Effects of intramedullary reaming and nailing on blood flow in rat femora

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The right femur in 40 rats was reamed, and in 40 others it was additionally nailed. Analysis of bone blood flow was performed by the distribution of radiolabeled microspheres at different postoperative time intervals. Blood-flow measurements were accompanied by analyses of hydroxyproline and calcium contents. Immediately after reaming, the blood flow of the diaphyseal part of the femur was reduced to approximately one third of that of the intact femur, whereas the contents of hydroxyproline and calcium were reduced by 10 percent. Within 1 week, the blood flow was normal. This study provides evidence that the presence of a nail does not interfere with the restoration of bone blood flow. Restoration of blood flow in bone apparently is a rapid process. The replacement of hydroxyproline and calcium contents seemed to be linked to flow, as no increase in these constituents were found until the blood flow had approximated the level of the intact femur.

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The combined procedures of reaming and nailing are followed by a period of circulatory deprivation (Trueta and Cavadias 1955, Rhinelander 1974, Pfister 1979, Kessler 1986, Indrekvam et al. 1991c).

We have explored the immediate effect of reaming on femoral blood flow, the recovery rate following reaming, and whether or not the presence of a nail interfered with the above. Also, the hydroxyproline and calcium contents were determined to monitor the rates of bone repair and magnitude of bone mass.

Materials and methods

Eighty—10 groups of 8 animals—male Wistar rats (Mol:WIST, Møllegaard Breeding Center, Ejby, Denmark) were used. The right femur was reamed and nailed in 40 rats, whereas in the other 40 rats, the right femur was reamed only. One group of animals with nailed femora and one group with reamed femora were evaluated immediately after the operation, whereas the remaining groups were studied 3, 7, 21, or 42 days postoperatively (Figure 1). The median body weight was 329 (321–339) g at the beginning of the experiment. The animals were given a standard maintenance rat diet (RM1 Expanded, Special Diets Services, Witham, England) and water ad libitum.

The right femora of the rats were reamed and nailed, or reamed only as described by Indrekvam et al. 1991c. The stiffness of the nails was about 1.5 times the stiffness of the intact rat femur of the actual age.

The guidelines of Heymann et al. (1977) were followed during blood-flow determination, which was performed under anesthesia with a mixture of fentanyl/fluanisone and midazolam. The body temperature of the animals was maintained at 38 °C with a heating pad.

The carotid arteries were isolated for catherization with polyethylene tubing (3FG, OD 0.75 mm, Portex Limited, Hythe, England). The right carotid catheter was passed into the left ventricle, and a correct position was demonstrated by a characteristic left ventricular pressure trace. The other catheter was inserted approximately 5 cm, and was used for collecting the timed reference blood sample.

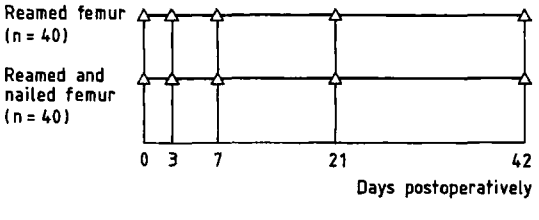

Figure 1. Timetable for evaluation following operation on Day 0. △ 8 rats evaluated.

Microspheres were labeled with ^{141}Cr or ^{46}Sc (15 μm in diameter), and suspended in 10 percent dextran with 0.01 percent Tween 80 (Du Pont Company, Biotechnology System, Wilmington, DE, U.S.A.). Spheres were dispersed with a shaker 1 min before infusion. A 0.43 mL suspension containing on an average 640,000 spheres (carrying an activity of 0.01–0.02 mCi) was infused into the left ventricle for 20 s, and was then flushed with 1 mL saline. The reference sample was withdrawn into heparinized syringes at a rate of 0.3 mL/min using a constant rate pump. Blood collection started immediately before the microsphere infusion and continued for 2 min. Left ventricular blood pressure was monitored before and after the infusion to ensure a stable hemodynamic situation.

