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Transforming growth factor-ß enhances fracture healing in rabbit tibiae

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The ability of exogenous Transforming Growth Factor-ß (TGF-ß) to stimulate bone formation in fracture healing was investigated. TGF-ß was continuously applied in doses of 1 and 10 µg/day for 6 weeks to 2 groups of adult rabbits with unilateral plated midtibial osteotomies. A group receiving solvent without TGF-ß served as control. Fracture healing was evaluated by mechanical tests, bone morphometry and bone densitometry. Increased maximal bending strength and callus formation were demonstrated in the groups receiving TGF-ß. TGF-ß had no effect on bending-stiffness, bone mineral content, cortical thickness or haversian canal diameter. We conclude that local application of exogenous TGF-ß may enhance fracture healing in rabbits.

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Transforming growth factor-ß (TGF-ß) is a 25,000-dalton polypeptide growth factor which is shown to have multifunctional stimulative and regulating effects on bone tissue in vitro (Roberts and Sporn 1988, Centrella et al. 1991). Local injection of TGF-ß into soft tissues has stimulated fibrosis and promoted healing of wounds in rats (Roberts et al. 1986, Mustoe et al. 1987, Lynch et al. 1989). Daily injections of 20–1000 ng TGF-ß in fetal rodent calvaria have initiated and stimulated bone formation leading to a 6-fold increase in thickness (Noda and Camilliere 1989, Marcelli et al. 1990, Mackie and Trechsel 1990). When injected subperiosteally in rat femur doses of 20–200 ng/day caused formation of cartilage and new bone (Joyce et al. 1990). In a recent study, slow release of 5 µg TGF-ß from a polysaccharide carrier during 4 weeks stimulated bony healing of large calvarial defects in rabbits (Beck et al. 1991). These results indicate a potent ability of TGF-ß to promote bone formation. We report that continuous local dosage of exogenous TGF-ß can promote healing of rabbit tibia osteotomies.

Material and methods

Experimental design

30 New Zealand white rabbits were randomly allocated to 3 groups (Table 1). All groups were sex- and weight-matched. The rabbits were 9 months old and bone mature, weighing between 3.5–4.5 kg. All animals were given unilateral plated mid-tibial osteotomies. Animals in Groups I, II, III received 0 µg, 1.0 µg and 10 µg TGF-ß/day, respectively. The group receiving only the solvent served as control group. The observation period was 6 weeks.

Operative procedures

During general anesthesia (Hypnorm, Janssen Pharma, Holland) in a dose of 0.5 mL/kg and under antibiotic cover (Ampicillin 0.5 g intramuscularly), unilateral midtransverse tibial osteotomies were performed through the tibiofibula junction with an oscillating saw. The osteotomy was stabilized using a four-hole AO DCP plate (52 × 7.5 × 2.0 mm) and 4 screws (2.7 mm) positioned on the anterolateral surface of the

Table 1. Experimental design

Group	Daily dose of TGF-ß (µg/day)	No. of animals entering the study	No. of animals completing the study
I (Control)	0	10	8
II	1.0	10	9
III	10.0	10	6

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PLATED MID-TIBIAL UNILATERAL OSTEOTOMY

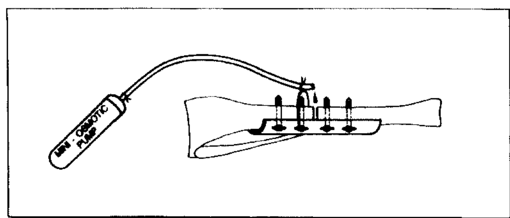

Figure 1. The experimental model consisted of a plated unilateral mid-tibial osteotomy. The continuously application of growth factor was accomplished by a mini-osmotic pump.

right tibia. A subcutaneous mini-osmotic pump (Alzet 2ML4, pumping rate 3.0 μL/hour) was connected to the osteotomy line via a 0.5 mm inner diameter polyvinyl catheter (Figure 1) (Keller et al. 1992). All pumps were exchanged during general anesthesia after 3 weeks. To minimize variation in the stability of the osteotomy, all operations were performed by the same surgeon. The animals were killed after a total of 6 weeks, using an intra-cardiac overdose of barbiturate. The tibiae were then dissected free from soft tissue for further analysis.

In the control group and the 10 μg TGF-ß group, 1 and 3 animals, respectively, were excluded due to fracture through the distal drill holes. In all 3 groups 1 animal died of unknown causes. The animals had an average weight loss of 250 g after 6 weeks of observation. The weight loss did not differ between the groups.

