Dose response of growth hormone on fracture healing in the rat

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The effect of different doses of biosynthetic human growth hormone on the mechanical properties of tibial fractures and intact bones was studied in a rat model; a three-point bending test was applied 40 days after fracturing. Ninety-day-old female rats received a daily dose of 0, 0.08, 0.4, 2.0, or 10 mg of growth hormone/kg body weight starting 1 week before fracture and continuing until mechanical testing. In the animals given 2.0 and 10 mg of hormone, the ultimate load sustained by the fractures, stiffness, and energy absorption at ultimate load increased, while the ultimate stress increased only in the latter group. In the intact bones, ultimate load of the bones increased in the same groups, while stiffness and energy absorption at ultimate load increased only in the group given the highest dose of hormone.

We have recently shown that biosynthetic human growth hormone stimulates fracture healing in the rat (Bak et al. 1988). We now report the effect on fracture healing of different doses of this hormone.

Materials and methods

Ninety 3-month-old female Wistar rats (Møllegaard, Lille Skensved, Denmark) were used for the experiment. In this breed of rats, the animals are sexually mature after approximately 50 days. The animals were randomized into six groups: no injections, saline injections, and biosynthetic human growth hormone 0.08, 0.4, 2.0 or 10 mg/kg body weight/day (Nordisk Gentofte A/S, Gentofte, Denmark; specificity: 1 mg = 3 I.U.) was injected subcutaneously in the nape of the neck in two daily doses (between 8 and 10 a.m. and between 4 and 6 p.m.). The injections commenced 1 week before the bones were fractured. All the groups were injected with the same volume by supplementing with isotonic saline. The animals were weighed and the doses of hormone and saline adjusted once a week. The animals had free access to tap water and pelletized food (Altromin diet 1314, Chr. Pedersen Ltd., Ringsted, Denmark), and were caged 3 animals per cage with a cycle of 12 hours of light and 12 hours of darkness.

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Fracture technique

The animals were anesthetized with pentobarbital (50 mg/kg, i.p.). A standardized, closed fracture was produced using the technique described by Bak and Andreassen (1988) 2 mm above the tibiofibular junction by three-point bending using specially designed adjustable forceps with blunt jaws. To minimize soft tissue damage, care was taken not to displace the fracture. Closed medullary nailing was performed with a 0.73 mm Kirschner wire (ultimate load 36 N, ultimate stiffness 62 N/mm, tested under the same conditions as the bones) through an entry hole in the proximal medial aspect. Radiographs were taken immediately after nailing, and animals with fractures outside the specified diaphyseal area or with displaced nails were excluded. Unprotected weight bearing was allowed.

Mechanical analysis

The animals were anesthetized with pentobarbital and killed by exsanguination. Both tibiofibular bones were removed by stripping all soft tissues including the periosteum. The bones were stored in a buffered Ringer's solution (4 °C, pH 7.4) until the three-point bending test was conducted within 3 hours (Bak and Andreassen 1988) after resection of

Table 1. The effect of different daily doses of growth hormone on the mechanical properties of the healing tibial fractures. Growth hormone injection was started one week before fracture. Doses in mg per kg body weight per day given in two injections. Mean *SEM*

Experimental group	n	Ultimate load (N)	Ultimate stress (N/mm^2)	Stiffness (N/mm)	Deflection at ultimate load (mm)	Energy absorption at ult. load (Nmm)	Body weight (g) at start	Body weight (g) at test
Control	23	32 *3.4*	27 *4.3*	148 *19*	0.39 *0.05*	4.9 *0.4*	216 *2*	260 *4*
Hormone								
0.08	8	31 *5.8*	19 *3.6*	155 *32*	0.32 *0.04*	4.5 *0.6*	215 *2*	259 *4*
0.4	13	33 *5.7*	27 *6.5*	185 *32*	0.33 *0.06*	4.2 *0.6*	214 *2*	259 *6*
2.0	10	55 *10.2***	35 *5.5*	220 *32**	0.35 *0.05*	9.9 *2.5** 1)*	220 *2*	306 *11****
10	12	74 *7.6****	46 *5.4**	347 *20***	0.28 *0.03*	11.4 *2.6*** 1)*	220 *2*	420 *7****
One-way ANOVA	*P*	0.0001	0.03	0.0001	NS	0.001	NS	0.0001
Linear regression	*P*(b=0)	0.0001	0.003	0.0001	NS	0.0005	NS	0.0001

