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Research article

Growth hormone effects on healing efficacy, bone resorption and renal morphology of rats: histological and histometric study in rat calvaria

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ABSTRACT

Previous reports demonstrated the utility of systemic application of growth hormone (GH) in the treatment of bone defects. Very few studies correlated bone repair efficacy with hepatic and renal side effects promoted by locally-delivered GH. The objectives of this study were to assess the bone repair properties along with hepatic and renal adverse effects promoted by local application of GH in a rat model. Thirty-two rats were randomly divided (4 groups; n = 8/group), as follows: (i) AB (autogenous bone + local application of saline solution [SS]), (ii) AB+ (autogenous bone + SS local application + SS irrigation), (iii) AB/GH+ (autogenous bone + SS local application + GH irrigation) and (iv) AB/GHL+ (autogenous bone + GH local application + GH irrigation). Critical-sized defects (diameter = 5.0 mm) were surgically created by a single operator in the calvaria of rats. Defects were filled with ground autogenous bone. Defects pertaining to AB+ and AB/GH+ received a mixture of autogenous bone and a SS-saturated (0.02 mL) collagen sponge covered with bovine cortical membrane. Defects in group AB/GHL+, were filled with the same biomaterials saturated with GH (0.02 mL). SS (0.1 mL) or GH (0.1 mL, equivalent to 0.4 IU) were applied locally on alternate days (8 weeks) in animals in groups AB, AB+ and AB/GH+ or AB/GHL+, respectively. Bone repair properties was determined in hematoxylin/eosin-stained slices using traditional histologic and histomorphometric techniques along with optical microscopy and digital image analysis. Statistical differences among groups was determined using Kruskal-Wallis and Tukey post hoc tests ($\alpha = 0.05$). Histology results indicated that AB and AB+ displayed greater presence of autogenous bone as compared to AB/GH+ and AB/GHL+. Histomorphometric results indicated significantly higher osteoid matrix formation in AB and AB+ when compared to AB/GHL+ ($p = 0.009$). Kidneys and livers were found to have their glomeruli preserved in AB and AB+. Strong glomeruli necrosis and large areas of protein deposition were found in AB/GH+. Abnormal small-sized glomeruli were found in AB/GHL+. The utilization of autogenous bone graft associated with local application and irrigation with GH was shown to not improve the bone repair in calvarial critical-sized defects in a rat model.

1. Introduction

Healing of critical-sized bone defects still represents a significant challenge in modern dentistry. Several tissue engineering studies have been conducted to develop novel stimuli-responsive biomaterials displaying osteoconductive and/or osteoinductive properties, that upon placement, are capable of triggering, upregulating and optimizing the formation of new bone [1, 2, 3, 4]. According to current and compelling scientific evidence, autogenous bone is the most appropriate type of grafting material due to its high-efficiency, biosafety and donor site availability [5, 6, 7]. However, the need to obtain grafting material from a donor site has been shown to be a critical limitation of techniques based on autogenous bone grafts, because they represent additional morbidity to the patient (second surgical site) and donor sites are typically limited in terms of their number and quality of bone, which further limits their utilization for the repair of critical-sized

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defects. More recently, the systemic administration of substances capable of accelerating the process of bone regeneration have attracted the attention of both the manufacturing and research communities. Modern strategies to accelerate the process of bone regeneration include the utilization of GH to physiologically modulate (increase) osteoblast activity [1, 8, 9, 10, 11].

Hormone replacement therapy in adults has been previously shown to result in significant increases in bone and muscular densities, and patients displaying improved muscular performance and better quality of lives [12]. In vivo animal studies investigating the efficacy of GH therapy in tendon healing and bone remodeling processes have demonstrated that GH administration promoted the attainment of significant increases in the cortical mass [9] and mechanical properties of bones [10, 11, 13]. In addition, other studies have underscored that GH therapy was able to improve bone defect regeneration (speed and quality) [14, 15] and the primary stability of titanium-based endosseous dental implants [16] due to the stimulation (direct or indirect) of osteogenic cells. Recent studies have evaluated topical applications of GH associated with calcium phosphate cements for the repair of critical-sized defects surgically created in the tibias of rabbits. The results reported have demonstrated that this modified approach significantly favors bone regeneration process [4].

In vivo animal models investigating bone-healing utility of GH when used either as an irrigation solution during implant placement [3], or as a tooth socket-filler powder [17, 18], demonstrated that both approaches resulted in improved bone healing and superior implant stability. Only one study has investigated the positive impacts of locally delivered (infiltrative injection) GH therapy as a material capable of promoting cartilage growth in a rabbit model of the temporo-mandibular joint. Results reported have shown that such approach was capable of inducing higher levels of cartilage growth in a very short period of time [19]. The majority of the current literature regarding the utilization of GH therapy indicates that promising results can be attained with strategies based on both local (topic or irrigation) and systemic delivery methods. According to Danna et al. [20] GH application has been successfully used in tissue engineering approaches for regenerative medicine in pilot studies. However, despite these encouraging results, very few studies have been conducted to investigate the effects of GH therapy as bone regeneration materials locally delivered to extraoral regions (craniofacial). Moreover, a recent study [21] was not able to demonstrate positive results from the utilization of GH on the modulation of mesenchymal stem cells (MSC) and on the improvement of short-term healing in a rabbit chondral defect model.

