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Homologous growth hormone accelerates bone healing—a biomechanical and histological study

S. Kolbeck,[a,*] H. Bail,[a] G. Schmidmaier,[a] M. Alquiza,[a] K. Raun,[b] A. Kappelgard,[b] A. Flyvbjerg,[c] N. Haas,[a] and M. Raschke[d]

[a] *Trauma and Reconstructive Surgery, Medical Faculty Charité, Virchow Clinic, Humboldt University Berlin, Germany*
[b] *Health Care Discovery-Endocinology, Growth Hormone Biology, Novo Nordisk A/S, Copenhagen, Denmark*
[c] *Institute of Experimental and Clinical Research, University of Aarhus, Denmark*
[d] *Trauma, Hand and Reconstructive Surgery, Medical Faculty, Westfälische Wilhelms Universität Münster, Germany*

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Abstract

The purpose of this study was to prove whether homologous growth hormone has a beneficial effect in the early phase of bone healing. Therefore the left tibias of 24 Yucatan micropigs were osteotomized and stabilized by plate fixation. The treatment group (12 animals) received 100 μg of recombinant porcine growth hormone (rpGH)/kg body w/day sc, whereas the control pigs (12 animals) received 1 ml sodium chloride as placebo. After a healing period of 4 weeks the animals were sacrificed and destructive torsional testing was performed. For histological evaluation 6-μm serial slices of the tibiae were stained with von Kossa. The total area of callus formation (CA) and the mineralized bone area (BA) were quantified by image analysis. The fraction of mineralized bone tissue within the callus area, the bone density (BD), was calculated as follows: BD = (BA/CA) $\times$ 100. Torsional failure load was 91% higher and torsional stiffness 61% higher in the treatment group than in the control group ($P < 0.05$). The histomorphometric measurements revealed an advance for the CA (GH: 127.6 $\pm$ 38.9 mm^2; placebo: 75.9 $\pm$ 50.7 mm^2; $P < 0.005$) as well as for the BA (GH: 89.3 $\pm$ 25.8 mm^2; placebo: 55.9 $\pm$ 38.5 mm^2; $P < 0.001$) for the GH-treated animals in comparison to the control animals. The BD was similar in both groups (GH: 70.6 $\pm$ 8.4%; placebo: 74.0 $\pm$ 6.24%; $P = 0.28$). These data indicate that administration of homologous GH stimulates callus formation and ossification in the early phase of bone healing, which consequently results in an increased mechanical strength and stiffness.

Keywords: Fracture healing; Osteotomy model; Recombinant growth hormone; Biomechanical testing; Histomorphometry

Introduction

Growth hormone (GH) is an important regulator of postnatal skeletal growth [1]. In the congenital absence of GH, long bone growth is severely compromised [2] and bone mineral density is significantly decreased by growth hormone deficiency during puberty [3]. Furthermore GH stimulates growth of cartilage and other tissue by increasing the number of cells rather than increasing the cell size [4–6]. In vitro it is well documented that GH increase bone growth by stimulating cartilage and bone cells to differentiate, proliferate, and produce extracellular matrix [7,8].

However, the effect of exogenous administration of GH in the process of skeletal repair remains controversial. GH has been reported to stimulate bone formation in osseous defects [9,10] and to enhance fracture healing in different animal species [11–18], whereas others have found that GH fails to alter callus formation in bone repair [9,19–24] (Table 1).

In a previous study a positive effect by application of recombinant homologous GH in a distraction ostegenesis model in micropigs could be observed [16]. Bone formation under distraction osteogensis follows different pathways than bone formation during secondary fracture repair. In

* Corresponding author. Trauma and Reconstructive Surgery, Virchow Clinic, Charité, Augustenburger Platz 1, 13353 Berlin, Germany. Fax: +49-30-450552901.

E-mail address: stefan.kolbeck@charite.de (S. Kolbeck).

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Table 1
The effect of GH-treatment in different animal models

