No effects of local somatomedin C on bone repair

Continuous infusion in rats

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We studied the effect of continuous local application of human recombinant somatomedin C (110 µg/100 g/day) on bone repair in a rat femoral osteotomy model. The mechanical strength and various metabolic variables were evaluated four weeks postoperatively. We did not find any effect in the treatment group as compared to controls on torsional strength, deformation, or stiffness. Radiographic healing was similar in both groups. Nor was there any difference in callus weight, callus vascularization, or mineralization.

Our results suggest that local administration of this dose of somatomedin C does not promote the early phase of diaphyseal repair in long bones.

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Somatomedin C is a growth factor for mesenchymal stem cells in vitro (Zapf et al. 1984). This peptide hormone also stimulates mitosis and differentiation of primitive chondroblasts and osteoblasts in vitro, and increases matrix formation (Froesch et al. 1985). The proliferation and differentiation of osteogenic cells form the basis of bone repair. Thus, there is substantial theoretical support for the hypothesis that somatomedin C might enhance bone repair. We have earlier been unable to show any effect of systemic administration of somatomedin C on bone repair in a rat osteotomy (Kirkeby and Ekeland 1990). We have now studied the effect of somatomedin C administration directly into the osteotomy site.

Material and methods

20 adult outbred Wistar rats (450–500 g) were operated on using fentanyl-fluanisone-midazolam anesthesia. The middle part of the femur was surgically exposed. An osteotomy was made with a rotating dental saw under continuous saline irrigation. The major trochanter was osteotomized and an intramedullary nail inserted into the femoral condyles. A 21-gauge needle was also inserted through the medulla into the area of the osteotomy for local application of somatomedin C (Figure 1).

The somatomedin C used in this experiment was recombined from *E. coli* (produced and supplied by KabiGen AB, Stockholm). The product is identical with native human somatomedin C and at least 90 percent pure. The specific activity is higher than 14,000 U/mg.

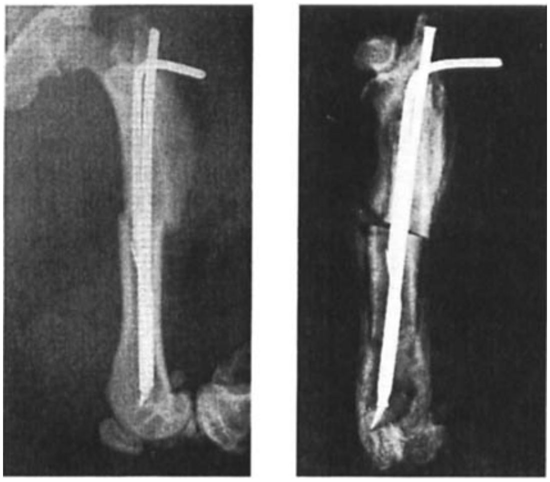

Figure 1. To the left, a rat femur with an osteotomy in the middle part (postoperative control). An intramedullary nail has been inserted, and a needle introduced into the osteotomy area. The needle is connected to a subcutaneously positioned minipump delivering vehicle or somatomedin C 110 µg/100 g body weight/day. The nails consist of a thin needle (21 gauge) running through the whole bone, a thin needle (23 gauge) for somatomedin infusion in the proximal part, and a thicker needle (18 gauge) outside (and locked to) the needle in the distal part, in order to make it more closely fit the marrow cavity.

To the right, dissected specimen four weeks postoperatively. There was extensive callus formation in both groups, but no differences in radiographic appearance between the two groups.

An osmotic pump (Alzet Model 2ML4) was used for the administration of the somatomedin C. The pump delivered 2.5 µL/h during 28 days. It was filled with 20 mg somatomedin C in 2.2 mL 0.1 M acetic acid, or 2.2 mL 0.1 M acetic acid. A tube filled with the solution was connected to the top of the pump which in turn was connected to the intramedullary needle. The pump was positioned in a subcutaneous pouch in the back of the animal.

The treatment group (10 animals) received 110 µg/100 g/day somatomedin C directly into the osteotomy site and the control group (10 animals) received the vehicle.

Three days before killing the animal, 1 mCi/100 g $^{85}SrCl_2$ was given intraperitoneally. The animals were anesthetized and a catheter was introduced into the ascending aorta through the right carotid artery. A bolus of 1.5 million ^{141}cerium-labelled microspheres (NenTrac), 15-µm diameter, in 1 mL saline, was injected. The animals were killed by bleeding 28 days postoperatively.

Both femora were dissected out and cleaned from all soft tissue, care being taken to leave the callus intact. The integrity of the delivery system was tested for all animals. The femora were examined in the frontal and lateral positions with a standard X-ray unit at 50 kV, 20 mAs, and 60-cm tube-target distance using Kodak occlusal Ultra-speed D film. The widest part of the calcified callus and the gap between callus ends over the osteotomy were measured, and the average between frontal and lateral views used as a measure for callus formation.

The femora were stored at –20 °C until removal of the intramedullary nail and mechanical testing. Following mechanical testing the distal 5 mm and the proximal 7.5 mm of the femora were removed.

