Effects of lesion between bone, periosteum and muscle on fracture healing in rats

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We assessed the effects of periosteal detachment from bone and musculature on the healing of diaphyseal fracture. In 30 male Wistar rats we produced a partial osteotomy, which was manually broken in the middiaphysis of the left femur. All fractures were reamed and stabilized with an 1.6 mm steel pin. The animals were randomly assigned to 3 groups. In group 1, a subperiosteal detachment between cortex and periost was created in the middle third of the diaphysis. An extraperiosteal detachment between periost and the surrounding musculature was performed in group 2. In group 3, the periosteum was isolated from the musculature by an extraperiosteal detachment and application of an e-PTFE sheath (Gore-Tex® expanded polytetrafluoroethylene) around the shaft between the periost and the surrounding muscles. The rats were killed after 4 weeks and callus formation and mechanical characteristics were measured. All fractures healed by production of external callus. The callus area was significantly less in the group where periost was mechanically isolated from the surrounding muscles compared to the other groups. Bending moment, bending rigidity and fracture energy were less in this group than in groups 1 and 2. No differences were detected between the sub- and extraperiosteal groups, either in callus formation or in mechanical measurements. Our findings underline the importance of the muscle-periosteal connection for periosteal healing of diaphyseal fractures.

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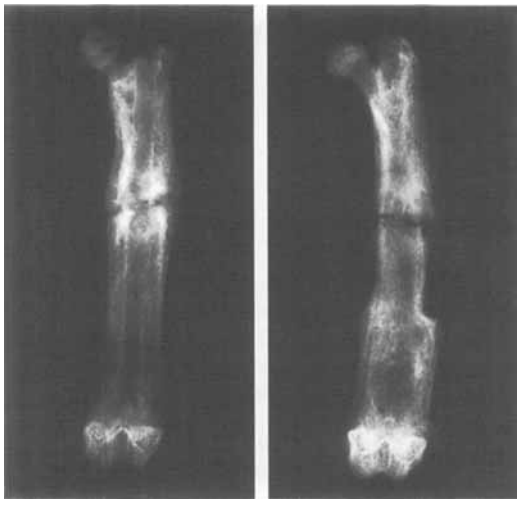
It is well recognized that the healing of diaphyseal fractures is related to the severity of damage to both bone and soft tissues at the time of injury (Oestern and Tscherne 1984). The fracture configuration, the presence of infection, the blood supply of the fragments and the stability of fixation all have an effect on healing (Gustilo and Anderson 1976, Court-Brown and Hughes 1985). Soft tissues surrounding a diaphyseal fracture are especially thought to play a role in the healing process as a vascular source (Rhinelander 1974, Trueta 1974), and severe soft-tissue loss with periosteal detachment and compromised vascularity are major determinants of the outcome (Gustilo et al. 1984). Surgical procedures for treatment of long bone fractures may further compromise the soft tissue structures and circulation.

In the first days after fracture, the periosteal cells are stimulated to proliferate and participate in the formation of callus (Einhorn 1992). The question of participation of cells from extraosseous tissues has, however, not been resolved (Hulth 1989). Some authors claim that healing is accomplished almost exclusively by the activitities of the periosteum and endosteum (Turek 1983), while others state that the callus forms mainly from pluripotent cells derived from several sources (Brand 1983, Rand and Bergquist 1986).

The question of participation of cells from extraosseous tissues in the healing of diaphyseal fractures is of general interest. Furthermore, knowledge of the outcome of selective interruptions of soft tissue contact to bone is of surgical importance. We examined the effects on fracture healing of periosteal detachment, detachment between periosteum and muscle and of isolation of the periosteum from surrounding soft tissue.

Animals and methods

30 18-week-old male Wistar rats (Møllegårds Avlslaboratorium, Eiby, Denmark) weighing 331–357 g were used. The rats were randomly assigned to 3 groups in which only the treatments of periost were different. Following intraperitoneal anesthesia (pentobarbital 5 mg/100 g body weight), a lateral incision of the left femur was performed. The medullary canal of the bone was entered from the greater trochanter by the use of an awl. The medullary cavity was then



Healing at 4 weeks of femoral osteotomy/fracture in rat after subperiosteal detachment (group 1) (left) and extraperiosteal detachment with application of an e-PTFE sheath between periosteum and muscle (group 3) (right). The intramedullary pins have been removed.

