Enhancement of bone formation in rabbits by recombinant human growth hormone

Anders Ehrnberg[1], Otte Brosjö[1], Peter Låftman[1], Olle Nilsson[3] and Lennart Strömberg[2]

We studied the effect of human recombinant growth hormone on diaphyseal bone in 40 adult rabbits. The diaphyseal periosteum of one femur in each animal was mechanically stimulated by a nylon cerclage band. The bands induced an increase in bone formation, bone mineral content, and maximum torque capacity of the diaphyseal bone at 1 and 2 months. Growth hormone enhanced the anabolic effect of the cerclage bands on bone metabolism, evidenced by a further increase in torsional strength of the femurs.

[1]Department of Orthopedics, and [2]Departments of Surgery and Experimental Surgery, Karolinska Hospital, Stockholm, and [3]Department of Orthopedics, Uppsala University Hospital, Uppsala, Sweden. Correspondence: Dr. A. Ehrnberg, Department of Orthopedics, Karolinska Hospital, S-104 01 Stockholm, Sweden. Tel +46-08 7292000.
Submitted 93-01-21. Accepted 93-08-15

In a previous study on the effects of recombinant human GH on the regeneration of experimentally atrophied bone in adult rabbits, the periosteal new bone, formed in response to surgical trauma, was increased 2–5 times in GH-treated animals, while the mechanical properties of the diaphyseal bone were not affected (Låftman et al. 1988). This effect was attributed to increased new bone formation by the periosteum in response to non-specific surgical trauma. We report the effects of recombinant human growth hormone on periosteal new bone formation in response to stimulation by the application of a plastic cerclage to one femur diaphysis in the rabbit.

Animals and methods

40 healthy adult rabbits (Swedish Land Breed) of both sexes, weighing 3.5–4.2 kg were used. The animals were housed in separate cages and given a standard diet and water ad libitum. The rabbits were randomized into 4 groups of 10. The groups had similar weights and sex. ^{45}Ca 8 μCi/kg body weight, and tritiated proline 16 μCi/kg body weight (Amersham AB, Stockholm, Sweden), were given as a single i.m. injection 2 days before death.

Plastic cerclage bands similar to those described by Partridge (1976) were used in order to achieve mechanical irritation of the periosteum. The bands (Sta-Straps, Panduit Corp., Tinby Park, IL, U.S.A.) are 2.4 mm wide and 0.9 mm thick, provided with a locking device. The inner surface, facing the periosteum, has elevations 1 mm high and 2 mm long, 3 mm apart. The material of the bands can be considered as biologically inert in this context.

All the animals survived surgery and the subsequent medication without any complications. At death, all cerclage bands were firmly attached to the femurs by closely appositioned ridges of newly-formed periosteal bone.

Surgery

The rabbits were anesthetized using Hypnorm Vet. (Jansen, Bruxelles, Belgium) and Diazemuls (KabiVitrum AB, Solna, Sweden) 1:1 given i.m. 1 mL/kg body weight. All operations were performed under strictly sterile conditions. A lateral incision was performed on one of the thighs, chosen at random. The femur mid-diaphysis was exposed by blunt dissection along the intramuscular septum posterior to the vastus lateralis muscle. An extraperiosteal tunnel was created at the mid-portion of the femur shaft. A cerclage band was applied around the femur diaphysis. The band was tightened to a loose fit by means of a device that creates a standardized tension. The fascia and skin were closed with absorbable and non-absorbable sutures, respectively. The contralateral femur was used as control, not subjected to surgery.

Growth hormone treatment

The rabbits in the 2 test groups were treated with daily s.c. injections of recombinant human growth hormone (Genotropin®, KABI AB, Stockholm, Sweden) 0.3 IE per kg body weight from the day of surgery until

death. The control animals received daily injections of 0.5 mL sterile saline solution as placebo.

After 1 and 2 months, the animals were killed with an i.v. dose of pentobarbital sodium and methanol. The femurs were immediately dissected free from all soft tissues and connecting bones, but all periosteal tissue was preserved, and the cerclage bands were left in place. The bones were kept moist with a physiological saline solution prior to bone mineral content (BMC) determinations, and then wrapped in saline-soaked gauze, put into plastic bags, and frozen. Prior to torsion tests, the bones in the bags were put in a physiological sodium solution, +40 °C. After 3 hours the bones had a temperature of about +35 °C and were tested. After the torsion tests, a segment of approximately 2 cm of the diaphyseal bone adjacent to the place of the nylon bands was processed for analysis of isotope incorporation.

