The role of hematoma and periosteal sealing for fracture healing in rats

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Bilateral closed femoral fractures were produced in two groups of rats. Reaming was made from the trochanteric area before fracture, and the fractures were stabilized by intramedullary pinning. In one (hematoma) group, both femurs were exposed subperiosteally at the midshaft prior to fracture. At one side, the hematoma was evacuated 30 min following fracture, at the other side the hematoma was left undisturbed. In the other (periosteal) group, the femur was exposed subperiosteally at one side, while the periosteum was left intact at the other side. The healing of the fractures was evaluated at 4 weeks. In the hematoma group, no differences were found in callus production, while bending moment and bending rigidity were greater at the side where the hematoma was not removed. No differences were found in fracture energy. In the periosteal group, marginal differences were found in the callus area. Bending moment, bending rigidity and fracture energy were greater at the side where the periosteum was left intact. A comparison of all four limbs, showed that the periosteal sealing was of particular importance for rapid healing.

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In a previous study we have shown that closed medullary nailing of femoral fractures in rats is conducive to better healing than open medullary nailing (Grundnes and Reikerås 1992) which indicates that other factors than blood supply may account for the difference between open and closed treatment. Open treatment inevitably causes removal of the fracture hematoma and enhanced destruction of the periosteal seal. The present study examined which of these factors are of major importance for fracture repair.

Material and methods

Two groups, each with 8 male Wistar rats (Møllegård Avlslaboratorium, Eiby, Denmark) weighing 338 (320–351) g were used. Under intraperitoneal anesthesia (fentanyl-fluanixone, 0.1 mL/100g body weight), the trochanteric area was exposed on both sides. The medullary canal was entered by an awl, and was successively reamed to a diameter of 1.5 mm. Another incision was made along the thigh. In one (hematoma) group of animals the midshafts of both femurs were exposed subperiosteally between the lateral vastus and the hamstring muscles. On the right side the incision was closed in two layers. 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 comminution of the fracture. The fractures were standardized to the distal end of the trochanteric rim, which easily could be felt under the skin. Both bones were then manually broken. Medullary pin insertion was performed by reduction with one hand while driving a 1.6 mm steel pin mounted on an electric drill from the trochanter area into the fragments. Fixation was achieved without roentgenographic control. Proper pin placement was confirmed roentgenographically at the end of the experiment. On the left side the hematoma was allowed to clot for half an hour before it was carefully removed, and remnants were rinsed out by saline irrigation. The thigh incision was then closed, as were the incisions over the trochanter areas.

In the other (periosteal) group, reaming was performed on both sides as described, and another incision was made along the thigh. On the left side the midshaft of the femur was exposed by subperiosteal elevation, on the right side the periosteum was left intact. The thigh incisions were closed in two layers, and bilateral fractures were performed and stabilized as previously described.

The animals were killed in a CO_2 chamber at 4 weeks following fracture. Soft tissue was carefully removed from both femurs and the bones were then radiographically examined. By use of a sliding caliper

Table 1. Cross-sectional callus area (mm^2), bending moment (Nm × 10^{-1}), bending rigidity (Nm/rad) and fracture energy (Nm × rad × 10^{-2}) of nailed femoral fractures with hematoma removed 30 min after fracture and hematoma intact at 4 weeks after fracture and of nailed femoral fractures with destroyed and intact periost at 4 weeks after fracture. Values are medians and 25–75 percentiles. N 8

	Hematoma removed	*P*-value	Hematoma intact	Periosteum elevated	*P*-value	Periosteum intact
Callus area	58 41–81	0.08	79 58–82	55 33–71	0.08	41 30–53
Bending moment	1.4 0.5–2.2	0.03	2.2 1.2–2.7	1.9 1.1–4.0	0.01	6.3 4.5–6.9
Bending rigidity	0.9 0.37–1.1	0.03	1.4 1.1–1.8	0.7 0.6–1.7	0.01	2.5 1.8–2.8
Fracture energy	0.59 0.1–1.2	0.2	0.61 0.3–1.4	1.1 0.5–2.6	0.1	4.0 2.5–4.9

(accuracy of 0.01 mm) the antero-posterior and transverse diameters of the callus mass were measured. The callus area was then 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 Engesæter 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. A 2-way Anova with repeated measurements was used for statistical evaluations. When statistical significance was found, the paired and non-paired Wilcoxon rank sum test was used. $P < 0.05$ was considered significant.