Source and application of TGF-ß

TGF-ß was extracted from human platelets by gelfiltration methods (Assoian 1987). The extracted TGF-ß more than 95 percent pure was freeze-dried and stored at –70 °C until use. TGF-ß was dissolved in isotonic NaCl solution containing phosphate buffer pH 7.4 and 0.1 percent bovine serum albumin. Biological activity of the produced TGF-ß was tested by an increase in collagen production in a fibroblast in vitro assay. TGF-ß was delivered by Alzet mini-osmotic pumps connected to the osteotomy lines via polyvinyl catheters. Also TGF-ß released from a mini-osmotic pump for 3 weeks at 37 °C was tested in the fibroblast in vitro assay. The produced TGF-ß was able to stimulate collagen production in the in vitro assay. TGF-ß released from a mini-osmotic pump at 37 °C for 3 weeks also showed intact biological activity.

Bone densitometry

The AO plate was removed from the tibia and the bone mineral content (BMC) was determined by photon absorptiometry (Gammatec Osteodensitometer Model GT 30). Bone mineral content (BMC) was determined in a standardized area from 8 mm proximal to 8 mm distal from the osteotomy line. The scanner determined BMC in 4 mm segments. BMC was calculated as the mean of the 4 scans. Coefficient of variation determined by 10 double measurements was 0.03.

Mechanical testing

All the screws were removed and the tibial shaft was loaded in a 3-point bending set-up on a universal testing machine (Instron Ltd., England) at a constant deformation rate of 5 mm/min. The tibia was placed on the posterior surface and the load was applied at the osteotomy site on the anterior edge of the bone. The distance between the supporting bars was 4 cm. The load-deflection curve was plotted by a X–Y writer. From the plot, maximal bending strength (F-max) and stiffness at the osteotomy were determined.

Bone morphometry

Transverse sections 100 μm in thickness were cut on a water-cooled diamond saw (Exact) 5 mm distally to the osteotomy line. From these sections histomorphometric evaluation was performed using a computer-assisted light microscope. Callus area was determined by computer tracing of the callus tissue lying on the cortical bone. Coefficient of variation determined by 10 double measurements was 0.03. Cortical thickness and haversian canal diameter were determined at 10 randomly selected places on each section using a digitizer connected to the microscope. Coefficients of variation determined by 10 double measurements were 0.02 for cortical thickness and 0.06 for haversian canal diameter.

Statistics

All data are presented as mean (SEM). One-way ANOVA followed by Fisher LSD-tests were used to compare differences between control and stimulation groups. P-values less than 0.05 were considered significant.

Table 2. Maximal strength (N) and stiffness (N/mm) in 3-point bending test after 6 weeks of TGF-ß stimulation. Mean *SEM*

Group	Maximal strength[a]		Stiffness[b]	
I	187	*14*	358	93
II	263	*20*	571	*149*
III	254	*17*	680	*134*
Intact tibia	515	*26*		

[a] *P* 0.03 for I vs II + III.
[b] Not significant

Table 3. Bone morphometry. Mean *SEM*

Group	n	Callus area (area unit)		Cortical thickness (μm)		Haversian canal diameter (μm)	
I	8	44.7	*5.5*	1232	*38*	32.9	*3.4*
II	9	77.5	*14.0*	1225	*41*	33.0	*2.0*
III	6	99.2*	*18.4*	1202	*17*	30.9	*2.1*

P 0.01 for callus area data (ANOVA).
*Group I vs III (0.05 < *P* < 0.01)

Results

The mechanical test showed a stronger maximal bending strength in the TGF-ß groups when compared with the control group (Table 2). In the group stimulated with 1.0 μg TGF-ß the result was significantly different from control. In the 10 μg TGF-ß/day group the *P*-value was 0.07. A tendency to increased stiffness of the osteotomy was observed, but the result was not statistically significant (Table 2).

The callus area was increased in both groups given TGF-ß, as compared to the control group (Table 3, Figure 2). No differences in cortical thickness and haversian canal diameter were found (Table 3). The histological appearance of the callus tissue in both control and TGF-ß groups was that of woven bone with an woven bone density between 50 –70 percent.

BMC. No differences in bone mineral content were demonstrated between the control and TGF-ß groups. The mineral content was 0.515 (0.023), 0.505 (0.026), 0.551 (0.029) g/cm in Groups I, II and III, respectively.

