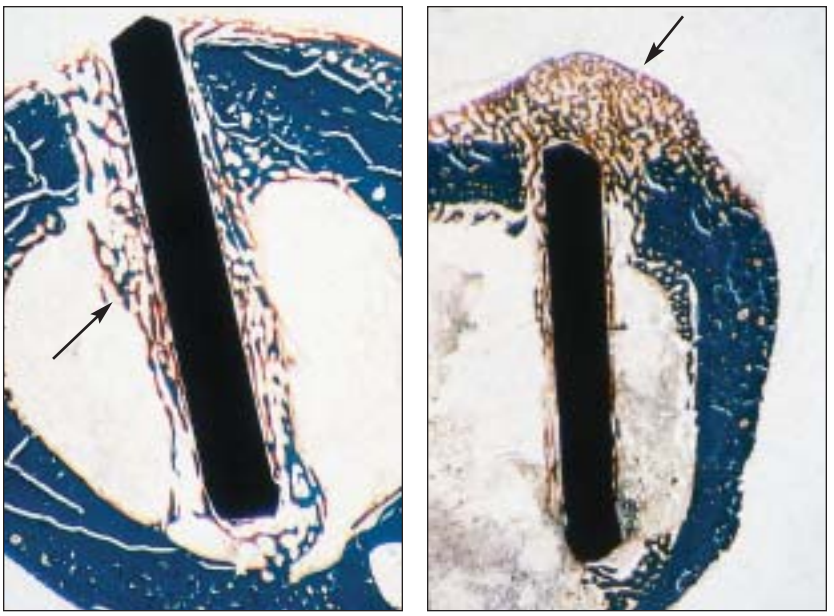

Fig 3 Sections obtained 2 weeks after surgery. The section without GH *(left)* showed a poor periosteal reaction. The specimen with GH *(right)* showed a greater periosteal reaction. In this case, the peri-implant medullar reaction was greater in the control group specimen than in the experimental one (Masson; original magnification ×12).

Densitometric Analysis

Mean values of bone mineral density ± SEM were calculated in g/cm^2. The densitometric data showed slighty greater BMD in the experimental group with respect to the control group (0.326 ± 0.01 g/cm^2 vs 0.322 ± 0.01 g/cm^2) after 1 week, but after 2 weeks, less BMD was found in the experimental group than in the control group (0.333 ± 0.01 versus 0.352 ± 0.01), although this difference was not statistically significant. At weeks 3 and 6, the differences in BMD between the 2 groups were minor (Fig 11). No statistically significant differences were found between the 2 groups in regard to BMD using the Student *t* test.