Bone mineral content

Bone mineral content determinations were performed on intact bones, with the bands in situ. The whole femur was scanned transversely in 1 mm increments. The single photon bone mineral scanner (Nuclear Data, Uppsala, Sweden) used ^{125}I as the radiation source. The section of the test bone with increased mineral content could be clearly delineated from a graph of the scan data (Figure 1). The ratio of the BMC values from this section and the corresponding control bone were calculated.

Mechanical properties

The cerclage bands were removed and the femurs were tested to final failure on inward twist at 10° per second. The tests were performed using a computerized test equipment with high precision (mean test error < 0.9 percent), as described by Strömberg and Dalén (1976 a, b) and Låftman et al. (1980). All but 2 of the hormone-treated bones failed by a spiral fracture through the mid-segment with some intermediate fragments. The bones which fractured outside the mid-segment were not excluded from the study. Maximum torque capacity (Nm), accumulated energy before final fracture (joule), and stiffness (Nm per degree) were recorded.

^{45}Ca and ^{3}H-proline Incorporation

Estimates of bone formation rates were made by measuring the incorporation in the femurs of ^{3}H-proline, reflecting the synthesis of bone matrix, and ^{45}Ca as a marker of the mineralization. Several samples were taken from the diaphyseal bone as close as possible to the site of the cerclage band, and from the corresponding part of the control bone.

Samples were ashed in a muffle furnace at 600 °C for 24 h, weighed and dissolved in 0.6 M HCl about 5mL/100 mg ash. To 0.5 mL samples were added 4 mL ATC scintillation solution (New England Nuclear). The samples were counted for ^{45}Ca in a Beckman LS 1702 liquid scintillation counter. The 2–300 mg (wet weight) samples processed for ^{3}H analysis were demineralized in 20 mL 0.6 M HCl for 24 h, lyophilized and weighed. The organic residues were hydrolyzed in a mixture of perchloric acid/hydrogen peroxide 1:2, 1.5–3.0 mL at 70 °C for 1 h, followed by the addition of 1 mL of the hydrolysate to 15 mL of scintillation fluid containing toluol/cellusol 2:1 and 6 mg PPO (2,5-diphenyloxazole) per liter toluol, prior to counting. The ^{3}H-proline and ^{45}Ca activities were determined as the means of 2–3 samples and were calculated as CPM per mg dry weight or ash weight, respectively. The activity (percent of given dose/g ash or dry weight) of each of the 2 isotopes was calculated and given as a ratio of cerclage-treated to control.

Statistics

The ratios for cerclage-treated to control bones were calculated for the different parameters in each rabbit. The effects of the cerclage band were calculated using the two-tailed Wilcoxon rank sum test for paired samples in the 4 groups. The groups of GH-treated and control animals were then compared at 4 and 8 weeks, using the Wilcoxon two-tailed rank sum test. Prior to testing, it was decided that $P < 0.05$ should be considered significant.

Results

The mid-diaphysis of the cerclaged femurs displayed a 7–8 percent increase in BMC in all groups ($P < 0.05$) compared to the control bone (Table 1). The increased BMC was localized within a well defined segment of the bone, corresponding to the band and its immediate surroundings (Figure 1). In most animals, ridges with increased BMC could be delineated at the proximal and distal margins of the band. The increase in BMC was consistent in all groups and the variations were small within the groups.

Calcium and tritium isotope activities were increased by 58 and 42 percent, respectively, at 1 month, and by 38 and 33 percent at 2 months ($P < 0.05$). In contrast, the mechanical properties of the cerclage-treated bones were not affected, compared to the

Table 1. Ratios (cerclage band-treated bone to the control) of bone mineral content and isotope activities. Mean, *SD*

	1 month		2 months	
	Saline	GH	Saline	GH
BMC	1.07 *0.02*	1.08 *0.02*	1.08 *0.02*	1.09 *0.02*
^{45}Calcium activity	1.58 *0.69*	1.50 *0.37*	1.38 *0.51*	1.46 *0.44*
^{3}H activity	1.42 *0.39*	1.50 *0.28*	1.33 *0.29*	1.44 *0.33*

