Blood flow and mechanical properties of healing bone

Femoral osteotomies studied in rats

Oliver Grundnes and Olav Reikerås

In male Wistar rats, a transverse osteotomy at the midshaft of the femur was made, and the acute effects on bone flow were measured before and after reaming. Flow and mechanical variables in the healing bones were measured at 4, 8, and 12 weeks following osteotomy. Osteotomy reduced total bone blood flow by about 50 percent, and cortical flow in the diaphysis by approximately 40 percent. Cortical flow was equally diminished in the middiaphysis and in the osteotomy area, and no differences between the proximal and distal diaphyseal flows were found. Reaming of the osteotomized bones did not lead to any further flow reduction. At 4 weeks, total bone flow was more than doubled; increases were found in every segment of the fractured bone, and a more than 10-fold increase in the callus area was seen. At the end of the experiment, the femurs had regained 83 percent of their normal strength, 88 percent of normal rigidity and 78 percent of normal fracture energy. At this time total bone flow was marginally increased, flows in the proximal and the distal diaphyses were almost normalized, while a nearly 3-fold increase was still found in the callus area. Flow in the callus area gradually decreased during healing, and regression analysis demonstrated a negative correlation between callus flow and mechanical properties.

Department of Orthopedics, Institute of Clinical Medicine, University Hospital, N-9012 Tromsø, Norway
Tel +47-83 26000. Fax -83-26042

Submitted 91-09-11. Accepted 92-04-09

Current knowledge of changes in bone blood flow during fracture healing is mainly based on microangiographic and histologic studies (Gøthman 1961, Trueta 1963, Rhinelander 1974). These methods are advantageous for an anatomical description of the vascular system, but give little information about the dynamic pattern of blood flow. Washout (Paradis and Kelly 1975) and microsphere techniques (Morris and Kelly 1980, Li et al. 1989) have been used, but none of the studies so far has related changes in blood flow to mechanical variables. Further, the majority of studies on this topic have focused on blood supply to the tibia.

We examined blood flow patterns in relation to mechanical properties of healing bone in osteotomized rat femurs.

Materials and methods

Animal model

40 male Wistar rats (Møllegård Avlslaboratorium, Eiby, Denmark) weighing 319 ±10 g were used. Following intraperitoneal anesthesia (fentanyl-fluanixone, 0.1 mL/100 g body weight) the left femur was exposed through a lateral incision. An elevator was passed underneath the periosteum of the middle part of the femur for protection of the soft tissues. A partial transverse osteotomy at the midshaft of the bone was made with a fine-toothed circular saw blade mounted on an electrical drill and then manually broken. The medullary cavity was successively 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. A steel pin with a diameter of 1.6 mm was introduced from the fracture site, and by use of the electrical drill it was driven through the greater trochanter. The osteotomy was then reduced, and the pin was driven into the distal fragment. The wound was closed in two layers.

Blood flow measurements

Microspheres (New England Nuclear, Boston, MA, U.S.A.) labeled with ^{141}Ce and ^{85}Sr of 15.5 ±0.1 μm diameter were used, and each injection consisted of 5 x 10^5 spheres homogeneously suspended in 0.9 percent saline. The spheres were mixed on a whirl mixer for 2 min prior to injection. A heparinized polyethylene catheter (PE-50) was introduced via the carotid artery and placed in the aortic root for injection of microspheres. The microspheres were injected over a period of 15 sec, and the catheter was then flushed with 0.5 mL saline. The caudal artery was cannulated with a heparinized polyethylene catheter (PE-10) and connected to a Harvard infusion-withdrawal pump for reference sampling. Flow rate in the reference organ was set at a rate of 195 µL/min. Withdrawal started 15 sec prior to injection of the microspheres and continued for 30 sec after the injection was finished.

To examine the acute effects of osteotomy and reaming on bone blood flow, a separate group of 10 animals was used. After osteotomy, the antero-posterior and transverse diameters of the medullary cavity were measured at the osteotomy site (1.43 ±0.08 mm and 2.09 ±0.11 mm). In this group the first injection (^{141}Ce) was done 20 min following osteotomy, and the second (^{85}Sr) immediately after reaming. Correction for spillover between the two isotopes was made when these bones were counted. 10 rats were used at each time interval for measurements of flow at 4, 8 and 12 weeks after osteotomy, in these rats the use of one isotope (^{141}Ce) was sufficient. During the surgical preparation for blood flow measurements, one rat in the 4-weeks group and one rat in the 8-weeks group died.