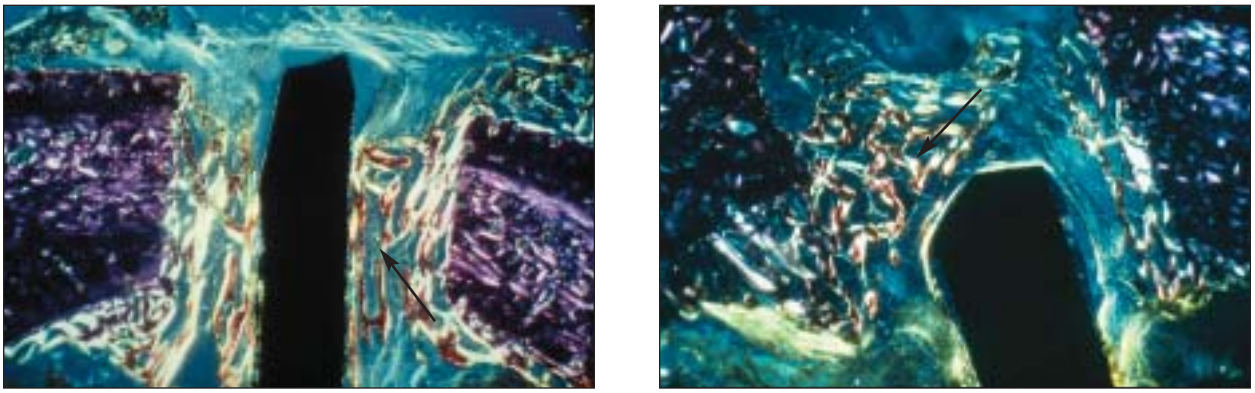

Fig 4 Sections obtained 2 weeks after surgery. The specimen without GH *(left)* showed birefringent newly formed trabeculae parallel to the implant surface (the collagen fibers are birefringents when polarized). The specimen with GH *(right)* showed birefringent newly formed trabeculae that were thicker and more irregular than those seen in the control group specimen (Sirius; original magnification ×15).

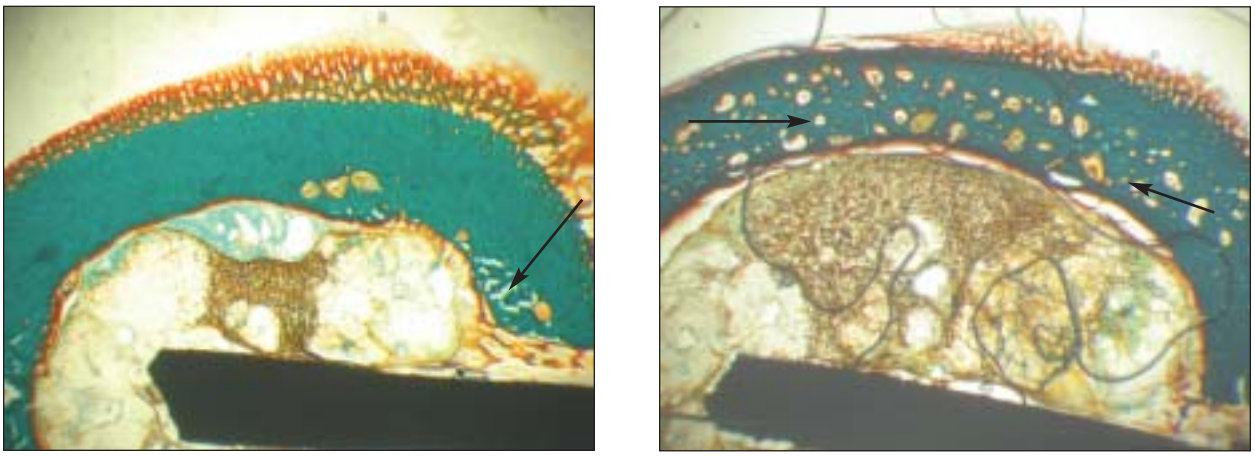

Fig 5 Sections obtained 1 week after surgery. The specimen without GH *(left)* showed fewer cavities of bone resorption *(arrow)* in the cortex. The specimen with GH *(right)* showed more cavities of bone resorption *(arrows)* in the cortex (Masson; original magnification ×12).