Author	Species (sample size)	Model	GH preparation	Dose per injection, frequency of injection	Follow up time	Treatment period	Method of evaluation	Effect
Pankratiew (1932)	Rabbit (n = 13)	Closed metatarseal II and III fracture, plaster cast	Epiphyseal extract from unknown source (pituikrin A)	Two Injections	Mean 4 weeks; 2 groups: pituikrin only (gh: n = 2, c: n = 5), pituikrin + other drugs (gh: n = 6, c: n = 5)	5 days	Qualitative X-ray	Pituikrin A-treated animals healed faster, compared to control
Boeminghaus (1933)	Dog and rabbit (n not known)	Radial bone defect, no fixation	Bovine	0.05 g, once	Individual follow up until consolidation	1 day	Qualitative X-ray	No effect
Silberberg (1935)	Guinea pigs (n = 24)	Closed tibia and fibula fracture, wooden splint, plaster cast	Bovine	Once per day	4 groups (each group: gh: n = 4, c = 2): 6, 10, 14 and 21 days	complete follow-up time	Qualitative histology	Faster ossification of callus in GH-treated animals
Koskinen (1959)	Rat (n = 40)	Closed tibia fracture, no fixation	Human	30 tibia units, once per day	5 groups (each group: gh: n = 4, c: n = 4); 6, 10, 14, 18 and 22 days	complete follow-up time	Histomorphometry X-ray planimetry	Significant increase in callus area in GH-treated groups
Shepanek (1953)	Mouse (n not known)	Tibia fracture	Bovine	50 gamma, once per day	4 groups, (each group: gh: n = 10, c = 10), 5, 10, 15, 20 days	Complete follow-up time	qualitative histology, m, quantitative X-ray	No effect
Zadek, Robinson (1961)	Dog (n = 23)	3-cm bone defect, ulna, intramedullary fixation	Bovine	1 mg/kg bw/3 times per week	follow up time not known, (c: n = 17, gh: n = 6)	4 weeks	Method not known	None of the 17 controls healed, almost closing of discontinuity in 4 GH-treated dogs, healing in two GH-treated dogs
	Rat (n = 50)	Tibia fracture, no fixation	Bovine	50 μg, daily	28 days (gh: n = 25; c: n = 25)	Complete follow-up time	m: loads needed to disrupt the fracture	No effect
Herold et al. (1971)	Rabbit (n = 32)	1.5-cm bone defect in both radii, no fixation	Three groups; 5 animals: bovine; 5 animals; porcine, 22 animals as control	bovine: 4 USP, twice per week: porcine: 10 I.U. twice per week	4 months	Bovine GH: 3 weeks, porcine GH: 4 weeks	Qualitative X-ray	30% unions in controls, 60% unions in GH-treated, no statistical significant difference
Harris et al, (1975)	Rabbit (n not known)	open osteoclasis, plaster cast	Not known	0,46 IU, once per day	5 groups: 1,2,3,4, and 5 weeks, number of animals in each group not known	complete follow-up time	m: maximum tensile failure load	No difference between treatment groups
Northmore-Ball (1980)	Rat (n = 65)	closed femoral fracture, intramedullary nailing	Bovine	5 mg, 5 per week	5 groups: gh and control 2,3,4 weeks, controls only: 5,7 weeks, n in groups not known	complete follow-up time	m: torsional testing	Moderate stimulation in the early phase, no effect afterwards
Bak et al. (1990)	Rat (n = 36)	closed diaphyseal tibia fracture, intramedullary nailing	Recombinant human	1 mg/kg bw, twice per day	2 groups: 20 days (c: n = 11, gh: n = 9), 40 days (c: n = 8, gh: n = 8)	complete follow-up time	m: destructive three-point bending	20 day group: no effect, 40 day group: stimulation (gh-treated 400% max, stiffness and ultimate load compared to controls)
Bak et al. (1991)	Rat (n = 48)	closed diaphyseal tibia fracture, intramedullary nailing	Recombinant human	1.35 mg/kg bw, twice per day	2 groups: 40 days (c: n = 12, gh: n = 13), 80 days (c: n = 12, gh: n = 11)	complete follow-up time	m: destructive three-point bending	40 day group: no effect, 80 day group: stimulation (gh-treated 63% increase in max. stiffness, 78% increase in ultimate load compared to controls)
Carpenter et al. (1992)	Rabbit (n = 27)	standardized tibia osteotomy, external fixation	Recombinant human	150 μg/kg bw, 5 times per week	3 groups: 4 weeks (gh: n = 4, c: n = 5), 6 weeks (gh: n = 6, c: n = 4), 8 weeks (gh: n = 3, c: n = 3)	complete follow-up time	m: destructive four-point bending, In vivo compliance: quantitative X-ray	No difference between treatment groups
Raschke et al. (1999)	Minipig (n = 30)	tibia osteotomy, external fixator, distraction osteogenesis	Recombinant porcine	100 μg/kg bw once per day	25 days	complete follow-up time	m: nondestructive torsional In vivo testing and destructive torsional testing post sacrifice	Significant increase for torsinal stiffness in vivo and for torsional failure load and ultimate torsional stiffness in the gh-treated group

Note. Animal models with pituitary deficiency were not considered; m, mechanical testing; h, GH-treated group: c, control group: bw, body weight.

contrast to secondary fracture healing, distraction osteogenesis is predominantly achieved by intramembraneous bone formation that is characterized by direct differentiation of mesenchymal cells into osteoblasts without the occurence of cartilage tissues [25,26]. This differs principally from secondary bone healing where perivascular mesenchymal cells are induced to differentiate to chondroblasts [27] and with further development of the callus undergo endochondral ossification [28]. In a second study using a defect model in the same animal species we found that homologous GH application accelerates bony bridging of the defect [17].

In both studies systemic application of rpGH resulted in a marked increase of serum IGF-I levels. Although a direct effect of GH in skeletal tissue has been described [29], it is well established that the GH has an indirect, IGF-I-mediated effect on mesenchymal tissue [30]. The increase of IGF-I demonstrated an intact GH-IGF-I axis. This is in contrast to a study by Carpenter et al., who reported that administration of recombinant human GH did not accelerate the healing of tibia osteotomies in a rabbit model [20]. In this study, GH application did not effect a significant increase of IGF-I serum levels in the GH-treated group compared to the control group.

Although our former studies demonstrate the stimulating effect of GH in bone regeneration, it remains unclear whether this effect of GH could be utilized for simple situations of bone healing. The question was whether it is possible to measure an relevant advantage caused by GH application in a model comparable to the clinical situation of an simple fracture. Consequently in the present investigation we used an osteotomy model of the tibia that represents a standardized model of secondary bone healing and is also comparable to the clinical situation of a simple fracture.

For this purpose we assessed the bone healing by biomechanical parameters and performed a histomorphometrical analysis of the osteotomy area in two groups of micropigs, one serving as control and the other treated with rpGH.

