The importance of the hematoma for fracture healing in rats

Oliver Grundnes and Olav Reikerås

In 3 groups of rats, bilateral femoral fractures were produced. The fracture hematoma was removed on one side after 30 min, 2 days and 4 days in the 3 groups, respectively. The fractures were mechanically tested at 4 weeks.

When the fracture hematoma was removed early, callus production, bending moment, and fracture energy were decreased. Removal of the hematoma at Day 2 or 4 impaired fracture healing even more, as both bending moment, bending rigidity and fracture energy were greatly decreased compared to the control fractures. We conclude that the fracture hematoma favors healing; removal of the hematoma after some days is more harmful to healing than when the hematoma is removed in the initial phase.

Department of Orthopedics, Institute of Clinical Medicine, University Hospital, N-9012 Tromsø, Norway
Tel +47–83 26000. Fax –83 26042
Correspondence: Dr. Oliver Grundnes, Dept. of Surgery, Harstad Hospital, N-9400 Harstad, Norway. Tel +47–82 15000. Fax –82 15127
Submitted 92-03-16. Accepted 92-08-28

In a previous study we have found that the hematoma seems to be of some importance for fracture union, as removal of the hematoma during operative stabilization impaired the initial phase of healing (Grundnes and Reikerås 1992). Furthermore, it should be of interest to know the consequences of removal of the hematoma after some days when it has been organized into a fibrin clot, as this often is the clinical situation when fractures are to be operated/reoperated. We therefore designed an experiment to determine the mechanical consequences of removing the fracture hematoma in the immediate situation and at 2 and 4 days following fracture.

Material and methods

24 male Wistar rats (Møllegård Avlslaboratorium, Eiby, Denmark) weighing 342 (320–378) g were randomly divided into 3 groups. Under intraperitoneal anesthesia (fentanyl-fluanixone, 0.1 mL/100g body weight), the trochanteric areas were exposed on both sides. From the trochanteric groove the medullary cavity was entered by an awl, and with steel burrs mounted on an electrical drill successively reamed to a diameter of 1.5 mm. Another incision was made along the thigh, and the midshaft of both femurs was exposed subperiosteally between the lateral vastus and the hamstrings muscles. Bilateral fractures were produced by first driving an awl percutaneously through the bone at the midshaft of the femur to weaken it and to avoid communition of the fracture. Both bones were then manually broken. The fractures were standardized to the distal end of the trochanteric rim, which easily could be felt under the skin. Closed medullary pin insertion was performed by closed reduction with one hand while driving a 1.6 mm steel pin mounted on an electrical drill from the trochanteric area through the fragments. Fixation was achieved without radiographic control. Proper pin placement was confirmed by radiograms at the end of the experiment; all the fractures healed by the production of external callus and proper pin placement was confirmed in all rats.

In Group A the thigh incision on the right side was closed in two layers prior to fracture. On the left side the hematoma was allowed to clot for 30 min before it was carefully removed and remnants rinsed out by saline irrigation. The thigh incision then was closed, as were the incisions over the trochanteric areas.

In Groups B and C both thigh incisions were closed in two layers before the bones were broken and stabilized as described. On the second (Group B) and fourth days (Group C) following fracture, again under intraperitoneal anesthesia, the thigh incisions were opened on both sides. On the left side, the fracture area was exposed and the organized hematoma carefully removed and remnants rinsed out by saline irrigation. On the right side, the fracture area was exposed, however, the organized hematoma was left undisturbed. The wounds were once again closed in two layers.

Table 1. Cross-sectional area of callus mass (mm^2), bending moment (Nm x 10^{-1}), bending rigidity (Nm/rad) and fracture energy (Nm x rad x 10^{-2}) of nailed femoral fractures 4 weeks after fracture. Hematoma removed 30 min (Group A), 2 days (Group B), and 4 days (Group C) after fracture and left intact. Values are medians and 25–75 percentiles. n 8

