Growth hormone promotes healing of tibial fractures in the rat

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The effect of administering growth hormone for different periods of time on the mechanical properties of healing rat tibial fractures was investigated after 40 days of healing. Biosynthetic human growth hormone, 2.7 mg/kg body weight/day, was administered to three groups of rats for 1, 2, or 3 weeks following fracture, whereas isotonic saline was administered to a control group for 3 weeks. The ultimate load values and maximum stiffness of the fractures increased in the groups injected with growth hormone for 2 or 3 weeks; linear regression analysis revealed a high probability of a positive linear relationship. In the intact bones an increase in ultimate load, maximum stiffness, and energy absorption at ultimate load was found in the group injected with growth hormone for 3 weeks, with linear regression analysis again showing a high probability of a positive linear relationship.

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Growth hormone increases longitudinal bone growth by stimulating cell differentiation in the germinal zone of the growth plate (Isaksson et al. 1982, Schlechter et al. 1986, Nilsson et al. 1987, Isaksson et al. 1988). Callus formation in healing diaphyseal fractures shows many similarities with the bone formation of the growth plate (Mindell et al. 1971, Page et al. 1986). Therefore, the influence of growth hormone on fracture healing has often been investigated, but the results have been rather diverse (Bak et al. 1991). However, as growth hormone has become accessible in substantial amounts, it has been shown that growth hormone promotes mechanical strength of rat tibial fractures (Bak et al. 1990a, b, 1991).

We investigated the influence of growth hormone on the mechanical properties of healing rat tibial fractures when growth hormone was administered during the first 1–3 weeks of a 6-week healing period.

Materials and methods

Sixty-five, 3-month-old female Wistar rats (Møllegaard, Lille Skensved, Denmark) were randomly divided into four groups. Three groups were injected with biosynthetic human growth hormone (b-hGH) (Nordisk Gentofte, Denmark; specificity: 1 mg = 3 IU) at a dose of 2.7 mg/kg body weight/day for 1, 2, or 3 weeks following fracture. After the end of hormone administration, saline injections were continued, so all the groups were injected for 3 weeks. A fourth group served as a control and received isotonic saline injections throughout the 3 weeks. All the injections were given twice daily in the nape of the neck. The rats were weighed, and the doses of hormone and saline were adjusted once a week. The body weights increased when the rats received hormone injections (Figure 1). When the hormone administration stopped, the body weight stagnated or decreased. The rats were housed under

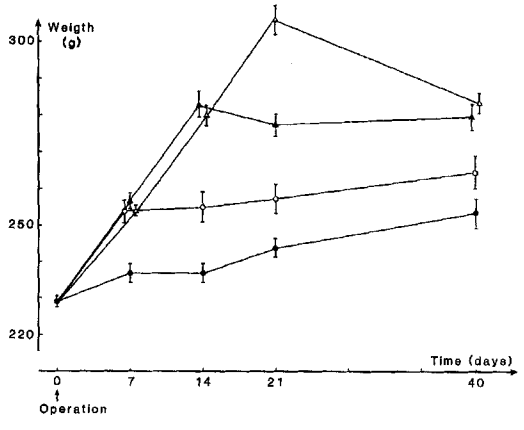

Figure 1. Changes in body weight during the fracture-healing experiment (mean ± SEM). ● saline 21 days; ○ b-hGH 7 days and saline 14 days; ▲ b-hGH 14 days and saline 7 days; △ b-hGH 21 days.

controlled conditions, with a cycle of 12 hours of light and 12 hours of darkness, and they had free access to tap water and pellet food.

Fracture technique

The animals were anesthetized with 50 mg/kg pentobarbital intraperitoneally. A unilateral, standardized closed fracture was produced above the tibiofibular junction in the right tibia by three-point bending (Bak and Andreassen 1988, 1989). Closed medullary nailing was performed with a 0.79-mm Kirschner wire. The skin was closed with monofilament sutures. The operations were performed under sterile conditions. Contact radiographs were taken immediately after nailing. Animals with fractures located less than 2 mm or more than 6 mm above the tibiofibular junction or with displaced nails were excluded. Unprotected weight bearing was allowed; the animals resumed normal activity immediately after recovery from the anesthesia.

