

Fracture healing in rats inhibited by locally administered indomethacin

Lars B. Engesæter¹, Bjørn Sudmann¹ and Einar Sudmann²

We studied the inhibitory effect of indomethacin on fracture healing in 135 young, male rats after oral administration compared with local application into the fracture. A closed mid-diaphyseal fracture of the left femur was performed in all the rats. The fractures were not immobilized. In one experiment, half of the animals received indomethacin via a stomach tube (2 mg/kg/day) for 10 days; the controls received only the vehicle. In another experiment, 0.5 mg of indomethacin, contained in a bioerodible polyorthoester

gel, was injected into the fracture area in half the rats; in the controls, only the gel was injected. In both experiments, random animals were killed on Days 0, 5, 10, and 20. As assessed by radiographs and manual testing, the same inhibition of fracture healing was found regardless of whether indomethacin was given orally or locally. However, the amount of indomethacin that was applied locally was only one fourth of the total dose given orally; no indomethacin was detected in the serum.

¹Surgical Research Laboratory, Department of Orthopedics, Haukeland Hospital, and ²Hagavik Orthopedic Hospital, University of Bergen, Norway

Correspondence: Dr. Einar Sudmann, Hagavik Orthopedic Hospital, N-5220 Hagavik, Norway

Tele +47-5 30 80 10. Fax +47-5 30 82 04

Submitted 91-06-29. Accepted 91-12-07

Nonsteroidal anti-inflammatory drugs (NSAIDs) have been shown to inhibit fracture healing in both humans (Sudmann and Hagen 1976) and animals (Sudmann 1975, Rø et al. 1976, Sudmann et al. 1979, Allen et al. 1980, Elves et al. 1982, Törnkvist et al. 1984). It is not known whether orally administered NSAIDs impair bone healing directly at the fracture site or indirectly via hormones.

We have used a new, sustained drug delivery system for application of indomethacin directly into the fracture area. To test whether or not indomethacin inhibits fracture healing directly in the fracture itself, the effect of locally versus orally administered indomethacin on fracture healing was assessed.

placebo was applied into the fracture (Table 1). In Series A, ether was used for anesthesia, whereas fluanisone (Hypnorm Vet, Leo, Sweden) was used in Series B.

At the start of the experiments, a closed, mid-diaphyseal fracture of the left femur was performed in all the animals by three-point bending with a special forceps (Ekeland et al. 1981). The fracture was left to heal without immobilization. Random rats were killed on Days 0, 5, 10, and 20; on Day 0, they had been killed for measurement of bone length (Table 1).

Materials and methods

Inbred male Wistar SPF rats (Møllegaard, Denmark) were used. The animals were housed five in each cage and given water and standard pellets ad libitum.

Two separate series were studied (Table 1); in both series the rats were divided into weight-matched groups, and were given indomethacin and placebo, respectively. In Series A, 70 rats, weighing 75 (67-82) grams at the start of the study, were given indomethacin or placebo orally. In Series B, 65 rats, with an initial weight of 58 (52-64) grams, indomethacin or

Table 1. Design of the experiment. Number of rats

Series	n	Treatment	Days			
			0	5	10	20
A Oral	70	Indomethacin 2 mg	-	10	10	10
		Control	10	10	10	10
B Local	40	Indomethacin 0.5 mg	-	6	5	6
		Control	6	5	6	6
	25	Indomethacin 0.5 mg	-	-	-	13
		Control	-	-	-	12

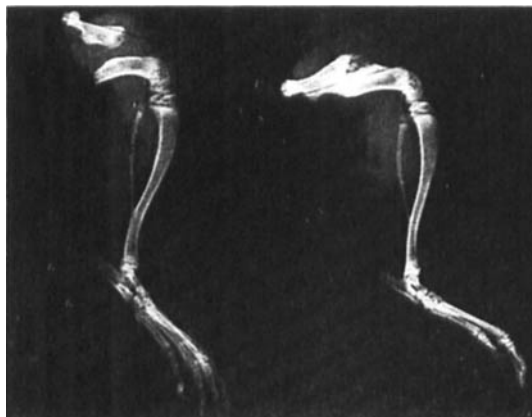


Figure 1. Lateral radiographs of the left leg on Day 20 in a rat treated with local indomethacin (left) and in 1 control-treated rat with the polyorthoester gel only (right). No callus is seen in the indomethacin-treated fracture, whereas the placebo-treated fracture is healed.

