Indomethacin and bone trauma

Effects on remodeling of rabbit bone

Johnny Keller[1], Per Kjærsgaard-Andersen[2], Ingrid Bayer-Kristensen[3], and Flemming Melsen[3]

The influence of indomethacin on remodeling activity in normal trabecular and cortical bone and its influence on cortical bone close to a midtibial drill hole, 2 mm in diameter were histomorphometrically evaluated. Eight rabbits were treated with indomethacin (12.5 mg/kg/day), and another 8 rabbits served as controls. After 3 days, the mean plasma indomethacin level was 542 ng/mL, resulting in an almost complete inhibition of prostaglandin synthesis as reflected by the serum levels. In the control rabbits the remodeling activity after 6 weeks was increased 1 mm away from the drill hole but not at 3 and 8 mm.

In conclusion, indomethacin had no effect on the activated remodeling process in cortical bone neighboring a small drill hole or on remodeling in nontraumatized cortical and cancellous bone. This suggests that the inhibitory effect of indomethacin on the remodeling process following local trauma to bone depends on the extent of the trauma.

Animal studies have shown that indomethacin inhibits fracture healing (Keller et al. 1987, Rø et al. 1976, Sudmann et al. 1979) and remodeling in cortical bone neighboring a tibial osteotomy (Keller et al. 1989, Sudmann and Bang 1979), whereas remodeling in nontraumatized bone seems unchanged after indomethacin treatment (Keller et al. 1989). Törnkvist et al. (1984) found the recovery of bone strength after a 2.3-mm-diameter drill hole in rabbit femoral bone to be inhibited by indomethacin.

We have studied the influence of indomethacin on remodeling nontraumatized cortical and trabecular bone and in cortical bone close to a minor bony lesion.

Materials and methods

16 adult rabbits weighing about 3.5 kg, kept in separate cages, were fed 150 g/day of ordinary dry laboratory diet and 300 mL of water. 8 rabbits were given indomethacin (12.5 mg/kg per day) in the drinking water beginning 4 days before surgery until they were killed. 8 more rabbits served as controls. To ensure that the correct amount of indomethacin was consumed, the animals were given 50 mL of water less than the daily mean water intake determined from daily pilot experiments. Blood samples (Table 1) collected prior to and on Days 3 and 38 after making the drill hole, all taken in the morning

Table 1. Plasma indomethacin (ng/mL) and plasma prostaglandin E_2 and I_2 (ng/mL) 24 hours after the last medication measured before, 3, and 38 days after making a midtibial drill hole. (N 6). Mean *SEM*

	Indomethacin treatment		
	Before	Day 3	Day 38
Indomethacin	0	542 *147*	139 *46*
Prostaglandin E_2	4.4 *0.6*	2.5 *0.4*	1.2 *0.1*
Prostaglandin I_2	23 *13*	2.0 *0.5*	0.0 *0.0*

University Department of Orthopedics[1] Århus Amtssygehus, The Orthopedic Hospital[2], University of Århus, and University Institute of Pathology,[3] Århus Amtssygehus, Århus, Denmark

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

Table 2. Remodeling in traumatized and nontraumatized cortical bone near a midtibial 2 mm drill hole. Mean *SEM*

	Distance (mm) from drill hole			Control tibia
	1	3	8	
Formative foci per field of view				
I	47 *11**	18 *6*	16 *7*	18 *5*
P	53 *22**	22 *5*	11 *5*	11 *3*
Resorptive foci per transverse section				
I	24 *6**	19 *5*	18 *5*	14 *4*
P	33 *10*	15 *3*	14 *3*	10 *2*
Porosity (percent)				
I	2.4 *0.3*	2.1 *0.3*	2.4 *0.2*	2.5 *0.2*
P	2.4 *0.3*	2.1 *0.2*	2.4 *0.2*	2.5 *0.3*
Bone formation rate (µm/day)				
I	2.2 *0.1*	2.3 *0.3*	2.1 *0.2*	2.1 *0.1*
P	2.3 *0.2*	2.2 *0.1*	1.7 *0.2*	2.0 *0.1*

I indomethacin group (n 6); P placebo group (n 6).
* significant ($p < 0.05$) compared with control tibia.

