

Indomethacin and bone remodeling

Effect on cortical bone after osteotomy in rabbits

Johnny Keller¹, Ingrid Bayer-Kristensen², Bue Bak¹, Cody Büniger¹, Per Kjærsgaard-Andersen¹, Ulf Lucht¹ and Flemming Melsen²

Remodeling in cortical bone close to a plated tibial midshaft osteotomy was histomorphometrically evaluated in 32 rabbits. The animals were divided into two groups, one being treated with indomethacin (10 mg/kg per day) and the other receiving placebo. In the placebo-treated group, the remodeling activity was higher in the osteotomized leg compared with the intact leg. Two and 6 weeks after osteotomy, the number of resorptive and formative foci was reduced in the indomethacin-treated group compared with the placebo group. Porosity did not differ between the groups after 2 weeks; but after 6 weeks, it was reduced in the indomethacin-treated animals. Throughout the study, the bone formation rate did not differ between the two groups.

This study demonstrates that indomethacin inhibits the remodeling of traumatized bone.

Whether or not prostaglandins in traumatized tissues (Dekel et al. 1981) are a mediator of repair reactions remains unclear, though there is an indication that prostaglandins enhance such processes (Shih and Norridin 1986). The non-steroidal anti-inflammatory drug indomethacin is known to inhibit the synthesis of prostaglandins (Vane 1971) and therefore may indirectly influence bone repair. Such a mechanism may explain why indomethacin inhibits fracture healing (Keller et al. 1987, Rø et al. 1976, Sudmann et al. 1979).

We have studied the effect of indomethacin on bone remodeling after osteotomy.

Animals and methods

In 32 adult Belgian rabbits, weighing 4.9 (4.3-6) kg, a midtransverse tibial osteotomy was performed on the right hind limb. The operation was made with an oscillating saw, and fixation was done by using a six-hole AO DCP plate (52.0 x 7.5 x 2.0 mm) and six screws (2.7 mm). The animals were kept in separate cages and fed 200 g of ordinary dry laboratory diet and 300 ml of water daily. Seventeen rabbits were given indomethacin (10 mg/kg per day) in the drinking water from 4 days before the operation until they were killed. Fif-

teen rabbits served as controls. To ensure that the daily amount of indomethacin was consumed, the animals were given 50 ml of water less than the mean water intake observed in a pilot experiment. The animals were killed after either 2 or 6 weeks. Blood samples were collected 1 week prior to death and analyzed for indomethacin (Jensen 1978).

Intravital bone labeling was performed by intravenous injection of oxytetracycline (15 mg/kg body weight) under Hypnorm[®] anesthesia 9 and 3 days before death.

Both tibiae from the rabbits were dissected free and fixed in 70 percent alcohol. Using a diamond band saw (ExactR[®]), a transverse section (50-µm thick) was cut 1 mm away from each of the two central and two peripheral drill holes, sections being procured from the innermost side of each drill hole relative to the osteotomy (Figure 1). Corresponding peripheral sections were cut in the intact leg.

The histomorphometric evaluation of the sections was performed "blind" using fluorescence and conventional light microscopy.



Figure 1. Central (CE) and peripheral (PE) bone sections after tibial osteotomy.

University of Århus, Institute of Experimental Clinical Research¹, and Institute of Pathology², Århus County Hospital Århus, Denmark

Correspondence: Dr. J. Keller, Agrivej 11, DK-8240 Risskov, Denmark

Remodeling activity was estimated by the following measurements, with each result representing the mean of the two peripheral or two central sections.

Resorptive foci: Number of haversian canals with increased diameter per transverse section at a magnification of 100x. An increased diameter of a haversian canal was defined by a value $> \text{mean} + 2 \text{ SD}$ of haversian canals in an uninjured rabbit leg.

Formative foci: Mean number of tetracycline-labeled canals per field of view for 10 randomly selected fields at a magnification of 160x.

Porosity: Mean percentage of points in a 100-point integrating reticle situated in haversian or Volkman canals per field of view for 10 random fields at a magnification of 100x.

Bone formation rate: Mean distance between double tetracycline labels using a stage micrometer at a magnification of 400x and divided by the labeling interval. To allow for the influence of oblique cutting, only the smallest distance was measured around each canal.

Statistics: Wilcoxon's paired rank test was used to compare results from the injured leg with results from the intact leg. For comparison of the groups, the one-tailed Student *t*-test was used on logarithmic-transformed data after homogeneity had been checked by the *F*-test. To compare loosening of the plate between the groups, the chi-square test was used. A *P*-value < 0.05 was considered significant.

Eight rabbits in the indomethacin group did not complete the study, 6 because of loosening of the plate, and 1 because of a vertebral fracture, whereas 1 rabbit died of unknown cause. In the placebo group, 1 rabbit also died of unknown cause.