Therefore, the objective of the present study was to assess, by histomorphometric and histological analyses, the bone regeneration efficacy of locally delivered GH therapy combined with autogenous bone graft in critical size calvarial defects in a rat model. The scientific justification for the execution of the present study using the experimental design reported is based on the necessity to have higher local concentrations of GH to upregulate bone repair in critical sized defects while avoiding undesired effects hepatic and renal toxicity associated with the systemic administration of GH, which is considered a significant drawback to systemic approaches.

2. Material & methods

2.1. Institutional animal care and use committee

The present study was approved by Ethics Committee on Animal Use of the Positivo University (protocol number # CEUA 290, appendix 1). Thirty two male rats (Rattus norvegicus, Albinus Wistar; age: 5–6 months, average weight: 430 g) were selected to participate in the present study to avoid issues with sexual dimorphism on the outcomes reported as previously demonstrated by previous studies.

2.2. Experimental design

Participating animals had *ad libitum* access to water and to a standard laboratory diet. The animals were then randomly divided into four groups (n = 8/group, Table 1), as follows: (i) AB (autogenous bone + local application of saline solution [SS]), (ii) AB+ (autogenous bone + SS local application + SS irrigation), (iii) AB/GH+ (autogenous bone + SS local application + GH irrigation) and (iv) AB/GHL+ (autogenous bone + GH local application + GH irrigation).

2.3. Surgical procedure and GH administration

Surgical procedures were performed according to a protocol previously published [22]. In brief, the animals were sedated using oxygen and isoflurane 3 L/min (Cristália, Brazil) and then anesthetized by intramuscular injection (back of thigh) using Xylazine (10 mg/kg; Vetbrands, Brazil) and Ketamine 80 mg/kg (Vetbrands). Anesthesia was maintained by isoflurane vaporization. After that, the dorsal region of the cranium was subjected to trichotomy procedures and aseptically prepared for surgery. Following, an U-shaped incision was performed (scalpel blade: 15c, Advantive, China) to allow access to the calvarial area. A full-thickness flap was raised and a critical-size cranial defect (diameter = 5 mm) was then manufactured anteriorly to the occipital bone (1.0 mm) and centered along the median sagittal suture by osteotomy using a trephine bur (5 mm, Neodent, Brazil) under profuse irrigation with sterile saline solution.

Critical size defects pertaining to AB were filled with autogenous ground bone (from the defect) and no further treatments were performed. Defects from AB+ were filled with autogenous ground bone and a resorbable hemostatic collagen sponge (diameter = 5 mm, Hemospon, Technew, Brazil) that was saturated with sterile saline solution (0.02 ml). AB+ defects were also covered with a bovine cortical membrane (8 × 8 mm; GenDerm, Baumer, Brazil). Defects from the AB/GHL+ received the same treatment described for AB+, but the hemostatic collagen sponge was saturated with GH (0.02 mL, 4 IU). Soft tissues at surgical sites were then repositioned and sutured with 4-0 silk thread (Ethicon Inc., USA). The animals received a single intramuscular dose of antibiotic (0.1 ml of Pentabiotic 24,000 IU/kg of body wt) and subcutaneous morphine (0.1 ml) immediately after surgery. For the following 3 days, paracetamol dissolved in water (20 drops/400 ml) was given to the animals. Immediately after the completion of the surgical procedure, animals in AB, AB+ and AB/GH+ groups received local administration of sterile saline solution (0.1 mL) that was delivered by a syringe with an insulin needle on alternate days until the completion of the experimental time. Animals from AB/GHL+ received local application of GH (0.1 mL, 0.133 mg or 0.4 IU) using the same protocol as above. At 60 days post-surgery, animals were euthanized using a CO_2 chamber (5–10 min). The original calvarial surgical defects were then retrieved and fixed in 10% buffered formalin solution for 24 h after the removal of surrounding soft tissues. Kidneys and livers were also removed and fixed in 10% formalin to allow for the observation of cellular and macroscopic changes resulting from the utilization of the experimental treatments investigated. The rationale for the dosage and type of administration of GH is based on a previous publication [22] that investigated the positive effects of the local administration of parathyroid hormone on the healing of calvarial bone defects in rats.

2.4. Tissue preparation

Resin blocks and organs retrieved were subjected to histotechnical preparation prior to histological and histomorphometric analyses. Individual sections (n = 3/block, 3 μm-thick, serially sectioned, longitudinally from the center of surgical defect) were then stained with hematoxylin and eosin (HE) and were analyzed using visible light stereo-microscopy. Immediately after euthanasia procedures, tissues from the calvaria, liver and kidneys were collected from each

Table 1. Comparative results of the defect areas of each group in terms of mean and standard deviation values.