Mechanical testing technique

After the animals were killed in a CO_2 chamber, both femurs were dissected free from all soft tissue, wiped dry and weighed. Antero-posterior and transverse diameters of the callus mass were measured by a sliding caliper (accuracy of 0.01 mm), and the quantity of the callus was expressed as the cross-sectional area, assuming it to be elliptical. The bones were then radiographically examined.

The healing osteotomies were tested in a cantilever bending machine. The proximal end was fixed with a clamp, a metal pin was used as a fulcrum at the osteotomy site, and was fractured by dorsal deflection of the distal half of the femur, as described by Engesæther et al. (1978). The length of the intact femurs was measured by a sliding caliper as the distance from the medial condyle to the tip of the femoral head, and according to this fractured at the midpoint when tested. The hydraulic testing machine was run at a constant rate of 0.04 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 a 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.

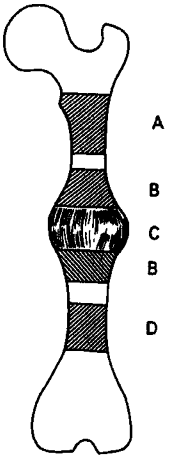

Figure 1. The bones were divided into segments for flow analysis. A proximal diaphysis, B callus near-area, C callus area, and D distal diaphysis.

Bone blood flow analysis

The bones were placed in counting vials and together with the reference samples counted in a multichannel analyzer (Packard Auto-Gamma Scintillation Spectrometer). Specimens were counted for 5 min and the sample count was calculated as the average of three countings. After the total bone flow was calculated, the fractured bones were separated as shown in Figure 1. The intact femora were divided into a proximal and a distal part for flow calculations. The bone segments were then weighed and counted together with their reference sample. Finally, the medullary cavity was rinsed and the spongious bone removed, leaving a segment of compact cortical bone. The bone segments were then once again weighed and counted for cortical flow analysis. Bone blood flow 20 min following osteotomy and reaming were measured in the proximal and the distal diaphyses, as well as in a 5 mm bone segment adjacent to the osteotomy site.

Statistics

Data are expressed as mean (*SEM*). For statistical evaluations in the acute study the paired *t*-test was used throughout. In the chronic study, the one-way analysis of variance was used when comparisons between the time intervals and different bone segments were made. The Student *t*-test was used when significant differences were found. For comparisons between osteotomized and intact bone at each time interval the paired *t*-test was used. $P < 0.05$ was considered significant. For regression analysis the ordinary product moment correlation coefficient was used.

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^{-1}) of nailed femoral osteotomies and intact femora at 4, 8, and 12 weeks after osteotomy. Mean *SEM*

	4 weeks n 9	8 weeks n 9	12 weeks n 10
Fractured bone			
Callus area	55.7 *3.25*	58.9 *8.71*	45.3 *3.86*
Bending moment	1.96 *0.42*	6.60 *0.55*	9.29 *0.60*
Bending rigidity	0.91 *0.20*	1.49 *0.10*	2.79 *0.19*
Fracture energy	0.32 *0.10*	1.28 *0.12*	1.57 *0.13*
Intact bone			
Bending moment	7.89 *0.26*	9.16 *0.28*	11.2 *0.66*
Bending rigidity	1.45 *0.13*	1.91 *0.07*	3.16 *0.26*
Fracture energy	2.22 *0.13*	2.13 *0.14*	2.01 *0.09*

Table 3. Combined cortical-medullary bone blood flow (mL/min x 100 g^{-1}) in fractured and intact bone at 4, 8, and 12 weeks after osteotomy. Mean *SEM*