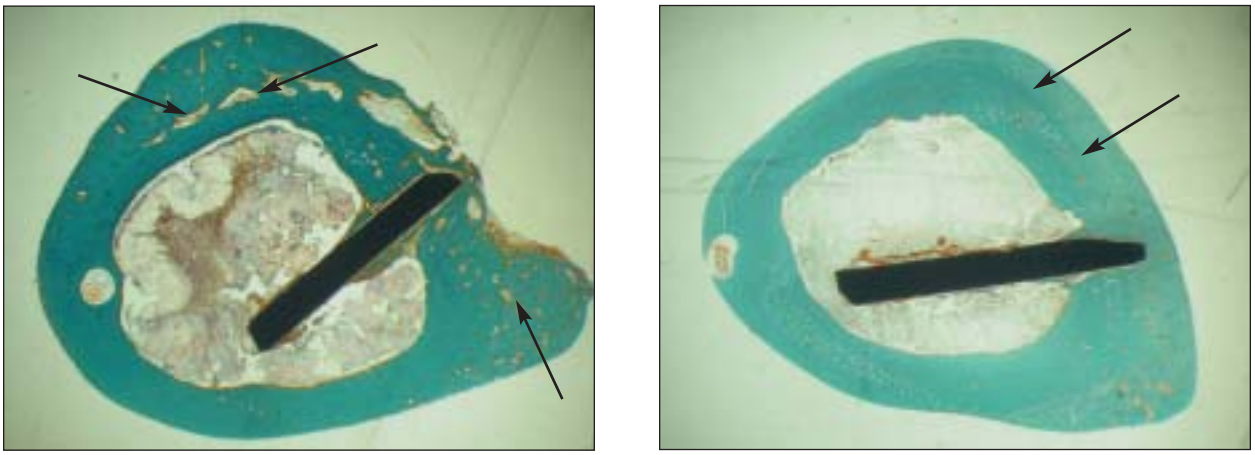

Fig 6 Sections obtained 6 weeks after surgery. The specimen without GH *(left)* showed more cavities in the cortex and more immature trabeculae. The specimen with GH *(right)* showed less resorption and more maturation of the cortex, with regular, mature osteons (Masson; original magnification ×10).

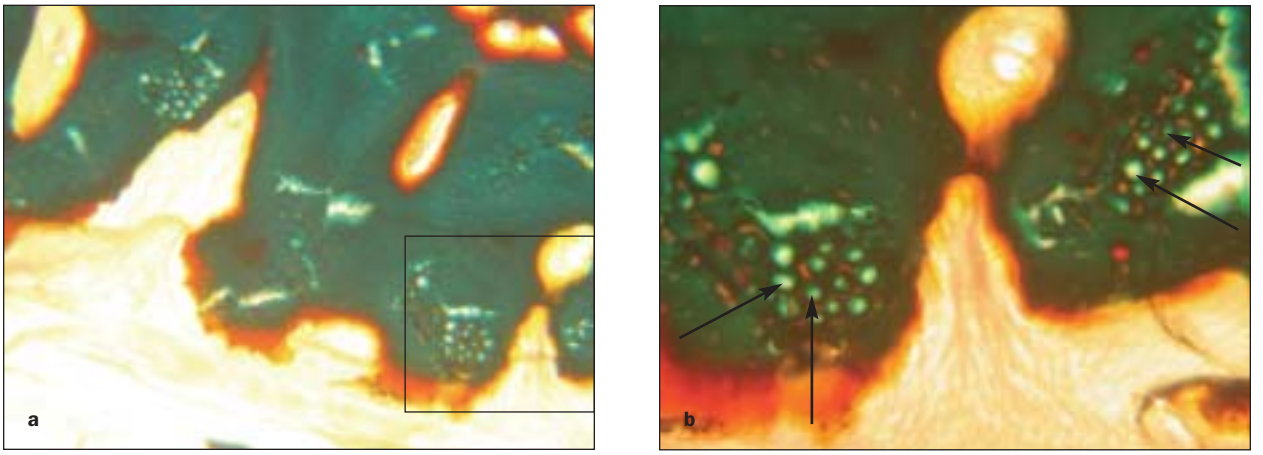

Fig 7 *(a)* Section obtained 2 weeks after surgery, from a specimen with GH, showing chondrocyte nests in the cortex (Masson; original magnification ×20). *(b)* Chondrocytes shown in *(a),* seen at a higher magnification (Masson; original magnification ×25).