Groups	AB		AB+		AB/GH+		AB/GHL+	
	Mean (mm^2 and %)	S.D. (+/-)	Mean (mm^2 and %)	S.D. (+/-)	Mean (mm^2 and %)	S.D. (+/-)	Mean (mm^2 and %)	S. D. (+/-)
AT	0,16 100%	0,06	0,16 100%	0,05	0,13 100%	0,03	0,18 100%	0,06
OM	0,05* 31,25%	0,01	0,05+ 31,25%	0,01	0,01 7,69%	0,002	0,01*+ 5,55%	0,005

* means $p = 0.021$ and + means $p = 0.009$.

animal and fixed (48 h) in formalin 10% (50 mL/vial; 4 °C; neutral, buffered, Sigma-Aldrich, São Paulo, Brazil). Samples from calvarial tissues were decalcified using trichloroacetic acid (TCA; 25 °C, 15 days) before being embedded in paraffin (Alphatec, Curitiba, Brazil) using an automated tissue processor (TP1020, Leica Biosystems, Nussloch, Germany). Three longitudinal sections were obtained from each tissue sample using a microtome (RM2235, Leica Biosystems, Nussloch, Germany) starting from the center of the original surgical defect/organ before being stained (hematoxylin and eosin, HE) and imaged using an optical microscope (BX 41, Olympus Optical Company, Japan).

2.5. Histological analysis

One previously trained and calibrated operator performed all histological analysis of the tissues retrieved from each animal (3 slides/animal). Three slides stained with HE/animal were analyzed. Experimental parameters of interest included (i) bone defect area (edges), (ii) connective tissue characteristics, (iii) presence of osteoid matrix, (iv) presence of inflammatory infiltrates (chronic or acute), (v) type of healing and (vi) thickness of newly-formed tissues. Animals' kidneys and livers were carefully assessed to check if animals subjected to experimental treatments with local application of GH would experience any type of renal or hepatic adverse effects.

2.6. Histomorphometric analysis

Obtained slides (3 slides/animal, total = 96 slides) were photographed to display the edges of each defect and offer a "panoramic" view. The ImageJ® software (Wayne Rasband NIH, Bethesda, MA, USA) was used to perform the following histomorphometric measurements: (i) total area (TA; bone defect total area), (ii) newly-formed bone area (NBA; measurement of the newly formed bone area), (iii) soft tissue area (STA; non-mineralized connective tissue measurement obtained by subtracting NBA from TA; STA = TA – NBA). Spatially-calibrated digital measurements of area for TA, NBA and STA were performed on acquired images using the modified oval selection tool in ImageJ® (available at: https://imagej.nih.gov/ij/docs/guide/146-19.html#toc-Subsection-19.2). TA, NBA and STA were measured (mm^2) and TA was considered to be 100% of the area to be analyzed (Figure 1).

2.7. Statistical analysis

The values of mean, standard deviation and coefficient of variation that were obtained from the histomorphometric measurements were assessed for normality and homoscedasticity using the Shapiro-Wilk and Levene tests, respectively. The non-parametric Kruskal-Wallis and Tukey post hoc tests were then used to determine the significance of differences among experimental groups investigated. Statistical analyses were performed with the Statistical Package for Social Science software (SPSS; version 20.0; SPSS Inc., USA) with 5% significance level.

3. Results

The main objective of the present study was to assess the efficacy of GH, applied locally and associated with autologous bone, as a bone-healing inductor material. However, the results of the present study have demonstrated that experimental groups treated with GH (AB/GH+ and AB/GHL+) under the experimental conditions and concentrations

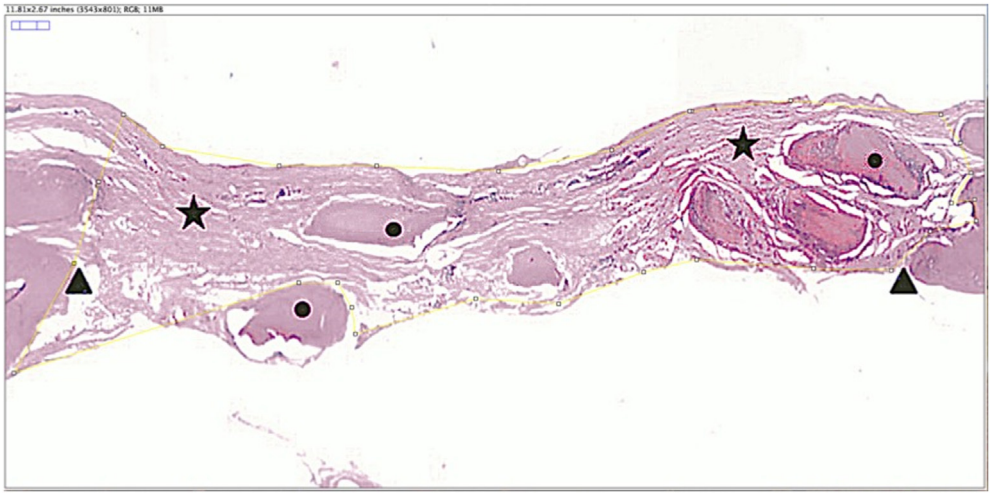

Figure 1. Spatially-calibrated digital measurements of area for TA, NBA and STA were performed on acquired images using the modified oval selection tool in ImageJ® (available at: https://imagej.nih.gov/ij/docs/guide/146-19.html#toc-Subsection-19.2). TA, NBA and STA were measured (mm^2) and TA was considered to be 100% of the area to be analyzed (Figure 1).

