

# Bone repair inhibited by indomethacin

## Effects on bone metabolism and strength of rabbit osteotomies

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We measured mineral content, maximum bending strength, and regional blood flow after tibial osteotomy fixed with a small metal plate in 38 rabbits. Half of the animals were treated with indomethacin (10 mg/kg/day) while the other half served as controls.

After 2 and 6 weeks, the bone mineral content and maximum bending strength were lower in the indomethacin group when compared with the controls. Compared with the controls, the blood flow at the osteotomy site was decreased after 2 weeks and increased after 6 weeks in the indomethacin-treated animals.

Inhibition of blood flow increase by indomethacin medication in the early period following osteotomy, as well as retarded bone healing, are probably caused by inhibition of the inflammatory reaction.

Nonsteroidal anti-inflammatory drugs have been reported to influence bone metabolism (Rø et al. 1975, Sudmann & Bang 1979, Törnkvist et al. 1985). Indomethacin, which is one of the most widely used of these drugs, is claimed to decrease inflammatory reaction by inhibiting prostaglandin synthesis (Vane 1971). The inflammatory reaction is probably of great importance in the early phase of bone healing (Simmons 1985). To obtain further knowledge of the effect of nonsteroidal anti-inflammatory drugs on fracture healing, we studied the influence of indomethacin on regional blood flow, bone mineral content (BMC), and mechanical strength after tibial osteotomy.

### Materials and methods

Thirty-eight adult Belgian rabbits weighing 4.9 (4.3–6.0) kg, kept in separate cages, were fed on

200 g of ordinary dry laboratory diet and 300 ml of water daily. Twenty rabbits were given daily oral doses of 50 mg indomethacin in the drinking water beginning 4 days before the operation. To ensure that all the indomethacin was consumed each day, the animals were given 50 ml less than the mean water intake in a pilot experiment. Blood samples collected on Day 35, just before a new water supply, was analyzed for indomethacin (Jensen 1978). Eighteen rabbits served as controls.

Two weeks after the osteotomy, the rabbits had a mean weight loss of 0.4 and 0.3 kg in the indomethacin and the control group, respectively. After 6 weeks, both groups had gained 0.1 kg in mean weight. The drug plasma level 24 hours after the last medication was 284 (52–977) ng/ml.

*Osteotomy.* Under antibiotic cover (ampicillin 0.5 g), a mid-transverse tibial osteotomy was performed with a small oscillating saw. A six-hole AO-DCP plate® (52.0×7.5×2.0 mm) was fixed with 2.7 mm screws to the anterolateral surface of the right tibia. Half of the animals were killed after 2 weeks and the remaining ones, 6 weeks after the operation.

*Blood flow.* The microsphere method with reference blood sampling was used to measure regional

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blood flow in both tibiae. NEN-TRAC microspheres (New England Nuclear), 15  $\mu\text{m}$  in diameter, were labelled with  $^{51}\text{Cr}$  and suspended in 10 per cent Dextran. A volume of 4 ml microsphere solution, containing approximately  $4.5 \times 10^6$  spheres, was used. To secure homogeneous dispersal of the spheres, the batch was agitated 5 min on a Whirlimixer<sup>®</sup> before injection.

Using halothane anesthesia a catheter (4.0 F, pigtail) was inserted into the left cardiac ventricle, via the left carotid artery, for sphere injection. Correct position of the catheter was checked by an image intensifier. Blood pressure was recorded and the arterial blood gases were analyzed to ensure a steady state. The  $\text{pO}_2$  was kept above 14.5 kPa. A polyethylene catheter (Venflon<sup>®</sup>) was advanced into the brachial artery for reference blood sampling. Blood was allowed to flow into a preweighed plastic cup just before the sphere injection.

The spheres were injected over a period of 15 s, followed by flushing with 5 ml (37° C) heparin-saline. The reference blood sampling was continued for 2 min after sphere injection. During flow measurements the animals were placed in the supine position with the hips and knees in 90° flexion. After the last flow measurement, the animals were killed with an overdose of barbiturate. The tibiae were dissected free and BMC measurement and mechanical testing were immediately performed. Then, both tibiae were cut into several standardized specimens and the marrow removed for separate analysis. All the tissue samples were placed in preweighed plastic vials for gamma radiation counting. The regional blood flow rate (RBF) in each specimen was calculated according to Bünger et al. (1983).

**BMC.** The DCP plate was removed and screws were inserted into the two end holes. For every 4-mm segment between the two screws and at the corresponding sites in the intact leg, BMC was determined by photon absorptiometry (Gamma-tec Osteodensitometer Model GT 30, Hareskov, Denmark).

