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Bone healing stimulated by plasma Factor XIII

Osteotomy experiments in sheep

Sheep had their right metatarsals osteotomized midshaft and stabilized by plate and screws. One group was injected with 1250 units of Factor Thirteen for 9 days postoperatively, and a control group received placebo injections. After 8 weeks, the bones were evaluated biomechanically, histomorphologically and by densitometry. The bones of the treated group had a significantly higher tensile strength than the bones of the control animals. The correlation of biomechanical and morphological results demonstrated that the tensile strength increased with an increasing number of osteons crossing the osteotomy gap. The hydroxyapatite content of the bone healing zone was 7.3 per cent higher for the treated bones than for the control bones.

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Changes in the biological status of the fracture haematoma, notably decreased Factor Thirteen, may delay wound and bone healing (Moussawi et al. 1978). Experimental and clinical investigations (Behring Information 1981, Gierhake et al. 1970, Salzman 1976) have shown that healing processes may improve after intravenous injection of Factor Thirteen. The efficacy of this factor is due to its ability to enhance fibrin cross-linking and to stimulate fibroblast proliferation (Beck et al. 1961). Benfer & Struck (1977) found that Factor Thirteen produced higher tensile strengths and histological evidence of accelerated healing of rat fractures, whereas Hellerer et al. (1980) in a similar animal model found no effects on callus formation and mineral salt density.

Our study was performed to provide additional data on the possible effect of Factor Thirteen on fracture healing.

Material and methods

Animal experiments

The experiment was performed in 22 adult male sheep with an average weight of 55 kg. All operations were done under general anaesthesia administered through an endotracheal tube. A medial incision was made through the skin. Preserving the periosteum, the right metatarsal diaphysis was osteotomized with an oscillating saw and stabilized with a 6-hole plate (Figure 1); the round-hole plates were pre-bent and fixed to the dorsal surface with six 3.5 mm cortical screws. By bending the plates slightly, it was possible, in each animal, to achieve good contact on the anterior side of the bone and hence to ensure stable fixation. The sheep were allowed to walk immediately after surgery.

A radiograph of the metatarsal was taken immediately and at 4 weeks postoperatively. Eight weeks after surgery, the animals were killed, the metatarsals were harvested, and radiographs were again taken (Faxitron 43805 N X-ray System, Hewlett Packard).

After removing the screws and plates, the diaphyseal part of the metatarsal was cut into three segments of equal length (Figure 2). The middle segment, containing the osteotomy zone, was longitudinally sectioned into halves. The medial half was

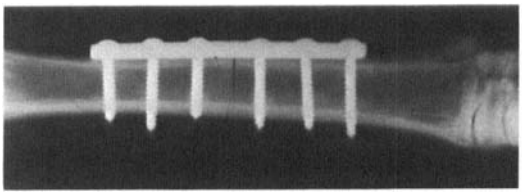

Figure 1. A metatarsal with a transverse osteotomy stabilized by a 6-hole internal fixation plate.

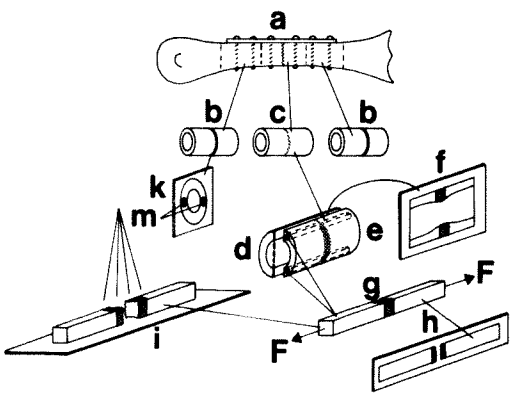

Figure 2. Diagram showing the processing of the sheep's metatarsals after explantation. a: Metatarsal with plate and screws. b, c: After removal of the implant, the bone is sawn into three segments. d, e, f: The middle segment (c) is sawn in half longitudinally; the medial half is embedded for longitudinal histological bone sectioning (f) while two bone samples (g) are taken from the lateral half (e). g, h, i: After tensile strength testing, one-half of the bone samples is embedded for longitudinal histological sectioning and microradiography (h), and the second half is for radiography (i). k, m: After embedding undecalcified bone, cross-sections are cut from the distal and proximal cylinders, and the number of osteons are counted at two fields (m).

tested biomechanically. The lateral half, together with the intact proximal and distal segments, was embedded in methylmethacrylate for undecalcified bone histology.

Factor Thirteen treatment

Eleven sheep were intravenously injected with 1250 units of human Factor Thirteen (Fibrogammin®, Behring, Marburg) preoperatively, immediately postoperatively, and on the first, third, fifth, seventh and ninth postoperative days. Factor Thirteen was supplied with albumin in the lyophilized form and dissolved in Ringer's saline solution immediately before the intravenous administration. The 11 control animals were given albumin placebo injections at the same time intervals.