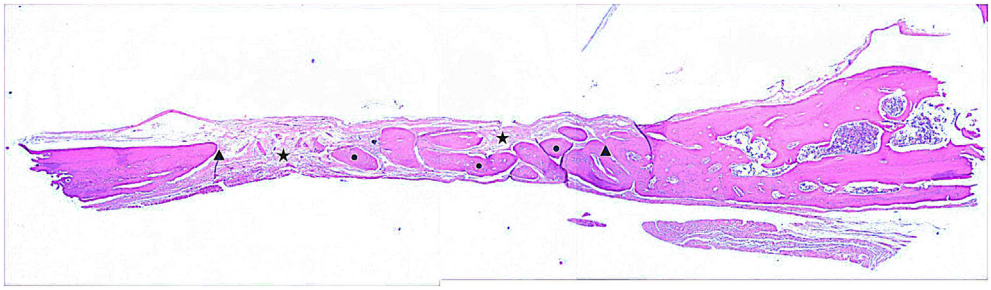

Figure 2. Representative histologic HE stained image (40x magnification) from AB (Control group) 60 days post-surgery displaying the edges of the surgical defect (triangles), the presence of autologous bone particles (spheres) and fibers of connective tissue (star) within the defect.

investigated, did not result in higher levels of newly-formed bone deposition and shorter healing processes.

3.1. Histological analysis

Results obtained have demonstrated that the majority of defects in AB were completely filled with autogenous bone particles surrounded by a dense and disorganized layer of connective fibrils, as denoted by the presence of reversal basophilic lines (Figure 2). Specimens pertaining to AB+ were characterized by the presence of particles of ground autogenous bone that were embedded in a well-organized and dense mesh of connective tissue rich in cells and blood vessels. In addition, it is also possible to observe the presence of bovine cortical membrane remnants encapsulated by pathological fibrosis within the defect area (Figures 3 and 4). The utilization of the hemostatic collagen sponge in AB+ seemed to induce the formation of dense collagen fibrils and intense collagenose activity. These findings combined suggest the formation of a tissue that mimics keloid and also displayed signs of the presence of reversal lines.

Specimens pertaining to the AB/GH+ group were characterized by the presence of few particles from the ground autogenous bone that were embedded in a dense connective tissue displaying high collagenose activity and no traces of the bovine cortical membrane (Figure 5). In the majority of specimens from AB/GHL+, the bone defect was completely filled by dense connective tissue. Such tissue was associated with the presence of multinucleated giant cells and high osteoclast activity. These findings combined suggest the occurrence of a low-intensity inflammatory process. In addition, very few particles of autogenous ground bone were observed to be present at the vicinity of the defect walls, as denoted in Figures 6 and 7.

The results from the histological analysis of the kidneys and livers of participating animals are shown in Figures 8 and 9. In the images from kidneys it is possible to observe that the cortex and glomerulus were well preserved in AB (Figure 8A) and AB+ (Figure 8B), which suggest that both experimental treatments did not adversely impact the physiology or the structure of the organs assessed. In the opposite direction, animals in the AB/GH+ displayed strong glomerulus necrosis (Figure 8C), whereas animals in AB/CHL+ were associated with large areas of protein deposition at the level of the proximal contorted tubules (Figure 8D). These findings indicate that animals in those groups displayed increased glomerular area with abnormal small-sized glomerulus and large areas of protein deposition mimicking the behavior of Bence Jones protein condition. Figure 9 shows representative histological images from livers demonstrating that, independently of the experimental group considered, all specimens analyzed displayed histological features and structures that were associated with normal and physiological liver function. In addition, it is possible to observe the presence of hexagonal hepatocytes in cordon patterns with sinusoid capillaries of normal aspect.

3.2. Histometric analysis

The histometric analysis indicated that decreasing amounts of newly-formed bone tissues were observed among the experimental groups investigated, where AB > AB+ > AB/GHL+. The bone formation in AB was significantly higher than that of AB+ ($p = 0.021$) and AB/GHL+ ($p = 0.009$), as denoted in Table 1.

4. Discussion

Previous published studies available in the literature have reported that systemic administration of GH enhances the healing of bone fractures in the tibia and femur [10, 11] and in calvarial defects in rats [1]. In addition, some studies have shown that local application of GH (either topical or irrigation), may also promote a direct and specific effect on bone tissues that seems to stimulate both bone deposition and the osseointegration of implants [3, 4, 16, 17, 18, 23].

