Healing of segmental and simple fractures in rats

Stein Erik Utvåg[1], Oliver Grundnes[1] and Olav Reikerås[2]

We studied the healing of segmental and simple middiaphyseal fractures in male Wistar rats. In one group we produced two standardized partial osteotomies with an 8 mm intermediary fragment in the femoral diaphysis and in the other group a simple partial osteotomy. The osteotomies were then manually broken, retaining the periosteal and muscular attachment on the medial side. The fractures were stabilized with a 1.6 mm steel pin, and the animals were allowed free movement. After 4, 8, and 12 weeks, 8 rats in each group were killed, and callus formation, mechanical parameters and bone blood flow were evaluated.

There were no differences in callus production between the simple and segmental fractures throughout the experimental period. The mechanical parameters increased in both groups, and the healing patterns were the same. No differences were found in the total bone blood flow, but the callus blood flow in the segmental fractures was lower after 8 and 12 weeks than that in the simple fractures.

Our findings indicate that closed segmental fractures treated by intramedullary nailing can regain strength in the same manner as simple fractures.

Department of Orthopedics, [1]Institute of Clinical Medicine, University Hospital, N-9038 Tromsø and [2]Sophies Minde Orthopedic Hospital, N-0570 Oslo, Norway. Correspondence: Dr. Olav Reikerås, Sophies Minde, N-0570 Oslo, Norway.
Tel +47 22-954300. Fax -378541
Submitted 93-07-17. Accepted 94-06-18

A large diaphyseal, segmental bone fragment is not an uncommon problem in orthopedic practice. Segmental fractures of the femoral shaft can be difficult to treat, but most investigators have agreed that intramedullary nailing is the treatment of choice for this injury (Winquist and Hansen 1978, Wiss et al. 1990, Butler et al. 1991). However, little is known about the healing pattern of double diaphyseal fractures, as most animal studies have dealt with the healing of simple fractures (Sarmiento et al. 1977, Mølster 1984, Goodship and Kenwright 1985, Reikerås 1990, Grundnes and Reikerås 1992). In this study we have investigated the healing of a segmental fracture, as compared to that of a simple fracture of the femur in rats.

Animals and methods

48 male Wistar rats (Møllegårds Avlslaboratorium, Eiby, Denmark) weighing 352–379 g were used. Following intraperitoneal anesthesia (pentobarbital 5 mg/100 g body weight), the left femur was exposed between the lateral vastus and hamstrings. The muscles were carefully elevated in the lateral part, and care was taken to avoid any periosteal or muscular stripping on the medial aspect of the femur. In the first group a partial, transverse osteotomy at the shaft of the bone was made 12 mm from the top of the greater trochanter with a fine-toothed circular saw blade mounted on an electrical drill and then manually broken. The medullary canal was gradually reamed from the osteotomy site in proximal and distal directions to a diameter of 1.5 mm, using steel burrs mounted on the electrical drill. In the second group the left femur was exposed as in the first group, but, in addition to the first osteotomy, a second osteotomy was made 8 mm distal to the first. The osteotomies were then broken. In this way a middiaphyseal segmental fracture was made. By careful inspection it was ascertained that the segment had a muscle attachment on the medial aspect. The fractures were reduced, and a 1.6 mm steel pin was inserted from the trochanter area for stabilization. Proper pin placement was confirmed by radiographs taken at the end of the experiment. Bone debris from sawing and reaming were rinsed out. The wounds were closed in two layers. All rats tolerated the operation well, resumed activity within a day and full weight bearing after 1–2 weeks.

At 4, 8, and 12 weeks after fracture, 8 rats in each group were anesthetized. In short, for blood flow measurements, microspheres labeled with ^{85}Sr were used. We have earlier described the technique in detail (Grundnes and Reikerås 1992). A polyethylene catheter (PE 50), introduced via the carotid artery and placed in the aortic root, was used for injection of the microspheres. The caudal artery was cannulated with a catheter and connected to an infusion/withdrawal pump for reference sampling.

