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EFFECTS OF PARATHYROIDECTOMY AND VITAMIN D ON FRACTURE HEALING

Fracture Biomechanics in Rats After Parathyroidectomy and Treatment with 1,25-dihydroxycholecalciferol

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Fracture healing was studied in male, adult Sprague-Dawley rats. Closed bilateral tibial fractures were observed to be clinically stable after 3 weeks. Parathyroidectomy (PTX) resulted in impaired fracture healing and several delayed unions. Fracture tensile strength, elastic stiffness and failure energy were significantly lower at the beginning of the healing period compared to that of control fracture rats. Treatment with low doses (60 ng/kg/day) of 1,25-dihydroxycholecalciferol ($1,25(OH)_2D_3$) increased early fracture bone formation and mineralization. However, these events did not result in a corresponding increase of tensile strength or failure energy compared with that of the controls. Increased bone turnover seemed to be the dominant characteristic and resulted in early resorption of periosteal callus. Toward the end of the healing period, fracture strength measured as tensile strength and failure energy actually decreased compared to that of the control rats. Elastic stiffness initially rose above control values due to increased mineralization, but declined later to control values.

Key words: fracture; 1,25-dihydroxycholecalciferol; parathyroidectomy; rat; vitamin D

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Bone fracture healing may be influenced by a variety of biological factors (cf. Penttinen et al. 1972). Among these, growth hormone, thyroxin, insulin, vitamin D_2, anabolic steroids and electric current have been reported to promote fracture healing. On the other hand, corticosteroids, alloxan-induced diabetes, high doses of vitamin D_3, vitamin D deficiency and delayed manipulation have been shown to retard bone healing.

In an unpublished study on the mineralization of experimental fractures in adult rats, we did not find any major reduction of minerals incorporated into the callus after parathyroidectomy. The present study deals with the biomechanical properties of the fracture under the influence of selective parathyroidectomy or treatment with low doses of $1,25(OH)_2D_3$.

METHODS

Animals and housing

A total of 149 male, 12-month-old rats of the Sprague-Dawley strain was used. Their body weight varied between 442 and 691 g (mean: control 535, PTX 586, $1,25(OH)_2D_3$ 506 g). All the animals were fed a standard laboratory diet (Ewos, Södertälje, Sweden), containing 1.16% calcium, 1.00% phosphate and 0.019% zinc on a dry weight basis (Ca/P ratio 1:0.86). The contents of vitamin D_3 were approximately 150 IU per 100 g diet. Food and deionized water were supplied *ad libitum*. The animals were housed in acrylic resin cages

covered with a stainless steel net, with four rats in each cage. They were not exposed to direct sunlight but there was electric light from 8 a.m. to 8 p.m.

Fracture

At the start of the experiment, the right and the left tibiae of all the animals were broken manually under ether anaesthesia. The fractures were accomplished in a standardized way. Roentgenographically, the fractures were nearly always situated at the border between the proximal and the middle third of the tibia. They were left to heal without fixation. No fracture was open.

The animals were divided into three main groups:

a) In 51 rats, the fractures were left to heal without any treatment and these rats served as controls.

b) Selective parathyroidectomy (PTX) was performed under ether anaesthesia using diathermy on 49 rats (cf. Ahlgren & Larsson 1975).

c) Forty-nine animals were given daily intraperitoneal injections of 30 ng (78 pmol) 1,25-dihydroxycholecalciferol (Vitrum AB, Stockholm, Sweden) ($1,25(OH)_2D_3$ in 0.1 ml 95% ethanol) 5 days a week from the start of the experiment until sacrifice.

Experimental period

In the animals in the three different groups the fractures were left to heal for 7, 14, 21, 27, 32, 39 and 48 days. The animals were killed by bleeding from the femoral artery under ether anaesthesia.

Testing procedure

The whole tibia was carefully removed without wrapping off muscles and periosteum. Testing was performed immediately using an Instron model 1026 hydraulic strength tester (Instron Ltd., Bucks, England, load cell 20 kg). The bone was held by clamps which were placed on the epiphysis at identical sites for all samples. Testing of the bones was performed in a plastic chamber at a constant temperature of 22°C and at 95–100% relative humidity.

The load was recorded continuously during constant rate elongation (50 mm/min). Maximum tensile strength, elastic stiffness and energy to failure were calculated from the graphs. The equipment was calibrated with known weights before use. Reference values were obtained by testing tibiae from intact, normal adult rats.

