Calcitonin effects on rabbit bone

Bending tests on ulnar osteotomies

Theofilos Karachalios[1], George P Lyritis[1], Dionyssis G Giannarakos[1], George Papanicolaou[2] and Kostas Sotopoulos[1]

30 adult male New Zealand white rabbits underwent osteotomy and plate fixation of the left ulna for 6 or 12 weeks. The right ulna served as control. In half of the rabbits 6 IU calcitonin was given daily until death, and in the other half placebo injections in the same manner. A three-point bending test was performed on both ulnae. Sections of bone, covered or uncovered by the plates, were examined histologically. Long-term administration of calcitonin increased the mechanical properties of both fractured and intact bones and reduced porosity of the cortex under the plate.

[1]Athens University Th. Garofalidis Research Center, Orthopedic Department, Kat Hospital, Kifisia, and [2]Polytechnical School of Athens, Greece. Tel +30–1 80 87 617. Fax –1 80 18 122
Correspondence: Dr. G. Lyritis, Th. Garofalidis Research Center, 10 Athinas Str., Kifisia, GR-145 61 Athens, Greece
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Increased bone remodeling beneath internal fixation plates leads to a negative bone balance and regional osteoporosis. It has been shown experimentally that this phenomenon is apparent 4 weeks after osteosynthesis and reaches its maximum 2–3 months later (Uhthoff and Dubuc 1971). Early removal of the plates may cause a refracture due to insufficient bone healing. On the other hand, delayed removal can cause a refracture due to structural weakness of the bone and reduced bone mass (Frankel and Burstein 1968, Uhthoff and Finnegan 1983).

Bone remodeling can be influenced by a wide range of chemical substances. The hormone calcitonin is one of these. It inhibits the absorption of bone (Martin et al. 1966, Munson 1976), mostly by inhibiting osteoclasts (Rasmussen and Bordier 1974). It has been suggested that calcitonin has a stimulating effect upon osteoblasts, although this specific action is under investigation (Toccafondi 1985).

We have investigated short- and longer-term influence of calcitonin upon the mechanical strength and porosity of the internally-fixed bone.

Material and methods

30 New Zealand white 3 kg rabbits, 6–7 months old, were kept under normal experimental conditions and were allowed unrestricted access to dried food and water.

General anesthesia was induced by intramuscular administration of 0.5 mg of Midazolam and 50 mg of Ketalar. The left ulna was osteotomized at the midshaft with a thin oscillating saw. Osteosynthesis was performed with a 6-hole, self- compressing stainless steel plate (miniset). The right ulna was used as a control.

The rabbits were divided into 4 groups. Rabbits in Groups A and B (8 rabbits each) received 6 IU of salmon calcitonin per day subcutaneously. Treatment started the first postoperative day and continued until death. Rabbits in Groups C and D (7 rabbits each) received placebo injections in the same way. The animals in Groups A and C were killed 6 weeks postoperatively, using 0.5 gr sodium pentobarbitone iv, while those in Groups B and D were killed 12 weeks postoperatively.

Mechanical testing

Both forelegs were removed and cleansed of soft tissue. The plate was carefully removed from the left ulna and the bones were immersed in Ringer's solution. Mechanical testing (INSTRON device—three-point bending method) took place within one hour after death. To avoid rotation, the ulnae were placed on a base with a 3 cm distance between the two points of support. The bending load was applied to the concave surface of the bone at the osteotomy site. On the right intact ulna the load was applied at the midshaft. The velocity of the moving parts was 10 mm/min. The fracture load (N) and the deflection at fracture (mm) were read from the load-deflection curves, while the stiffness (N/mm) was calculated and the toughness (J) estimated as indicated in Figure 1.

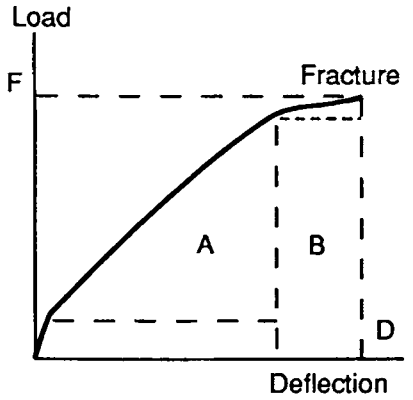

Figure 1. Load deflection curve during three-point bending. F is the fracture load. D is the deflection at fracture. The stiffness was calculated as the average slope at the load-deflection curve between 20 and 70 percent maximal force. The energy absorption until fracture (toughness) was estimated from areas A + B.

Histomorphometry

Using an electrical saw, three samples were taken after mechanical testing: from the midshaft of the left ulna at the site of the osteotomy; from the bone beneath the end of the plate that was closest to the fracture; and from the midshaft of the intact, right ulna. The specimens were immersed in 2% water solution of $AgNO_3$ for 48 hrs. After repeated sequential immersion in NaOH and $Na_2S_2O_3$ the specimens were decalcified in formic acid for 14 days and paraffin-embedded (Tripp and MacKay 1972). 4-µ-thick bone sections were placed on glass slides. The sections were stained using the von Kossa technique. A semiautomatic method was used. A Reichert-Jung photomicroscope was connected with a magnetic plate, a special pen and a Kontron computer. Computer-generated values of porosity (in percent) of bone beneath the plate and opposite the plate were obtained.

The following parameters were calculated for each sample: P1 is the percentage of the osteoporotic cross-sectional area in the bone covered by the plate; and P2 is the percentage of the osteoporotic cross-sectional area in the bone opposite the plate.