Even though there are several well-known and accepted strategies for the administration of GH in the literature, only one study has assessed the bone regeneration potential of locally-delivered GH [19]. The results reported by the authors have demonstrated a significant and positive influence of local administration of GH on the regeneration of condylar cartilage in a rabbit model. With this in mind, the present study had the objective to assess the impact of the local administration of GH associated with the utilization of autogenous bone graft in calvarial critical size defects in a rat model. The quantity and quality of newly-formed bone tissues in defects have been demonstrated to be influenced by several factors such as defect stability and size, anatomical localization, healing period, experimental methodology, animal (species and age) and the assessment criteria [24].

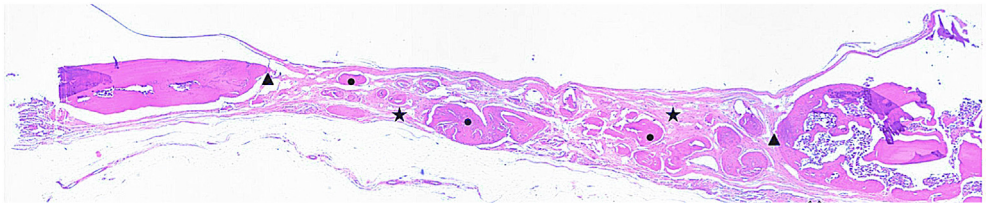

Figure 3. Representative histologic HE stained image (40x magnification) from AB+ 60 days post-surgery displaying the edges of the surgical defect (triangles), the presence of autologous bone particles (spheres), connective tissue fibers (stars) and remnants of the bovine cortical membrane at the top of the defect.

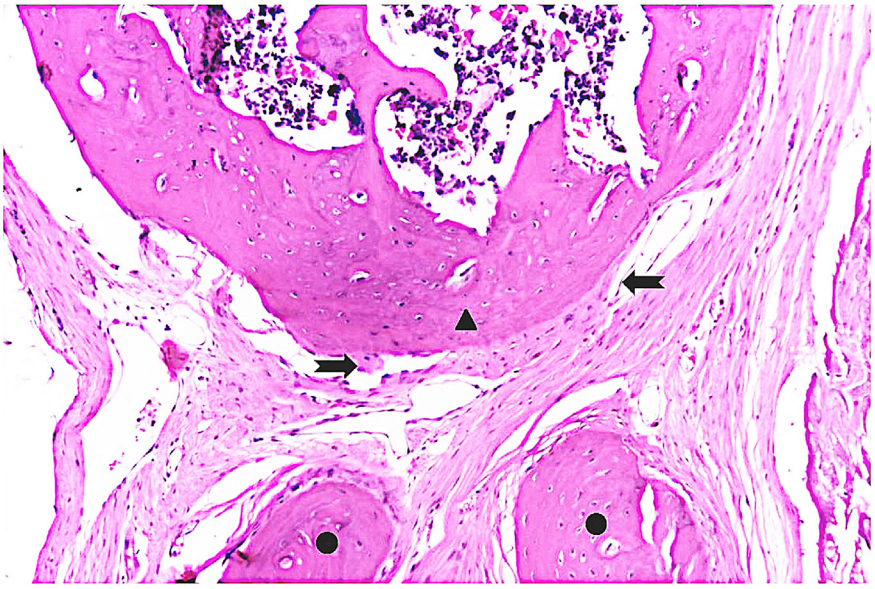

Figure 4. Representative histologic HE stained image (100x magnification) from AB+ 60 days post-surgery displaying the edge of the surgical defect (triangle), autologous bone particles (spheres) and osteoclast activity (arrows).

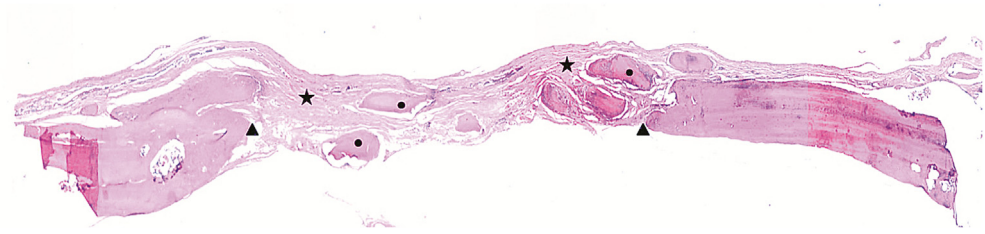

Figure 5. Representative histologic HE stained image (40x magnification) from AB/GH+ 60 days post-surgery displaying the edge of the surgical defect (triangle), few autologous bone particles (spheres) and dense connective tissue (stars) within the defect.