Materials and methods

Animals

All procedures were undertaken in compliance with the guidelines for the care and use of animals as described in the *American Journal of Physiology*. Study protocols were reviewed and approved by the local governmental animal rights protection authorities and supervised by the local animal protection officer. Twenty-four mature female Yucatan minipigs with a mean age of 13 months (11–17 months) were used. Every animal was weighed before surgery. The mean body weight for the rpGH-treated animal was 39.0 kg and for the control animals 37.6 kg. The animals were matched by age and weight prior to surgery. Animals were housed in groups of six during the experimental period and fed a standard pellet cereal foodstuff (300 g/day) and water ad libitum.

Surgery

Prior to surgery all animals received a subcutaneous implantable port system (Vascular Access Port, Access Technologies, Skokie, IL) to provide easier access for blood sampling and intravenous injections. Animals received 2 mg metomidate hydrochloride intravenously as preanesthetic sedation and were then induced intravenously with sodium thiopental. The micropigs were intubated and muscle relaxation was achieved with pancuronium bromid. General anesthesia was maintained with sodium thiopental and fentanyl dihydrogenecitrate for the duration of surgery. The right hind limb was prepared in the usual sterile fashion and a skin incision was made in the middle of the tibia using an anteromedial approach. After preparing the subcutaneous tisue a 9- or 10-hole 3.5-mm dynamical compression plate (Synthes, Bochum, Germany) was contoured to the medial surface of the intact tibia under fluoroscopic control. The plates were provisionally held to the tibia and all holes were predrilled with a 2.5-mm drill bit. Using an oscillating saw a 1.0-mm osteotomy was created. Without suturing the periosteum the plate was attached with 3.5-mm corticalis screws. The subcutaneous tissue was closed with absorbable suture, the skin with nonabsorbable suture. A sterile dressing was then applied. Posteroanterior X-rays were performed after the operation and the animals returned to their cages (Fig. 1).

Perioperative antimicrobial prophylaxis, consisting of amoxicilline, clavulanate, and depot benzylpenicilline, was administered. Full weight bearing was allowed immediately after surgery. Analgesia was maintained by intravenous administration of flunixine meglumine for 3 postoperative days. The animals were visited twice daily. Wound inspections, temperature measurements, and radiographic examinations were performed the entire study period until killing (Fig. 2).

Recombinant porcine growth hormone

Minipigs in the treatment group received a daily subcutaneous injection of 100 μg of recombinant porcine GH (rpGH)/kg body wt (met-pGH, Bresa Gen Ltd., Adelaide, Australia), whereas minipigs in the control group received sodium chloride as a placebo. The injections were given in a marked neck skinfold every day between 8.00 and 10.00 A.M., starting on the day of surgery and continuing until the day of sacrifice.

Measurement of serum IGF-I

After sedation and intubating of the animals before implantation of the port system 20 ml of blood was drawn from an ear vein to obtain initial values of the serum IGF-I. The animals were sedated every fourth day (4, 8, 12, . . . ,

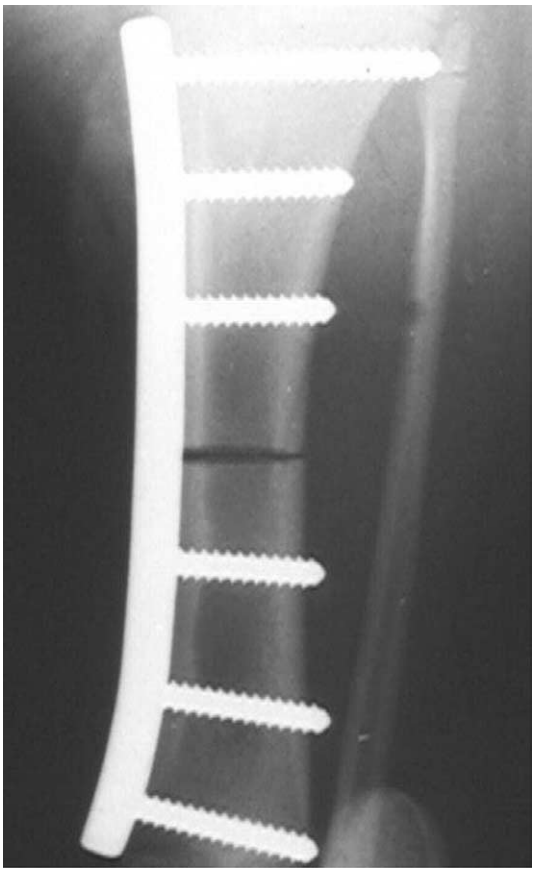

Fig. 1. Posteroanterior radiograph after surgery showing the osteotomy and the plate on the medial side of the tibia.

28) after surgery with intravenous metomidate for blood drawing and radiography.

The blood was immediately centrifuged and the serum frozen at −80°C. Acid ethanol was used to remove the IGF-binding proteins [31]. Serum extracts were diluted in assay buffer (final dilution, 1 in 1000) and total IGF-I serum levels were determined with a noncompetitive time-resolved immunofluorometric assay (TR-IFMA) as previously described [32]. Briefly, two sets of monoclonal IGF-I antibodies were used: the first was immobilized on microtestplate wells (IGF-I MAB from Novo-Nordisk A/S, Denmark), and the second was labeled with europium (Eu^{3+}) (IGF-I, Diagnostic System Laboratories Inc., Webster, TX, USA). Biosynthetic human IGF-I (hIGF-I, Amgen Biologicals, CA, USA; Distributor: Amersham Int., Amersham, Bucks, UK) served as standards. Detection limit was 0.0025 μg/liter, and the operating range was 0.005 to 2.5 μg/liter. The calibration curve was linear in this interval. Intraassay and interassay coefficients of variation were less than 5 and 10%, respectively.

