Poor muscle coverage delays fracture healing in rats

Stein Erik Utvåg[1], Knut Børge Iversen[2], Oliver Grundnes[3] and Olav Reikerås[4]

Departments of Orthopedic Surgery, [1]Institute of Clinical Medicine, NO-9038 University Hospital of Tromsø, [2]Diacones Hospital, NO-5009 Bergen, [3]Ullevål Hospital, NO-0450 Oslo, [4]National Hospital, NO-0570 Oslo, Norway. Correspondence: Dr. Olav Reikerås. E-mail: olav.reikeras@rikshospitalet.no
Submitted 01-10-13. Accepted 02-02-05

ABSTRACT – We undertook this study in rats to ascertain the influence of muscle coverage on tibial fracture healing. 30 rats were randomly assigned to three intervention groups. Following a mid-diaphyseal osteotomy in the left tibia, reamed nailing was performed in all animals. In one group (A), the antero-lateral muscles were detached from the fractured bone, while the antero-lateral compartment was excised in another group (B). In the third group (C), the muscle compartment was resected, and the superficial gluteal muscle was mobilized and transposed over the fractured area. Muscle intervention, like that in group A and C, had no effect on the blood flow. The fibular nerve was resected in all the rats. At 4 weeks, we studied the healing bones in each group clinically, radiologically and mechanically.

At 4 weeks, radiographs in two planes revealed a clearly visible fracture line in the three experimental groups. Mechanical testing of the healing fractures showed significantly lower bending moment and bending rigidity in group B than in groups A and C. No difference in mechanical characteristics was detected between the healing bones in groups A and C. This animal study indicates that in tibial fractures, an extensive muscle tissue defect may have negative effects on early bone healing.

It has been generally accepted that the soft tissues surrounding fractures are important in the healing of bone (Gustilo and Anderson 1976, Whiteside and Lesker 1978, Gustilo et al. 1984, Oestern and Tscherne 1984, Wu et al. 1984), especially those around a diaphyseal fracture, which are thought to play a role as a source of vascularity (Rhinelander 1974, Trueta 1974) by supplying oxygen and nutritional factors. The vascular supply of immature mesenchymal cells from extra-osseous tissue may also contribute significantly to callus formation (Brighton et al. 1992, Diaz-Florez et al. 1992). In fact, early soft tissue coverage has been advocated as an integral part of the treatment of tibial fractures in cases of severe damage to soft tissues (Byrd et al. 1981, Hertel et al. 1999).

Although muscle flaps have been widely used in trauma care, their effect on the strength of osseous union has attracted less attention (Hertel et al. 1999), and there is some dispute about the relative importance of the contributions of various muscle flaps to fracture healing. The periosteum also helps to head fractures by supplying cellular components (Ritsila et al. 1989, Reynders et al. 1999), while some consider the musculature an important source of progenitor cells. This experimental study was undertaken to focus on early fracture healing in the tibia. We denuded a segment of muscle and periosteum and then compared treatment with a transposed thigh muscle flap to a viable in situ muscle flap.

Animals and methods

We used 51 male 18-week-old Wistar rats (Møllegårds Avlslaboratorium, Eiby, Denmark) weighing 356 (330–377) g. They were housed in cages with two animals in each and received a standard rodent diet (Special Diet Services, U.K.; R.M. 1) containing 0.71% calcium and 0.5% phosphorous and given tap water ad libitum. The light/dark cycles were 12h/12h. The experiment conformed to the Norwegian Council's Animal Research Code

for Care and Use of Animals for Experimental Purposes.

30 rats were randomly assigned to 3 groups in which only the soft tissue procedure differed. After intraperitoneal anesthesia (pentobarbital 5 mg/100 g body weight), the middle third of the left tibia was subperiosteally exposed through a ventral incision. A transverse osteotomy just distal to the tuberositas tibia (between 15 and 16 mm below the tibial plateau) was performed with a fine-toothed circular saw blade mounted on an electric drill. A steel pin of 1.1 mm was inserted proximal to the tibial tuberosity in the midline through the proximal and distal fragments using the drill. The 1.1 mm steel pin had a bending rigidity of 2.72 Nm/rad.