Series A

The rats were given an indomethacin suspension (Confortid[®], Dumex Ltd., Denmark) via a stomach tube once daily (2 mg/kg/day) for 10 days, or the vehicle only (Table 1). Thus, each of the indomethacin-treated rats received totally about 2 mg of indomethacin.

Series B

The fractures were exposed through a longitudinal, lateral incision. In the indomethacin group, 0.5 mg of indomethacin in 0.08 mL of a viscous bioerodible polyorthoester gel (Alzamer[®], Alza Corp., Palo Alto, CA, U.S.A.) was injected into the fracture area. The control rats were treated in the same way, but the polyorthoester gel lacked indomethacin (Table 1). To avoid leakage of the gel, the quadriceps fascia was closed with a pursestring suture.

On the day after the operation, 2 of the 6 rats treated with indomethacin-polyorthoester gel, which were scheduled for killing on Day 20, were found to have leakage of the gel through the skin incision. Therefore, for a better statistical assessment of the results, this group was supplemented with 25 rats, which had a median starting weight of 62 (60-65) g.

Evaluation

All the assessments were carried out without knowledge of the group identity (indomethacin or placebo).

Before killing the rats on Days 5 and 10 in both series, blood was collected under anesthesia by puncturing the aorta at the iliac bifurcation. Sera for indomethacin assessment were frozen at -20 °C until analysis (Jensen 1978).

Lateral radiographs of the left leg (disarticulated at the hip) were taken of all the rats killed on Days 5, 10, and 20 (Figure 1), and the dorsal angulation of the fractures was measured using a protractor (Rø et al. 1976).

In dissected left femurs, the maximal interfragmentary movement in the sagittal plane (fracture instability) was manually tested and measured with a protractor (Sudmann et al. 1979). When no instability could be detected, the fracture was classified as stable; when the fracture was also radiographically bridged by mineralized callus, the fracture was classified as healed.

The lengths of dissected right femurs from the femoral head to the distal end of the medial condyle were measured with a digital sliding calipers.

A median with 25- and 75-fractiles was used to express the average and the dispersion of the measured values. Statistical significance was evaluated by the Wilcoxon test for two independent samples (two-tailed test) and the Fischer exact probability test. Differences were considered significant when $P < 0.05$.

Results

The body weight of the animals increased normally during the investigation; there was no difference between those treated with indomethacin and the controls. No effect of indomethacin on the longitudinal growth of the right femur could be detected.

By oral treatment, the concentration of indomethacin in the serum 24 hours after the last medication was 0.52 (0.33-0.72) µg/mL on Day 5 and 0.42 (0.28-0.62) µg/mL on Day 10. In contrast, after local application of indomethacin, no detectable amount of the drug could be found in the serum, i.e., less than 10 ng/mL.

In both series the angulation of the distal fragments was greater in the rats receiving indomethacin than in the controls on Days 10 and 20 (Figure 2).

The fracture instability was higher in the indomethacin-treated rats than in the controls on Days 5, 10, and 20 in Series A and on Days 5 and 20 in Series B (Figure 3). In the indomethacin-treated rats, no fractures healed. On Day 20, all the fractures in the orally treated control rats healed, while only three fractures healed out of 18 in the locally treated controls ($P < 0.001$).

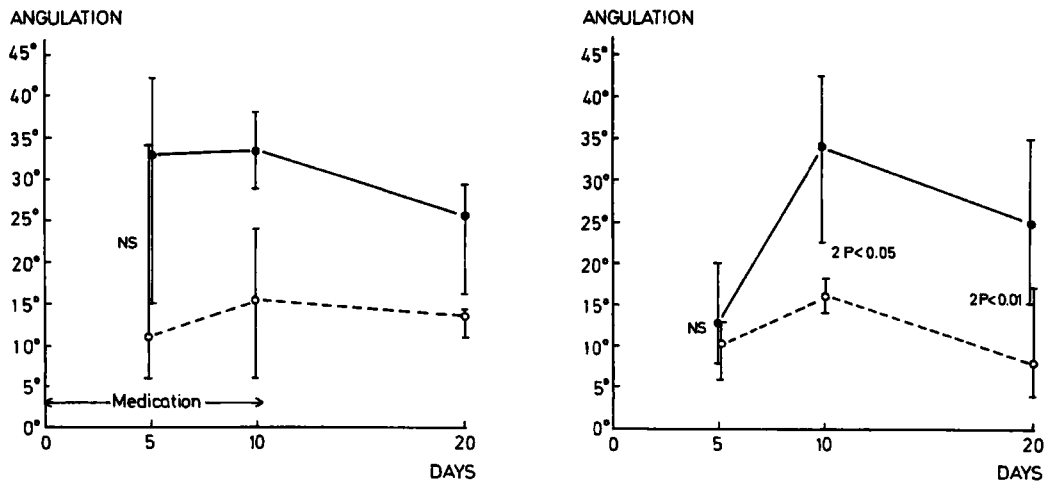


Figure 2. Angulation of the left femur fragments in the lateral radiographs of the indomethacin-treated rats and the controls (dashed line) in Series A (oral medication—left) and in Series B (local application—right). Median with 25- and 75-fractiles.