Table 3. Bone formation rate in nontraumatized trabecular bone. Mean *SEM*

	Lumbar vertebrae	Left trochanter	Left femoral head
I	1.0 *0.1*	1.1 *0.1*	1.4 *0.1*
P	1.1 *0.1*	0.9 *0.1*	1.4 *0.2*

I indomethacin group (n 6); P placebo group (n 6).

just before a new water supply, were analyzed for indomethacin (Jensen 1978) and prostaglandins E_2 and I_2 (Richelsen 1987). A midtransverse tibial drill hole was made through both cortices in the right tibia. The rabbits were allowed to move freely immediately after the operation. Under Hypnorm® anesthesia, 9 and 3 days before killing, oxytetracycline (15 mg/kg body weight) was given intravenously for intravital labeling of formative mineralizing surfaces. The rabbits were killed 6 weeks after the operation. The fourth and fifth lumbar vertebrae, the left femur, and both tibiae were dissected free from soft tissue and fixed in 70 percent alcohol. A sagittal section was then cut from the middle of both vertebral bodies using a diamond band saw (Exact®). In the proximal left femur a section was made to include the middle of the head and the trochanter region. In the right traumatized tibia, transverse sections were obtained at 1-, 3-, and 8-mm intervals from the drill hole both proximally and distally. In the left nontraumatized tibia the transverse sections were obtained corresponding to the location of the 1-mm sections in the right tibia. The histomorphometric evaluation of the sections was performed using fluorescence and conventional light microscopy. In the femoral neck and the vertebral bone, the rate of trabecular bone formation was determined as the mean distance between tetracycline lines using a stage micrometer at a magnification of 400x and divided by the labeling interval. No correction for obliquity was performed. In the tibial cortical bone, all the parameters were estimated as the mean of the proximal and distal sections obtained at 1-, 3-, or 8-mm intervals from the drill hole, respectively. Resorptive and formative foci and cortical bone formation rate were determined as we described previously (Keller et al. 1989). The periosteal callus area was obtained from division by the cortical cross-sectional area using a planimeter at a magnification of 15x. Two placebo-treated and 2 indomethacin-treated rabbits were excluded from the study, 3 due to death from unknown causes and 1 due to technical failure during preparation of the specimens. Wilcoxon's paired rank test was used to compare results obtained in the same rabbit from various sections in the traumatized leg, and for sections obtained at the 1-mm interval in both legs. For comparison of groups, the two-tailed Student's *t*-test was used after homogeneity of variances had been tested by the *F*-test. A *P*-value below 0.05 was considered significant.

Results

Evaluated from rabbits in the placebo group, the number of formative foci was increased in sections obtained at a 1-mm interval from the drill hole when compared with the results from the nontraumatized leg (Table 2). At intervals 3 and 8 mm from the drill hole, no differences in the numbers of resorptive and formative foci could be observed, when compared with the results obtained from the 1-mm interval sections in the nontraumatized leg. The bone formation rate and porosity were not influenced by the drill hole. Small amounts of periosteal callus appeared in the area neighboring the drill hole.

Effects of indomethacin

No difference was shown in remodeling in the traumatized cortical bone between the indomethacin

group and the control group (Table 2). Periosteal callus formation 1 mm from the drill hole was slightly reduced in the indomethacin group (mean 4.9 percent; SE 1.1 percent) compared with the control group (mean 7.4 percent; SE 1.6 percent). Nontraumatized cortical and trabecular bone were not affected by indomethacin treatment (Tables 2 and 3).

Discussion

Our study demonstrates that an almost complete inhibition of prostaglandin synthesis occurs after 3 days' treatment with indomethacin. Nonspecific interaction of plasma albumin with the antigen-antibody reaction in the radioimmuno-assay determination of prostaglandin E_2 may explain residual low levels of prostaglandin E_2 after 42 days of indomethacin treatment (Richelsen 1987). The plasma-indomethacin levels obtained in our study corresponded well with those reported in other studies using the rabbit (Rø et al. 1976, Sudmann and Bang 1979), although we administered the drug in the drinking water rather than via a stomach tube.

Burstein et al. (1972) reported that a drill hole through both femoral cortices in rabbits initially reduces the mechanical strength of the bone. After 4 to 8 weeks the bone recovers mainly due to filling in of the defect by woven bone. Our study confirmed this; at the end of our study all the rabbits had woven bone in the defect and minor amounts of periosteal callus. Only in the region close to the drill hole could an increased remodeling be recorded, i.e., the so-called regional acceleratory phenomenon (Frost 1983). In contrast to a fracture, which generally is irregular and breaks the continuity of all the Haversian canals in the cortical bone, a drill-hole defect is smooth and regular and only breaks the continuity of some of the Haversian canals. This may explain why the cortical remodeling in the present study was increased only close to the drill-hole defect. However, the observed threefold-to-fivefold increase of the number of resorptive and formative foci close to the drill hole corresponded well with the observed changes 6 weeks after a plated midtibial osteotomy (Keller et al. 1989). In that same study, it was shown that 2 weeks after the osteotomy, the number of formative and resorptive foci had, at most, doubled, making the 6-week period convenient for remodeling studies after trauma to cortical bone in rabbits.