Statistical analysis

All data were fed a statistical program (SPSS) for non-linear multiple regression. The curves were considered polynomial as a simplification. Polynomial terms were introduced in steps, testing the null hypothesis at each step that higher polynomials were not significant. In this way, it was possible to find the point where no substantial improvement of fit was obtained. Testing the null hypothesis between groups was performed by a non-parametric test (Mann-Whitney-Wilcoxon).

RESULTS

Tensile strength

The tibial fractures of *the control animals* were found to be clinically stable after 3 weeks. The tensile strength increased rapidly, particularly during the first 3 weeks after fracture. The increase then became less rapid, but from the fifth week onward a further continuous increase was recorded (Fig. 1).

In *the group of PTX animals*, seven delayed unions occurred in contrast to none in either the control group or the $1,25(OH)_2D_3$-treated group. The non-united fractures were so unstable that no meaningful tests could be performed. At repeated examinations, one delayed union was apparent after 3, two after 4, and four after 7 weeks. As to tensile strength, the stable fractures of the remaining PTX rats showed quite a slow increase ($P < 0.01$) between the first and the following 2 weeks. From then on, the tensile strength showed a steady increase but did not reach the level of the control animals. The mean value obtained for all

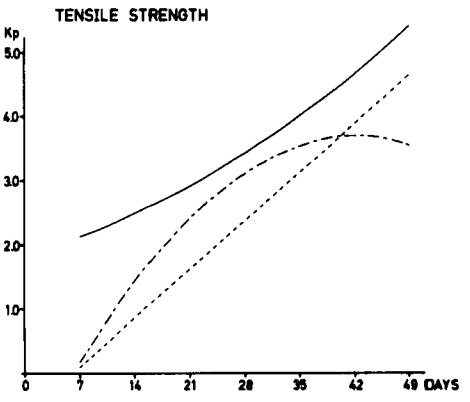

Figure 1. Regression curves of tensile strength for fractures at different intervals. The correlation coefficients for the curves were: 0.63 Controls (——), 0.61 PTX (– – –), 0.82 $1,25(OH)_2D_3$ (– · –).

the PTX rats was significantly lower ($P<0.001$) than that of the controls.

The tensile strength of the *1,25(OH)$_2$D$_3$-treated animals* showed the same rapid increase as in the control animals from the end of the first week. However, from the fifth week after fracture, the tensile strength evidently declined to values which were lower ($P<0.01$) than those of the control fracture rats. The mean value obtained from all the 1,25(OH)$_2$D$_3$-treated animals was lower ($P<0.05$) than that of the controls.

Elastic stiffness

An almost linear increase of fracture elastic stiffness was obtained for *the control rats* (Figure 2). After 7 weeks the values had reached a level approximately one third that of the reference values which were obtained for intact rat tibiae. *The PTX rats* showed low values initially compared with those of the control rats ($P < 0.01$). However, an increase was recorded after 2 weeks and later on, a level was reached that did not differ significantly from that of the control rats. The values obtained for *the 1,25(OH)$_2$D$_3$-treated rats* showed a very rapid increase initially compared to the controls ($P<0.05$), but a decline was then recorded towards the end of the experimental period. The final values were significantly lower than those obtained for the control fracture rats ($P<0.01$).

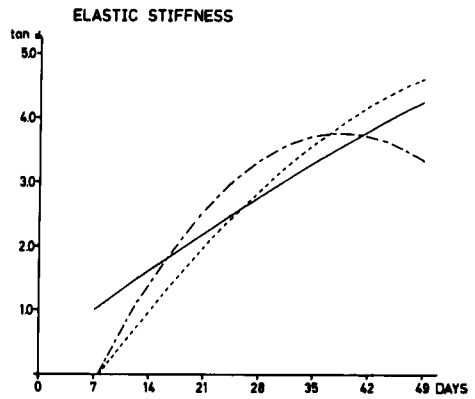

Figure 2. Regression curves of elastic stiffness for fractures at different intervals. The correlation coefficients were: 0.75 Controls (——), 0.58 PTX (– – –), 0.81 1,25(OH)$_2$D$_3$ (– · –).

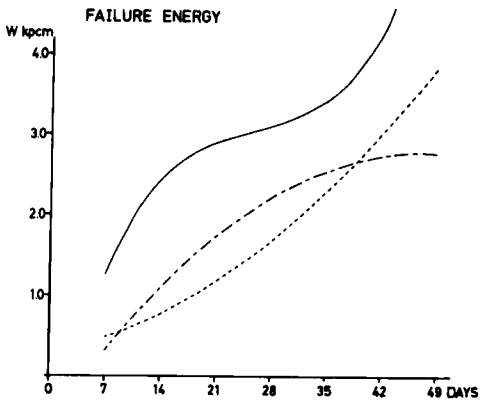

Figure 3. Regression curves of failure energy for fractures at different intervals. The correlation coefficients were: 0.57 Controls (——), 0.53 PTX, 0.79 1,25(OH)$_2$D$_3$ (– · –).