Results

Two weeks after 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, the mean weight loss was 0.1 and 0.0 kg in the two groups, respectively. The indomethacin plasma levels 24 hours after the last medication was 260 (52-481) ng/ml.

There was no loosening of the plates in the control group, but this was frequent in the indomethacin group.

After 2 and 6 weeks, both the number of resorptive foci and the porosity were increased in the osteotomized leg as compared with the intact control leg. Both the number of formative foci and the bone formation rate were unchanged after 2 weeks, but increased after 6 weeks.

Influence of indomethacin (Table 1). After 2 and 6 weeks, both the number of resorptive and formative foci were reduced in the indomethacin group. Porosity did not differ between the two groups after 2 weeks, but was reduced after 6 weeks in the indomethacin group. The bone formation rate did not differ between the two groups after 2 weeks, nor after 6 weeks.

Finally, after both 2 and 6 weeks, none of the recorded histomorphometric parameters differed when the noninjured leg of the indomethacin-treated rabbits was compared with the noninjured leg of control rabbits.

Discussion

The changes in cortical bone following plated osteotomy demonstrate a rapid internal reorganization of bone. The increased porosity in the traumatized leg after 2 weeks followed by an increased number of formative foci after 6 weeks accords with a previous report (Frost 1983), showing that bone resorption precedes bone formation. In the remodeling sequence, the osteoclasts and the osteoblasts are closely related. Resorption probably stimulates the differentiation of precursor cells into osteoblasts and thereby stimulates bone formation (Frost 1983).

Our study demonstrates that indomethacin inhibits the regional acceleratory phenomenon by reducing the number of resorptive and formative foci both 2 and 6 weeks after osteotomy. This confirms the observations of Sudmann and Bang (1979) that indomethacin caused a decrease in haversian remodeling after osteotomy in rabbit ulnar bone.

After 2 weeks of indomethacin treatment, the number of formative foci was more reduced than the number of resorptive foci, whereas after 6 weeks, they were equally reduced. This may explain why the porosity was unchanged after 2 weeks, but reduced after 6 weeks of indomethacin treatment. We suggest that indomethacin inhibits the remodeling sequence without influencing the coupling between bone resorption and bone formation.

Prostaglandins, the synthesis of which is known to be inhibited by indomethacin, are important mediators in the inflammatory response and probably stimulate bone healing and remodeling (Vane 1971). These assumptions are supported by studies of heterotopic bone formation after implantation of demineralized bone (Törnkvist et al. 1985, Nilsson et al. 1986), in which an effect of indomethacin was only seen if treatment was started within the first week after injury. In several animal studies, indomethacin has been shown to inhibit fracture healing (Keller et al. 1987, Rø et al. 1976, Sudmann et al. 1979). This is supported by the increased frequency of plate loosening after indomethacin treatment in our study.

Table 1. Remodeling changes in cortical bone neighboring a plated tibial osteotomy in rabbits. I indomethacin group, P placebo. Mean (SE)

	2 weeks		6 weeks	
	I n 5	P n 6	I n 4	P n 8
Resorptive foci per transverse section				
Central location	46.8 (10.3) *	85.6 (9.0)	130 (40.1) *	236 (10.5)
Peripheral location	69.5 (7.8) *	126 (16.2)	136 (17.9) *	190 (21.4)
Control leg	49.5 (16.5)	61.0 (10.2)	49.5 (8.8)	50.3 (19.4)
Formative foci per field of view				
Central location	2.1 (0.6) *	7.6 (2.1)	22.3 (1.0)	35.2 (0.3)
Peripheral location	3.2 (1.2) *	8.8 (2.8)	18.5 (6.2) *	46.4 (10.4)
Control leg	8.3 (3.7)	11.3 (1.6)	2.4 (1.1)	5.1 (1.6)
Porosity in percent				
Central location	5.95 (0.42)	6.83 (0.35)	5.81 (0.45) *	8.19 (0.64)
Peripheral location	6.45 (0.47)	6.49 (0.27)	6.06 (0.53) *	9.72 (1.09)
Control leg	6.03 (0.47)	5.48 (0.49)	4.53 (0.43)	5.09 (0.34)
Formation rate in $\mu\text{m/day}$				
Central location	1.5 (0.1)	2.0 (0.4)	3.7 (0.3)	3.4 (0.3)
Peripheral location	1.7 (0.6)	3.1 (0.4)	2.8 (0.2)	3.3 (0.4)
Control leg	2.0 (0.6)	2.9 (0.3)	2.3 (0.7)	2.0 (0.5)

* $P < 0.05$.

Remodeling was unchanged in the nontraumatized leg in the indomethacin group compared with the placebo group, which suggests that indomethacin only inhibits the stimulus to remodeling caused by trauma,

but does not influence remodeling away from the site of trauma. This is in agreement with Nilsson et al. (1986) and another study in which indomethacin did not influence normal growth (Sudmann et al. 1982).

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