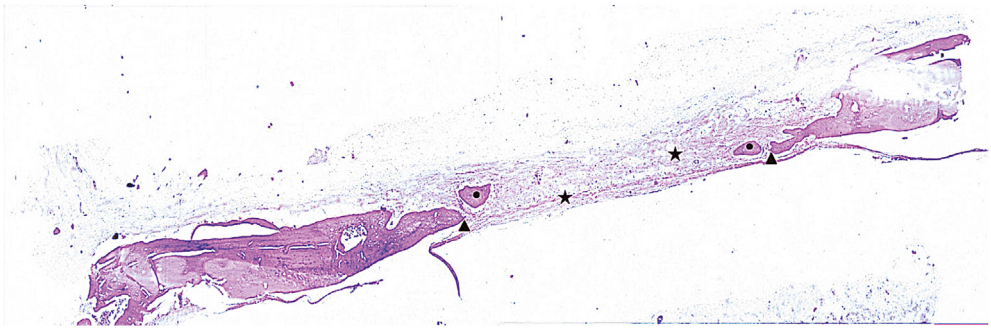

Figure 6. Representative histologic HE stained image (40x magnification) from AB/GHL+ 60 days post-surgery the bone defect was completely filled by dense connective tissue (stars) and it can be observed very few autologous bone particles (spheres). The edge of the surgical defect (triangle).

The rationale for the selection of animal model and type of defect (critical size calvarial) was based on the fact that this model has been widely used in several previous studies, and because the animal model of choice is easy to manipulate, displays fast tissue response and is cost-effective [1, 4, 24, 25]. The critical defect approach mimics a very difficult healing situation where the utility of novel regenerative approaches, such as the one investigated in the present study, can significantly improve the healing process.

The choice for the utilization of a bovine cortical membrane was based on a previous publication that has shown the importance of graft stability during the process of bone healing [24]. In addition to these important mechanical properties, the bovine cortical membrane has also been demonstrated to act as a selective barrier that physically blocks the penetration of undesired cells and tissues into the defect site, while promoting the exchange of important ions and micron-sized molecules, to upregulate the activity and migration of both stem cells and cells from the autogenous bone grafts [26]. These reports corroborate the findings of the present study, where specimens pertaining to AB+ were shown to display higher quantities of bone particulates derived from the autogenous graft when compared to specimens of AB where the bovine cortical membrane was not utilized. One study investigating the bone regeneration efficacy of calcium phosphate cements combined with platelet growth factors and human growth hormone has shown that physical

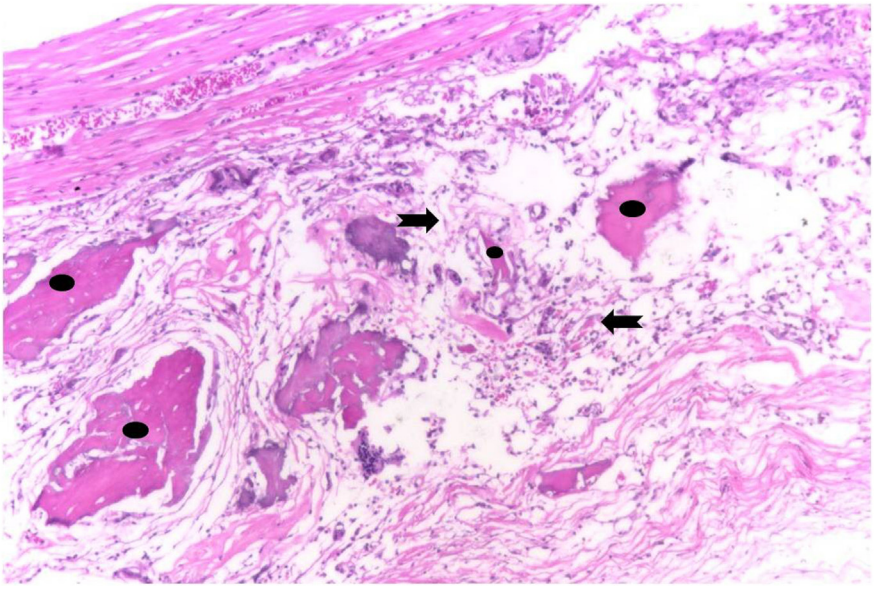

Figure 7. Representative histologic HE stained image (100x magnification) from AB/GHL+ 60 days post-surgery where it can be observed very few autologous bone particles (spheres) and intense osteoclast activity (arrows).