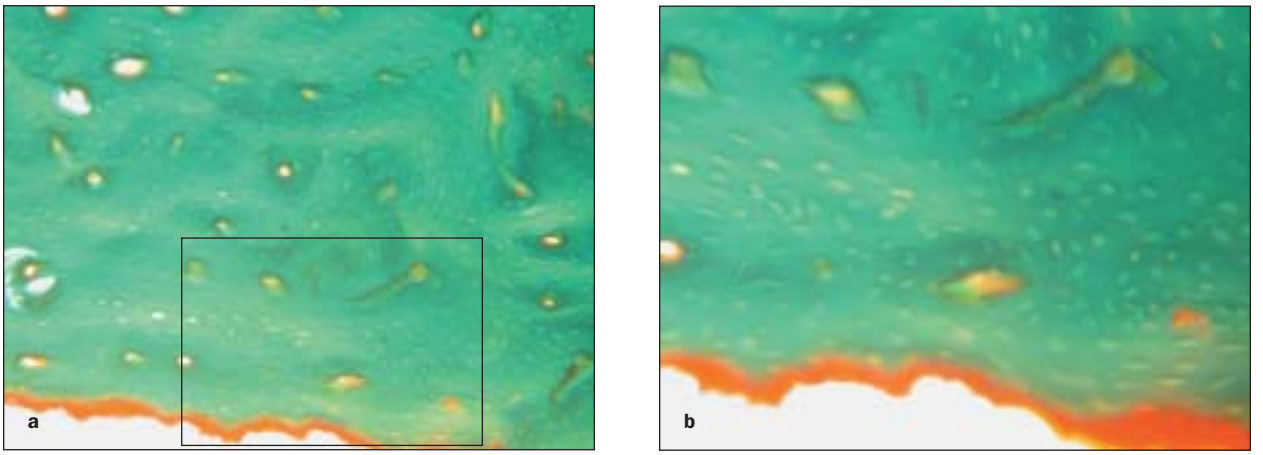

Fig 8 *(a)* Section obtained 2 weeks after surgery, from a specimen without GH, without chondrocyte nests in the cortex (Masson; original magnification ×20). *(b)* Section of *(a)* seen at a higher magnification (Masson; original magnification ×25).

DISCUSSION

In recent years, GH has been administered systemically as an anabolic agent to treat several systemic diseases. For example, it has been used to improve pulmonary function in patients with chronic obstructive lung disease[23] and to improve the hemodynamic management and the negative nitrogen balance of the catabolic patient with sepsis.[24] In patients with hypopituitarism with GH deficiency, life expectancy is shortened because of an increased risk of cardiovascular diseases.[25] GH is also used to increase the ratio of muscle mass to fat mass, since it increases protein synthesis, exercise capacity, and lipolysis, reducing the atherogenic lipid profile. GH has been observed to produce an insulin-like effect in the short term and an anti-insulin effect over the long term.[26,27] As aging is associated with a reduction of serum GH levels, systemic administration of GH may reverse some of the changes associated with senescence, such as increasing skin thickness and skin collagen or increasing lean body mass, muscle mass, and bone mass.[28,29]

Systemic administration of GH has been previously documented to stimulate bone fracture repair[12–14] and to increase bone mass.[15] However, GH has rarely been administered locally to enhance periimplant bone growth. Few articles have documented the effects of local application of GH. Hedner and colleagues[30] observed that GH, administered locally in

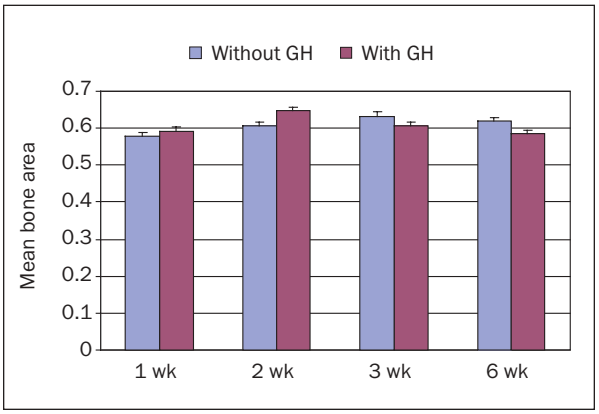

Fig 9 Mean values of bone area ± SEM. No statistically significant differences were found among the groups.

