

No effect of systemic administration of somatomedin C on bone repair in rats

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The effect of somatomedin C on the bone repair process was studied in a rat femoral osteotomy. We used continuous systemic administration of human recombinant somatomedin C (110 µg/100 g per day). Radiographic evaluation after 4 weeks showed no effect of somatomedin C on the healing. There was no effect on mechanical strength, and no effect was found on callus vascularization or mineralization. The weight of the callus was slightly reduced in the somatomedin C treated rats. The results were not suggestive of any acceleration with systemic somatomedin C treatment in the early phase of cortical bone repair.

Somatomedin C (insulin-like growth factor I) is a polypeptide with a chemical structure related to insulin and insulin-like biological effects. Somatomedin C has been shown to be a potent growth stimulus for many different cells in culture including chondrocytes and bone cells (1). Somatomedin C probably directly effects the differentiation of osteoblast-like calvaria cells (2). The effect of somatomedin C on immature cells from skeletal tissue is directed towards both an enhancement of differentiation and an increased proliferation (2).

Information on the effects of somatomedin C in vivo is scanty because of the small quantities of the peptide available until recently. Subcutaneous continuous infusion of pure human somatomedin C in both hypophysectomized and normal rats promotes body weight, tibial epiphyseal width, and thymidine incorporation into costal cartilage (3, 4).

Somatomedin C has been shown to stimulate the proliferation and differentiation of mesenchymal stem cells, primitive chondroblasts, and osteoblasts in vitro. We have established a bone-repair model in the rat to investigate whether subcutaneously administered somatomedin C can stimulate the process of bone repair.

Material and methods

A total of 19 adult outbred male Wistar rats (450-500 g) were used. The rats were kept 1 in each cage postoperatively, and they were given standard rat pellets containing 0.9 percent calcium and 0.7 percent phosphorus. Mature rats were used because bone repair in younger rats is so rapid that an acceleration of the process might be difficult to show.

The animals were operated on under fentanyl anesthesia. A skin incision was made from the great femoral trochanter to the supracondylar area of the right femur. The middle part of the femur and the trochanter area were dissected out. An osteotomy was made with a rotating dental saw under continuous saline irrigation and protection of the soft tissues. The major trochanter was osteotomized with a pair of cutting nippers, and an intramedullary nail was inserted into the femoral condyles. The nail was made of an 18 G needle (distal part), a 21 G needle (whole length), and a 23 G needle (locking the proximal part; Figure 1). The nail gave axial, but no rotational, stability.

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

An osmotic pump (Alzet osmotic pump, 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.

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Figure 1. Rat femur with an osteotomy in the middle part. An intramedullary nail made of injection needles has been inserted.



Figure 2. Rat femur 4 weeks after osteotomy. There were extensive callus formation and endosteal resorption, especially in the distal part, in both groups. There were no differences in radiographic appearance between the groups.

The pump was positioned in a subcutaneous pouch in the back of the animal. The continuous application of somatomedin C seemed the most physiologic in light of the stable serum concentration and the lack of diurnal variation of the peptide (2). The pump delivered 110 µg/100 g per day somatomedin C subcutaneously in the treatment group (10 animals), and only the vehicle in the controls (9 animals). This dosage has been shown to stimulate bone growth in vivo (3).

All the animals were killed 28 days postoperatively. Three days before 1 mCi/100 g $^{85}\text{SrCl}_2$ in saline was given intraperitoneally. All the animals were anesthetized with fentanyl. PE-10 catheters were introduced into the ascending aorta through the right carotid artery. A bolus of 1.5 million $^{141}\text{cerium}$ -labeled microspheres (NenTrac), with a diameter of 15 µm, in 1 mL saline, was injected into the ascending aorta. The animals were killed by bleeding. Adequate emptying of the pump was ascertained for each animal.

Both femora were dissected out and cleansed of all the soft tissue, taking care to leave the callus intact. They were examined in the frontal and lateral position 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 with a pair of calipers, and the average between frontal and lateral projections was used as a measure of 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.