Results (Table 1)

When analysis of variance was done, no differences in cross-sectional callus area were seen. Significant differences were found in bending moment and bending rigidity. The differences in fracture energy were significant.

Removal of the fracture hematoma resulted in a 26 percent reduction in cross-sectional callus area. Decreases were seen in bending moment and bending rigidity at 4 weeks following fracture. No differences were found in fracture energy between the two limbs.

Periosteal elevation resulted in a 34 percent increase in callus production. In mechanical testing the median bending moment was 3 times greater and the median bending rigidity was 3.5 times greater at the fracture site in the bones where no surgical damage to the periost was done.

There were no differences in mechanical parameters between the periosteal elevated group and the intact hematoma group. The bones in which the periosteum was left intact in the periosteal group showed significantly better healing than the other bones.

Discussion

Fracture healing under unstable conditions is characterized by the proliferation of mesenchymal cells with the formation of external callus. The first step in the reparative process is the organization of the fracture hematoma. This has hardly any mechanical role in immobilizing the fracture, but serves as a fibrin network for the proliferation and migration of the osteogenic cells (Cruess and Dumont 1975, Brighton 1984). Mizuno et al. (1990) demonstrated different properties of the fracture hematoma at different intervals following fracture. In our study, the hematoma was removed at 30 min after fracture without any significant effect on callus production, while bending moment and rigidity were reduced. However, the reduction of mechanical parameters was minor as compared to the differences in the periosteal group. At the acute stage when the hematoma was removed, it was not fully organized and differentiated. Therefore, we may presume that a new hematoma was produced at the fracture site with at least some osteoinductive potential intact.

The influence of the periosteum on new bone formation has been addressed by many authors (Siffert 1955, Finley et al. 1978). It is now generally accepted that the periosteum alone does not induce new bone formation, but does so in conjunction with the fracture

hematoma. In secondary union, the periosteum is the principal source of blood vessels (Rhinelander et al. 1968, Mindell et al. 1971). In our previous study (Grundnes and Reikerås 1992) we found that callus flow in the openly-treated fractures at 4 weeks was greater than callus flow in closed nailed femoral fractures. This observation indicates that reduced blood flow to the bridging callus cannot fully explain the impaired healing by the open nailing. On the other hand, additional damage to the periosteum due to surgical stripping by open methods, may be critical for ischemic bone cells and lead to excessive necrosis at the fracture site. Furthermore, it has been shown that even small variations in the microenvironment around the fracture influence the healing process (Bassett 1962). Among other factors, low oxygen tension leads to the formation of cartilage instead of bone tissue (Heppenstall et al. 1976).

Fractures associated with extensive soft tissue trauma are known to heal slowly. This is most probably due to high energy damage to the soft tissue envelope around the fracture. In addition to fracture healing, mesenchymal cells also must aid in the healing of the soft tissues (Hulth 1989). Kernek and Perry (1981) compared open versus closed pin insertion in tibial fractures, and found better healing with the closed method in low energy fractures, but not with severely displaced fractures. They suggested that in high-energy fractures surgery probably does not add any significant further damage to the soft tissues. In our study the periosteal stripping we performed by surgery obviously was greater than what was caused by the controlled low energy fracture.

The importance of an intact periosteum has been demonstrated by Macnab and De Haas (1974). They showed that when the periosteal seal was destroyed, fibrous tissue derived from the soft tissue surroundings could infiltrate between the bone ends, with a tendency for fibrous union. Destruction of the periosteal seal also makes it possible for the local hematoma to escape into the soft tissue with diffusion of mesenchymal cells. Our results are in full agreement with such suggestions.

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