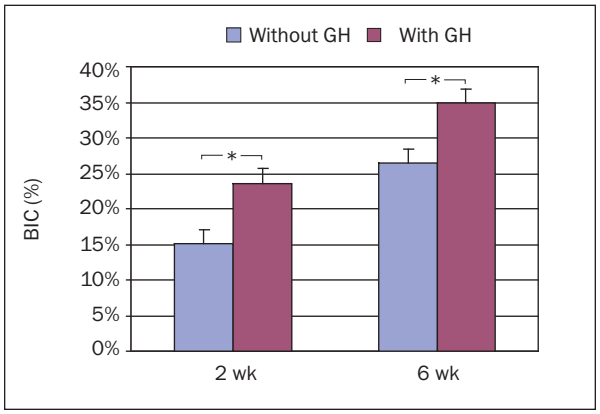

Fig 10 Mean BIC ± SEM were calculated in the samples obtained at 2 and 6 weeks postsurgery. BIC was significantly greater in the GH-treated group at both 2 and 6 weeks ($P < .05$).

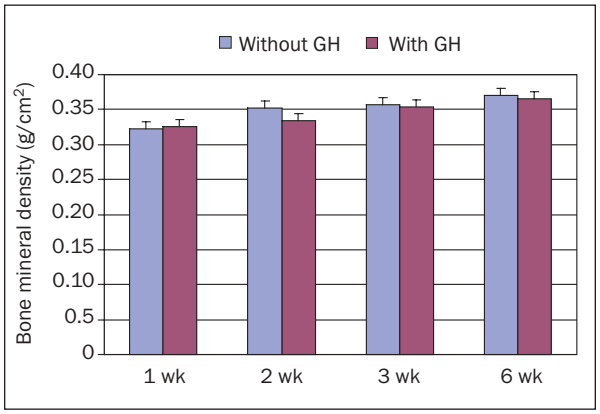

Fig 11 Mean BMD ± SEM at 1, 2, 3, and 6 weeks. No statistically significant differences were found with the Student *t* test.

the mandibles of adult rats, significantly stimulated local bone formation compared to saline; GH was administered in conjunction with the use of expanded polytetrafluororethylene membranes. Guicheux and coworkers[16] demonstrated that local GH was able to enhance bone neoformation using a phosphate calcite matrix GH release system in rabbits. The authors' previous data showed an increase in peri-implant bone with local administration of GH in an osteoporotic rabbit model[17] and a nonosteoporotic rabbit model.[18]

Systemic GH increases the cortical mass, primarily by periosteal apposition, but most authors have not documented modifications in the endosteal response.[15,30–33] However, the authors' histologic results showed that local GH increased the cortical response from periosteal and endosteal reactions. This periosteal response was more irregular next to the implant and was more marked in the GH-treated animals sacrificed in the second week. However, Jorgensen and associates[34] observed newly formed bone after the systemic and serial administration of GH in growing rats, and concluded that it had the characteristics of normal bone, with concentric lamellae forming osteons and with collagen fibers in the same direction.

The authors' data showed more disorganized osteons in the GH-treated rabbits in the first phases of bone repair, while these osteons were more organized in the sixth week. In the callus of the GH-treated animals, connective tissue, irregular trabeculae, and partially calcified osteoid tissue can be seen. In the samples of the first and second week "chondrocyte nests" could be seen inside the periosteal

callus in the GH-treated group but not in the control group. This exaggerated and fast periosteal response could be explained by direct action of GH on the pluripotential mesenchymal cells in the first steps of the repair process. The GH accelerated this process in what has been called the "impulse effect,"[17,18] stimulating osteoblasts, chondroblasts, and fibroblasts. As the dose is relatively high for local application, it could produce an anarchic stimulation of the osteoprogenitor cells that would form new and more irregular bone trabeculae.