The distribution of the sheep between experimental and control groups was randomized. Before each intravenous injection, a blood sample was taken for the Factor Thirteen Rapid Test (Behring) (Bohn & Haupt 1968).

Sequential polychrome labeling

To study the rate of bone remodeling, sequential polychrome labeling was performed (Rahn & Perren 1975). Intravenous injections of fluochrome labels were given in the first week (tetracycline, Reverin, 15 mg/kg) and fourth week (calcein green 20 mg/kg) (Rahn & Perren 1975).

Biomechanics

Two bone samples of approximately 25 mm length were taken from the medial half of the middle segment and machined to 2 × 2 mm in cross-section. One sample was taken from the plated dorsal cortex, and the other from the ventral cortex; both included the healing zone in the middle of the specimens. To assess the tensile strength at these sites, the samples were tested to failure on a materials testing machine (Zwick 1454) with a strain rate of 1 mm/min. The tensile force (F) and the elongation (ΔL) of the test samples were measured and recorded simultaneously. From these data, in conjunction with the cross-section area (A) and initial length (L) of the bone samples, stress (σ) – strain (ε) curves were plotted from the tensile tests ($\sigma = F/A$), ($\varepsilon = \Delta L/L$).

From the stress-strain curves (Figure 3), the tensile strength (S) was derived. The tensile modulus of elasticity (E) of the bone was determined from the slope of the initial linear part of the curve ($E = \tan \alpha$); where α is the angle with the strain axis.

Histomorphology

In order to assess bone healing, undecalcified, longitudinal 100 µm thick sections were cut from the lateral half of the middle segment and from the bone samples used for the biomechanical examination; they were stained using the trichrome Goldner method. Transverse sections of the same thickness from the distal and proximal segments were stained with Paragon stain. Microradiographs of all histolog-

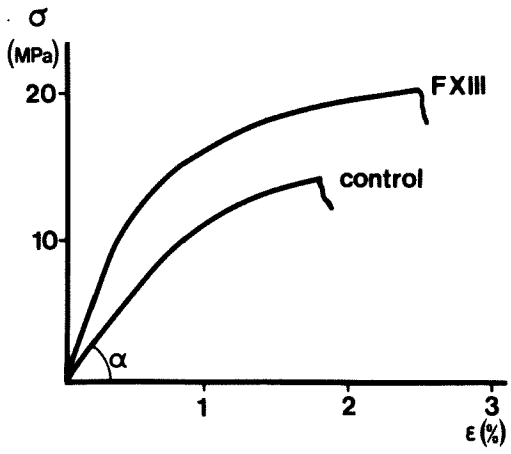

Figure 3. Typical stress-strain diagram of tensile strength testing of bone samples.

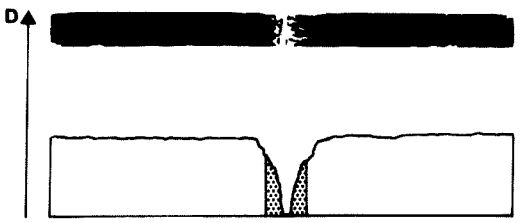

Figure 4. Typical plot of the density distribution curves of bone samples. Density determination was carried out 0.5 mm proximal and distal to the fracture line (tensile testing) by planimetry of the surfaces under the density curve (stippled areas).

ical sections were taken (Faxitron 43805 N X-ray system, Hewlett Packard, Kodak Glass Plates).

From the longitudinal sections of each of the bone samples used in the tensile tests, the number of osteons crossing the healing zone was determined. Using the transverse sections from the proximal and distal segments of bone, the number of newly formed osteons was counted. Distinctions were made among those osteons laid down by the end of the first week (tetracycline), the first and fourth weeks (tetracycline and calcein green), and during the fourth week (calcein green). For this purpose, 8.5 × 2 mm fields from two areas were used, one on the medial and the other on the lateral cortical surface of the bone. It was not possible to make standardized determinations of the occurrence of the various fluorescent colour bands in the vicinity of the healing osteotomy using the longitudinal sections.

Mineral density

After biomechanical testing, the remaining bone samples were used for radiography. For indirect determination of hydroxyapatite concentration in the osteotomy zone (Figure 4), optical densities of the radiographic images were measured 0.5 mm proximal and distal to the fracture site (Microdensitometer MK 3CS Joyce-Loebl). The apatite content of the healed bone was calculated from the radiographic density by the method of Meema et al. (1964); this method compares the density of the bone with the density of a liquid wedge of K_2HPO_4 which has a known hydroxyapatite equivalent radiographic density.