In a previous study in rabbits, indomethacin caused a 50-percent reduction in the maximum tibial bending strength when evaluated 6 weeks after a plated osteotomy (Keller et al. 1987). The degree of inflammation is believed to be correlated with the extent of the trauma, and may explain why no effect of indomethacin on the repair processes after a tibial drill-hole could be shown in the present study, although there was a trend for reduced remodeling and periosteal callus after indomethacin treatment. In accordance with this, Törnkvist et al. (1984) found full-strength recovery 6 weeks after a femoral drill hole was made when comparing the traumatized leg with the intact leg of placebo-treated rabbits and reduced strength recovery in indomethacin-treated rabbits, but they found no difference when comparing the traumatized legs of the indomethacin-treated rabbits with the traumatized legs of the placebo-treated rabbits. Moreover, Elves et al. (1982) found similar small changes in strength after drill-hole repair in vertebrae. For nontraumatized trabecular bone, we found no change in bone formation rate between the indomethacin and control groups. This accords with Sudmann et al. (1982) and Nilsson et al. (1986), who demonstrated indomethacin to be without effect on ordered growth in nontraumatized cortical bone in rats.

The present study suggests that in rabbits indomethacin has no effect on the activated remodeling process in cortical bone neighboring a minor drill-hole. Moreover, remodeling in nontraumatized cortical and cancellous bone is not influenced by treatment with indomethacin. The inhibitory effect of indomethacin on the activated remodeling process following local trauma to bone seems to depend on the extent of the trauma.

Acknowledgements

The analyses of plasma indomethacin were performed by Dumex Ltd, Copenhagen. The prostaglandin analyses were performed by Bjørn Richelsen, Division of Endocrinology and Metabolism, Medical Department III, Århus County Hospital.

References

Burstein A H, Currey J, Frankel V H, Heiple K G, Lunseth P, Vessely J C. Bone strength. The effect of screw holes. J Bone Joint Surg (Am) 1972; 54(6): 1143–56.

Elves M W, Bayley I, Roylance P J. The effect of indomethacin upon experimental fractures in the rat. Acta Orthop Scand 1982; 53(1): 35–41.

Frost H M. The regional acceleratory phenomenon: a review. Henry Ford Hosp Med J 1983; 31(1): 3–9.

Jensen K M. Determination of indomethacin in serum by an extractive alkylation technique and gas liquid chromatography. J Chromatogr 1978; 153(1): 195–202.

Keller J, Bayer Kristensen I, Bak B, Bünger C, Kjaersgaard Andersen P, Lucht U, Melsen F. Indomethacin and bone remodeling. Effect on cortical bone after osteotomy in rabbits. Acta Orthop Scand 1989; 60(1): 119–21.

Keller J, Bünger C, Andreassen T T, Bak B, Lucht U. Bone repair inhibited by indomethacin. Effects on bone metabolism and strength of rabbit osteotomies. Acta Orthop Scand 1987; 58(4): 379–83.

Nilsson O S, Bauer H C, Brosjö O, Törnkvist H. Influence of indomethacin on induced heterotopic bone formation in rats. Importance of length of treatment and of age. Clin Orthop 1986; (207): 239–45.

Richelsen B. Factors regulating the production of prostaglandin E2 and prostacyclin (prostaglandin I2) in rat and human adipocytes. Biochem J 1987; 247(2): 389–94.

Rø J, Sudmann E, Marton P F. Effect of indomethacin on fracture healing in rats. Acta Orthop Scand 1976; 47(6): 588–99.

Sudmann E, Bang G. Indomethacin induced inhibition of haversian remodelling in rabbits. Acta Orthop Scand 1979; 50(6): 621-7.

Sudmann E, Dregelid E, Bessesen A, Mørland J. Inhibition of fracture healing by indomethacin in rats. Eur J Clin Invest 1979; 9(5): 333–9.

Sudmann E, Tveita T, Hald J Jr. Lack of effect of indomethacin on ordered growth of the femur in rats. Acta Orthop Scand 1982; 53(1): 43–9.

Törnkvist H, Lindholm T S, Netz P, Strömberg L, Lindholm T C. Effect of ibuprofen and indomethacin on bone metabolism reflected in bone strength. Clin Orthop 1984; (187): 255–9.