Mechanical analysis

The mechanical properties of both fractured and nonfractured tibiae were tested after 40 days of healing. The animals were killed with an overdose of pentobarbital, and both tibiofibular bones were dissected free and stored in a buffered Ringer's solution (4 °C, pH 7.4). Contact radiographs of all the fractures were obtained. The fibula was resected and the intramedullary nail was removed. The mechanical properties of the healing fractures were analyzed using a destructive three-point bending procedure within 3 hours. The bone was placed on two rounded bars at a distance of 15-mm in a materials testing machine (Alwetron 250, Lorentzen and Wettre, Stockholm, Sweden) and deflected by another rounded bar on the fracture at the opposite side of the bone with a constant speed of 2 mm/min. All the bones were oriented alike, with the concave facet of the lateral tibial condyle resting on one of the supporting bars so that the bone was loaded from the medial side. The left unfractured tibia was tested at the same level of the bone as the fracture in the corresponding right tibia, using exactly the same procedure. The load and deflection were recorded continuously by transducers coupled to measuring bridges, and the signals were fed to an x-y recorder. The load-deflection curves obtained were read by a digitizer into a calculator, and the following parameters were calculated: ultimate load, maximum stiffness, deflection at ultimate load, and energy absorption at ultimate load (Oxlund and Andreassen 1980, Bak and Andreassen 1988). The external transverse and anteroposterior diameters of the bone at the point of loading (the fracture line and the corresponding level at the intact bone) were measured with a sliding caliper.

The transverse diameter of the marrow space was measured in a projection microscope from the contact radiographs using the diameter of the nail as a reference. The cross-sectional area was calculated, approximating it to an elliptical configuration with a circular center hole. Stress values could then be calculated from the bending moment and second moment of area (Kenedi 1980). In the nonfractured tibiae, Young's modulus was calculated from ultimate stiffness, from the distance between the supporting bars in the bending procedure, and from the area moment of inertia assuming that (a) the cross-sectional area of the bone was constant during loading, (b) the shape and area of the cross section was constant between the supporting bars, (c) the extent of deflection was small, and (d) the composition of the bone was homogeneous (Kenedi 1980).

Of the 65 rats used for the experiment, 21 were excluded: of these, 4 had fractures outside the specified area, 2 had comminuted fractures, 7 had infection around the nail, and 8 animals died while under anesthesia.

Statistics

The groups were tested for normal distribution (G1 and G2) and homogeneity of variances (Bartlett's test). Linear regression of the mechanical parameters on the square root of the injection periods was calculated. The linearity of the regression analysis was tested by comparing the magnitude of deviations of array means from the regression line and within-arrays variation (Armitage and Berry 1987). Also one-way analysis of variance followed by Fischer's LSD test were performed; finally, $2P < 0.05$ was considered significant.

Results

The ultimate load values of the fractures increased by 48 percent in the groups given hormone for 2 or 3 weeks, and maximum stiffness increased by 25 and 34 percent, respectively, compared with the

Table 1. The effect of injection with biosynthetic human growth hormone (b-hGH; 2.7 mg/kg body weight/day) on the mechanical properties of healing tibial fracture in the rat. Mean *SEM*

Experimental groups Saline/b-hGH (Weeks)	n	Ultimate load (N)	Ultimate stress (N/mm^2)	Maximum stiffness (N/mm)	Deflection at ultimate load (mm)	Energy absorbed at ultimate load (Nmm)	Cross sectional area (mm^2)
3/0	12	49 *6.1*	46 *6.1*	251 *21*	0.22 *0.02*	6.4 *1.3*	10 *0.9*
2/1	10	62 *7.6*	50 *6.0*	256 *31*	0.27 *0.02*	9.3 *1.4*	11 *1.3*
1/2	10	73 *9.2**	46 *5.6*	313 *14**	0.25 *0.03*	11.1 *2.4*	13 *1.1*
0/3	12	73 *8.6**	49 *3.8*	337 *17**°	0.24 *0.02*	10.4 *2.2*	12 *0.9*
One-way ANOVA, *P*		0.11	NS	0.013	NS	NS	NS
Linear regr., *P* (b = 0)		0.02	NS	0.004	NS	NS	NS