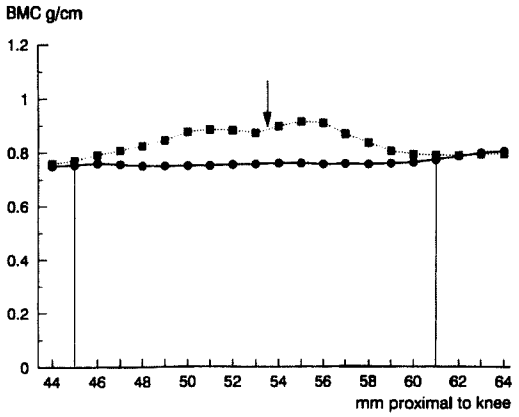

Figure 1. Mineral content of femur diaphyses in 1 rabbit treated with recombinant human growth hormone for 4 weeks after the application of a plastic cerclage band to 1 femur diaphysis. The location of the center of the cerclage band is marked. The vertical lines delineate the area used for calculation of cerclage-treated (■) to control (●) sides.

Table 2. Maximum torque capacity. Ratio of the cerclage band-treated bone to the control in each animal

	1 month		2 months	
	Saline	GH	Saline	GH
	1.07	1.38	1.08	1.27
	1.0	1.30	1.0	1.42
	1.16	1.48	1.10	1.25
	1.40	1.11	1.01	0.86
	1.07	1.79	0.95	1.16
	1.36	1.08	1.07	1.41
	1.07	1.16	1.0	1.27
	0.99	1.37	1.06	1.15
	0.70	0.73	1.28	1.11
	1.07	1.30	1.08	1.25
Mean	1.09	1.27	1.12	1.22
SD	*0.10*	*0.17*	*0.09*	*0.19*

contralateral side, although there was a tendency towards increased strength (Table 2).

Effects of recombinant human growth hormone

In GH-treated rabbits BMC was further elevated 1–2 percent. This difference was not significant when the 1 and 2 month groups were analyzed separately. Uptake of the 2 isotopes was not affected by GH-treatment.

The maximum torque capacity of the cerclage-treated bones was further increased by GH-treatment; at both timepoints the strength exceeded that of the control bones by approximately 25 percent ($P < 0.05$). The stiffness of the cerclage-treated bones was affected in the same way as the torque capacity, but the difference was not significant.

Discussion

Maximum torque capacity was chosen to estimate the strength of the bones, since a change in the geometry of the bone has less influence on the torsional strength than on other commonly studied strength parameters; the torque is constant in every section of the twisted bone. This is especially important since the periosteal new bone induced by the nylon band was not homogeneously distributed. In contrast, stiffness and accumulated energy are affected even by small variations in the bone geometry and are also dependent on a very firm fixation of the bone ends to the test machine. Thus, these parameters show a higher degree of variation than the maximum torque capacity.

Growth hormone is necessary for the normal development and growth of bone (Albrektsson-Wikland 1986, Wilton and Gunnarsson 1988). It has also been reported to increase turnover of the skeleton in adult dogs, resulting in increased bone mass (Harris et al. 1972, Mankin et al. 1978). Systemic administration of GH stimulates the proliferation of precursor cells of bone and enhances osteoblastic activity (Harris et al.

1972, Reddi and Sullivan 1980). Furthermore, GH has been reported to enhance the healing of fresh fractures (Bak et al. 1991) and fractures showing signs of delayed union or non-union (Ahl and Kalén 1979, Koskinen et al. 1979). In a previous study in adult rabbits, we found no accelerating influence of GH on the recovery process in atrophied plated diaphyseal bones as BMC, cross-section geometry, or torsional strength (Låftman et al. 1988). However, GH increased the periosteal new bone formation in both atrophied tibias and in sham-operated bones, indicating an increased activity of the periosteum. These findings prompted the present investigation.

Many species have a specificity for GH, but most mammals, including the rabbit, are sensitive to human growth hormone (Geschwind 1966, Posner et al. 1974, Hughes 1979, Hughes et al. 1983, Wittbjer et al. 1983, Skottner et al. 1984). The recommended clinical systemic dose for human GH in the treatment of short stature is 0.1 I.U. per kg body weight (Albrektsson-Wikland 1986, Wilton and Gunnarsson 1988). A similar dose has been calculated for use in the rabbit (Wittbjer et al. 1983). In the present study, recombinant human growth hormone (Genotropin®) was effective in doses of 0.3 I.U. per kg body weight. This preparation has been found to be equipotent to the human native GH (Crescormone®) previously used (Goodman 1984, Skottner et al. 1984, Låftman et al. 1988).