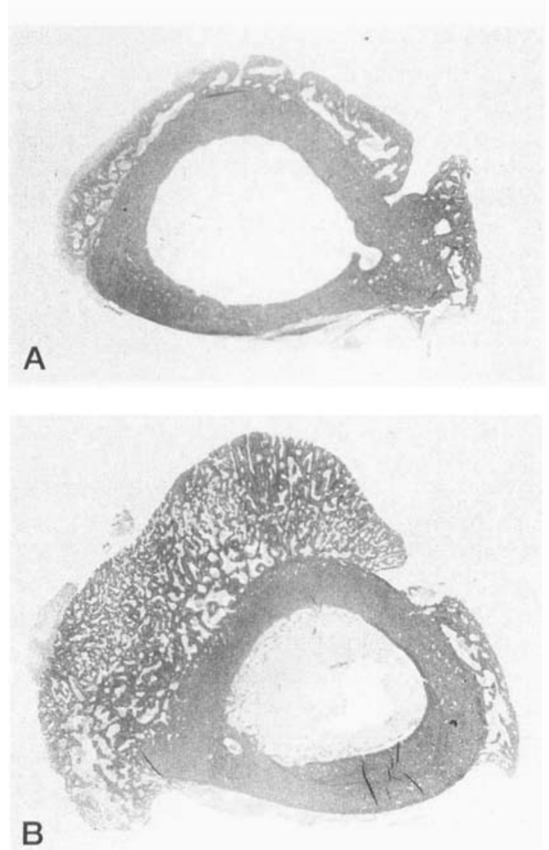

Figure 2. Decalcified transverse section 5 mm from the osteotomy line showing diaphyseal cortical bone and callus formation in a specimen from the control group (A) and 10 μg/day group (B). A moderate callus formation is seen in the control and massive callus formation in the stimulated specimen. The callus-free area represents the AO-plate position (HE × 5).

Discussion

The general stimulatory effect of TGF-ß on fracture healing may be ascribed to the greater callus formation around the osteotomy. The increased callus formation found in the present study is in agreement with several previous investigations. In vitro studies on callus tissue have shown a stimulatory effect of several growth factors. Joyce et al. (1989, 1991), and Noda and Camilliere (1989), found up to six-fold increase in calvarial thickness in rats given local calvarial injections of 1 μg TGF-ß, and Joyce et al. (1990) found a considerable stimulation of new bone and cartilage formation when TGF-ß was injected subperiosteally in newborn rats. In the present study we found an increased callus formation with increased doses of exogenous TGF-ß. Although callus formation was enhanced by 10 μg TGF-ß/day no increase in mechanical strength was demonstrated by the bending test. One explanation might be that the extensive callus formed was still too immature to enhance the mechanical strength of the osteotomy. We investigated remodeling in the cortical bone using the parameters cortical thickness and

haversian canal diameter. No changes in these parameters were shown in the groups stimulated with TGF-ß. Thus this study did not demonstrate any effect on cortical bone remodeling due to exogenous TGF-ß stimulation. Bone mineral content in callus tissue and cortical bone around the osteotomy were not altered in the groups stimulated with TGF-ß. The most probable reason for this finding is that the cortical bone contained most of the mineral, and since cortical bone remodeling was not influenced by TGF-ß stimulation, a significant increase in bone mineral content could not be expected. Our finding of increased strength of the osteotomy supports the theory that TGF-ß stimulates differentiation of the cell types involved in the healing process (Roberts and Sporn 1988, Joyce et al. 1991). Most previous in vivo studies with TGF-ß have been made in immature rodents with a greater growth potential than the mature rabbits used in the present study. Moreover, a species-related difference in reaction to TGF-ß stimulation could be present. However, a study by Beck et al. (1991) has shown that low doses of TGF-ß (70 ng/day) released from a polysaccharide matrix was able to stimulate bone formation in calvarial defects in mature rabbits. Other growth factors are present in callus tissue and may be able to influence the growth of this tissue. Experimental evidence shows that TGF-ß and Platelet-Derived Growth Factor (PDGF) are able to stimulate cell growth in callus tissue (Joyce et al. 1991). A combination of growth factors might be able stimulate fracture healing even further. The doses used in the present study were chosen from previous in vivo studies using injectional application (Noda and Camilliere 1989, Joyce et al. 1990, Marcelli et al. 1990). In these studies doses from 0.2 μg to 5.0 μg TGF-ß/day were found to stimulate new bone formation in young rats. These studies, however, used rodents as experimental animals and, since rabbits are considerably larger doses of 1 and 10 μg were used in the present study.

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