Biomechanical testing

After 28 days the animals were narcotized with intravenous sodium thiopental, and circulation was halted with potassium chloride. After killing both tibiae were harvested, all soft tissue was dissected, and the plates were carefully removed. The tibia were embedded into polymethylmetacrylate, keeping a constant distance of 7 cm between the embedded bone ends and mounted in a material testing machine (Zwick 1455, Zwick GmbH, Ulm, Germany). Each specimen was kept moist through biomechanical testing. A preload of 1 Nm was applied and the construct was then loaded in torsion under displacement control of 10°/min until failure. Torsional failure load (yield load) and torsional stiffness were determined from the load displacement curve.

Histomorphometrical analysis

Histological preparation

After biomechnical testing the tibiae were prepared for histological evaluation. The osteotomy zone and 2 cm of adjacent cortical bone was divided equally in 3-mm-thick sagittal sections using a precision diamond grinding saw (Exakt, Norderstedt, Germany). These sections were embedded in methylmethacrylate (Technovit 9100, Kulzer, Germany). Six-micrometer serial slices were produced using a hard-cutting microtome (Polycut, Leica, Cambrige, UK). For assessment of the calcified tissue, the sections were stained due to the modified von Kossa method.

Image analysis

The microscopic image of the region of interest was digitized by a 3-Chip CCD color camera and processed using the LEICA Quantimet image analysis work station (Leica, Bensheim, Germany). The measurements were performed within a standardized region of interest (ROI). This region was defined by a line parallel to osteotomy proximally and distally. The distance of this line was determined by halfening the bone diameters at the osteotomy zone (Fig. 3). The cortical bone ends were excluded from this ROI and measured separately. In each animal three locations were evaluated: The center slice and two slices situated 3 mm laterally and medially, respectively. From each location measurements were performed using two slices in a distance of 30 μm. In total, the means from 6 measured slices were calculated in each animal. The ROIs were digitized and the calcified structures were binarized using image segmentation. With specially developed algorithms the following parameters were determined by a pixel-based measurement: the total area of callus formation (CA, in square millimeters) and the bony tissue within the callus area, the mineralized bone area (BA, in square millimeters). From these parameters we calculated the fraction of mineralized bone tissue within the total area of the callus, the bone density, as follows: $BD = (BA/CA) \times 100$

Statistical analysis

The Kolmogoroff–Smimov goodness-of-fit test was used to ensure that data were normally distributed. A repeated-