The weight of the callus was determined by subtracting the weight of the opposite intact segment from the weight of the callus sample. Total radioactivity of each isotope in the samples was measured by placing the sample in the center of a Packard 5221 Autogamma scintillation spectrometer with the windows set over the highest energy peak of each isotope. Individual isotope activity of the samples was calculated with correction for background, cross-talk, and physical decay. The specific activity of strontium (counts per minute/gram) on the test side relative to the intact side was used to express the mineralization rate. The relative vascularization of the osteotomy area was calculated by the formula:

$$\frac{\text{callus cpm/mg}}{\text{intact segment cpm/mg}}$$

The torsional tests were performed in a hydraulic testing machine. The force was applied at a constant deformation rate, 2.5°/second. The strength of the bones was defined as the ultimate torsional moment, read as the y-coordinate from each load deformation curve. The corresponding x-coordinate was defined as the ultimate torsional deformation. The torsional stiffness was measured from the slope of the linear portion of the curves. The technique and the calculations have been described in detail previously (Ekeland et al. 1982). Values from the healing osteotomy were in all cases compared with those of the opposite intact femora.

Table 1. Radiographic, mechanical and metabolic healing of 4-week-old osteotomies in rats treated with continuous local injection of somatomedin C (110 µg/100 g/day) and controls (mean, *SD*). Relative values for healing of fracture/intact bone are given for torsional and metabolic tests

	Somatomedin C group (n 10)		Control group (n 10)	
Body weight (g)	463	*30*	469	*34*
Radiographic assessment				
Callus width	8.09	*1.3*	8.55	*1.0*
Callus gap	1.95	*1.7*	1.01	*0.9*
Torsional tests				
Strength	0.20	*0.13*	0.21	*0.06*
Deformation	2.81	*1.20*	2.37	*0.97*
Stiffness	0.29	*0.29*	0.24	*0.08*
Metabolic tests				
Relative vascularization	2.72	*1.05*	2.89	*0.60*
Relative mineralization	4.99	*1.31*	5.31	*1.43*
Callus weight (mg)	1.02	*238*	1.03	*245*

Statistical evaluation was done with Student's *t*-test.

Results

All healing bones were in good alignment. Radiographically there was extensive callus formation along the femoral shaft, but there was a clear gap between callus ends in all rats (Figure 1). Radiographic measurements of callus formation showed no differences between the groups (Table 1).

There were no significant differences between the groups as regards ultimate strength, deformation and stiffness of the healing side, but a tendency was seen towards decreased strength in the somatomedin C-treated group.

There were no differences between the groups in specific cerium or strontium activity in the healing or the intact sides.

Nor were there any differences between the groups concerning the healing bone relative to the intact bone.

Discussion

Continuous infusion into the marrow cavity might affect the bone circulation and intramedullary pressure, and thus change the conditions for osteotomy healing. Comparison with an earlier experiment on systemic effects of somatomedin C, however, shows similar values to the present results for all variables (Kirkeby and Ekeland 1990). Significant disturbance of the healing process due to local infusion therefore seems unlikely.

We have used only one dose of somatomedin C in the present study. Previous results from other groups do not give information concerning which dosages would be relevant in our model. Dose-response studies would therefore be very important in order to definitely answer the question of bone healing and somatomedin C. This was not possible in our study for practical reasons (limited supply of somatomedin C). The results should therefore be interpreted with this shortening in mind.

Somatomedin C is strongly bound to carrier proteins in the serum, and this binding can probably affect the availability of the peptide to the callus following systemic administration (Sara and Hall 1990). Direct infusion into the osteotomy site should provide a high local concentration of free somatomedin C in the callus, and theoretically this should be a more effective method of administration. Purified human somatomedin C has earlier been shown to act directly at a target tissue (cartilage) following local application (Russel and Spencer 1985, Isgaard et al. 1986). We were, however, unable to show any effect of local somatomedin C treatment on callus mechanical strength, rate of mineralization, or callus blood flow. We have earlier found a decrease in callus weight following systemic somatomedin C treatment (Kirkeby and Ekeland 1990). This could not be confirmed using local administration, perhaps because local and systemic administrations provide different concentrations of somatomedin C at the healing site. That the decrease after systemic administration was coincidental cannot be entirely excluded, because the difference was significant at a low level ($0.025 > P < 0.05$) (Kirkeby and Ekeland 1990).

The effect of somatomedin C on bone healing has been evaluated previously by Aspenberg et al. (1989). They applied a continuous local infusion to callus in a titanium chamber in rabbit tibia. They found a decreased bone mineral turnover but no effect on the amount of bone after 14 days of healing. We have used four weeks of healing and different parameters for evaluation, but we agree that local application of somatomedin C does not increase bone formation in vivo.

Growth hormone-deficient animals have been used in most studies on the effect of somatomedin C in vivo (Van Buul Offers et al. 1988). Effects of exogenous somatomedin C in normal animals may be modulated by intact regulating mechanisms. Bone repair and callus development are closely regulated. Attempts to accelerate this process may be hampered by local regulatory mechanisms. Earlier studies using calcitonin, growth hormone, estrogen, thyroxine, phosphate, or vitamin D to promote bone repair have largely been unconvincing (Koskinen 1959, Udupa and Gupta 1965, Wray and Goldstein 1966, Harris et al. 1975, Northmore Ball et al. 1980, Ekeland et al. 1983). It is probably difficult to promote bone repair in the normal animal. The present study joins the list of unsuccessful experiments attempt to stimulate bone repair by exogenous hormone supplements.

Acknowledgements

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