The rats were then killed in a CO_2 chamber, and the left femur was carefully dissected free from all soft tissue and weighed. Anteroposterior and transverse diameters of the callus area of the proximal double fractures and of the simple fractures were measured with a sliding caliper (accuracy of 0.01 mm). The quantity of the callus was expressed as the cross-sectional area, assuming it to be an ellipse. Furthermore, the anteroposterior and transverse diameters of the distal diaphysis were measured 10 mm proximal to the medial condyle (about 10 mm distal to the segmental fracture), assuming the cross-section to be elliptical.

The bones were then radiographically examined, and the intramedullary pin was removed. The healing of the simple fracture and the healing of the proximal of the double fractures were tested in cantilever bending. The proximal end was fixed with a clamp, the cam of a rotating wheel engaged the femoral condyles, and a fulcrum at the osteotomy site was the third point of force application. Refracture was then 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 values were transformed to a chart recorder displaying the load deformation curve. The strength was calculated as the bending moment necessary to produce refracture. The bending rigidity was determined from the slope of the linear part of the curve. The fracture energy was defined as the energy absorbed during loading to refracture.

The bones and reference samples were counted in a multichannel analyzer (Packard Auto Gamma Spectrometer) for 10 min. After the total bone blood flow was calculated, the bones were cut into segments for further analysis. For the simple fractured bones, it included the callus area and a distal diaphyseal segment, as previously described (Grundnes and Reikerås 1992). For the double-fractured bones, this included the proximal callus area and a distal diaphyseal segment. The content of the medullary canal was rinsed out prior to recounting.

Data are presented as median values with 25 and 75 percentiles. For testing the time effects within the groups, one-way analysis of variance (Kruskall-Wallis test) was used. For testing the differences between the groups, two-way analysis of variance (Friedman's test) was applied. When statistical differences were found, the Wilcoxon rank sum test was used. $P < 0.05$ was considered significant.

Figure 1. Healing at 8 weeks of simple (left) and double (segmental) (right) femoral osteotomy/fracture (arrows) in rats. The intramedullary pin has been removed.

Results

The osteotomies were radiographically visible at 4 and 8 weeks after fracture, but scarcely after 12 weeks (Figure 1). In the double-fractured bones there was a decrease in the proximal callus area during the experimental period. This was not found in the simple fractures (Table 1). The differences in callus area between the double- and simple-fractured bones were not significant. There were no changes in cross-sectional area of the distal diaphysis with time in either the simple- or segmental-fractured bones. At 12 weeks the distal diaphyseal area was greater in the double-fractured bones than in the simple ones.

The mechanical characteristics of the simple and the proximal of the segmental fractures improved during the healing period. At each time interval, there were no differences in mechanical properties between the simple and segmental fractures (Table 2).

Total bone blood flow was reduced during the healing period in both the simple and segmental fractured bones. Callus flow and distal diaphyseal flow

Table 1. Cross-sectional area of callus mass and distal diaphysis (mm^2) in simple and segmental fractures at 4, 8, and 12 weeks after fracture. Median and 25 and 75 percentiles

			Time after operation		
	4 weeks	*P*	8 weeks	*P*	12 weeks
Callus area					
Simple fractures	58 (48–70)	0.09	50 (40–59)	0.09	37 (32–50)
P	0.2		0.2		0.2
Segmental	59 (54–66)	0.2	63 (53–82)	0.01	44 (41–52)
Diaphyseal area					
Simple fractures	23 (20–27)	0.06	25 (22–30)	0.06	20 (20–21)
P	0.03	0.2			0.03
Segmental	29 (25–36)	0.3	36 (27–39)	0.3	29 (25–34)

Table 2. Bending moment ($Nm \times 10^{-1}$), bending rigidity (Nm/rad) and fracture energy ($Nm \times rad \times 10^{-1}$) in simple and segmental fractures at 4, 8, and 12 weeks after fracture. Median and 25 and 75 percentiles