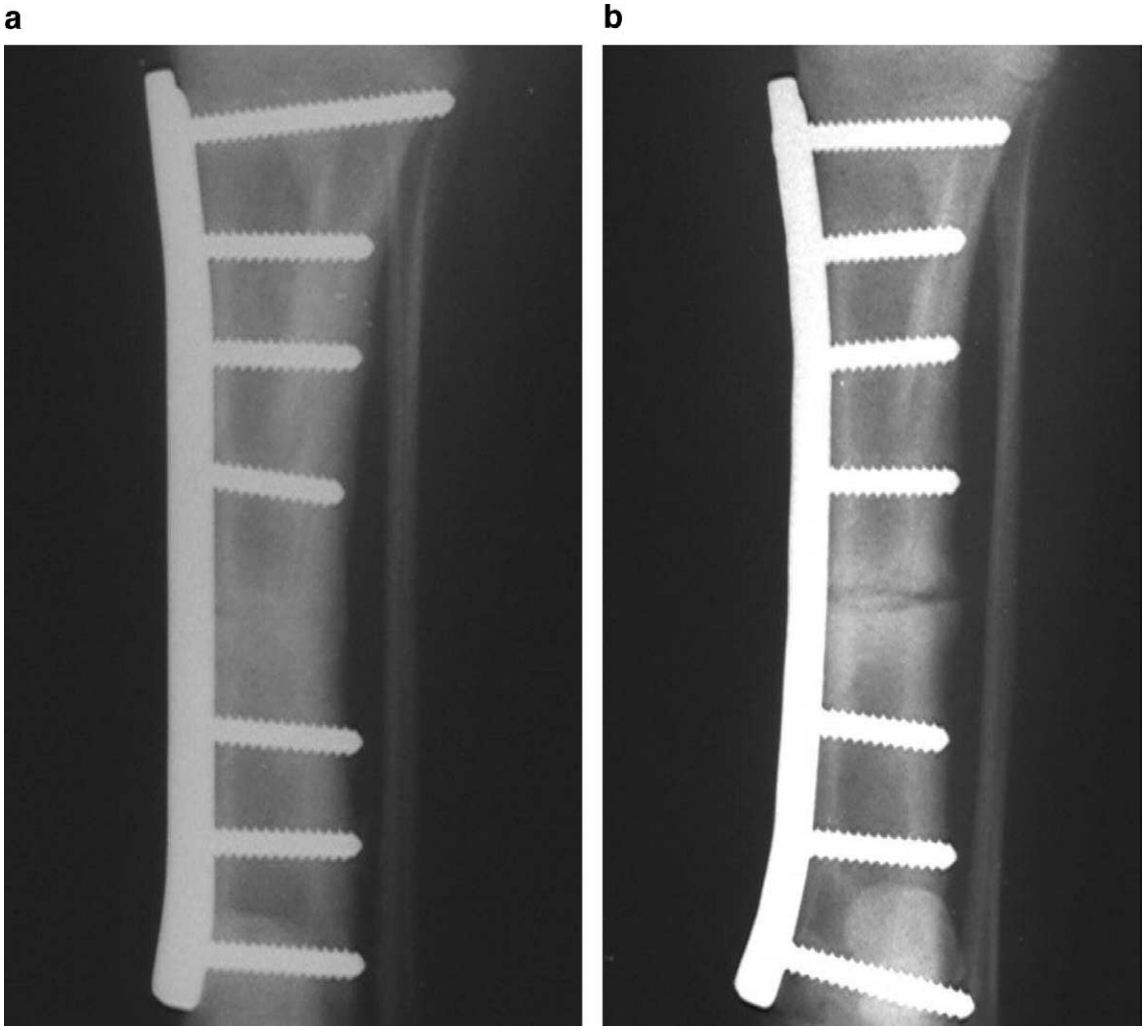

Fig. 2. Conventional X-ray the day before sacrifice. (a) GH animal: the osteotomy is completely bridged; (b) control animal: the osteotomy gap is still visible.

measures analysis of variance (ANOVA) was performed between IGF-I data of both groups. An analysis of variance was performed to test the influence of covariates: supplied drugs, age, and preoperative weight. An independent sample *t* test was used to determine differences between the groups in CA, BA, BD, torsional stiffness, and maximum torsional failure load. Data are expressed as the mean ± standard deviation. All statistical analysis was carried out using the SPSS software package (Statistical Package for Social Sciences, SPSS Inc.).

Results

Clinical data

No animal was excluded from the study. All pigs appeared healthy throughout the experiment. Postoperatively they tended to limb for 1–3 days and then they loaded their legs fully for the time up to sacrifice. No wound infection was observed. The regularly measured body temperature revealed no significant difference between the rpGH-treated and control animals. In both groups a slight increase of the mean body weight was seen. That if the rpGH-treated group rose from 39.0 kg preoperatively to 43.2 kg at the day of sacrifice, whereas that of the control group rose from 37.6 to 40.8 kg. A statistically significant effect for the mean body weight by application of rpGH was not observed.

Radiographic impressions

In the regularly performed posteroanterior X-rays an earlier callus formation could be observed in the rpGH-treated animals. In most of these animals a remarkable callus formation in the osteotomy gap was visible between day 12 and day 16, whereas the control animals showed only slight or no callus at these time points. At day 28 most of rpGH-treated animals demonstrated a completely bridg-

Fig. 3. Six-micrometer slice stained by von Kossa demonstrating the standardized region of interest (ROI). This region was defined by lines parallel to the osteotomy in a distance of half of the bone diameter at the osteotomy. The cortical bone ends were excluded.

ing of the osteotomy, whereas in the control animals the osteotomy gap was still visible (Fig. 2).

Serum IGF-I levels

The mean level of serum IGF-I increased in the GH-treated animals from the pretreatment level (197 ± 22 ng/ml) to 1046 ± 65 ng/ml at day 12 and then remained nearly constant (day 28: 1149 ± 76 ng/ml), whereas the IGF-I levels of the control group decreased from the pre-treatment level (309 ± 28 ng/ml) to 198 ± 15 ng/ml at day 12 and then remained nearly unchanged (day 28: 241 ± 14 ng/ml). A repeated-measures ANOVA showed a significant difference between both groups starting at day 4 for every examination point ($P < 0.001$) (Fig. 5).

Biomechanical testing

During biomechanical testing the tibiae were observed and the location of the fracture was documented. In every case the fracture line was inside the osteotomy gap. In no case the fracture fails through a screw hole. Concerning the intact contralateral tibiae we found for both groups torsional fractures in the diaphysis of the tibiae.

The tibiae of the rpGH-treated group exhibited 91% higher mean torsional failure load than the tibiae of the placebo group (GH: 17.14 ± 4.66 Nm; placebo: 8.96 ± 4.91 Nm; $P = 0.001$). The mean torsional stiffness was 61% higher in the treatment group (2.54 ± 0.92 Nm/°) than in the control group (1.58 ± 0.90 Nm/°) ($P < 0.05$) (Table 2).

In relation to the intact contralateral tibia, the tibia in the treatment group and the control group reached 67.66 ± 24.37 and 29.45 ± 14.16% of the torsional failure load, respectively ($P = 0.001$). The values for torsional stiffness were 130.87 ± 43.17% for the GH group and 76.77 ± 43.60% for the control animals ($P = 0.002$). (Fig. 6)

Histomorphometrical analysis

The histomorphometric measurements revealed an 68% advance for the total area of callus formation for the GH-treated animals in comparison to the control animals (GH CA: 127.6 ± 38.9 mm^2; placebo CA: 75.9 ± 50.7 mm^2; $P < 0.005$). The mineralized bone area for the GH treated animals was 60% higher than in the control group (GH BA: 89.3 ± 25.8 mm^2; placebo BA: 55.9 ± 38.5 mm^2; $P < 0.001$) for the GH-treated animals (Figs. 4 and 7).