Results

One sheep from the Factor Thirteen-treated group was excluded from the study because of an infection. All other fractures healed without complications. The animals were fully weight bearing on the operated leg 2–3 weeks after the operation. They maintained their weight during the experimental period.

Factor Thirteen serum analyses

Postoperatively, the sheep in the control group showed no detectable decrease in plasma Factor Thirteen levels (Figure 5); all had 120–140 per cent of levels found in standard human plasma, i.e. the normal range of Factor Thirteen in sheep plasma. In the sheep given Factor Thirteen intravenously, the plasma Factor Thirteen levels increased steadily during the first week to 240–280 per cent. After the last injection on the ninth postoperative day, levels declined to within the normal range within 1 week.

Biomechanics

During tensile testing, all the bone samples failed at the osteotomy site. There was no difference between the mechanical properties of the specimens taken from the dorsal and ven-

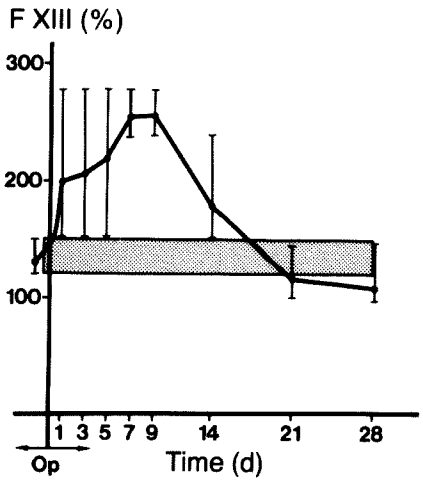

Figure 5. Plasma Factor Thirteen levels (curve: treated group, mean ± maximal and minimal value, stippled area: normal range) during the first 4 weeks of the experiment.

Table 1. Bone healing of metatarsal osteotomy in sheep stimulated by Factor XIII (mean ± SD)

	Biomechanics (MPa)		Histomorphology (No. of osteons)				Hydroxy-apatite content (mg/cm^3)
	Ultimate tensile str.	Tensile modulus	Distal and proximal segment cross section			Osteotomy segment longitudin. section	
			1st week	1–4th week	4th week		
Factor XIII	19.5±2.0	2590±1640	1.1±0.9	6.7±5.7	6.2±7.7	2.8±1.6	397±20
Controls	13.5±1.9	1240± 360	0.02[a]	6.7±5.6	3.8±3.7	1.4±0.7	370±21

[a]Only one value.

tral sides of the bone healing zone. Therefore, for the statistical analysis, mean values were determined using specimens from both locations to represent the mechanical properties of the bone. Bone samples from the animals treated with Factor Thirteen had higher ultimate tensile strengths than the controls (Figure 3, Table 1). The calculated modulus of elasticity also clearly showed higher values for the Factor Thirteen-treated animals than for the controls.

Sequential polychrome labeling

In the early phase of bone remodeling, i.e. during the first postoperative week, there were more tetracycline-labeled osteons in the Factor Thirteen group than in the controls (Table 1). Tetracycline/calcein green labeled (first-fourth week) osteons occurred with equal frequency in both groups. However, in the Factor Thirteen group there were more celcein green-labeled (fourth week) osteons.

There were thus more newly formed osteons in the Factor Thirteen-treated group than in the control group.

Histomorphology

Morphological evaluation of the undecalcified bone sections and microradiographs revealed forms of contact and gap healing in the osteotomy zones with minimal endosteal and periosteal callus formation (Figure 6). Haversian remodeling of the cortical bone was observed. The number of osteons crossing the osteotomy was determined for all specimens that were previously used for the tensile testing (Figure 7). The tensile strength increased with the number of osteons crossing the osteotomy site. The samples having the highest tensile strength and the largest numbers of longitudinally arranged osteons all belonged to the group that had been treated with Factor Thirteen (Figure 8).

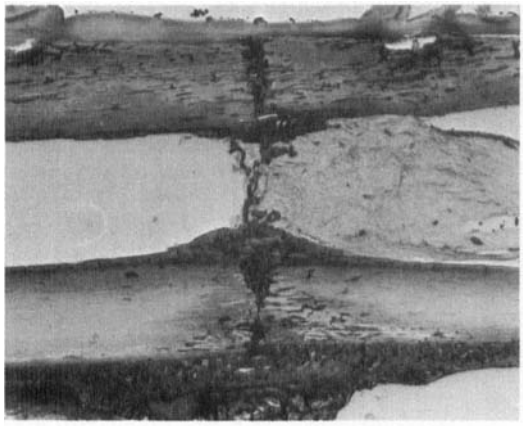

Figure 6. Undecalcified longitudinal section through the middle of the diaphysis of a sheep's metatarsal. The osteotomy is in contact on the anterior side (bottom) and shows a gap filled with new bone on the dorsal side, which is underneath the bone plate (top).