**Mechanical testing.** All the screws were removed and the tibial shaft was loaded in a three-point bending set-up (Alkwetron 250, Lorentsen & Wettre) at a constant deformation rate of 5 mm/min. The tibia was placed on the posterior

surface and load was applied at the osteotomy site on the anterior edge of the bone. The distance between the supporting points was 4 cm. The intact contralateral tibia was tested similarly.

**Statistics.** Mean values and standard errors were calculated for all the recordings. Comparison of the means was performed by the two-tailed Student's *t*-test. Comparison of loosening of the DCP plate between the groups was performed by the chi-square test. A *P* value <0.05 was considered significant.

## Results

Of 20 rabbits receiving indomethacin, 9 did not complete the experiment, 7 because of loosening of the plate proximal to the osteotomy and 1 because of fracture of the back. Another rabbit died for unknown reasons. Of 18 control rabbits, 1 was killed because of plate loosening and 1 because of technical failure at surgery. Loosening of the plate was more frequent in the indomethacin group.

After 2 and 6 weeks, periosteal callus was present and the marrow space between the two screw holes in the middle of the tibia was macroscopically replaced by callus.

In the osteotomized leg in the control group, but not in the indomethacin group, the regional blood flow in cortical bone below the plate was elevated after 2 and 6 weeks compared with the intact leg. In the control animals, the regional blood flow of the osteotomy area had increased 4.1 times after 2 weeks and 0.46 times after 6 weeks when compared with the intact leg. In the indomethacin group, the regional blood flow increased 0.67 times and 1.13 times after 2 and 6 weeks, respectively.

After 2 weeks, the blood flow in the cortical bone of the osteotomy area was lower in the indomethacin group compared with the control group (Figure 1), whereas it was higher after 6 weeks (Figure 2). Blood flow in the bone marrow close to the osteotomy did not differ between the two groups.

After both 2 and 6 weeks, the BMC ratio between the osteotomized and the intact leg was lower at all the sites in the indomethacin group,

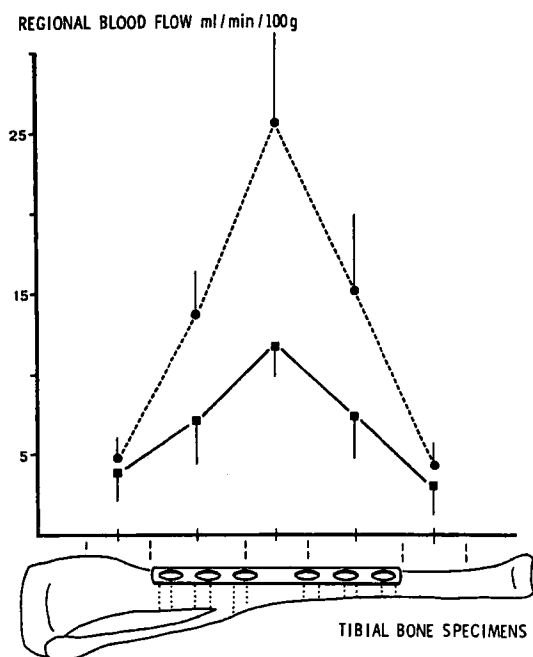


Figure 1. Blood flow (Mean  $\pm$  SE) in tibial specimens two weeks after osteosynthesis. Region analyzed in relation to DCP plate is also given.  $\bullet$ — control group (n/7),  $\blacksquare$ — indomethacin group (n/5).

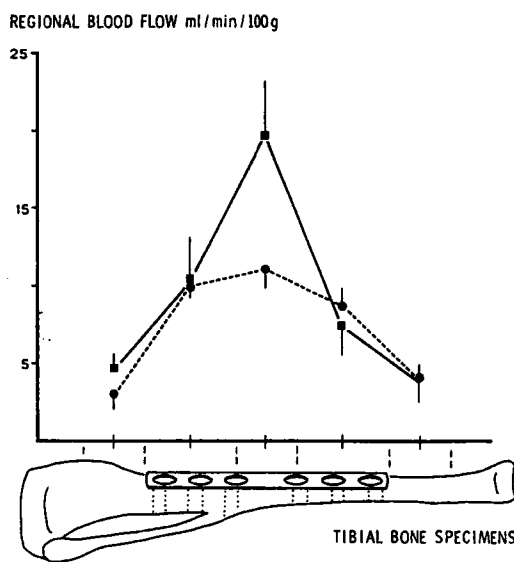


Figure 2. Blood flow (mean  $\pm$  SE) in tibial specimens 6 weeks after osteosynthesis.  $\bullet$ — control group (n/8),  $\blacksquare$ — indomethacin group (n/6).