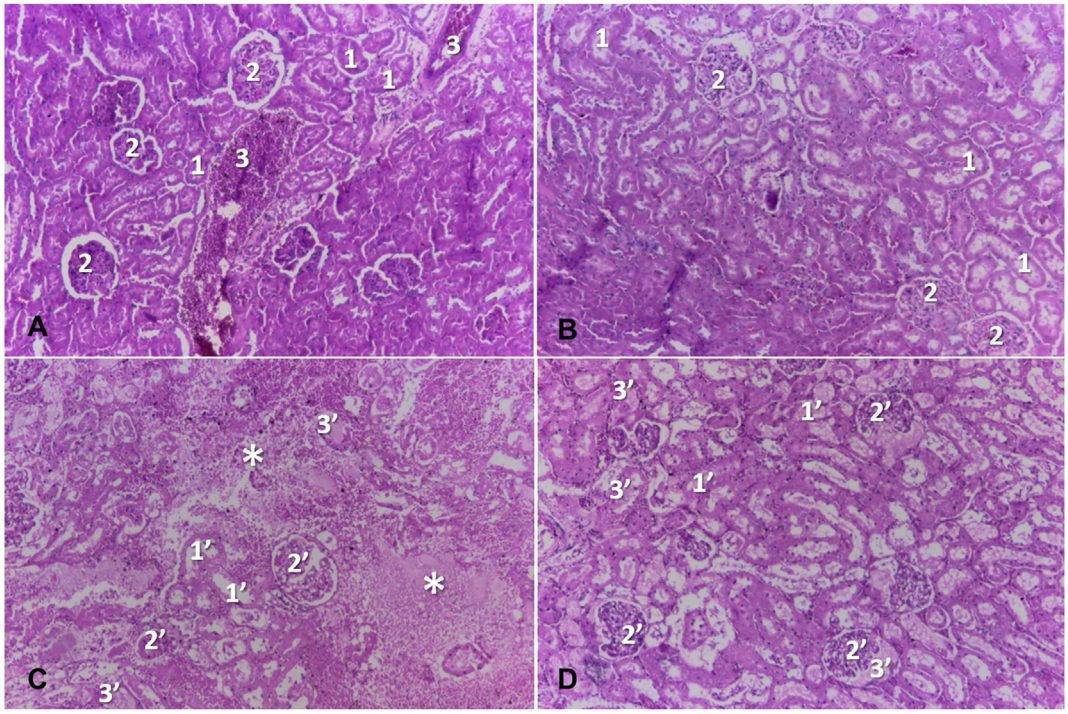

Figure 8. Representative histological HE stained images (100x magnification) from the kidneys of animals treated with (A) AB, (B) AB+, (C) AB/GH+ or (D) AB/GHL+ at 60 days post-surgery. 1 = renal tubules, 2 = renal corpuscles, 3 = large blood vessels; 1' = abnormal renal tubules, 2' = abnormal renal corpuscles, 3' = deposits in cast nephropathy, * = area of necrosis.

carriers are made necessary to immobilize liquid agents and to increase their local availability and concentration.

With this in mind, we decided to use a porcine hemostatic collagen sponge as a physical carrier for GH (groups AB/GH+ and AB/GHL+). This sponge has been previously demonstrated to be biocompatible and bioresorbable (within 15 days), accelerate the healing process and stabilize the blood cloth, while physically protecting the bone defect site [27]. Despite these relevant properties, published results have also indicated that the utilization of these sponges leads to the formation of dense collagen fibrils with intense collagenase activity, which partially explains the results observed in specimens from the group AB/GH+. Other studies have shown that such type of behavior might be associated with an antibody-antigen type of reaction [28].

The histomorphometric and histological results from the present study suggest that under the experimental conditions and GH concentrations (0.133 mg) investigated, the local administration of GH immobilized in a porcine hemostatic collagen sponge did not improve the healing of critical size calvarial defects in an in vivo rat model. Specimens pertaining to each one of the groups where the GH was used either as a locally administered irrigation solution (AB/GH+ and AB/GHL+), displayed very few remnants of bone particles from the autogenous ground bone graft, no indication of the presence of the bovine cortical membrane