The fraction of mineralized bone tissue within the total area of the callus, the bone density was similar in both groups (GH BD: 70.6 ± 8.4%; placebo BD: 74.0 ± 6.24%; $P = 0.28$) (Fig. 8).

Discussion

Conflicting results have been published since 1930s concerning the influence of exogenous growth hormone administration on healing fractures and bone defects. Starting with Pankratiew in 1932, who observed a stimulatory effect on callus formation in healing metatarsals of rabbits by injection with an extract from anterior pituarities [15], different results in various animal models occurred in the following decades [18,19,23,33]. The first stimulation of growth hormone on fracture healing in humans was described by Cordebar, who reported a positive effect of GH in a series of case histories [34]. Koskinen demonstrated increased callus formation and faster stabilization in rat tibial fractures when

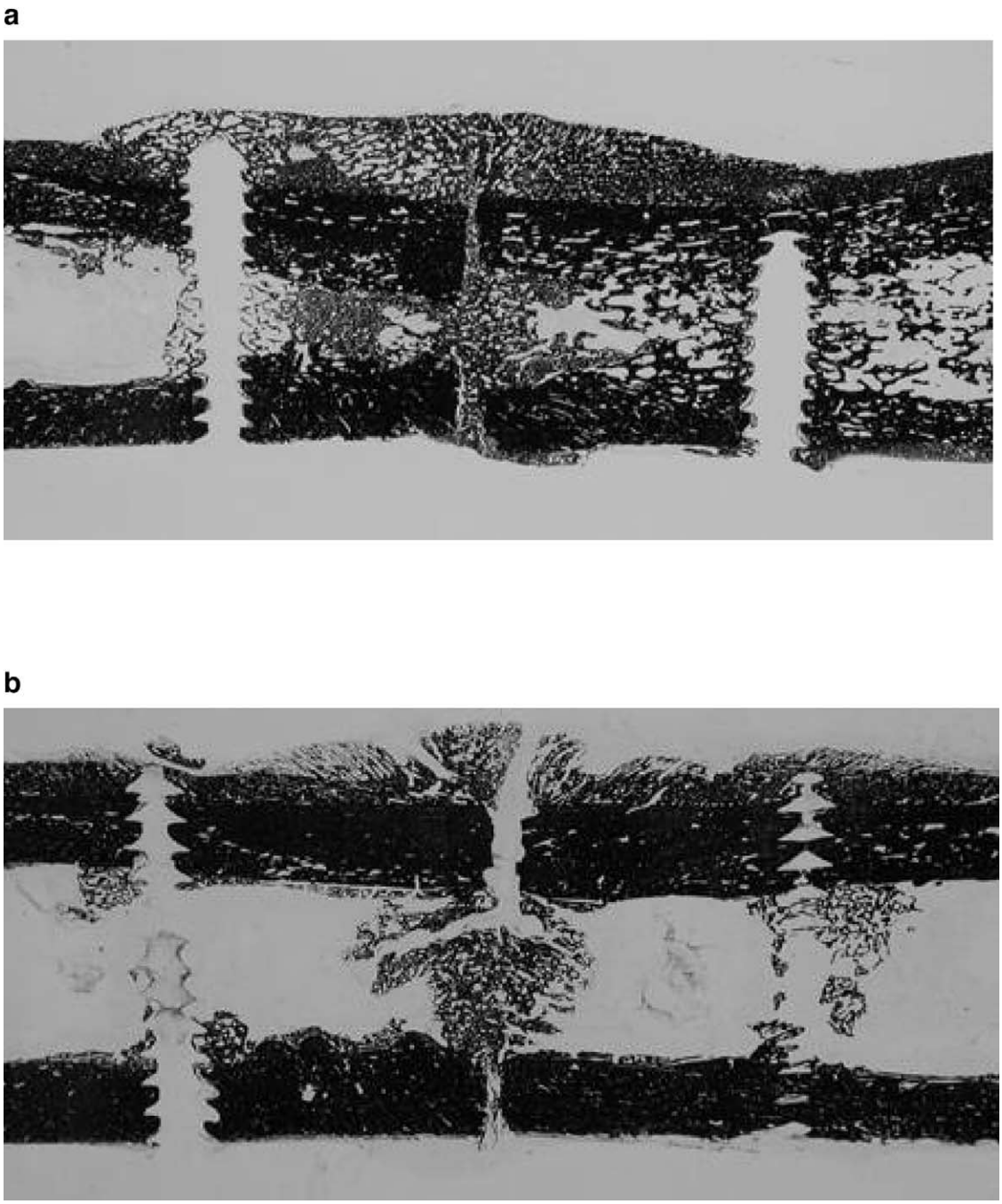

Fig. 4. Six-micrometer slice stained by von Kossa. (a) GH animal: complete bridging of the osteotomy; (b) control animal: no bony callus formation inside the osteotomy.

growth hormone was given daily [14]. In subsequent clinical studies Koskinen found that growth hormone treatment stimulated the healing of fractures and pseudarthrosis [35]. The significance of theses results are impaired by the inclusion of a variety of fractures without randomization. In the following years several studies demonstrated the beneficial effect of GH on fracture healing [24,36–39], whereas a number of other studies reported that GH has no stimulatory effect on repair of fractures [9,21,22,40]. Due to the lack of availability of GH until the 1980s the early published studies are characterized by inhomogenity of the treatment and control groups, different sources of GH, and insufficient animal gender and age. Because genetically produced GH is available the more recent experiments using recombinant GH focus on biomechanic parameters and histological results. But the effect of GH on fracture healing is still controversial. Bak et al. found a significant increase of torsional stiffness and strength in different fracture models using recombinant human GH in rats [11,12]. This is in contrast to Carpenter, who did not find an effect of recom-