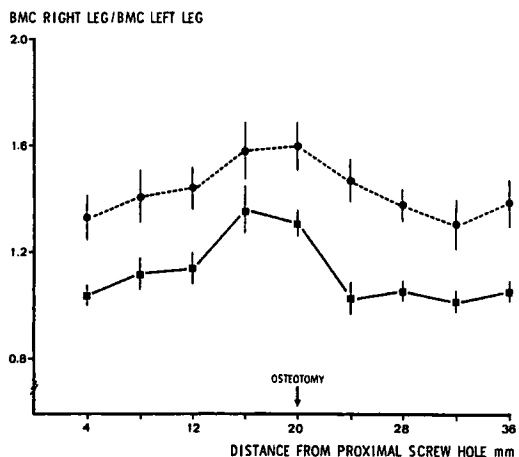


Figure 3. Ratio of BMC (mean  $\pm$  SE) between the osteotomized and intact legs after six weeks.  $\bullet$ — control group (n/9),  $\blacksquare$ — indomethacin group (n/6).

Table 1. Postoperative maximum bending strength of the tibia. I = indomethacin group, C = control group. Mean (SE)

	2 weeks		6 weeks	
	I	C	I	C
	n 5	n 4 <sup>a</sup>	n 4	n 8
Osteotomized tibia (kilopounds)	2.4 (0.5)	4.6 (1.2)	25 (6.7)	43 (5.1)
Control tibia (kilopounds)	68 (4.6)	69 (4.4)	90 (4.9)	77 (5.9)
Osteotomized tibia/control tibia $\times$ 100	3.8 (0.9)	6.4 (1.1)	27 (5.9)	61 (11.4)

<sup>a</sup> Two specimens with no measurable bending strength are excluded.

although the differences were only significant after 6 weeks (Figure 3).

All the osteotomized legs broke at the osteotomy site at testing. The bending strength was low after 2 weeks in both groups. In the indomethacin group the maximum bending strength of the osteotomized tibia was lower after 6 weeks compared with the control group (Table 1).

## Discussion

By supplying indomethacin in the drinking water, we obtained steady-state plasma levels in the lower range of the therapeutic interval (Rane et al. 1978). The plasma values corresponded well with those reported by Sudmann & Bang (1979), who squirted a drug suspension of the same amount into the mouth of their rabbits each day.

The increase in blood flow in the osteotomized leg is suggested to be part of the inflammatory reaction in bone healing. The observed increase in blood flow at 2 and 6 weeks after osteotomy accords with the value obtained by Rand et al. (1981) using  $^{85}\text{Sr}$  clearance in plate-fixed tibial osteotomies in dogs. The increase of BMC in bone beneath the DCP plate in 6 weeks in the osteotomized leg compared with the control leg indicates that not only osteotomy, but also plating affects the mineral content of the entire bone.

Our demonstration of reduced blood flow 2 weeks after osteotomy in indomethacin-treated animals suggests that indomethacin inhibits the inflammatory reaction and probably bone metabolism. This was further supported by the concomitant decrease in bone mineral content and mechanical strength at the osteotomy in the indomethacin group as compared with the control

group, and also more frequent loosening of the DCP plate. These observations are in agreement with the findings of Sudmann et al. (1979), who proposed that the inhibitory effect of indomethacin on fracture healing is caused by analgetic effect, increased fracture hematoma, and reduced inflammatory response.

Reduced mechanical strength after indomethacin treatment is in accordance with Rø et al. (1976), who found decreased mechanical strength in rat femurs throughout 24 days after closed, nonimmobilized fracture. At 6 weeks the osteotomy site in our control group had regained more than half of the mechanical strength as compared with the intact leg, indicating healing (Terjesen 1984). Increased blood flow in the indomethacin group at 6 weeks, but lower mechanical strength, indicates that metabolism was still elevated and healing retarded.

Inhibition of bone resorption by indomethacin and stimulation of bone resorption by prostaglandins have been demonstrated in vitro (Lerner 1984, Klein et al. 1970) and in vivo (Keller et al. 1986, Sudmann & Bang 1979). However, it has not been clarified whether these alterations are caused by decreased numbers of osteoclasts or impaired function of the cells. Also, osteoblasts seem to be inhibited by indomethacin. Thus, Törnkvist et al. (1985) found inhibition of bone formation induced by demineralized bone when indomethacin treatment was started with a week after implantation, but not when treatment was started later.

In conclusion, indomethacin inhibits bone healing and normal increase of blood flow in the early period following osteotomy, probably by inhibition of the inflammatory reaction.

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