At the end of the experiment, the rat was killed with KCl injected intracardially. The femora and tibiae were removed and mechanically cleansed. The femora were divided into two parts by separation along the distal epiphyseal line. The bones were placed in a counting vial, weighed, and activities were counted in a scintillation gamma counter (1282 Compugamma, Wallac, Turku, Finland). Residual activity was determined by counting the remaining activity in the flushed syringe.

Blood flow rate in the bones was determined by the equation

organ flow/activity in organ =
reference-sample flow/activity in reference sample.

Blood flow was calculated in the diaphyseal part of the femur, the femur condyles, and the tibia in both limbs. Cardiac output for the rats was determined by the equation

cardiac output/total amount of activity injected =
reference-sample flow/activity in reference sample

(Heymann et al. 1977). The number of microspheres in lung tissue was determined to detect eventual shunting of the spheres.

Chemical analyses were performed as previously described (Indrekvam et al. 1991b). The collagen content was measured by analyzing hydrolyzed bone for hydroxyproline in an amino-acid analyzer (Biotronik Aminoacid Analyzer LC 7000, Wissenschaftliche Geräte GmbH, Frankfurt, Germany) with an integrator (Spectra Physics SP 4200 Computing Integrator, San Jose, CA, U.S.A.). Calcium analysis was carried out by means of continuous-flow spectrophotometry (Technicon SMAC System, New York, NY, U.S.A.). Mineralization was expressed as the calcium/hydroxyproline ratio (mmol/mmol).

Statistics

All the variables are presented as absolute values for operated on femur and as the ratio between operated on and intact femur. Median values with 0.25 and 0.75 fractiles were used to express the average and the dispersion of the measurements. The Mann-Whitney test was used to analyze differences between the two groups of reamed and nailed femora. The Wilcoxon signed rank test was applied to test for differences from 1.00 for median values (Minitab; Ryan et al. 1985). Tests were considered significant when $P < 0.05$.

Results

In both experimental groups, there was an immediate reduction of blood flow in the diaphyseal part of the femur to about one third of that of the intact femur, which was 0.27 (0.24–0.37) mL/min × g (Table 1). Three days postoperatively, the operated on/intact femur flow ratio was about 0.9, and within 1 week the blood flow of the operated on femur had reached a level close to that of the intact femur. Then followed a period with higher flow values in the operated on than in the control femora; and at the evaluation after 6 weeks, the flow was again of the same magnitude in the two femora. There were no differences between reamed and nailed femora with respect to blood flow throughout the study.

Apparently, neither reaming nor nailing affected flow in the femoral condyles or tibia. Pooling the two groups, however, gave a reduction in tibial blood flow to 0.93 of that in the intact side 3 days postoperatively ($P = 0.02$).

The percentage of the cardiac output distributed to the intact left femur was 0.15 (0.12–0.19), and to the tibia 0.12 (0.09–0.15).

The hydroxyproline content in the operated on femur followed a similar trend in reamed and nailed bones. In the diaphyseal part of the femur, the hydroxyproline content immediately after operation was reduced to about 0.9 of that of the intact femur ($P = 0.02$), and showed a similar ratio 3 days postoperatively ($P = 0.009$). Within 3 weeks the amount of collagen in operated on femora seemed to be restored.

The contents of calcium was reduced to about 0.9 of that in intact femur 0–3 days postoperatively for the reamed group ($P = 0.05$ and $P = 0.02$), as well as for the nailed group ($P = 0.02$ and $P = 0.05$). Also the calcium content was fully restored within 3 weeks postoperatively. Although there was no difference between reamed and nailed bones at any evaluation

Table 1. Blood flow, hydroxyproline and calcium content in reamed femora (RF) and reamed/nailed femora (RNF). Ratios of intact femora and absolute values. Median and 0.25–0.75 fractiles