*Fisher's LSD test with *2P < 0.05, **2P < 0.01, versus saline-injected group. °2P < 0.05, versus b-hGH for 1 week.*

Table 2. The effect of injection with biosynthetic human growth hormone (b-hGH; 2.7 mg/kg body weight/day) on the mechanical properties of the nonfractured tibiae in the fracture healing experiment. Mean *SEM*

Experimental groups Saline/b-hGH (Weeks)	n	Ultimate load (N)	Ultimate stress (N/mm^2)	Maximum stiffness (N/mm)	Young's modulus (10^3 N/mm^2)	Deflection at ultimate load (mm)	Energy absorption at ultimate load (Nmm)	Cross sectional area (mm^2)
3/0	12	89 *2.8*	267 *6*	255 *13*	12 *4.8*	0.45 *0.02*	24 *1.2*	3.8 *0.1*
2/1	10	89 *2.3*	271 *1*	267 *10*	13 *5.2*	0.44 *0.02*	24 *1.3*	3.7 *0.1*
1/2	10	98 *3.7**	275 *5*	296 *18*	13 *5.2*	0.42 *0.01*	24 *1.3*	4.0 *0.1*
0/3	12	101 *2.8**°°	281 *6*	300 *10**°	13 *4.8**°	0.46 *0.01*	28 *1.2**°+	3.9 *0.1*
One-way ANOVA, *P*		0.006	NS	0.037	NS	NS	0.03	NS
Linear regr., *P* (b = 0)		0.002	NS	0.006	NS	NS	0.04	NS

Fisher's LSD test with *2P < 0.05, **2P < 0.01, versus saline injected group. °2P < 0.05, °°2P < 0.01 versus b-hGH for 1 week. + 2P < 0.05 versus b-hGH for 2 weeks.

control group (Table 1). A 31 percent increase in maximum stiffness was found in the group given growth hormone for 3 weeks when compared with the 1-week group. Linear regression analysis of ultimate load and maximum stiffness values on the square root of the period of hormone administration revealed a high probability of a positive linear relationship.

In the intact bones the ultimate load at fracture increased by 11–14 percent in the groups given hormone for 2–3 weeks compared with the control group (Table 2). Maximum stiffness similarly increased by 13–18 percent. Energy absorption at ultimate load increased by 19 percent in the 3-week hormone group. Linear regression analysis of ultimate load, maximum stiffness, and energy absorption at ultimate load on the square root of the period of hormone administration showed a high probability of a positive linear relationship. No change in deflection at ultimate load was found.

Discussion

In a recent study, we found that growth hormone increased the strength of healing rat fractures when given during the first 3 weeks of a 6-week healing period. No further stimulation was achieved if the hormone administration was continued for the entire healing period, and no effects were seen when hormone was given during the last 3 weeks of the healing period (Bak et al. 1991). These results suggest that the initial phases of the fracture repair process are most sensitive to exogenous growth hormone administration or that there is a delay before the effects of growth hormone are seen. The present experiment shows that the period of growth hormone administration cannot be reduced to less than 2–3 weeks with the dose and frequency of administration that we used.

The results of our studies do not explain the stimulating effect of growth hormone on fracture repair. Nonosteonal fracture repair in rats is mainly dependent on the formation of external cartilaginous

callus and endochondral ossification, which have many features in common with the ossification process in the physis (Urist and McLean 1941, Slätis and Rokkanen 1965, Henricson et al. 1987). Cartilage appears in the callus from the second to the fourth day after a tibial fracture (Urist and McLean, 1941). The dual effector theory proposed by Green et al. (1985) suggests that growth hormone acts on skeletal tissues directly by stimulating the differentiation of stem cells and indirectly by increasing the responsiveness of the cells to IGF-I and by stimulating the local production of IGF-I (Isaksson et al. 1988). The finding that exogenous growth hormone stimulates fracture repair when given during the early phases of healing suggests that the action of growth hormone on the callus tissue is parallel to that on the growth plate.

Acknowledgements

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