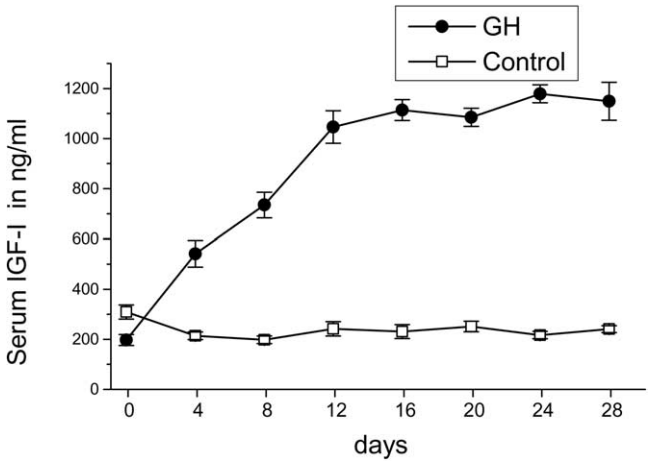

Fig. 5. Results of the IGF-I measurements. Statistical analysis showed significant differences ($P < 0.001$, ANOVA model for repeated measurements) between the rpGH-treated and the control groups (mean values ± SEM).

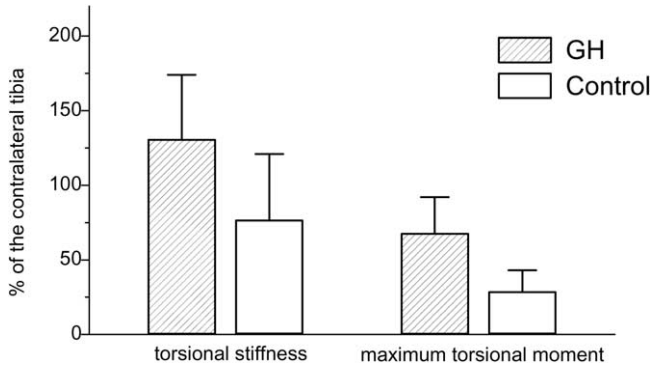

Fig. 6. Results of the biomechanical testing. Statistical analysis showed significant differences ($P < 0.05$; independent-sample t test) between the rpGH-treated and the control group.

binant GH in a rabbit osteotomy model determining bending stiffness and strength [20].

Like Carpenter we used a well-defined osteotomy model for secondary bone healing in the present study. But in contrast to his results we could demonstrate that systemic application of recombinant homologous GH promotes bone healing substantially in our micropig animal model. The biomechanical parameters, torsional stiffness, and torsional failure load of the osteotomy tibia in the rpGH-treated group were 61% respectively, 134% higher than in the placebo group. We found that the mean torsional stiffness of the osteotomy tibiae in the rpGH-treated group was 135% higher than in the contralateral tibia (placebo group: 84%). The torsional failure load in the rpGh-treated group was 68% of the contralateral tibia in comparison to 29% in the placebo group. The fact, that torsional failure load is lower than the torsional stiffness has been described by Connolly, who found that rigidity in weight-bearing bones returns more rapidly than strength [41].

Concerning the effect of rpGH application on the intact contralateral tibiae a negative effect in terms of the biomechanical parameters could not observed. The mean torsional failure load of the contralateral tibia in the rpGH-treated group was slightly less than in the control group, but not statistically significant, whereas the mean torsional stiffness was almost identically.

Although it has been found that GH stimulates osteoclastic resorption through both direct and indirect actions on osteoclast differentiation and indirect activation of mature osteoclasts [42], in the presented study we found no effect on the contralateral tibiae. This might due to the relative short application (28 days) in our study. To evaluate long-term effects of GH adminstration further studies with different dosages and longer follow-up times are necessary.

In contrast to most other studies where the influence of GH on fracture healing in long bones was evaluated either by biomechanical parameters or histologically analysis, we performed both methods. The histomorphometric measurements revealed the reasons for the advantage of the GH-treated pigs concerning the biomechanical parameters. The mean callus area (CA) of the GH-treated animals was significantly higher (68%) than in the placebo animals, indicating that GH has an initially stimulatory effect on callus formation. A similar result was achieved for the mean bone area (BA) where the GH treated group was 60% higher than in the placebo group, indicating a faster ossification in the GH-treated group. The bone density (BD) values for the GH-treated groups and the placebo groups were similar. In our opinion these data demonstrate that the structure of the callus tissue in both groups is comparable. This is in con-

Table 2
Results of final biomechanical testing[a]

	rpGH ($n = 11$)	Placebo ($n = 11$)	Significance level[b]
Torsional failure load (Nm) of defect tibia	17.14 ± 4.66	8.96 ± 4.91	$P = 0.001$
Torsional failure load (Nm) of contralateral tibia	26.39 ± 4.52	30.15 ± 3.27	ns
Torsional failure load (Nm) of defect tibia in percentage of contralateral tibia	67.66 ± 24.37	29.45 ± 14.16	$P = 0.001$
Torsional stiffness (Nm/°) of defect tibia	2.54 ± 0.92	1.58 ± 0.90	$P < 0.05$
Torsional stiffness (Nm/°) of contralateral tibia	2.01 ± 0.73	2.03 ± 0.46	ns
Torsional stiffness (Nm/°) of defect tibia in percentage of contralateral tibia	130.87 ± 43.17	76.77 ± 43.60	p = 0.002

[a] Data presented as mean ± SD.
[b] Independent sample t test (ns, not significant).

trast to the histological data from Mosekilde et al., who found in a fracture model in rats also a larger amount of callus after GH application, but the new formed callus presented a more loose structure compared with placebo animals [43].