In group A, the muscles comprising the lateral anterior compartment were detached with the periosteum from the bone in a distal direction, but the proximal and distal tendon insertions were left intact. It was then left in situ, but the fibular nerve was resected. In groups B and C, the muscles comprising the compartment were cut just distal to their proximal insertions and then excised with the periosteum and fibular nerve along the entire antero-lateral margin of the bone. In group C, the proximal and dorsal insertions of the superficial gluteal muscle were mobilized and the muscle transposed over the denuded fracture segment and sutured to the medial tibial fascia. The arterial blood supply of the gluteal muscle, derived from branches of the iliac artery, was always preserved. Free and unrestricted movement of the knee joint was evaluated after transposition. All wounds were closed in layers.

At 4 weeks, the rats were killed in a carbon dioxide chamber, and both hindlimbs dissected free of all soft tissues from the tibia. We measured the antero-posterior and transverse diameters of the callus and the amount was expressed as the cross-sectional area, assuming an elliptical symmetry. Radiographs of the bones were taken in two planes (Siemens S 06 x-ray tube, Munich, Germany). The medullary nail was removed before mechanical testing. The bones were preserved at –70 °C between removal and mechanical testing.

Before the experiments, 7 rats were used for baseline data on dimensions and mechanical properties of the tibial bone and 14 other rats for data on muscle blood flow 30 minutes after the two types of muscle intervention at the fracture site.

Microspheres (Du Pont, Bad Hamburg, Germany) labeled with ^{85}Sr of 15.5 ± 0.1 μm in diameter were used to measure muscle blood flow. Each injection consisted of 750,000 spheres homogeneously suspended in 0.9% saline. The spheres were vortexed in a whirl mixer for 2 minutes before injection. A heparinized polyethylene catheter (PE 50) was introduced via the carotid artery and inserted in the aortic root for injection of microspheres. The microspheres were injected over a period of 30 sec, and the catheter was then flushed with 0.5 mL saline. The caudal artery, cannulated with a heparinized polyethylene catheter (PE 10), was connected to a Harvard infusion/withdrawal pump for reference sampling. The flow rate in the reference organ was set at the rate of 195 L/min. Withdrawal started 15 sec before injection of the microspheres and continued 30 sec after the injection was finished. We then killed these rats in a carbon dioxide chamber and the superficial gluteal and tibialis anterior muscles were isolated and placed in counting vials. The specimens and reference samples were placed in a Packard Auto Gamma Spectrometer for 5 minutes which has a counting error less than 1%. Standard test tubes with a certain number of microspheres were counted with the test tubes and provided data on the number of spheres in each specimen. The number of spheres in each specimen exceeded 400, and the variation caused by random distribution of microspheres was therefore less than 3% within a 95% confidence interval, as described by Buckberg et al. (1971).

We tested mechanically the intact and healing bones in a cantilever bending machine. The proximal end of the tibia was fixed with a clamp, the cam of a rotating wheel engaged the distal metaphyseal region, and a fulcrum at the fracture site was the third point of force application. The bone was fractured 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 values were documented on a chart recorder showing the load-deformation curve. The strength was calculated as the bending moment necessary to produce a fracture. The bending stiffness was determined from the slope of the

Cross-sectional area of callus (mm^2), bending moment ($Nm \times rad \times 10^{-1}$), bending stiffness (Nm/rad) and fracture energy ($Nm \times rad \times 10^{-3}$) in group A (local muscle flap), group B (tibialis anterior muscles excised) and group C (distant muscle flap) 4 weeks after tibial osteotomy and soft tissue injury. Median, 25th and 75th percentiles