Statistics

ANOVA was used to establish the difference between groups. The nonparametric Wilcoxon rank sum test of significance was used to study the differences between individual groups. The level of significance was set at $P < 0.05$.

Results

The 12-week calcitonin group showed a higher fracture load ($P < 0.01$) and stiffness ($P < 0.01$) than the 12-week noncalcitonin group (Table 1). Moreover, for the 12-week calcitonin group the parameter of stiffness was higher than that of the 6-week calcitonin group ($P < 0.01$).

At 6 weeks, the calcitonin group showed a higher fracture load ($P < 0.05$) and toughness ($P < 0.05$) than the control group. Comparing the intact right ulnae we found that the 12-week calcitonin group showed a higher fracture load ($P < 0.01$) and stiffness ($P < 0.01$) than the 12-week noncalcitonin group.

Cortical porosity under the plate was lower in the 12-week calcitonin group in comparison with the 6-week calcitonin and the 12-week noncalcitonin groups ($P < 0.05$).

Cortical porosity was greater in the 6-week groups than in the 12-week groups ($P < 0.05$). There was more woven bone at the site of the osteotomy in the noncalcitonin groups and at the edge of the plate in the 6-week noncalcitonin group compared to the calcitonin groups.

Table 1. Mechanical parameters. A and B treated groups, C and D controls. Mean, *SD*

	6 weeks				12 weeks			
	Osteotomy		Control		Osteotomy		Control	
	Group A	Group C	Group A	Group C	Group B	Group D	Group B	Group D
Calcitonin	+	−	+	−	+	−	+	−
Fracture load (N)	57 *24*	50 *19*	102 *19*	102 *12*	58 *22*	48 *16*	115 *19*	106 *16*
Stiffness (N/mm)	51 *15*	55 *20*	80 *21*	56 *12*	58 *23*	46 *26*	97 *23*	85 *22*
Toughness (J)	40 *24*	31 *16*	65 *9*	99 *34*	33 *13*	31 *14*	73 *27*	72 *26*
Deflection (mm)	1.2 *0.5*	1.0 *0.2*	1.3 *0.2*	1.9 *0.5*	1.1 *0.2*	1.4 *0.4*	1.3 *0.4*	1.3 *0.0*

The 12-week calcitonin group had less porosity in the cortex opposite the plate compared with the noncalcitonin groups ($P < 0.05$).

The cortex opposite the end of the plate showed lower porosity in the 12-week groups compared with the 6-week groups ($P < 0.05$). During mechanical testing the bones fractured at the end of the plates at this specific area of increased porosity.

The Haversian systems both at the level of the osteotomy and at the edge of the plate in the 12-week calcitonin group were larger than in all the other groups. The amount of woven bone in the 12-week calcitonin group was smaller than that found in the 6-week calcitonin group and less than that seen in the noncalcitonin groups both at the level of the osteotomy and the level of the edge of the plate.

Discussion

Several experimental studies have analyzed the phenomenon of regional osteoporosis under internal fixation plates. Much of this work relies more on histological evaluation and less on mechanical testing.

The bone loss under the plates of osteosynthesis has been investigated quantitatively in humans (Cordey and Perren 1984). The time it takes for the bone to regain the normal Haversian architecture and mechanical strength after removal of plates is still unknown and is possibly longer than was previously thought (Perren et al. 1988).

The late results of long-term implantation are not clear. It is known that the presence of plates delays the remodeling process and leads to loss of bone mass. These effects are greater with stainless-steel plates than with titanium plates (Uhthoff and Finnegan 1983). Since the time required for recovery of the mechanical strength is influenced by the time of implantation, early removal has been suggested (Låftman et al. 1980). Moreover, early removal of the materials promotes a vigorous reconstructive activity which quickly restores the normal cortical architecture, while delayed removal causes decreased activity (Uhthoff and Finnegan 1983).

Our study focused on mechanical strength in relation to the histological changes beneath and away from the material of osteosynthesis during long-term administration of a safe dose of calcitonin. In designing the study we took into consideration the following: the usual experimental dose of calcitonin for rabbits weighing 3 Kg is 3 to 4 IU (Schatzher 1979). Doses higher than 10 IU have not resulted in any side-effects (Lyritis et al. 1986). It is also known that primary bone healing in rabbits takes place in 9 weeks (Ashhurst 1986).

It is important that in our study there was no close relation between the histological changes and the mechanical parameters due to the administration of calcitonin. It is suggested that calcitonin influences the quality (mechanical properties) rather than the quantity of the bone (i.e., the architecture of the Haversian systems, mineralization of the collagen fiber and crystallization of the hydroxyapatite).

We found a mechanically and histologically weak area, located in the cortex opposite the end of the plate in comparison with the cortex under the end of the plate. This phenomenon was more obvious in the groups which had undergone long-term plating and was independent of the administration of calcitonin. These findings suggest that internal fixation exerts a negative influence on the cortex not covered by the plate. One possible explanation for this phenomenon is that the presence of the plate causes abnormal load transmission and thus a stress-shielding effect in the cortex.

In the groups which had undergone shorter-term plating, the bone under the plate showed higher porosity compared with the groups with late removal. These findings differ from those published by Uhthoff and Finnegan (1983).

The finding that long-term administration of calcitonin improved the mechanical properties of internally-fixed bones (late removal of the plates) is important. The same phenomenon in a lesser degree, is shown in cases of early removal of the plates.

The finding that long-term administration of calcitonin improves the mechanical properties of the intact ulnae is also important. This is further evidence to support the suggestion that calcitonin influences the quality (structure) rather than the quantity of the bone.

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