Fisher's LSD test with $2P < 0.05^{*}$; 0.01^{**}; 0.001^{***}
1) Statistical analysis performed on logarithmic transformed values.

the fibula and removal of the intramedullary nail. The left, nonfractured tibia was tested at the same level after resection of the fibula. The load-deflection curves obtained were read by a digitizer into a calculator, and the following parameters were derived from the curves: ultimate stiffness, ultimate load, deflection at fracture, and energy absorption at fracture.

The external transverse and anteroposterior diameters of the bone at the point of loading were measured by a sliding caliper (0.05-mm resolution). The transverse diameters of the marrow space were measured in a projection microscope from postoperative, anteroposterior contact radiographs using the diameter of the nail as a reference. The cross-sectional area and area moment of inertia were calculated assuming the cross section to be elliptical with a centrally located circular hole. Stress values could then be calculated from bending moment and area moment of inertia (Kenedi 1980).

The elastic modulus was calculated from ultimate stiffness, distance between the supporting bars in the bending procedure, and the area moment of inertia assuming (a) that the cross-sectional area of the bone was constant during loading, (b) that the shape and area of the cross section was constant between the supporting bars, (c) that the extent of deflection was small, and (d) that the composition of the bone was homogeneous (Nielsen 1974, Kenedi 1980).

Of the ninety 3-month-old animals used, 24 were excluded: 5 because of comminuted fracture or fracture outside the specified area, 6 because of technical failure in the osteosynthesis, 3 because of resorption at the fracture site, 2 because of bending of the nail, and 8 because they died of anesthesia.

Statistical analyses

The groups were tested for normal distribution and homogeneity of variances (G1, G2, Kolmogorov-Smirnov's test and Bartlett's test) followed by one-way analysis of variance. For *post hoc* analysis the Fisher LSD test was used. Accordingly, $2\ P < 0.05$ was considered significant. Linear regression analysis was applied for further description of the dose-response relationship.

No differences were observed between the saline-injected animals and the animals receiving no injections. These two control groups were therefore combined.

Results

The body weights of the animals increased only in the two groups of animals given the highest hormone doses (Table 1). Linear regression revealed a high probability for a linear relationship between the body weight and the square root of the hormone dose.

Fractures

The ultimate load values of the healing fractures increased to 176 percent and 235 percent of the ultimate load sustained by the controls in the 2.0- and 10-mg groups, respectively, while the maximum stiffness increased to 149 and 234 percent, respectively, compared with the corresponding values of the controls (Table 1). In the 10-mg group, ultimate

Table 2. The effect of different daily doses of growth hormone on the mechanical properties of the nonfractured tibiae in the fracture healing experiment. Growth hormone injection was started one week before fracture. Doses in mg per kg body weight per day given in two injections. Mean *SEM*