reamed to 1.5 mm by steel burrs mounted on an electric drill. The lateral aspect of the diaphysis was then exposed between the lateral vastus and hamstrings. In group 1, a subperiosteal detachment between cortex and periost along the diaphyseal region from the lesser trochanteric level to the condylar area was performed in the whole circumference of the bone. In group 2, an extraperiosteal detachment between periost and muscles was created. A partial transverse osteotomy at the mid-diaphyseal level was then performed with a fine-toothed circular saw blade mounted on an electric drill. The osteotomy was then manually broken. Reposition of the fracture was performed manually, while a steel pin of 1.6 mm was inserted from the trochanteric area through the proximal and distal fragments to the level of the condyles, using an electric drill. In group 3, the soft tissue procedure was carried out as in group 2, and in addition a 16-mm long Gore-Tex® e-PTFE (expanded polytetrafluoroethylene) sheath was sutured around the shaft (Prolene 4.0). Thus, the e-PTFE sheath provided a mechanical barrier between periost and muscles. Proper pin placement was confirmed by radiographs taken at the end of the experiment. The wounds were closed in two layers. All the rats tolerated the operation well and resumed walking on the first postoperative day and full weight bearing after 1–2 weeks. There were no dislocations of fractures or pins and all the e-PTFE sheaths remained in position.

The rats were killed in a carbon dioxide chamber after 4 weeks, and the left femur was dissected free from all soft tissue. Anteroposterior and transverse diameters of the callus area were measured with a sliding caliper (accuracy of 0.01 mm). In measuring the callus, slight manual pressure was applied, without indenting the callus. The quantity of callus was expressed as total cross-sectional area, the cross-section being assumed to be elliptical. The bones were then radiographically examined and the intramedullary pin was removed. The bones were preserved at –70 °C between removal and mechanical testing. The mechanical characteristics of the healing fractures were tested in a cantilever bending machine. The proximal end of the femur was fixed with a clamp, the cam of a rotating wheel engaged the femoral condyles, and a fulcrum at the fracture site was the third point of force application. Refracture was performed by deflection of the distal half of the femur, as described by Engesæter et al. (1978). The testing machine was run at a constant rate of 0.08 rad/sec. The load and deformation values were noted with a chart recorder displaying the load-deformation curve. The strength was calculated as the bending moment necessary to produce refracture. Bending rigidity was determined from the slope of the linear part of the curve. Fracture energy was defined as the energy absorbed during loading to refracture. In our testing situation, we found no slipping of clamps.

Data are presented as median values with 25th and 75th percentiles. For testing differences between the groups, one-way analyses of variance (Kruskall-Wallis test) was applied. When significant differences were found, the Wilcoxon rank sum test was used. The level of significance was set at $p < 0.05$.

Results

The osteotomies in groups 1 and 2 healed by production of external callus (Figure), whereas the callus production in group 3 was scanty and significantly less than in groups 1 and 2 (Table) ($p = 0.004$ and $p = 0.0002$, respectively). There were no significant differences in callus area between groups 1 and 2.

Bending moment, bending rigidity and fracture energy were lower in group 3 than in groups 1 and 2; $p = 0.002$ and $p = 0.007$, $p = 0.03$ and $p = 0.02$, $p = 0.03$ and $p = 0.01$, respectively. There were no differences in bending moment, bending rigidity or fracture energy between groups 1 and 2.

Discusson

Our study focused on the interrelationship between cortical bone, periost and surrounding musculature in

Cross-sectional area of callus, bending moment, bending rigidity and fracture energy 4 weeks after fracture. Median and 25th and 75th percentiles

	Group 1	Group 2	Group 3	P-value [a]
Callus area (mm^2)	72 (57–78)	55 (45–61)	25 (17–37)	0.02
Bending moment ($Nm \times 10^{-1}$)	1.4 (0.95–1.7)	1.6 (1.1–3.3)	0.32 (0.02–1.2)	0.01
Bending rigidity (Nm/rad)	4.2 (2.8–5.0)	4.7 (3.2–8.8)	0.94 (0.52–3.6)	0.02
Fracture energy ($Nm \times rad \times 10^{-3}$)	1.1 (0.9–1.3)	1.5 (1.2–2.9)	0.6 (0.3–1.1)	0.01

[a] Kruskall-Wallis test

fracture healing. We found no significant differences with regard to mechanical strength between subperiosteal and extraperiosteal dissection. The clinical implication of this result is that sub- and extraperiosteal dissection in a fracture seems to have the same consequences for healing, evaluated at 4 weeks. On the other hand, separation of the periosteum from surrounding muscles by an e-PTFE membrane significantly impaired fracture healing. This indicates that periosteal callus formation depends on a complex interaction between skeletal muscle and periosteum, and that the integrity of the soft tissues is an important prerequisite for the formation of periosteal callus.