		Days after reaming/nailing				
		0	3	7	21	42
Blood flow						
RF	(ratio)	0.28 (0.24–0.37)*	0.85 (0.66–0.90)*	1.02 (0.80–1.25)	1.11 (0.99–1.21)*	1.01 (0.86–1.22)
	(mL/min g)	0.08 (0.06–0.09)	0.23 (0.18–0.28)	0.23 (0.19–0.34)	0.28 (0.18–0.34)	0.22 (0.21–0.26)
RNF	(ratio)	0.34 (0.32–0.39)*	0.94 (0.86–1.03)	1.06 (0.93–1.20)	1.05 (1.00–1.40)*	0.98 (0.88–1.14)
	(mL/min g)	0.11 (0.08–0.16)	0.26 (0.24–0.31)	0.34 (0.17–0.38)	0.29 (0.17–0.42)	0.24 (0.20–0.28)
Hydroxyproline						
RF	(ratio)	0.94 (0.90–0.96)*	0.92 (0.85–0.96)*	0.96 (0.89–1.02)	1.00 (0.95–1.08)	1.04 (0.92–1.09)
	(μmol)	58.8 (54.9–59.1)	57.3 (52.3–59.7)	59.7 (56.4–68.1)	69.7 (62.5–72.8)	75.0 (69.4–81.0)
RNF	(ratio)	0.92 (0.86–0.97)*	0.92 (0.91–0.95)*	0.97 (0.90–1.06)	0.98 (0.88–1.07)	0.98 (0.91–1.04)
	(μmol)	56.5 (52.3–63.3)	53.8 (52.2–54.9)	59.3 (56.4–63.5)	66.8 (59.3–70.1)	71.8 (67.2–77.7)
Calcium						
RF	(ratio)	0.95 (0.95–1.00)*	0.91 (0.86–0.92)*	1.00 (0.90–1.12)	1.02 (0.99–1.02)	1.02 (0.98–1.26)
	(mmol)	1.5 (1.4–1.6)	1.1 (1.1–1.4)	1.6 (1.4–1.6)	1.6 (1.4–1.8)	2.0 (1.9–2.1)
RNF	(ratio)	0.92 (0.86–0.97)*	0.92 (0.81–1.00)*	0.96 (0.82–1.02)	0.92 (0.89–1.09)	0.95 (0.89–0.99)
	(mmol)	1.3 (1.3–1.4)	1.2 (1.1–1.3)	1.3 (1.2–1.3)	1.4 (1.3–1.5)	1.6 (1.5–1.8)

*Ratio statistically different from 1.00.

time, the nailed rats as a group consistently had a lower calcium content in operated on femora than the reamed ones ($P = 0.04$).

Mineralization of bone was similar in operated on and intact femora during the whole experimental period in both the reamed and the nailed groups. However, again nailed animals as a group revealed lower mineralization than the reamed ones in the operated on femur: viz., 24 mmol calcium/mmol hydroxyproline for reamed femora and 22 mmol calcium/mmol hydroxyproline for nailed femora ($P = 0.03$).

The hydroxyproline and calcium contents in the femoral condyles were similar in operated on and intact femora, and did not change with time.

Discussion

Approximately two thirds of the blood vessels must have been destroyed by reaming the medullary canal. The trapped microspheres indicated that at least one third of the femoral vessels were functional immediately after reaming, and these were (due to the operation damage) mainly periosteal vessels. This ratio accords with about 70 percent disturbed cortical circulation found by intravital staining after reaming and nailing canine tibia (Klein et al. 1990). Also, others have reported that about one third to one fourth of the outer cortex in rabbit radius and canine femur is supplied by periosteal vessels (Trueta and Cavadias 1955, Rhinelander 1974), whereas some report that the ratio is one half to two thirds of the outer cortex of sheep tibia (Pfister et al. 1979).