			Time after operation		
	4 weeks	*P*	8 weeks	*P*	12 weeks
Bending moment					
Simple fractures	2.1 (1.7–2.8)	0.001	5.1 (4.6–7.9)	0.07	8.0 (6.1–9.3)
P	0.4		0.4		0.4
Segmental	2.4 (1.8–2.9)	0.005	4.9 (3.5–5.6)	0.02	9.2 (7.9–12)
Bending rigidity					
Simple fractures	0.98 (0.7–1.4)	0.01	1.8 (1.5–2.1)	0.9	1.7 (1.5–1.9)
P	0.3		0.3		0.3
Segmental	0.86 (0.7–1.2)	0.01	1.5 (1.3–1.7)	0.2	1.7 (1.5–2.1)
Fracture energy					
Simple fractures	0.22 (0.12–0.4)	0.002	0.77 (0.62–1.7)	0.08	1.6 (1.3–2.6)
P	0.7		0.7		0.7
Segmental	0.28 (0.16–0.48)	0.02	0.84 (0.44–1.2)	0.01	2.1 (1.7–3.3)

Table 3. Blood flow ($mL/min \times 100^{-1}g$) in simple and segmental fractures at 4, 8, and 12 weeks after fracture. Median and 25 and 75 percentiles

			Time after operation		
	4 weeks	*P*	8 weeks	*P*	12 weeks
Total bone flow					
Simple fractures	45 (34–91)	0.02	32 (23–34)	0.03	20 (13–25)
P	0.7		0.7	0.7	
Segmental	56 (41–92)	0.01	29 (21–35)	0.1	22 (17–25)
Callus bone flow					
Simple fractures	71 (47–136)	0.2	56 3(9–71)	0.2	61 (34–63)
P	0.9		0.03		0.03
Segmental	60 (37–139)	0.01	27 (24–51)	0.4	26 (22–30)
Diaphyseal flow					
Simple fractures	34 (19–59)	0.1	21.38 (12–34)	0.1	21 (12–27)
P	0.4		0.4		0.4
Segmental	43 (27–63)	0.4	32 (24–45)	0.03	16 (11–29)

showed a decline with time in the double-fractured bones, but not in the simple-fractured bones (Table 3). Callus flow was less in the segmental-fractured bones than in the simple-fractured bones at 8 weeks and at 12 weeks. Otherwise there were no differences in blood flow between the simple- and segmental-fractured bones.

Discussion

In this study the healing of a middiaphyseal, segmental fracture was compared to that of a simple fracture. The healing of a simple fracture in rat femoral bone is properly described in a previous paper (Grundnes and Reikerås 1992), and it takes about 12 weeks before normal characteristics are regained. During this time there were rather small reductions in callus area and also an inverse relationship between the mechanical properties and blood flow of the callus. These observations reflect the maturation of the callus mass during the healing period. In the present study, soft tissue attachments were retained on the medial side. The fractures were stabilized with intramedullary nails, which is the accepted method of treatment of femoral fractures (Kempf et al. 1985). Healing of both the simple and segmental fractures occurred with the production of external callus symmetrically around the fracture sites. Our experimental model, then, is comparable to segmental fractures with limited soft tissue damage in clinical practice. We found that under such circumstances the segmental fractures followed the same pattern of healing as simple fractures. On the other hand, it has been shown that fracture healing is impaired when periosteum and soft tissue attachment are totally absent (MacNab and De Haas 1974, Whiteside et al. 1978, Nather 1990a).

The cross-sectional area of the callus was larger in the segmental group after 8 weeks. The distal diaphyseal area was also larger in the double-fractured bones. This observation reflects an increased periosteal reaction in the segmental-fractured bones, probably because the area of periosteal elevation is larger.

The microsphere method is well documented for blood flow analysis (Morris and Kelly 1980, Gross et al. 1981, Li et al. 1989). Li et al. (1989) studied the effect of different numbers of spheres in bone specimens and found reliable data for a number exceeding 150. In our study, the number of spheres in each bone specimen was estimated to be more than 500. Our study showed a substantial vascular response in both segmental and simple fractures after 4 weeks. As the bones regained mechanical properties, the general vascular response subsided. Our results are similar to those in earlier studies on simple fractures (Grundnes and Reikerås 1992). At 8 and 12 weeks after fracture, proximal callus flow in the segmental fractures was less than half of that in the simple fractures. This observation is thought to show that the segmental area was less vascularized than the fracture ends of the simple fractures. Cortical bone flow in the distal diaphysis showed no differences, although the median value was higher in the segmental-fractured bones after 4 and 8 weeks. This probably reflects a generalized cortical/periosteal reaction through the whole femur due to an increased periosteal elevation caused by the segmental fracture.