These results are of clinical importance. The proper way to perform surgical exposure in diaphyseal fractures has been debated. Some authors have advocated extraperiosteal dissection to avoid disturbances of circulation between bone and periosteum (Anderson 1971), while others have advocated subperiosteal dissection to preserve collateral circulation between the periosteum and overlying muscles (Whiteside and Lesker 1978).

The Gore-Tex® Patch is an expanded, fibrillated polytetrafluoroethylene (e-PTFE) material. The Soft Tissue Patch (1 mm thick; pore size 22 m; Gore-Tex®, W. L. Gore & Associates Inc, Flagstaff, Arizona, USA) used in this experiment allows for tissue ingrowth into the membrane surface, with minimal capsule formation (Berman et al. 1986). A pore size of 30 m has been considered the limiting factor for tissue regeneration in e-PTFE. This size will not permit ingrowth of blood vessels, which suggests that nutrition is derived mainly from diffusion (Richter 1989). Thus, penetration of fibroblasts and immature mesenchymal cells into the lattice of e-PTFE is limited to the superficial layer of the sheath, and the pore size of 22 m does not permit ingrowth of blood vessels from the muscles to the periost. However, diffusion of oxygen through the membrane is possible, depending on pressure differences on the sides of the membrane (Boyce 1982).

Many authors have addressed the sources of osteoprogenitor cells in the fracture healing process, and two theories exist (McKibbin 1978, Hulth 1989). According to the first theory, osteoprogenitor cells occur only in close association with bone surface or the bone marrow (Oni 1996), and the osteogenetic potentials of the endosteum and periosteum are well documented (Einhorn 1992). The alternative view is that repair tissue does not arise from specialist cells, but rather from the activity of previously uncommitted mesenchymal cells that can develop the power of osteogenesis, if given the appropriate environmental stimulus (Brand 1983, Rand and Bergquist 1986). Cells derived from tissue outside the skeleton can form bone (Owen 1970) and implants of bone morphogenic proteins have induced bone formation in soft tissues (Urist 1965), as well as intramuscular transplantation of fracture hematoma (Mizuno et al. 1990). However, the direct contribution of extraosseous tissue in fracture healing has been difficult to demonstrate and previous investigators appear to exclude muscle tissue as a significant source of osteogenic cells for fracture healing (Oni 1996). It has been assumed that the role of surrounding tissues in diaphyseal fracture repair is to provide oxygen and nutrients (Rhinelander 1974). In our experimental set-up the e-PTFE sheath blocks invasion of pluripotent cells from the muscles to reach the periost at the fracture site. Furthermore, invasion of blood vessels from the muscles to the periost is avoided. Thus, both the cellular and vascular components were excluded from reaching the fracture site from the muscles.

Human tibial fractures are often associated with some degree of damage to the soft tissues surrounding the bone, even in closed fractures (Oestern and Tscherne 1984). Muscle has been shown to provide an important collateral source of blood to cortical bone in both clinical (Byrd et al. 1981) and experimental studies (Richard and Schemitsch 1989), and there is evidence that the periosteum and overlying muscles share circulatory channels and have collateral circulation (Whiteside and Lesker 1978). It has also been found that the vessels of the musculature dilate and proliferate after fracture, thereby participating in the healing process (Einhorn 1992). Reduction in the per-

fusion of muscle may delay union of diaphyseal fractures. Whiteside and Lesker (1978) documented delayed union, measured as mechanical strength in rabbit osteotomies superimposed with muscle trauma. Our results, indicating a marked reduction in mechanical characteristics after separation of the periost-muscle connection, further indicate the important role played by muscle in the healing process. Richards et al. (1991) compared skin and muscle coverage in a devascularized segment in osteotomized canine tibia and observed a marked reduction in mechanical characteristics in the low-perfused skin group. This indicates that the lack of perfusion by the muscle plays an important role in the mechanism behind the reduced healing observed in this study. However, whether the reduced perfusion from muscle to periost is the main mechanism underlying the observed reduction in healing remains open to discussion. It is also possible that the inability of fibroblast cells from muscle to reach the periosteal surface is part of the mechanism. These cells, which can transform to osteogenesis, may plah an important role, in the healing process (McKibbin 1978).

Our experiment indicates that in early healing, coverage of cortical bone by muscle might be of benefit to maximize the osteogenetic potential in medullary nailed fractures. This may be due to the ability of the musculature to reestablish periosteal blood flow or muscle contribution of pluripotent mesenchymal cells, which differentiate to osteogenetic cells in the fracture healing process.

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