

Local application of growth-factor IGF-1 to healing bone

Experiments with a titanium chamber in rabbits

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In order to study the effects of bone healing, insulin-like growth factor 1 (IGF-1) was continuously applied with a minipump to a healing bone callus located inside a titanium chamber in a rabbit tibia. After 2 weeks, ^{99m}Tc-MDP uptake and calcium content were measured. IGF-1 decreased ^{99m}Tc-MDP uptake, and thus the mineral turnover rate, but the amount of bone mineral was unchanged. Probably the normal endogenous production of IGF-1 is already optimal for healing in this model.

Although much studied *in vitro*, the effects of various growth factors *in vivo* on bone and bone healing are mostly unknown. Healing bone may produce growth factors in an autocrine fashion, as demonstrated for insulin-like growth factor 1 (IGF-1) in muscle (Jennische et al. 1987). In traumatized rat sciatic nerve, which produces IGF-1 itself (Hansson et al. 1986), regeneration is further increased by local addition of IGF-1 (Kanje et al. 1989).

We have attempted to study whether local addition of IGF-1 increases bone regeneration.

two 1-mm-diameter holes to coincide with a transverse semicircular groove across the inside end of the cylinder. Thus, when implanted in the tibia of a rabbit, a continuous canal, open at either end, receives ingrowth of bone that may then be repeatedly harvested after removal of the core without disturbing the outer cylinder.

Further, the removable core is provided with a 10- μ L cavity acting as a fluid reservoir linked to the bone-

Materials and methods

The drug-test chamber

The drug-test chamber (Aspenberg et al. 1988a; Figure 1) consists of a titanium cylinder closed at one end and open at the other to receive a removable titanium core. When the cylinder is implanted in the bone, its open end sticks out in the extraosseous tissue, so that the core can be removed when necessary without disturbing the bone. The external surface of the outer cylinder is threaded to ensure firm emplacement and pierced by

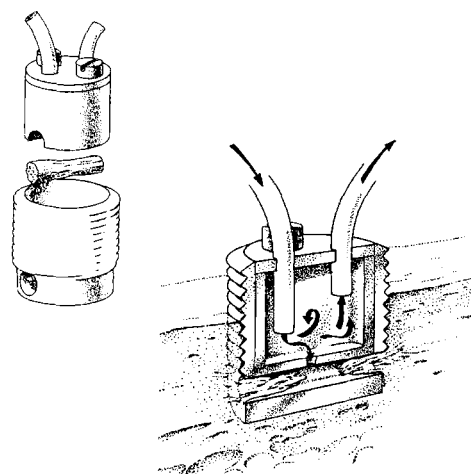


Figure 1. After the drug test chamber is osseointegrated, the bone within the bone ingrowth canal is removed and new bone fills the canal. During the latter process, IGF-1 was continuously applied from an implanted minipump.

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Table 1. $^{99}\text{TcMDP}$ activity and calcium yield in consecutive harvests from bilateral test chambers in a rabbit receiving IGF-1 alternately in the left and the right chamber

$^{99}\text{TcMDP}$ cpm $\times 10^{-3}$		Calcium μg		$^{99}\text{TcMDP}$	Calcium
IGF side	Control side	IGF side	Control side	IGF/Control	IGF/Control
32	36	—	—	0.88	—
32	41	—	—	0.78	—
24	29	152	188	0.84	0.81
23	22	132	144	1.05	0.92
2.3	3.0	188	208	0.77	0.90
13	15	292	360	0.87	0.81

Mean IGF/Control ratio for $^{99}\text{TcMDP}$: 0.86 SD 0.10 ($P < 0.05$).

ingrowth canal via a 1-mm-long, 0.25-mm-diameter diffusion capillary. For continuous infusion, 2 silicone tubes are connected to the reservoir. One tube is connected to a subcutaneous osmotic minipump (ALZET, model 2002, ALZA Corp., CA, USA). The other tube is open to the subcutis, thereby functioning as a drain for the reservoir. This allows a slow, continuous flow of the solution through the reservoir, creating a concentration gradient along the diffusion capillary. The percolation of solvent through the diffusion capillary to the bone is minimal due to the reservoir being drained to the subcutaneous tissues. Thus, the ingrowing bone was locally treated with an IGF-1 solution without the disturbance of a solvent flow.

Animals and operations

The chambers were implanted bilaterally into the medial aspect of the proximal tibia of 11 fully grown lop-eared rabbits under anesthesia with Hypnorm® (Leo, Helsingborg, Sweden) at a dose of 0.5 mL/kg body weight. At chamber implantation, the skin and fascia over the medial side of the proximal metaphysis were opened via separate curved incisions. The periosteum was removed via an 8-mm-diameter circular incision. Using a 6.2-mm trephine, the medial cortex was removed under profuse irrigation with a saline solution to minimize heat-induced damage to the implantation site. The hole thus made was threaded and the chamber screwed in place; care was taken to place the openings of the bone-ingrowth canal just below the bone surface. The skin was closed with a continuous intracutaneous suture. The chambers were harvested 6 weeks after implantation and then every second week.