Normally, the vascular supply to the cortex is largely through the medullary system, and the flow is centrifugal (Brookes 1971). Following devascularization of a bone segment by fracture, the repair is initiated by the surrounding soft tissue (Rhinelander 1974). The repair process seems to proceed from the periphery until the intramedullary circulation is reestablished. Although previous studies have dealt with the healing of segmental bone loss (Nather et al.1990a,b, Richards et al. 1991), the present study seems to be the first to demonstrate an improvement in mechanical characteristics in such fractures, as in simple fractures.

References

Brookes M. Blood supply of long bones. Br Med J 1971; 5364: 1064–5.

Butler M S, Brumback R J, Ellison T S, Poka A, Bathon G H, Burgess A R. Interlocking intramedullary nailing for ipsilateral fractures of the femoral shaft and distal part of the femur. J Bone Joint Surg (Am) 1991; 73 (10): 1492-502.

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.

Goodship A E, Kenwright J. The influence of induced micromovement upon the healing of experimental tibial fractures. J Bone Joint Surg (Br) 1985; 67 (4): 650–5.

Gross P M, Marcus M L, Heistad D D. Measurement of blood flow to bone and marrow in experimental animals by means of the microsphere technique. J Bone Joint Surg (Am) 1981; 63 (6): 1028–31.

Grundnes O, Reikerås O. Blood flow and mechanical properties of healing bone. Femoral osteotomies studied in rats. Acta Orthop Scand 1992; 63 (5): 487–91.

Kempf I, Grosse A, Beck G. Closed locked intramedullary nailing. Its application to comminuted fractures of the femur. J Bone Joint Surg (Am) 1985; 67 (5): 709–20.

Li G, Bronk J T, Kelly P J. Canine bone blood flow estimated with microspheres. J Orthop Res 1989; 7 (1): 61–7.

Macnab I, De Haas W G. The role of the periostal blood supply in the healing of fractures of the tibea. Clin Orthop 1974; 105: 27–33.

Morris M A, Kelly P J. Use of tracer microspheres to measure bone blood flow in conscious dogs. Calcif Tissue Int 1980; 32 (1): 69–76.

Mølster A O. Effects of rotational instability on healing of femoral osteotomies in the rat. Acta Orthop Scand 1984; 55 (6): 632–6.

Nather A, Balasubramaniam P, Bose K. Healing of non-vascularised diaphyseal bone transplants. An experimental study. J Bone Joint Surg (Br) 1990a; 72 (5): 830–4.

Nather A, Goh J C, Lee J J. Biomechanical strength of non-vascularised and vascularised diaphyseal bone transplants. An experimental study. J Bone Joint Surg (Br) 1990b; 72 (6): 1031-5.

Reikerås O. Healing of osteotomies under different degrees of stability in rats. J Orthop Trauma 1990; 4 (2): 175–8.

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

Richards R R, McKee M D, Paitich C B, Anderson G I, Bertoia J T. A comparison of the effects of skin coverage and muscle flap coverage on the early strength of union at the site of osteotomy after devascularization of a segment of canine tibia. J Bone Joint Surg (Am) 1991; 73 (9): 1323–30.

Sarmiento A, Schaeffer J F, Beckerman L, Latta L L, Enis J E. Fracture healing in rat femora as affected by functional weight- bearing. J Bone Joint Surg (Am) 1977; 59 (3): 369–75.

Whiteside L A, Ogata K, Lesker P, Reynolds F C. The acute effects of periosteal stripping and medullary reaming on regional bone blood flow. Clin Orthop 1978; 131: 266–72.

Winquist R A, Hansen S T. Segmental fractures of the femur treated by closed intramedullary nailing. J Bone Joint Surg (Am) 1978; 60 (7): 934–9.

Wiss D A, Brien W W, Stetson W B. Interlocked nailing for treatment of segmental fractures of the femur. J Bone Joint Surg (Am) 1990; 72 (5): 724–8.