The mode of action of GH on bone-forming cells is not known in detail, but it is generally accepted that it is, at least in part, mediated through the somatomedin system (Isaksson et al. 1985, Trippel et al. 1986, Isaksson et al. 1987). However, recent evidence suggests that GH may also directly affect the target cells (Isaksson et al. 1982, Isaksson et al. 1985, Russell and Spencer 1985, Isaksson et al. 1987). Most of the accumulated knowledge about GH concerns the effects on the development and growth of bone in immature individuals, while less is known about the effects on the adult skeleton. Furthermore, animal and clinical investigations into the effects of GH on the mature skeleton, and on fracture healing, have been contradictory. Some authors claim enhancing effects of GH on the healing of fractures exhibiting signs of delayed union (Ahl and Kalén 1979, Koskinen et al. 1979), increased callus formation (Zadek and Robinson 1961) and bone strength in experimental fractures (Bak et al. 1991), while others have been unable to identify such effects (Harris et al. 1975, Lindholm et al. 1977, Northmore et al. 1980, Wittbjer et al. 1983).

In the present study, GH did not further increase the BMC of the femur diaphyses induced by the plastic cerclage band. Likewise, no effects on the bone formation rates could be detected. The latter finding may be due to the fact that the cerclage bands had become fixed to the diaphysis by newly-formed periosteal bone and thereby lost their ability to further stimulate the periosteum. The presence, due to the sampling method, of not newly-induced bone in the samples lessens the probability of detecting a very small difference in metabolic rate and that could be another reason why none was found. But the similarity of the effects on matrix formation and mineralization indicates that the mineralization process was not affected by GH-treatment.

The most prominent effect of the GH-treatment in the present study was the increased maximum torque capacity of the femurs; the slight increase in strength and stiffness observed in cerclage-treated rabbits was enhanced by about 15–20 percent in GH-treated rabbits. Most of the new bone was located on the periosteal surface of the diaphyses in the region where the diaphyseal bone normally fractures under torque. Since alterations in the bone geometry have a relatively great influence on structural properties, these factors can explain how the comparatively slight increase in bone mass gave a more pronounced increase in strength (Akeson et al. 1976, Strömberg and Dalén 1976a, b, Låftman et al. 1980, Jonsson and Strömberg 1985). It has been shown that small variations in bone mineral concentration can have profound effects on the strength of cortical bone (Currey 1969, 1984). As we made no assessment of bone mineral concentration, but detected a tendency towards increased BMC in cerclage-treated femurs of GH-treated animals, we cannot rule out the possibility that the substantial effect of GH was mediated by an increase in mineral concentration. However, in view of the more pronounced increase in BMC by the cerclage treatment itself, we regard that as unlikely.

Acknowledgement

This study was supported by grants from the Swedish Medical Research Council (B90-06577-08B).

References

Ahl T, Kalén R. Effect of human growth hormone in the treatment of pseudarthrosis. Opuscula Med 1979; 24: 26–8.

Akeson W H, Woo S L, Rutherford L, Coutts R D, Gonsalves M, Amiel D. The effects of rigidity of internal fixation plates on long bone remodeling. A biomechanical and quantitative histological study. Acta Orthop Scand 1976; 47 (3): 241–9.

Albrektsson-Wikland K. Growth hormone treatment in short children. Acta Paediatr Scand (Suppl 325) 1986: 64–70.

Bak B, Jörgensen P H, Andreassen T. The stimulating effect of growth hormone on fracture-healing is dependent on onset and duration of administration. Clin Orthop 1991; 264: 295–301.

Currey J D. The mechanical consequences of variation in the mineral content of bone. J Biomechan 1969; 2: 1–11.

Currey J D. Effects of differences in mineralization on the mechanical properties of bone. Philos Trans R Soc Lond (Biol) 1984; 304 (1121): 509–18.

Geschwind I. Species specificity of anterior pituitary hormones. In: Pituitary Glands (Eds. Harris G W, Donovan, B T) Butterworths, London 1966; 2: 589–612.

Goodman H M. Biological activity of bacterial-derived human growth hormone in adipose tissue of hypophysectomized rats. Endocrinology 1984; 114 (1): 131–5.

Harris W H, Heaney R P, Jowsey J, Cockin J, Akins C, Graham J, Weinberg E H. Growth hormone: the effect on skeletal renewal in the adult dog. I. Morphometric studies. Calcif Tissue Res 1972; 10 (1): 1–13.

Harris J M, Bean D A, Banks H H. Effect of phosphate supplementation, thyrocalcitonin, and growth hormone on strength of fracture healing. Surg Forum 1975; 26: 519–21.