Group	A			B			C		
Hematoma	removed	*P*	intact	removed	*P*	intact	removed	*P*	intact
Callus	48.1 (41.9–78.0)	0.05	62.1 (57.7–81.9)	33.8 (28.6–50.2)	0.06	44.4 (37.5–66.3)	31.8 (30.0–41.4)	0.07	38.6 (35.0–53.6)
Moment	1.97 (0.66–2.39)	0.02	2.47 (1.69–2.8)	0.21 (0.13–0.43)	0.006	2.63 (2.03–3.08)	0.26 (0.20–0.39)	0.006	1.76 (0.97–2.39)
Rigidity	0.81 (0.55–1.18)	0.06	1.40 (0.95–1.53)	0.11 (0.05–0.28)	0.006	1.20 (0.73–2.07)	0.09 (0.04–0.19)	0.006	1.05 (0.51–1.43)
Energy	2.32 (0.36–2.64)	0.03	2.52 (1.08–3.48)	0.26 (0.10–0.82)	0.006	3.04 (2.12–4.28)	0.46 (0.40–0.94)	0.01	2.32 (1.16–3.74)

The animals were killed in a CO_2 chamber at 4 weeks following fracture. Soft tissue was carefully removed in both femurs and the bones were then radiographically examined. By use a of sliding caliper (accuracy of 0.01 mm) the antero-posterior and transverse diameters of the callus mass were measured and the callus area was calculated, assuming an elliptical shape. The bones were mechanically tested in a cantilever bending test with dorsal deflection of the distal end of the femurs, as described by Engesaether et al. (1978). The hydraulic testing machine was run at a constant rate of 0.08 rad/s. The load values were transferred to a chart recorder displaying the load-deformation curve. The strength was calculated as the bending moment necessary to produce fracture. The bending rigidity was determined from the slope of the linear elastic part of the curve. Fracture energy was defined as the energy absorbed during loading to fracture.

Data are presented with medians and 25–75 percentiles. For statistical evaluation within the groups we used the paired Wilcoxon signed rank test. To evaluate differences between the groups the Kruskall-Wallis test was used, and the non-paired Wilcoxon rank-sum test was used when statistical differences were found. $P \leq 0.05$ was considered significant.

Results (Table 1)

Removal of the fracture hematoma after 30 min decreased the callus area, bending moment and fracture energy, while bending rigidity was not different in the two limbs. In Group B where the hematoma was removed after 2 days, no differences in cross-sectional callus area were found. The median bending moment at the hematoma-removed side was 8 percent of the control side, median bending rigidity 9 percent, and median fracture energy 8 percent, respectively. In Group C where the hematoma was removed on one side after 4 days, no differences in the cross-sectional callus area were found. In this group, the median bending moment at the experimental side was 15 percent of the control side, median bending rigidity was 9 percent and median fracture energy 19 percent, respectively.

Analysis between the groups revealed that the cross-sectional callus area on the experimental side was greater in Group A compared to Group B (*P* 0.02) and Group C (*P* 0.004). The same differences were found at the sham-operated side. There were no differences in cross-sectional callus area between Group B and Group C (*P* 0.3).

Analysis of mechanical properties showed that in Group A both bending moment (*P* 0.02) and bending rigidity (*P* 0.001) were greater compared to Group B and Group C. No differences were found between Group B and Group C (*P* 0.4). There were no differences in fracture energy between the 3 Groups (*P* 0.06). There were no differences in mechanical parameters between the groups in the sham-operated limb (momemt; *P* 0.1, rigidity; *P* 0.6, energy; *P* 0.2).

Discussion

The sequence of events following a fracture has become gradually more understood, and the cellular responses are known to be complex, involving numerous substances (Hulth 1989). Immediately following fracture a hematoma accumulates at the fracture area. The hematoma is known to be of some importance, but its exact role is unclear. It is generally accepted that mesenchymal cells provided by the periosteum and bone marrow will differentiate and produce the external fracture callus (Kernek and Wray 1973, Owen 1980). In the very acute phase of healing the hemat-

oma has not become well organized and shows little differentiation (Henricson et al. 1987). Therefore, we may presume that when the hematoma is removed in the immediate stage, it may reproduce rapidly with some of the osteoinductive potential intact. On the other hand, when the fracture hematoma was removed after 2 and 4 days it was partly organized. Thus, most of the osteogenetic stimulus at the fracture site was removed, which resulted in prolonged fracture healing and less callus production.