Histologic study with Alcian blue stain, which indicates the presence of sulfated acid proteoglycans in zones of bone resorption, has shown different responses regarding the cortex resorption in control and experimental animals. In the GH-treated group, there was intense intracortical resorption at 7 days, with more cavities in the cortex, while at 42 days the resorption was less and the osteons in the cortex were more organized in the GH-treated group than in the control group. The newly formed bone in the GH group was more organized and could be regarded as more mature than that of the control group 6 weeks after surgery. This could be explained by the fact that locally administered GH apparently accelerates the remodeling process, stimulating first resorption[35] and later bone formation, as documented by Kassem and coworkers[4] and Bravenboer and associates.[36] Further studies are necessary to assess the GH-induced bone maturation process.

Regarding the quantitative analysis, morphometric results showed that only those animals in the GH-treated group that were sacrificed in the second week demonstrated a tendency toward increased bone area, a tendency that did not reach statistical significance, whereas no changes were observed during the first week. The bone area even decreased slightly later in the study. Nevertheless, BIC was significantly greater in the GH group than in the control group in weeks 2 and 6 ($P < .05$), which is in agreement with the authors' previous data.[18] This could be explained by the fact that both old and new bone were measured to calculate bone area, whereas BIC concerns only the newly formed bone around the sheet. In the second week there was more bone area and more BIC in the GH group than in the control group. By week 6, BIC had increased, but the bone area had decreased. Since local administration of GH could accelerate the remodeling process, stimulating the periosteal, transcortical, and endosteal reaction in the second week, with more disorganized trabeculae, by the sixth week the cortex could be more organized, with regular and mature osteons, and therefore with less bone area.

In the densitometric analysis, slightly more bone density was found in the GH group only in the first week. This difference was not statistically significant. In the remaining times, the untreated animals presented slightly more bone density. The morphometric and densitometric data for the 2 groups were similar. Bone area as well as bone density were slightly greater in the untreated animals. The fact that there was less bone area in the GH-treated rabbits at weeks 3 and 6 could indicate that GH had accelerated the remodeling process, stimulating resorption first[4,35] or accelerating the maturation process. GH could increase the remodeling space that results in an initial bone loss, as shown by Bravenboer and coworkers,[36] or, as Martínez and associates[33] showed, the administration of GH could produce changes in the cortical bone micro-organization, making it more porous and decreasing its initial density.

Data obtained from the GH-treated animals sacrificed at week 2 (who showed greater but less dense bone formation than the control animals) confirmed the results of most authors, who found that systemic administration of GH increases bone formation without increasing mineral density.[34,37] Thus, short-term GH treatments can initially decrease bone density instead of increasing it, as documented by Vandeweghe and colleagues.[38] However, Eschen and Andreassen,[39] Andreassen and Oxlund,[40] and Kidder and associates[41] suggested that GH could exert a positive effect on mineralization, even under lower calcium conditions. GH may not only increase calcium absorption in the digestive system[42] but also may stimulate local calcium availability to increase the mineralization of the newly formed osteoid tissue. Local GH may stimulate osteocyte osteolysis and thus increase calcium perilacunar availability (unpublished data, 2003).

However, the limited densitometric and morphometric differences found in the young animals of this study could be the result of the fact that young rabbits have high endogenous GH levels and are perhaps not the best experimental model. To obtain greater differences, *(1)* older animals and *(2)* a combination of local and systemic GH (maintenance) should be used; however, older rabbits are extremely sensitive to anesthesia and thus very difficult to utilize. The combination of local GH administration with systemic treatment could potentiate the acceleration of the remodeling process. Since GH also increases calcium absorption in the digestive system,[42] systemic maintenance of GH administration could stimulate the "impulse effect." Thus, the bone repair process could be accelerated, and the differences would be more representative.

CONCLUSIONS

Local administration of GH during the placement of a titanium sheet stimulated the periosteal and endosteal response 2 weeks after surgery. There was an initial decrease in BMD but increased BIC 2 and 6 weeks after surgery (significantly more in the GH-treated rabbits than in the control rabbits). These results suggest that local administration of GH accelerates the remodeling process. Further studies in experimental animals with low endogenous GH are necessary to assess the maturation process of the newly formed bone to clarify these results.

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