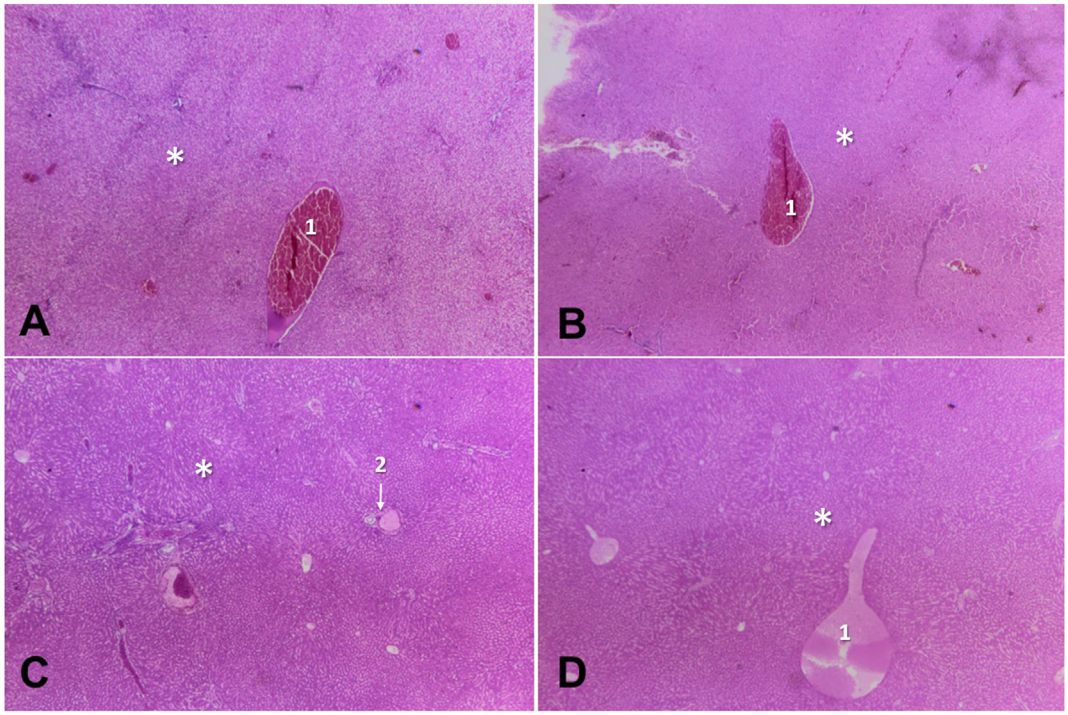

Figure 9. Representative histological HE stained images (40x magnification) from the livers of animals treated with (A) AB, (B) AB+, (C) AB/GH+ or (D) AB/GHL+ at 60 days post-surgery. 1 = central vein, 2 = portal vein, * = normal liver parenchymal aspect.

and high numbers of osteoclast cells, which indicates an intense and active bone degradation process.

A recently published study [29] investigated the systemic effect of GH over experimental orthodontic teeth movement in a rat model. The results reported have indicated that the utilization of GH delayed the formation of collagen while accelerating and intensifying the bone resorption process. In light of these results, the authors have hypothesized that GH may have had a catabolic effect, which combined with deficient local vascularization, resulted in a delayed process of bone formation. The results of the present study are well aligned with these findings where no significant bone formation or deposition was observed in specimens pertaining to experimental groups AB/GH+ and AB/GHL+. In an opposite direction [1], the healing effect of systemic administration of GH (1.35 mg/kg, 2x/day for 28 days) on calvarial critical size defects surgically created on parietal bones of rats, has been demonstrated by the systemic administration of GH combined with polytetrafluorethylene (e-PTFE) membranes, as denoted by significant improvements in the healing process and increased bone remodeling levels.

A detailed analysis of the current literature has indicated that no consensus has been reached with regards to the underlying mechanisms of action associated with either endogenous or exogenous types of GH. Despite that, it is generally understood that both GH types (endogenous or exogenous) are capable of expressing anabolic or catabolic activities because they display identical epitope amino acid sequences. It has been previously shown that the selection of GH-activity type seems to be determined in a concentration-dependent manner. However, the necessary dose to promote bone deposition and remodeling remains an open question.

It is generally understood that the hepatic production of IGF-1 is directly proportional to the concentration of GH in plasma. Previous studies have shown that the administration of high doses of GH results in high IGF-1 levels and may induce high expression of PTHrP. It has been previously demonstrated that PTHrP expresses its effects in autocrine and paracrine manners, and can imitate several actions that are typically triggered by PTH including calcium coaptation for the homeostasis of serum calcium in blood [30]. The intense bone resorption results reported in the present study, for experimental groups treated with GH (0.4 UI), have been corroborated by a previous publication investigating the signaling effects of PTHrP and PTH in osteoclasts. According to that report, the continuous administration of PTHrP or PTH induced bone resorption by significantly decreasing the activity of osteoblastic cells [31]. In addition, the authors have stated that activated PTHrP is capable of exerting catabolic activities because even though it binds to PTH-specific receptors, PTHrP does not induce inhibitory regulation through the negative feedback loop, as is observed during PTH regulation. Other studies, however, have contradicted the results of the present study and demonstrated significant depositions of bone using similar doses of GH [1, 32].

Horowitz and Stewart [33] have correlated PTHrP levels with the occurrence of malignant humoral hypercalcemia (MHH), which is a neoplastic syndrome characterized by the increased secretion of calcium-related factors. These factors are typically distributed to target tissues by the blood stream and tend to preferentially attack bones and kidneys. The hypercalcemia commonly observed in these cases is the consequence of intense and typical diffuse skeletal resorption [33].

Phosphaturia, hypophosphatemia and the increase in tubular calcium absorption, are considered the major consequences of high PTHrP accumulation in the kidneys, which was demonstrated to cause irreversible nephritic damages [34, 35], as illustrated by the results of the present study. The renal histologic results reported from GH-treated groups have indicated that the intense resorption of autogenous bone graft particles was the major factor and coincides with the renal alterations observed. Histologically, these renal alterations mimicked the behavior of Bence Jones' proteins, which is a behavior commonly occurring in multiple myeloma in MHH. Our findings suggested that GH displayed a catabolic activity, probably mediated by high PTHrP levels, that resulted in the intense resorption of the autologous bone graft.

5. Conclusion

Our working hypothesis that local administration of GH associated with autologous bone grafts would result in improved healing processes

in calvarial critical size bone defects in a rat model had to be rejected based on the results reported. Since no consensus has been reached in the literature, the present work offers additional information for conditions where the utilization of GH might not be relevant and may even adversely interfere with the normal healing process.

Declarations

Author contribution statement

Luis Henrique Chaves, Allan Fernando Giovanini, Carmen Lucia Mueller Storrer: Performed the experiments; Analyzed and interpreted the data.

Joao Cesar Zielak, Rafaela Scariot, Carla Castiglia Gonzaga, Sharukh Soli Khajotia, Fernando Luis Esteban Florez, Tatiana Miranda Deliberador: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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