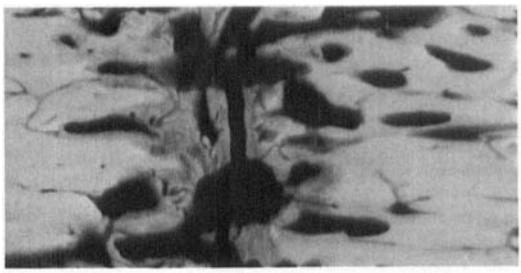

Figure 7. Osteotomy healing in sheep's metatarsal 8 weeks postoperatively. Microradiographs (× 20) from the bone samples used for tensile strength testing. Failure invariably occurred at the osteotomy zone in the middle of the figure.

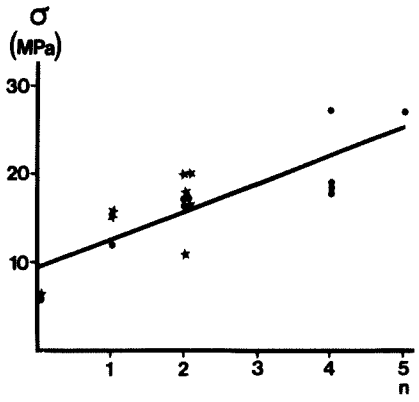

Figure 8. Correlation between the tensile strength (σ) of the healing osteotomy and the number (n) of osteons crossing the osteotomy gap. All of the bone samples with the highest tensile strength and the largest number of osteons belonged to the group treated with Factor Thirteen (linear regression R = 0.85). • Factor XIII; * Albumin.

Mineral density

In the Factor Thirteen-treated animals, the hydroxyapatite content of the bone within the healing zone averaged 397 ± 20 mg/cm^3, this being approximately 7.3 per cent higher than the average of 370 ± 21 mg/cm^3 found in the control group. The difference between the means was significant ($p < 0.05$, Wilcoxon test).

Discussion

We found that early bone healing in sheep was definitely accelerated by treatment with Factor Thirteen; 8 weeks postoperatively, the ultimate tensile strength of the healing osteotomies was, on average, 44 per cent higher ($p < 0.01$) in the Factor Thirteen-treated sheep than in the controls. The higher tensile strengths found in the Factor Thirteen group are apparently due to more advanced bone healing, this difference being reflected morphologically in the greater number of new longitudinally arranged osteons crossing the osteotomy site, and in the higher density of hydroxyapatite as compared with the controls in the microdensitometry studies. The fact that the Factor Thirteen-treated animals had increased numbers of newly formed osteons in the cortical bone remote from the osteotomy seems to indicate an enhanced tendency to new bone formation during the first 4 postoperative weeks.

In previous investigations, it was assumed that Factor Thirteen deficiency was caused by operations or trauma, and attempts were made to correct these deficiencies. However, there was no postoperative drop in Factor Thirteen level in the present experiment (Figure 5). For this reason, the doses of Fibrogammin®, instead of merely restoring the level to normal, caused a definite increase in Factor Thirteen level above the normal value for sheep. This increase persisted for approximately 14 days postoperatively (Figure 5).

Of all the parameters tested, the one most suitable for demonstrating the bone-healing differences between the Factor Thirteen-treated sheep and the controls was the tensile strength test. The test which showed the smallest difference was the determination of the hydroxyapatite content in the healing zone. These results provide a possible explanation for the findings of Hellerer et al. (1980) who were unable to demonstrate any differences in mineral content between treated and untreated animals. Benfer & Struck (1977) found significant differences with tensile strength testing. The parameter most closely correlated with bone strength was found to be the number of osteons crossing the osteotomy site, a value which clearly reflects the structure of the healing bone. The structural remodeling of immature woven bone into mature bone with an adequate number of longitudinally arranged osteons was found only in the group treated with Factor Thirteen.

The more rapid bone healing in the animals treated with Factor Thirteen is probably attributable to gains made during the first and second phases of fracture healing. It is in these phases that Factor Thirteen enhances fibrin cross-linking and fibroblast proliferation (Behring Information 1981). There might also be some influence on osteoblast activity or proliferation, but this requires further investigation.

Our data are in close agreement with the results obtained by Benfer & Struck (1977) on the influence of Factor Thirteen on fracture healing in rats; whereas we investigated the early phase of fracture healing, their studies showed that the difference in tensile strengths

between treated and untreated fractures decreased with time.

For normal fracture healing, the accelerating effect of Factor Thirteen is probably not essential. However, it might be useful in the treatment of delayed bone union and nonunion.

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