Failure energy

The control animals showed an evident biphasic curve pattern for regaining fracture strength (Fig. 3). After a rapid increase during the first 2 weeks, a plateau level was reached which was followed by a second increase from the fifth week. The values obtained for the animals of *the PTX group* were quite low initially but showed a rapid increase after the fourth week. In contrast, the values of *the 1,25(OH)$_2$D$_3$-treated rats* showed an increase initially but from the fifth week onward, an evident level-off was recorded. Testing the mean values obtained from the groups of PTX and 1,25(OH)$_2$D$_3$-treated animals against that of the control group gave significantly lower values both for the PTX rats ($P<0.001$) and the 1,25(OH)$_2$D$_3$-treated rats ($P<0.01$).

DISCUSSION

The control fracture rats in the present study started to bear weight on the fractured limbs after 1 week. After 3 weeks all the fractures were clinically stable. The curves obtained for tensile strength and energy failure were biphasic with quite a slow increase between the third and fifth week after fracture. Thereafter, there was a continuous increase. This pattern of events might de-

pend on the remodelling of the callus which occurs between the third and fifth week (Penttinen et al. 1972). Neither the PTX rats nor the $1,25(OH)_2D_3$-treated rats showed this characteristic biphasic curve pattern. This difference was certainly due to a disturbed remodelling of the callus. The fracture strength of the parathyroidectomized rats was significantly lower during the early part of the healing period as compared with that of the control rats. Microscopically, this was related to delayed maturation and differentiation of the callus, although mineralization of the callus was not greatly impaired (unpublished observations). These findings correspond well with the fact that bone turnover is low in hypoparathyrodism (Rasmussen & Bordier 1974).

During the early healing period, the callus of the 1,25-DHCC-treated rats was more voluminous and early mineralization seemed to be enhanced. This is in accordance with reports by Lindholm & Sevastikoglou (1978) of increased healing rate, enhanced mineralization of fracture callus and cortical bone formation on treatment with small doses of $1\alpha(OH)D_3$. The voluminous callus, as such, could have contributed to increased fracture strength by forming a periosteal callus collar during the early healing (Mølster et al. 1982). However, the rapidly growing callus in the $1,25(OH)_2D_3$-treated rats did not reach the strength of the control rat callus probably because of insufficient differentiation and orientation (cf. Glimcher & Crane 1968). The decreasing strength towards the end of the healing period was probably the result of increased resorption of the periosteal callus. These events are consistent with the findings reported by Marie & Travers (1980) of an effect of $1,25(OH)_2D_3$ primarily on bone turnover. The results of Galus et al. (1980) and Gallagher & Lawson (1980) further indicate that $1,25(OH)_2D_3$ acts as a bone remodeller primarily, while other vitamin D metabolites like $25(OH)D_3$ and $24,25(OH)_2D_3$ may act on bone formation more preferentially.

Early callus strength seems to be determined preferentially by the organic matrix and later by the mineral contents (Currey 1969). The elastic properties and strength of calcified tissues are determined predominantly by maturation of the collagen and by intermolecular collagen cross-linking (Less & Davisson 1977). The effect of $1,25(OH)_2D_3$ may have been an overall stimulation of callus bone turnover, resulting in a callus collar more disorganized and porous than normal, and with less strength.

Dietary zinc supplementation has been reported to give a more proliferative callus formation (Milachowski et al. 1980). There seems to be a direct relationship between the zinc status of the individual and collagen synthesis (Starcher et al. 1980). The zinc metalloenzyme alkaline phosphatase has been investigated as a possible primary line in fracture healing and is dependent on the zinc status (Starcher et al. 1979). However, the increased incorporation of zinc into callus of the $1,25(OH)_2D_3$-treated rats in the present study was not associated with a rise in alkaline phosphatase activity. On the other hand, low alkaline phosphatase activity was recorded in the callus of the PTX rats, although incorporation of zinc was normal (unpublished observations).

In conclusion, our results indicate that parathyroidectomy greatly impairs the normal process of fracture healing, probably by insufficient differentiation of osteoprogenitor cells and remodelling of callus. Administration of $1,25(OH)_2D_3$ seemed to increase callus bone turnover with early mineralization, which gave temporary increased elastic stiffness over controls but not increased tensile strength.

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