	Group A	Group B	Group C	P-value[a]	P-value A vs B	P-value B vs C	P-value A vs C
Callus area	22.6 (21.2–25.9)	23.1 (18.8–33.3)	26.7 (22.7–31.4)	0.6			
Bending moment	3.83 (2.94–5.02)	2.73 (2.05–3.37)	3.73 (3.36–4.28)	0.03	0.03	0.01	0.09
Bending rigidity	2.66 (2.51–3.71)	2.21 (1.53–2.34)	3.07 (2.65–3.39)	0.002	0.01	0.0003	0.8
Fracture energy	2.42 (2.18–3.77)	2.80 (1.40–3.67)	2.71 (1.93–3.79)	0.6			

[a] Kruskall-Wallis test
P-values between groups

linear elastic part of the curve. Fracture energy was defined as the energy absorbed during loading to fracture.

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

Results

At the start of the experiment, the internal anteroposterior diameter of the medullary cavity at the fracture site was 1.38 (1.35–1.40) mm and the transverse diameter 1.64 (1.62–169) mm. In the intact tibia, the maximal bending moment was 5.08 (4.56–6.21) Nm, bending rigidity 2.91 (2.81–2.97) Nm/rad and fracture energy 4.84 (4.35–5.82) $Nm \times rad \times 10^{-2}$.

In 7 rats in group A, blood flow measurements 30 minutes after intevention showed that the flow in the tibialis anterior muscles on the osteotomized left side was similar to that in the unaffected right side—i.e., 10.3 (5.74–24.6) and 8.93 (6.55–17.5) ml/min^{-1} ($p = 0.8$). In 7 rats in group C, the postoperative flow in the transposed superficial gluteal muscle was similar to that in the controls—i.e., 14.7 (7.33–22.8) and 12.0 (9.99–16.7) ml/min^{-1} ($p = 0.6$).

All the rats developed a permanent drop-foot on the operated side, and full weight bearing was impossible. They tolerated the operation well and, none of them had pin migration or infection.

Radiographs showed periosteal callus bridging the osteotomy gap, which was clearly visible in the three groups. We found no differences in the cross-sectional callus area between the intervention groups ($p = 0.6$) (Table). Mechanical testing revealed differences in bending moment and rigidity. Bending moment was higher in group A and C than in group B ($p = 0.03$ and $p = 0.01$), while no difference was evident between groups A and C ($p = 0.9$). Bending rigidity was also higher in groups A and C than group B ($p = 0.01$ and $p = 0.0003$) with no difference between groups A and C ($p = 0.8$). No significant differences in fracture energy were noted between the groups ($p = 0.6$).

Discussion

We compared transposition of a thigh-muscle flap to an in situ muscle flap over a fracture segment denuded of periosteum and muscle. We found a marked reduction in the rate of early bone healing after low perfused skin coverage, and fast periosteal healing after muscle flap coverage. However, whether or not muscle coverage was ensured by transposition of the superficial gluteal muscle or in situ coverage by the tibialis anterior muscles, the quality of early bone healing, was not affected if the muscle flap was adequately vascularized.

The vascularity on the operated side was similar in groups A and C. In group A, the tibialis anterior muscle was dissected between the periosteum and the cortex, leaving a thin membrane of periosteum attached to the muscle. In group C, there was no periosteal attachment to the flap. Periosteal grafts can induce the formation of new bone (Ritsila et al.

1989, Reynders et al. 1999). This proliferative and bone-forming capacity of the osteogenetic cells of the periosteal cambium layer may act as a stimulus in fractures associated with an extensive soft tissue lesion. In this study, we found no increase in the rate of healing due to the periosteal attachment to the tibialis anterior muscles or transposition of the superficial gluteal muscle which induced similar mechanical characteristics at 4 weeks.