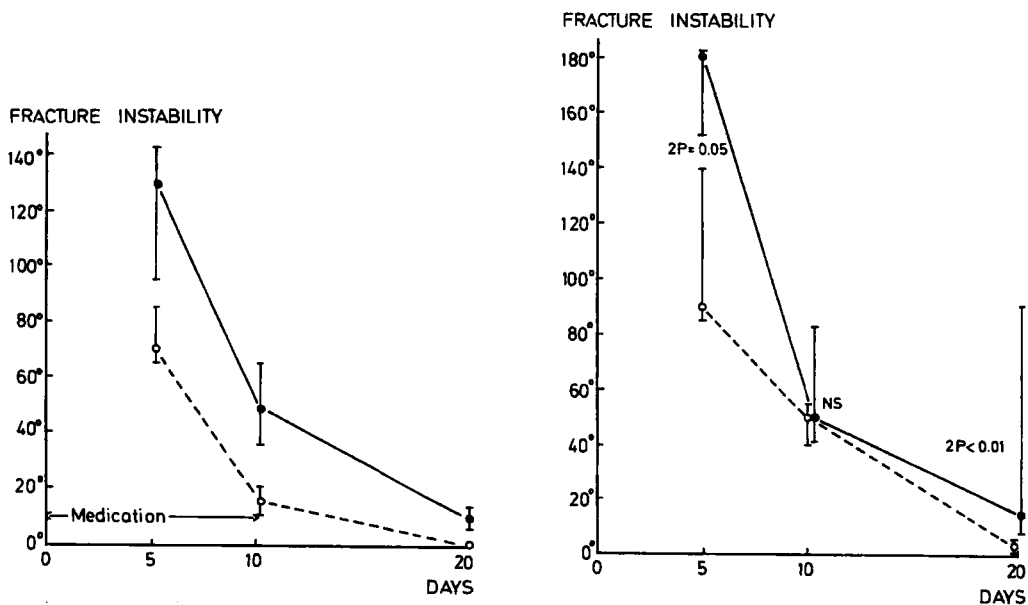


Figure 3. Fracture instability of the healing fracture of the left femur of the indomethacin-treated rats and the controls (dashed line) in Series A (oral medication—left) and in Series B (local application—right). Median with 25- and 75-fractiles.

Discussion

Our results show that indomethacin inhibits fracture healing in rats both when administered orally or locally. The inhibitory effect of oral administration of indomethacin accords with earlier studies (Sudmann 1975, Rø et al. 1976, Sudmann and Bang 1979, Sudmann et al. 1979, Allen et al. 1980), but it has not been demonstrated before that the same effect can be

achieved by local application of indomethacin. The polyorthoester, in which the indomethacin was contained, is hydrolyzed at body pH in the presence of water, releasing the drug of choice at a predictable rate (Capozza et al. 1978, Heller et al. 1981).

The inhibition of fracture healing by indomethacin did not differ regardless of whether or not the indomethacin is applied locally or administered orally (Figures 2 and 3). However, in lateral radiographs on

Day 5, the malalignment was less after local application than after oral treatment (Figure 2). These results suggest that the local injection of the viscous polyorthoester gel may function as an internal brace of the fracture; failing as the gel is absorbed, i.e., before Day 10, when the malalignment was about 34° in both the orally and the locally treated group (Figure 2). On Day 20, all the fractures had healed in the orally treated control rats, while only three out of 18 of the controls in the locally treated rats had healed. This difference could have been caused by the exposure of the fractures in the latter group. In addition, the gel itself might delay fracture healing until it is resorbed.

It is unclear how indomethacin impairs fracture healing (Sudmann 1975, Rø et al. 1976, Sudmann et al. 1979, Törnkvist et al. 1984, Newman and Ling 1985, Ritter and Sieber 1985, Solheim 1987). Because the polyorthoester may delay, but not inhibit, heterotopic and orthotopic bone formation (Sudmann et al. 1990, Pinholt et al. 1991, Solheim et al. 1992), these results support the contention that the direct local effect of indomethacin is the important one (Sudmann et al. 1979).