The hyperemic period that occurred about 3 weeks after the operative procedures may be related to both dilatation of intact vessels and revascularization. The present study cannot separate these effects. An interesting study in rabbit tibiae has shown that immobilization in a plaster cast produced a similar hyperemic period regardless of a healing fracture or tenotomy (Geiser and Trueta 1958). It is therefore possible that hyperemia, like that observed in our study, might be a general or unspecific vascular response to various types of remodeling, resorption included.

An important finding in our study was that the presence of a nail did not interfere with the restoration of blood flow. Thus, the implant itself apparently does not represent a major obstacle to revascularization, as was suggested by Kessler 1986. It has been reported that the size of the nail may affect the pattern of revascularization and thereby the remodeling of the

diaphysis of the femur. Rhinelander (1973) found that if the nail did not occupy the medullary canal completely, regeneration of vessels was more rapid and the amount of cortical necrosis decreased.

The proportion of cardiac output distributed to the intact left femur and tibia agreed with the results of Tothill and MacPherson (1986), who reported that in rats the two femora received 0.34 percent of the cardiac output and the tibiae received 0.23 percent.

Blood flow in the femoral condyles did not change with the passage of time. Neither did flow in the tibiae alter significantly, unless we pooled both the reamed and the nailed groups, which revealed a slightly reduced tibial blood flow 3 days postoperatively. One might speculate whether a greater amount of blood supply to the hindlimb was needed for repair processes in the femur at an early stage of the revascularization process. Aalto and Slätis (1984) found that increased tibial blood flow after osteotomy was accompanied by a slight decrease in overall skeletal circulation.

An important assumption for correct distribution of microspheres is that they are trapped in arterioles during the first passage through the circulation (Lunde and Michelsen 1970, Malik et al. 1976, Tothill 1984). If microspheres are not trapped, but only shunted past the capillary bed of bone, flow will be correspondingly underestimated. Spheres with a diameter of 15 μm are commonly used, and evidence has been provided that this is the optimal size for measuring blood flow in bone (Gross et al. 1979). According to our data from lung tissue, shunting of spheres did not take place to any great extent and did not alter during the experimental period. It is, however, acknowledged that femoral shunting cannot be specifically determined.

The restoration of blood flow preceded that of bone mass. Hydroxyproline content 3 days postoperatively was still reduced to the same extent as immediately after reaming, but it seemed to be restored within 3 weeks. These results corroborate a previous study that also revealed that the rate of synthesis of hydroxyproline in reamed and nailed femora 3 days postoperatively was reduced to 0.82 of that of the intact femur (Indrekvam et al. 1991c). This observation, according to the present study, may well be ascribed to vascular damage from reaming. Three weeks postoperatively, the synthesis rate was higher, which corresponds to the period of increased bone flow in the present study. Both blood flow and synthesis of hydroxyproline had regained normal levels within 6 weeks. These results suggest that repair and bone turnover are linked to changes in blood flow, a relationship that might well be causal. This interpretation is consistent with the observation that during in vitro incubation of bone in a medium with sufficient access to nourishment, collagen synthesis took place within 2 hours (Langeland 1975, Langeland and Teig 1975). Also, this accords with the finding that remodeling starts from the border of nonsupplied bone and progresses centripetally after medullary nailing of sheep tibia (Pfister et al. 1979).

Calcium content followed the same trend as hydroxyproline content. However, calcium content, and thus mineralization, remained lower in the nailed bones than in the reamed bones. This is an interesting observation because it may correspond to the common clinical observation of a fibrous layer formed around a nail. Thus, less of the collagen mass would be calcified.

In conclusion, intramedullary reaming immediately reduced blood flow in femoral bone by approximately 70 percent and reduced the hydroxyproline and calcium contents about 10 percent of that in the intact femur. Restoration of blood flow preceded that of bone mass. Blood flow in operated on femora regained the level of the intact side within 1 week, whereas the contents of hydroxyproline and calcium were fully restored within 3 weeks. The presence of a nail did not interfere with the rate of blood flow restoration.

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