Experimental group	n	Ultimate load (N)	Ultimate stress (N/mm^2)	Stiffness (N/mm)	Young's modulus (10^3 N/mm^2)	Deflection at ultimate load (mm)	Energy absorption at ultimate load (Nmm)
Control	23	99 *2.0*	301 *8*	317 *6*	16.4 *0.6*	0.45 *0.02*	27 *2.1*
Hormone							
0.08	8	92 *2.5**	297 *9*	302 *9*	16.6 *0.6*	0.44 *0.03*	24 *2.0*
0.4	13	99 *1.9*	317 *9*	326 *9*	17.2 *0.8*	0.44 *0.01*	26 *1.3*
2.0	10	108 *3.7**	302 *8*	341 *14*	15.2 *0.7*	0.47 *0.02*	31 *2.5*
10	12	131 *2.4***	306 *8*	410 *9***	14.7 0.6	0.45 *0.01*	35 *1.5***
One-way ANOVA *P*		0.0001	NS	0.0001	NS	NS	0.009
Linear regression *P* (b=0)		0.0001	NS	0.0001	0.02	NS	0.0005

Fisher's LSD test with $2P < 0.05^{*}$; 0.01^{**}; 0.001^{***}.

stress increased to 167 percent and energy absorbed at ultimate load increased to 232 percent. Linear regression of ultimate load values, ultimate stress, stiffness, and energy absorption at ultimate load on the square root of the hormone dose revealed a high probability for a positive linear relationship.

Intact bones

In the 2- and 10-mg groups, ultimate load increased to 109 and 132 percent, respectively, compared with the corresponding values of the controls. In the 10-mg group, both stiffness and energy absorbed at ultimate load increased by one third (Table 2). In the 0.08-mg group, the ultimate load decreased only slightly. Linear regression of the values for Young's modulus on the square root of the hormone doses revealed a negative relation.

Discussion

Our present experiment showed dose-related stimulation of biosynthetic human growth hormone on the strength of healing tibial fractures. The increased ultimate stress in the 10-mg group indicated a qualitative change in the callus tissue. The hormone effect on the nonfractured bones with increased ultimate load only, but not stress, indicated that the former was a quantitative phenomenon related to the increased size of the bones, which was seen in the animals that had been given high doses of growth hormone. The negative relation between the hormone dose and Young's modulus might be a consequence of the increased amount of newly formed bone in the animals given high doses.

The results of the experiment accord with the results of Jørgensen and Andreassen (1987), who investigated the influence of growth hormone on formation and strength of granulation tissue, although they did not find any difference between the effect of the two highest doses used in their experiment. Lindahl et al. (1986) investigated the effect of different concentrations of growth hormone in suspension cultures on the colony formation of epiphyseal chondrocytes, and found that 10 ng/mL of human growth hormone potentiated colony formation, whereas 40 ng/mL gave maximum stimulation, and higher doses showed reduced potentiation of colony formation. Rokkanen and Kettunen (1972) found that 1.2 U.S.P. growth hormone from human pituitaries, given daily for 1 to 2 weeks to mature rats with full-thickness articular-cartilage defects, stimulated cartilage formation. Koskinen (1959) treated young adult rats with 30 tibia units of human pituitary growth hormone daily for 6 to 22 days and found stimulation of fracture healing already at 6 days. Zilkens et al. (1980) had similar results with growth hormone from human pituitaries, 2 I.U./kg body weight/day, for 13 days in rats.

In contrast, Northmore-Ball et al. (1980) did not find any effect on the healing of stabilized femoral fractures of 5 mg/day of bovine growth hormone (specificity not stated) given to mature rats. Neither did Harris et al. (1975), who investigated fractures in rabbits receiving growth hormone (type not specified) 0.46 I.U./kg body weight/day for 10 to 50 days.

In all of these animal experiments, the growth hormone was given in a single daily injection. Recent studies indicate that the frequency of administration is important as regards the effects of the hormone. The endogenous growth hormone secretion is

highly pulsatile; in the rat the secretory bursts occur at approximately 3-hour intervals (Tannenbaum and Martin 1976, Edén 1979, Clark et al. 1987). Jansson et al. (1982) found that the longitudinal bone growth and weight gain in hypophysectomized rats increased when equal daily doses of growth hormone were given in 2–8 daily injections rather than in one single injection. This, as well as the range of doses used, might be important factors in explaining the results of the present experiment.

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