The biomechanical and histomorphometrical advance of the GH-treated animals could be confirmed also radiographically. In the GH-treated group in most animals at day 28 the osteotomy gap was completely bridged with callus, whereas in the control group in most animals only a profuse bone formation was observed and the osteotomy was still visible.

The action of GH on bone cells is still not fully understood. A widely discussed question has been whether GH acts directly or whether the effect is mediated by a liver-derived growth factor, initially called sulfation factor, but later renamed IGF-I. In the original somatomedine hypothesis, GH stimulates skeletal growth by stimulating liver production of somatomedines, which, in turn, stimulates longitudinal bone growth in an endocrine manner [30,31,44]. In the early 1980s the somatomedine hypothesis was challenged. For the epiphyseal growth plate, it has been shown that GH has a direct effect, resulting in an increase longitudinal bone growth [29], and that both IGF-I mRNA [2] and IGF-I expression are increased in the growth plate [45]. Regardless of a direct or indirect effect of GH on bone growth or bone healing an intact GH-IGF-I axis is necessary for mesenchymal tissue response to GH.

In our study daily subcutaneous application of 100 μg rpGH/kg body wt resulted in a marked increase in IGF-I serum levels. The IGF-I levels of the GH-treated animals steadily increased during the first 12 days of the observation period and remained constant at a fivefold level relative to the preoperative baseline level. In contrast we found a significant decrease during the first 12 days of the IGF level in the placebo group relatively to the baseline level. Although the serum IGF-I levels may not reflect the local IGF-I level the increase of IGF-I after systemic application of GH indicates that the GH-IGF-I axis is intact and that the dosage for the GH therapy is efficient.

In this study we used homologous porcine GH. Therefore

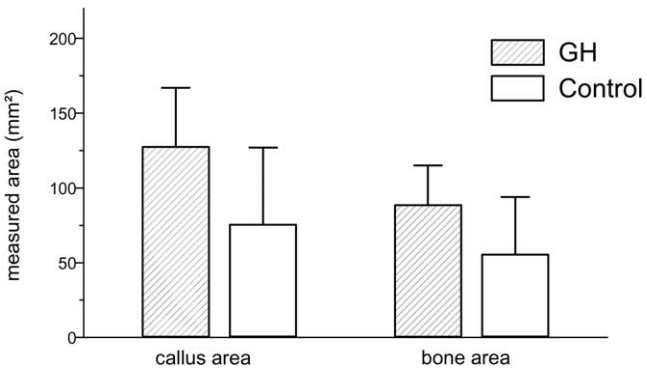

Fig. 7. Results of the histomorphometric analysis. Callus are a (CA) and bone area (BA) showed significant advance ($P < 0.05$; independent-sample *t*-test) of the GH-treated group.

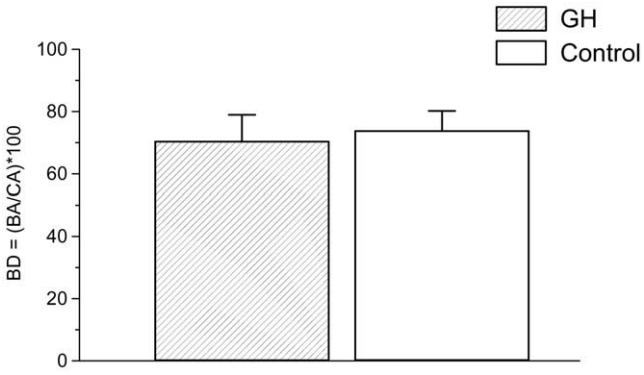

Fig. 8. The bone density showed no significant difference between the GH-treated and the control groups ($P = 0.28$; independent-sample *t* test)

the antibody formation against allogene GH that is described in the literature is very unlikely [10,39,46]. The results presented here are consistent with our previous studies. In the first study we found a pronounced acceleration of bone regenerate consolidation by rpGH administration in a distraction osteogenesis model [16]. In the second study we used a defect model representing a fracture with segmental bone loss. We could demonstrate the beneficial effect of GH in accelerating bone repair under these circumstances [17].

The purpose of the present study concerned whether GH application leads to a significant advance in a standardized model of bone healing that is comparable to the clinical situation of an simple fracture. The histomorphometrical data as well as the biomechanical results demonstrated that the potential of homologous GH in accelerating bone repair could be utilized even in simple situations of bone healing. In contrast to locally active growth factors (such as bone morphogenetic proteins or transforming growth factors) for the application of GH open access to the fracture is not necessary. The systemic approach of GH administration might therefore be viable alternative as a method for enhancement fracture healing. Although future studies especially concerning the dosage of GH are necessary our findings strongly suggest that recombinant GH administration could be used clinically in the future to accelerate the treatment of fractures.

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