Oral treatment with indomethacin inhibits recurrent partial closure of the epiphyseal plate in rabbits (Sudmann et al. 1982) and para-articular ossifications after hip arthroplasty (Dahl 1975, Almásbakk and Røysland 1977, Ritter and Sieber 1985, Kjærsgaard-Andersen and Schmidt 1986), i.e., primitive osteoblastic activity (Sudmann and Marton 1975). However, a material for releasing the drug at controlled rates, locally where needed, would be preferable to oral treatment to avoid systemic effects. Bioerodible polymers seem to have such potential.

Acknowledgements

We express our gratitude to the Alza Corporation for the polyorthoester and to Dumex Ltd. for the indomethacin and placebo suspensions, and for making the indomethacin analyses.

References

- Allen H L, Wase A, Bear W T. Indomethacin and aspirin: effect of nonsteroidal anti inflammatory agents on the rate of fracture repair in the rat. *Acta Orthop Scand* 1980; 51 (4): 595-600.
- Almásbakk K, Røysland P. Does indomethacin (IMC) prevent postoperative ectopic ossification in total hip replacement? (Abstract). *Acta Orthop Scand* 1977; 48: 556.
- Capozza R, Sendelbeck L, Balkenhol W. Preparation and evaluation of a bioerodible naltrexone delivery system. In: *Polymeric delivery systems* (Ed. Kostelnick, R J) Gordon and Breach Science Publ, New York 1978: 59-73.
- Dahl H K. Indometacin/ossifikasjonsprosesser: Kliniske observasjoner. In: *Symposium om Artrose*, Merck Sharp & Dohme, Drammen 1975: 37-46.
- Ekeland A, Engesæter L B, Langeland N. Mechanical properties of fractured and intact rat femora evaluated by bending, torsional and tensile tests. *Acta Orthop Scand* 1981; 52 (6): 605-13.
- 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.
- Heller J, Penhale D W H, Helwing R F, Fritzinger B K. Release of norethindrone from poly (ortho esters). *Polym Eng Sci* 1981; 21: 727-31.
- Kjærsgaard Andersen P, Schmidt S A. Indomethacin for prevention of ectopic ossification after hip arthroplasty. *Acta Orthop Scand* 1986; 57 (1): 12-4.
- Jensen K M. Determination of indomethacin in serum by an extractive alkylation technique and gas-liquid chromatography. *J Chromatogr* 1978; 153 (1): 195-202.
- Pinholt E M, Solheim E, Bang G, Sudmann E. Bone induction by composite of bioerodible polyorthoester and demineralized bone matrix in rats. *Acta Orthop Scand* 1991; 62 (5): 476-80.
- Ritter M A, Sieber J M. Prophylactic indomethacin for the prevention of heterotopic bone formation following total hip arthroplasty. *Clin Orthop* 1985; 196: 217-25.
- Rø J, Sudmann E, Marton P F. Effect of indomethacin on fracture healing in rats. *Acta Orthop Scand* 1976; 47: 588-99.
- Solheim E, Pinholt E M, Bang G, Sudmann E. Regeneration of calvarial defects by composite of bioerodible polyorthoester and demineralized bone in rats. *J Neurosurg* 1992; 76: 275-9.
- Solheim L F. Influence of nonsteroidal anti-inflammatory drugs on bone. A mechanical and biochemical study with acetylsalicylic acid and naproxen in rats. Thesis, University of Oslo, Oslo, Norway 1987.
- Sudmann B, Anfinnsen O G, Rait M, Bang G, Sudmann E. Use of a new hemostatic, bioerodible polymer versus bone wax made of beeswax—a clinical and experimental study. *Acta Orthop Scand (Suppl 237)* 1990; 61: 63-4.
- Sudmann E. Effect of indomethacin on bone remodelling in rabbit ear chambers. *Acta Orthop Scand (Suppl 160)* 1975; 46: 91-115.
- Sudmann E, Marton P F. Bone remodelling in rabbit ear chambers. A vital microscopical and histological study. *Acta Orthop Scand (Suppl 160)* 1975; 46: 65-90.
- Sudmann E, Hagen T. Indomethacin-induced delayed fracture healing. *Arch Orthop Unfallchir* 1976; 85 (2): 151-4.
- 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, Husby O S, Bang G. Inhibition of partial closure of epiphyseal plate in rabbits by indomethacin. *Acta Orthop Scand* 1982; 53 (4): 507-11.
- 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.