In the first series of experiments, IGF-1 was applied to a chamber in 1 rabbit and a control chamber in the contralateral tibia was treated with phosphate-buf-

fered saline (PBS). Both sides had minipumps. The experiment was then repeated six times, using the same side alternately as experimental and control side.

In the second series, pumps were applied on both sides in 9 rabbits, one containing IGF-1, the other only a control solution. One rabbit was used twice, with 2 weeks' intermission and side switch after the preceding experiment. Thus, totally 10 pairs were analyzed.

All the tubing and pumps were checked for leakage at harvest.

IGF-1

For the first series, we used human recombinant met-IGF-1 with a radioreceptor assay activity of 6,000 U/mg. For the second series, we used human recombinant IGF-1 with a radioreceptor assay activity of 13,500 U/mg. In both series the IGF-1 was dissolved in 0.1 M HAc, then diluted in PBS to yield 2,000 U/mL, with the pH adjusted to 6.2 and an osmolality of 235 mOsm/L. The control solution was identical, but lacked IGF-1. The pumps contained 250 μL and delivered 0.5 $\mu\text{L}/\text{h}$.

Evaluation

Fourteen days after the start of the experiment, the contents of the canals were harvested. The calcium was solubilized, and the calcium content was measured as previously described (Aspenberg et al. 1988a). Three hours before the chamber was harvested, 45 MBq intravenous injections of $^{99\text{m}}\text{Tc-MDP}$ were given; the activity of the harvested tissue was measured in a well counter. The ratios between the counts of the two sides in each animal were tested for difference from 1 with the Student's *t*-test.

Table 2. $^{99}\text{TcMDP}$ activity and calcium yield in 10 harvests from bilateral test chambers in 9 rabbits receiving IGF-1 in one chamber and control solution in the other

Rabbit no.	$^{99}\text{TcMDP}$ cpm $\times 10^{-3}$		Calcium μg		$^{99}\text{TcMDP}$ IGF/Control	Calcium IGF/Control
	IGF side	Control side	IGF side	Control side		
1	15	17	120	64	0.90	1.08
2	17	19	176	292	0.89	0.60
3	7.9	8.6	56	40	0.92	1.40
4	21	30	184	168	0.69	1.10
5	22	22	400	232	1.02	1.72
5	7.5	13	68	96	0.57	0.71
6	19	22	248	212	0.89	1.17
7	16	26	176	164	0.62	1.07
8	14	19	136	148	0.73	0.92
9	5.7	17	120	200	0.33	0.60

Mean IGF/Control ratio for $^{99}\text{TcMDP}$: 0.76 SD 0.21 ($P < 0.01$).

Exclusion of the rabbit that was used twice gives the ratio 0.75 SD 0.20 ($P < 0.01$).

If series 1 (Table 1) and 2 were combined, the ratio would be 0.80 SD 0.18 ($P < 0.001$).

Results

In both series the isotope activity was decreased on the IGF-1-treated side (Tables 1 and 2). No side differences of calcium content were observed.

Discussion

The drug test chamber is unique in that a fracture callus of a known age can be isolated in vivo, locally treated, and harvested in toto. Also, in each animal an untreated control callus can be studied for comparison. The feasibility of drug application with this chamber has been demonstrated (Aspenberg et al. 1988a). However, the absence of a stimulatory or inhibitory effect would be inconclusive, because local concentrations achieved are not under control. Further, it seems that bone healing in this model already has so favorable conditions that further stimulation is difficult to achieve, even with methods known to stimulate the healing of ordinary skeletal defects. e.g., bone matrix implantation (Aspenberg et al. 1988b).

The concentration in the bone ingrowth canal of a stable factor would be dependent on the supply rate (by diffusion through the capillary from the reservoir), and of the elimination rate by diffusion and capillary transport out of the canal. The supply rate would be reasonably constant or decreasing through the experimental period, whereas the elimination rate probably increases

when capillaries grow in with the new bone. Thus, the concentration is probably higher in the beginning. Judging from various preliminary experiments, we assume the local concentration in the canal towards the end of the period to be 0.01–0.001 of that in the pump. In case of IGF-1, the achieved local activity is almost impossible to predict, because it depends on unknown local concentrations of carrier proteins that may and may not inactivate the IGF-1 (Skottner 1988).

In the present study, however, IGF-1 was shown to decrease $^{99\text{m}}\text{Tc-MDP}$ uptake at 14 days. Thus, IGF-1 has reached the tissue in the bone ingrowth canal in sufficient amounts to exert an effect, but the amount of harvested bone was unchanged, as determined by the calcium content.

Because $^{99\text{m}}\text{Tc-MDP}$ uptake mainly reflects bone deposition (Russel and Fleisch 1976), but the amount of bone was unaffected, it would seem that bone resorption was diminished as well, or that IGF-1 has exerted a stimulatory effect at the beginning of the experimental period followed by an inhibition.

We chose an experimental period of 2 weeks because at that time the bone formation rate should be maximal: bone has usually grown at least halfway from the openings of the canal, but has not yet met in the middle, which occurs after about 3 weeks.

Because many regenerating tissues are thought to produce growth factors for their own use, the absence of stimulatory effect in our model might indicate that the endogenous supply of IGF-1 is already optimal. This is not necessarily the case in other bone fractures.

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