Early soft tissue reconstruction in severe tibial fractures is thought to play a favorable role in union, infection and final outcome (Hertel et al. 1999). Our results support this view. After a fracture, flow increases gradually to a peak after 2–4 weeks (Rand et al. 1981). Transposition did not reduce muscle blood flow since no differences in flow were found between the two muscle flaps. Thus, the muscle bellies were considered to have the same vascular ability to provide oxygen and nutrient factors as well as cellular components. We consider this model reliable for studies comparing bone healing after tibial fractures with a soft tissue defect treated with a local muscle flap as compared to a more distant flap.

Our findings, indicating a marked reduction in mechanical characteristics after skin coverage over the fracture segment alone, support the view that muscles play an important role in the healing process. However, we do not know whether the reduction in the supply of oxygen and nutrients to the fracture site is the main mechanism underlying the reduction in healing. The inability of fibroblast cells from the muscle to reach the fracture hematoma may also be involved. These cells, with their ability to become transformed and take part in osteogenesis, may be an important factor in the healing process.

Our findings indicate that in tibial fractures, an extensive soft tissue defect may delay early bone healing, but transposition of a viable muscle flap over the fracture segment induces fast periosteal healing. However, coverage over the fracture region by a local or more distant muscle flap does not seem to influence fracture healing significantly, if the muscular flow to bone is adequate.

No funds have been received to support this study.

Brighton C T, Lorich D G, Kupcha R, Reilly T M, Jones A R, Woodbury R A. The pericyte as a possible osteoblast progenitor cell. Clin Orthop 1992; 275: 287-99.

Buckberg G D, Luck J C, Payne D B, Hoffmann J I, Archie J P, Fixler D E. Some sources of error in measuring regional blood flow with radioactive microspheres. J Appl Physiol 1971; 31: 598-604.

Byrd H S, Cierny G III, Tebbetts J B. The management of open tibial fractures with associated soft tissue loss: external pin fixation with early flap coverage. Plast Reconstr Surg 1981; 68: 73-9.

Diaz-Flores L, Gutierrez R, Lopez-Alonso A, Gonzales R, Varela H. Pericytes as a supplementary source of osteoblasts in periosteal osteogenesis. Clin Orthop 1992; 275: 280-96.

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

Gustilo R B, Anderson J T. Prevention of infection in the treatment of 1,025 open fractures of long bones: Retrospective and prospective analyses. J Bone Joint Surg (Am) 1976; 58: 453-8.

Gustilo R B, Mendoza R M, Williams D N. Problems in the management of type III (severe) open fractures. J Trauma 1984; 24: 742-6.

Hertel R, Lambert S M, Muller S, Ballmer F T. On timing of soft-tissue reconstruction for open fractures of the lower leg. Arch Orthop Trauma Surg 1999; 119: 7-12.

Oestern H J, Tscherne H. Pathophysiology and classification of soft tissue injuries associated with fractures. In: Fractures with soft tissue injuries. (Eds. Tscherne H, Gotzen L). Berlin, Springer Verlag, 1984: 1-9.

Rand J A, Nan K, Chao EYS, Kelly P J. A comparison of the effect of open intramedullary nailing and compression-plate fixation on fracture-site blood flow and fracture union. J Bone Joint Surg (Am) 1981; 63: 427-42.

Reynders P, Becker J H R, Broos P. Osteogenic ability of free periosteal autografts in tibial fractures with severe soft tissue damage: An experimental study. J Orthop Trauma 1999; 13 (2): 121-8.

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

Ritsila V A, Santavirta S. Alhopuro S et al. Periosteal and perichondral grafting in reconstructive surgery. Clin Orthop 1989; 302: 259-65.

Trueta J. Blood supply and the rate of healing of tibial fractures. Clin Orthop 1974; 105: 402-18.

Whiteside L A, Lesker P A. The effects of extraperiosteal and subperiosteal dissection. I. On blood flow in muscle. II. On fracture healing. J Bone Joint Surg (Am) 1978; 60: 23-30.

Wu J-J, Shyr H S, Chao E Y S, Kelly P J. Comparison of osteotomy healing under external fixation devices with different stiffness characteristics. J Bone Joint Surg (Am) 1984; 66: 1258-64.