The trauma caused by fracture and reaming are thought to elict stimuli to the pluripotent mesenchymal cells derived from periosteum and bone marrow which, together with inflammatory cells, form granulation tissue in the acute stage. The effect of single and double trauma on the mitotic activity of bone marrow was studied by Johnell and Hulth (1979). They showed that when two traumatic injuries took place only 2 days after each other, the cell response to the second trauma became completely abolished. Therefore, when the fracture hematoma was removed 2 and 4 days following fracture, the bone marrow would have been refractory to the new stimuli elicited by the second operation. Mizuno et al. (1990) studied the osteogenetic potential of the fracture hematoma. They found that a two-day hematoma did not inherit the osteogenetic potential and that osteoinduction in this hematoma may result from the periosteum. The four-day hematoma, however, possessed osteogenetic potential as new bone formation was seen when the hematoma was transplanted to an intramuscular site. In the present study, removal of the hematoma at Day 4 did not provoke any further impairment in fracture healing compared to removal of the hematoma after 2 days. Thus, from a practical point of view, it is immaterial at what time an organized fracture hematoma is removed in the acute phase after fracture. Our results indicate that it is the fracture hematoma itself that possesses the osteogenetic potential from the fracture trauma. When the hematoma is removed, the fracture area is partly incapable of regaining this property.

In Group B and Group C the callus areas in the sham-operated side were significantly reduced compared to Group A. Many authors have proposed that the cells forming the external callus derive from the surrounding musculature (McLean and Urist 1968), and microangiographic studies have demonstrated that much of the vascular supply to the callus area derives from the surrounding soft tissue (Gøthman 1961, Rhinelander 1974). A sufficient supply of external callus in unstable fractures is probably dependent on relatively intact surrounding soft tissue (Whiteside and Lesker 1978, Henricson et al. 1987). Therefore, the reduced callus production in our study may result from diminished soft tissue vascular supply due to the sham operation. However, the reduced callus area did not provoke any changes in mechanical characteristics of the bones.

We conclude that fracture hematoma has an important role in fracture healing, and that removal of an organized hematoma some days after fracture impairs the healing rate more seriously than when the hematoma is removed in the immediate phase.

References

Engesaether L B, Ekeland A, Langeland N. Methods for testing the mechanical properties of the rat femur. Acta Orthop Scand 1978; 49 (6): 512–8.

Grundnes O, Reikerås O. The role of hematoma and periosteal sealing in fracture healing. An experimental study in rats. Acta Orthop Scand 1993; 64 (1): 47–9.

Gøthman L. Anterial changes in experimental fractures of the monkey's tibia treated with intramedullary nailing. Acta Chir Scand 1961; 121: 56–66.

Henricson A, Hulth A, Johnell O. The cartilaginous fracture callus in rats. Acta Orthop Scand 1987; 58 (3): 244–8.

Hulth A. Current concepts of fracture healing. Clin Orthop 1989; 249: 265–84.

Johnell O, Hulth A. The effect of single and double trauma on the mitotic activity of bone marrow and thymus. Acta Chir Scand 1979; 145 (2): 73–5.

Kernek C B, Wray J B. Cellular proliferation in the formation of fracture callus in the rat tibia. Clin Orthop 1973; 91: 197–209.

McLean F C, Urist M R. Bone. Fundamentals of the physiology of skeletal tissue. 3rd ed. University of Chicago Press, Chicago, 1968.

Mizuno K, Mineo K, Tachibana T, Sumi M, Matsubara T, Hirohata K. The osteogenetic potential of fracture haematoma. Subperiosteal and intramuscular transplantation of the haematoma. J Bone Joint Surg (Br) 1990; 72 (5): 822–9.

Owen M. The origin of bone cells in the postnatal organism. Arthritis Rheum 1980; 23 (10): 1073–80.

Rhinelander F W. Tibial blood supply in relation to fracture healing. Clin Orthop 1974; 105 (0): 34–81.

Whiteside L A, Lesker P A. The effects of extraperiosteal and subperiosteal dissection. II. On fracture healing. J Bone Joint Surg (Am) 1978; 60 (1): 26–30.