The femoral segments were counted for strontium and cerium radioactivity in a gamma scintillation counter. The specific activity of strontium (c.p.m. per gram, "osteogenic index") of the test side relative to the intact side was used to express the mineralization rate as described by Elves (5). The relative vascularization of the osteotomy area was calculated in the same manner as:

^{141}Ce -microsphere radioactivity of callus sample per gram/radioactivity of intact segment per gram.

The torsional tests were performed in a hydraulic testing machine. The force was applied at constant deformation rate of 2.5°/s. 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 (6, 7). Values from the healing bone were compared with those of the opposite intact femur.

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

Results

All the healing bones were in good alignment.

Radiographically, there was extensive callus formation along the femoral shaft, but there was a clear gap at the osteotomy site between callus ends in all the rats (Figure 2). Measurements of callus formation on the radiographs showed no differences between the groups (Table 1).

Somatomedin C did not affect the torsional properties of the healing osteotomies or the intact bones.

The values for ultimate strength, deformation, and stiffness of the healing bone relative to the intact side did not differ between the groups, except for a tendency towards decreased strength in the somatomedin C treated group.

No differences were found between the groups in specific cerium or strontium activity in the healing or the intact sides. Neither were there any differences between the groups concerning the healing bone relative to the intact bone (Table 1).

The only difference found was a decreased callus weight in the somatomedin C treated animals ($P < 0.05$; Table 1).

Discussion

Our results showed that the size and the mechanical strength of the callus were not affected by systemic somatomedin C treatment. Neither did this treatment affect the rate of mineralization of the callus, nor the blood supply to the osteotomy area. To reduce variation because of individual differences between rats, all the values were expressed as a ratio between the healing and the contralateral femur. The lack of effect cannot be explained by increasing strength of the intact side in the somatomedin C treated group, because no such effect was found. The only significant difference was a slightly decreased callus weight in the somatomedin C treated animals. The radiographic appearance, including callus width and gap between callus ends, was similar in both groups. Extensive callus formation and endosteal resorption, especially in the distal part, were common in both groups.

In their study of the effect of somatomedin C on bone healing in vivo, Aspenberg et al. (1989) found that local application to callus inside a titanium chamber decreased bone-mineral turnover, but had no effect on the amount of bone mineral after 14 days of healing. Our model is not directly comparable because we have used systemic administration, a 4-week healing period, and different evaluation pa-

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

	Somatomedin C (n 10)	Control (n 9)
Body weight	470 33	464 28
Radiographic assessment (mm)		
callus width	8.10 1.4	9.3 1.3*
callus gap	2.00 0.8	1.85 1.3
Torsional tests		
strength	0.27 0.1	0.30 0.2
deformation	2.42 1.42	2.59 1.31
stiffness	0.30 0.15	0.53 0.57
Metabolic tests		
relative vascularization	2.99 0.98	2.55 0.93
relative mineralization	4.53 1.4	4.61 1.1
Callus weight (mg)	848 280	1164 324*

* $P < 0.05$, * $P = 0.07$.

rameters. Still, our study confirmed the important point that high level of somatomedin C does not increase bone formation in vivo.

The somatomedin concentration in serum is mainly regulated by the level of growth hormone (1). Enhanced bone repair after systemic growth hormone treatment has been reported (9, 10), but the majority of studies find no evidence of a shortening in bone healing time (11, 12), or even an inhibiting effect on bone repair (13). The somatomedin hypothesis and available evidence state that the effect of growth hormone is mediated through the somatomedins (3, 14, 15). Growth hormone and somatomedin C may nevertheless have different effects on bone repair. Direct local effects of growth hormone on the rat tibial epiphysis have been demonstrated (16, 17). Growth hormone and somatomedin have different effects on cartilage formation as shown in tissue culture (18). Treatment with growth hormone and somatomedin C can therefore not be expected to give identical results.

Subcutaneous infusion of somatomedin C has been shown to affect bone growth and epiphyseal cartilage in vivo despite its strong binding to carrier proteins limiting the access to the tissues (1, 3, 4). The quantities available to osteogenic cells in the callus might, however, be too small to have any appreciable effect on healing.

Because our study focused on systemic somatomedin C treatment in the early phase of bone repair, it is not possible to evaluate whether it has any effect

on the later development and final result of the healing process. Nevertheless, our results suggest that little benefit might be expected from the systemic administration of somatomedin C on the normal repair process of long bones.

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