	4 weeks n 9	8 weeks n 9	12 weeks n 10
Fractured bone			
Total bone	27.5 *3.18*	22.2 *2.49*	12.0 *1.99*
Proximal	27.8 *5.46*	22.0 *3.81*	13.3 *0.96*
Callus near	39.4 *7.00*	30.1 *3.58*	16.3 *1.29*
Callus	107 *17.6*	52.1 *7.95*	25.6 *2.15*
Distal	47.5 *9.93*	28.9 *3.27*	14.1 *1.03*
Intact bone			
Total bone	11.8 *2.07*	8.06 *1.22*	8.00 *1.38*
Proximal	7.16 *1.44*	8.19 *0.73*	10.2 *1.11*
Distal	9.70 *1.31*	8.48 *1.26*	9.81 *0.89*

Table 2. Acute effects on bone blood flow (mL/min x 100g^{-1}) in total bone, cortical diaphysis and osteotomy area at 20 min following osteotomy and reaming and osteotomy. N 10. Mean *SEM*

	Intact	*P*	Osteotomy	*P*	Reaming and osteotomy
Total bone	12.5 *1.62*	< 0.01	6.00 *1.13*	NS	4.99 *1.07*
Cortical diaphysis	7.04 *1.11*	< 0.05	3.90 *0.83*	NS	2.37 *0.88*
Osteotomy area			4.01 *0.90*	NS	4.22 *1.03*

Table 4. Cortical bone blood flow (mL/min x 100g^{-1}) in different bone segments in fractured and intact bone at 4, 8, and 12 weeks following osteotomy. Mean *SEM*

	4 weeks n 9	8 weeks n 9	12 weeks n 10
Fractured bone			
Proximal	16.0 *2.02*	21.7 *2.36*	13.3 *1.31*
Callus near	27.8 *3.45*	26.8 *3.11*	16.0 *2.21*
Distal	22.2 *4.61*	18.9 *1.05*	11.7 *1.23*
Intact bone			
Proximal	7.11 *1.19*	8.74 *0.98*	9.14 *1.47*

Results

All rats tolerated the operation well, resumed normal activity within a few days, and full weight bearing after approximately 2 weeks. The osteotomies were unstably pinned, so they healed by the production of external callus. 4 weeks following osteotomy, the fractured femurs had regained 25 percent in strength and 63 percent in bending rigidity of intact bone. At 8 weeks bending moment was increased to 72 percent of normal and bending rigidity to 78 percent of the intact side. After 12 weeks these parameters were further increased to 83 and 88 percent, respectively. Fracture energy at the three time intervals were 14, 60 and 78 percent of normal value (Table 1).

Following osteotomy, reductions in total bone flow and cortical blood flow were seen. No difference in cortical flow was found between the middiaphysis and the area adjacent to the osteotomy. Reaming of the osteotomized bones reduced total bone flow by 17 percent, and cortical blood flow by 39 percent (Table 2). Flow in intact bones was not altered by surgical manipulation of the contralateral limb, 12.5 (*1.62*) mL/min × $100g^{-1}$ versus 10.4 (*1.89*) mL/min × $100g^{-1}$.

At 4 weeks, total bone blood flow was increased by 2.3 times ($P < 0.05$) in relation to intact bone flow. This increase remained practically unchanged at 8 weeks, and decreased from 8 to 12 weeks ($P < 0.01$). At the end of the experiment, total blood flow still was increased by 50 percent (*P* 0.06) in relation to intact bone (Table 3). Combined cortical-medullary flow was increased in every bone segment at 4 weeks. In the callus area, flow was approximately 12 times increased compared to the mean blood flow in the intact diaphysis. Between 4 and 8 weeks callus flow declined by 50 percent ($P < 0.05$), and at 12 weeks the initial increase was further halved ($P < 0.05$). However, the callus flow was still twice the flow in the proximal diaphysis and 2.5-fold increased compared to normal cortical flow at the end of the experiment ($P < 0.005$; Table 3).

Cortical blood flow was increased at 4 and 8 weeks ($P < 0.05$), but was not different from intact cortical bone flow at 12 weeks (Table 4). At 4 weeks the combined cortical-medullary flow was different from the cortical flow in the proximal, callus near, and distal diaphysis (Tables 3 and 4). During the experimental period, cortical flow in the diaphysis and in the callus near-area gradually subsided, decreases were found between 8 and 12 weeks.