Hughes J P. Identification and characterization of high and low affinity binding sites for growth hormone in rabbit liver. Endocrinology 1979; 105 (2): 414–20.

Hughes J P, Simpson J S, Friesen H G. Analysis of growth hormone and lactogenic binding sites cross-linked to iodinated human growth hormone. Endocrinology 1983; 112 (6): 1980–5.

Isaksson O G, Jansson J O, Gause I A. Growth hormone stimulates longitudinal bone growth directly. Science 1982; 216 (4551): 1237–9.

Isaksson O G, Eden S, Jansson J O. Mode of action of pituitary growth hormone on target cells. Annu Rev Physiol 1985; 47: 483–99.

Isaksson O G, Lindahl A, Nilsson A, Isgaard J. Mechanism of the stimulatory effect of growth hormone on longitudinal bone growth. Endocr Rev 1987; 8 (4): 426–38.

Jonsson U, Strömberg L. Torsional tests of long bones with computerized equipment. J Biomed Eng 1985; 7 (3): 251–5.

Koskinen E V, Lindholm R V, Nieminen R A, Puranen J, Attila U. Human growth hormone in delayed union and non-union fractures. Int Orthop 1979; 1: 317–22.

Lindholm R V, Koskinen E V, Puranen J, Nieminen R A, Kairaluoma M, Attila U. Human growth hormone in the treatment of fresh fractures. Horm Metab Res 1977; 9 (3): 245–6.

Låftman P, Sigurdsson F, Strömberg L. Recovery of diaphyseal bone strength after rigid internal plate fixation. An experimental study in the rabbit. Acta Orthop Scand 1980; 51 (2): 215–22.

Låftman P, Holmström T, Kairento A L, Nilsson O S, Sigurdsson F, Strömberg L. No effect of growth hormone on recovery of load-protected bone. Cortical bone mass and strength studied in rabbits. Acta Orthop Scand 1988; 59 (1): 24–8.

Mankin H J, Thrasher A Z, Weinberg E H, Harris W H. Dissociation between the effect of bovine growth hormone in articular cartilage and in bone of the adult dog. J Bone Joint Surg (Am) 1978; 60 (8): 1071–5.

Northmore Ball M D, Wood M R, Meggitt B F. A biomechanical study of the effects of growth hormone in experimental fracture-healing. J Bone Joint Surg (Br) 1980; 62 (3): 391–6.

Partridge A. Nylon straps for internal fixation of bone (letter). Lancet 1976; 2 (7997): 1252.

Posner B I, Kelly P A, Shiu R P, Friesen H G. Studies of insulin, growth hormone and prolactin binding: tissue distribution, species variation and characterization. Endocrinology 1974; 95 (2): 521–31.

Reddi A H, Sullivan N E. Matrix-induced endochondral bone differentiation: influence of hypophysectomy, growth hormone, and thyroid-stimulating hormone. Endocrinology 1980; 107 (5): 1291–9.

Russell S M, Spencer E M. Local injections of human or rat growth hormone or of purified human somatomedin C stimulate unilateral tibial epiphyseal growth in hypophysectomized rats. Endocrinology 1985; 116 (6): 2563–7.

Skottner A, Forsman A, Löfberg E, Thorngren K G. Comparison of methionyl human growth hormone and pituitary growth hormone on somatic growth of hypophysectomized rats. Acta Endocrinol (Copenh) 1984; 107 (2): 192–8.

Strömberg L, Dalén N. Influence of a rigid plate for internal fixation on the maximum torque capacity of long bones. Acta Chir Scand 1976; 142 (2): 115–22.

Strömberg L, Dalén N. Experimental measurement of maximum torque capacity of long bones. Acta Orthop Scand 1976; 47 (3): 257–63.

Trippel S B, Van Wyk J J, Mankin H J. Localization of somatomedin C binding to bovine growth-plate chondrocytes in situ. J Bone Joint Surg (Am) 1986; 68 (6): 897–903.

Wilton P, Gunnarsson R. Clinical experience with Genotropin in growth hormone-deficient children. Acta Paediatr Scand (Suppl 343) 1988: 95–101.

Wittbjer J, Rohlin M, Thorngren K G. Bone formation in demineralized bone transplants treated with biosynthetic human growth hormone. Scand J Plast Reconstr Surg 1983; 17 (2): 109–17.

Zadek R E, Robinson R A. The effect of growth hormone on healing of an experimental long bone defect. J Bone Joint Surg (Am) 1961; 43: 1261.