Medullary blood flow in intact bones was 15.0 (*2.33*) mL/min × 100 g^{-1}. Medullary blood flow rate was 0.57 (*0.14*) mL/min × 10^{-2}, and remained constant during the experimental period. This constituted 47 percent of cortical flow rate to the intact bone. In the osteotomized bones, medullary flow rate at 4 weeks was 9.93 (*2.80*) mL/min × 10^{-2} which represented 64 percent of cortical flow rate. At 12 weeks medullary flow rate had declined to 6.81 (*1.22*) mL/min × 10^{-2}, which was 42 percent of cortical flow rate in the osteotomized bones.

Regression analysis showed a correlation of –0.79 between callus flow and bending moment, –0.70 between flow and rigidity, and –0.69 between flow and fracture energy. All of these correlations were highly significant ($P < 0.01$). No correlation was found between the callus flow and the callus area (r 0.37, P 0.08).

Discussion

Our study demonstrates a substantial vascular response in fracture healing, and suggests that bone blood flow and fracture healing are closely related. These findings are in agreement with previous observations showing that blood flow is related to mineral deposition in fractured tibia (Paradis and Kelly 1975).

The microsphere method is well documented for bone blood flow analysis (Morris and Kelly 1980, Gross et al. 1981, Li et al. 1989). We used a medium-large dose of 5 × 10^5 microspheres in each injection (1.6 × 10^6 spheres/kg body weight). Li et al. (1989) studied the effects of different numbers of spheres in bone specimens in the dog tibia, and found reliable data when the number of microspheres per sample was more than 150. In the present study, no specific number of counts/sphere was done. However, the number of spheres in each bone segment of the control femurs was estimated at 150–250. Therefore, it may be that some of the specimens in the acute study contained a rather small number of microspheres with the potential risk of overlooking small differences.

An immediate reduction in total bone blood flow was observed following osteotomy, the cortical circulation being nearly halved. This has also been observed by previous authors studying such an effect in dogs (Rand et al. 1981, Smith et al. 1990). This reduction has been attributed to a physiological vasoconstriction in both the periosteal and the medullary vessels as a response to trauma regulated by alpha adrenergic receptors (Kelly et al. 1990). In the present experiment, no significant influence of reaming on blood flow was observed. On the other hand, this was hardly to be expected since much of the medullary circulation probably already was destroyed due to the osteotomy.

Intramedullary nailing of tibial fractures has been shown to cause damage to the medullary vessels resulting in large areas of avascularity of the endosteal bone (Dankwardt-Lilliestrȯm 1969). The intact tibial diaphysis receives its blood supply almost exclusively via the medullary circulation and the periosteal vascular supply is of less importance (Rand et al. 1981). In the femur, which has soft tissue attachments along the linea aspera, periosteal circulation should be abundant, and due to rich collateral circulation, the diaphyseal blood supply should be less affected after intramedullary nailing. Our results are in agreement with this view.

Blockade of the medullary circulation by intramedullary rods has been shown to induce compensatory mechanisms via the extraosseous periosteal circulation (Rand et al. 1981, Smith et al. 1990). In the present study the diaphyseal medullary flow constituted about 60 percent of cortical flow at 4 weeks, and significant differences between the combined cortical-medullary blood flow and cortical blood flow were found in the proximal and distal diaphyses. This indicates that the endostium is richly vascularized even in the early stage of fracture healing. This is most likely due to a reversal of flow in the perforating arteries from periost to endost, or from neovascularization of medullary vessels (Brånemark 1961, Whiteside et al. 1978). The present dynamic flow study shows that fractured femoral bone has a vascular reserve which may well compensate for loss of endosteal and medullary flow.

Both the fracture site and the fixation method will affect the blood supply in the healing process (Rand et al. 1981, Smith et al. 1990). By the ^{85}Sr-clearance techniques, the flow to intramedullary fixed fractures has been shown to be higher than those fixed with plates (Rand et al. 1981). This increase was thought to be associated with the degree of stability, the unstable fracture fixation with the intramedullary nailing has a higher blood flow as a result of the strong external bridging callus response. We were, however, not able to prove any correlation between callus area and callus flow. This indicates that vascular response in association with external callus production results from union phenomenons and not from the degree of stability. The close adjustments seen between callus flow and mechanical properties are in accordance with this view. However, when each time interval was analyzed separately, no correlation was found.

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