

INDOMETHACIN-INDUCED INHIBITION OF HAVERSIAN REMODELLING IN RABBITS

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The effect of indomethacin on haversian remodelling in the radius was studied in 16 rabbits. An osteotomy and a saw-cut almost through the bone were made in the middle and distal parts of the right radius, respectively. In order to obtain biological osteosynthesis in the osteotomy and the saw-cut the rabbits were not treated in any way during the following 3 weeks. They were then given either indomethacin or placebo suspensions per os, 10 mg/kg/day for 2 weeks, followed by 5 mg/kg/day for 4 weeks. The extent of haversian remodelling was estimated by calcein and oxy-tetracycline fluorochrome labels given at the start of the indomethacin treatment and 3 weeks later, respectively. After 2 weeks of indomethacin treatment (10 mg/kg/day) the indomethacin plasma levels were about 0.18 µg/ml 24 hours after the last dose. Indomethacin treatment significantly ($2P < 0.019$) inhibited haversian remodelling as estimated by fluorochrome labelling.

Key words: anti-inflammatory agents; bone; fractures; non-union; haversian remodelling; fluorochrome labelling; indomethacin; pseudarthrosis; rabbits

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Recent studies indicate that indomethacin may inhibit woven-fibred bone tissue formation both in experimental animals and in humans (Sudmann 1975 b, Rø et al. 1976, Sudmann & Hagen 1976). This contention has been supported by a prospective study of patients receiving indomethacin and showing significantly inhibited heterotopic ossification after hip surgery (Almåsbaek & Røysland 1977). As the healing of fractures immobilized by plaster casts depends mainly on periosteal woven-fibred bone formation, indomethacin treatment may inhibit healing in these cases (Sudmann 1975 b, Sudmann et al. 1979).

Rigid plate osteosynthesis with interfragmentary compression appears to induce a different type of fracture healing, which apparently depends mainly on

longitudinal haversian remodelling across the fracture, and periosteal callus is usually not seen (Schenk & Willenegger 1964). The problem is whether doses of indomethacin that inhibit periosteal fracture healing also inhibit haversian remodelling in rigidly immobilized fractures or osteotomies. The aim of this study was to investigate the effect of indomethacin treatment on this type of haversian remodelling in rabbits.

MATERIAL AND METHODS

Animals

Sixteen female Dutch rabbits were divided into two equal weight-matched groups. At the start of the experiment they were about 4½ months old

and their weights ranged from 2.0 to 2.7 kg. They were given an ordinary rabbit laboratory diet (Felleskjøpet, Trondheim, Norway) and water *ad libitum* from noon to the following morning. To make them take the indomethacin or placebo suspensions more readily at noon, they were deprived of water during the morning. They were kept separately in metal cages at 21°C, with 60 per cent humidity, and a 12-h light, 12-h dark cycle.

Experimental design

Operative procedure. Under neurolept anaesthesia (Hypnorm Vet. 0.3 ml/kg intramuscularly, Mecos, Helsingborg, Sweden) and sterile conditions a 0.12 mm wide transverse osteotomy was made in the middle of the right radius by means of a circular dental saw. Another cut was made almost through the bone 12 mm distal to the osteotomy (Figures 1, 2). The periosteum of the radius between the osteotomy and the cut was removed and the wound was closed in layers.

To enable a sheath of callus to form around the osteotomy, providing stability, the rabbits were not given any treatment for 3 weeks following the operation. In this way a biological osteosynthesis was achieved before the indomethacin or placebo regimens were started.

Indomethacin treatment. Three weeks after the operation, indomethacin suspension (Confortid, Dumex Ltd., Copenhagen, Denmark) or placebo (Vehiculum, Confortid suspension) was squirted into the rabbits' mouths once a day, 6 days a week (Sudmann 1975 b). The doses were 10 mg/kg/day for 2 weeks followed by 5 mg/kg/day for another 4 weeks. The rabbits were weighed once a week and the dosage adjusted accordingly.

Fluorochrome labels. On the first day of indomethacin treatment a single dose (5 mg/kg) of calcein (type 2315, Merck, Darmstadt, Germany) was given slowly intravenously under neurolept anaesthesia. The calcein was given as a 2 per cent solution dissolved in 2 per cent NaHCO₃. Three weeks later the animals were given a single dose (33 mg/kg) of oxytetracycline (OTC) (Oxy-Dumocyclin, Dumex Ltd., Copenhagen, Denmark) by the same method.

Evaluation

All the laboratory analyses and evaluations were carried out without knowledge of which groups

(indomethacin or placebo) the samples or histological sections belonged to.

Indomethacin analysis. Heparinized blood samples for indomethacin analysis were obtained from anaesthetized animals 12 and 43 days after the start of the indomethacin treatment. They were taken 24 hours after the daily dose of indomethacin or placebo. The blood samples were centrifuged and plasma samples were collected, frozen and stored at -21°C until analysed (Jensen 1978). The presence of the concomitant indomethacin metabolite desmethylindomethacin was not tested for by this method (Jensen 1978). The samples were analysed by Dumex Ltd., Copenhagen, Denmark.

X-rays. Radiographs of the right radius were obtained on Kodak dental film 3 weeks after the operation (Figure 2).

Histological examination. The animals were killed by a lethal intravenous dose of pentobarbital and the radius and the ulna were dissected free from the soft tissue and removed *en bloc*. The specimens were fixed in 4 per cent formaldehyde and sectioned transversely with a circular dental saw. The right radius and ulna were cut through at the levels of the osteotomy and the saw-cut and in the middle of the bone fragment between the two. The left radius and ulna were cut only once (Figure 1). The bone specimens were orientated and fastened with crown wax and 90 µm cross sections were cut in a thin sectioning machine (Gillings-Hamco, Hamco Machines Inc., Rochester, N.Y., USA). Those from the right side were numbered 1 to 6, as shown in Figure 1.

All sections were evaluated by incident-light

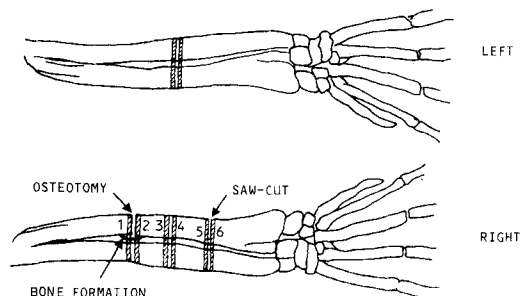


Figure 1. Schematic drawing of the right and left radius and ulna. The osteotomy is bridged by new bone on the ulnar side. At sacrifice one undecalcified 90 µm cross section was cut from each hatched area. The cross sections from the right side were numbered 1 to 6.

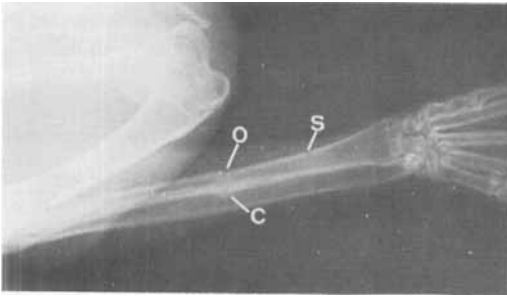


Figure 2. Radiograph of typical right forelimb. The osteotomy (O) is bridged by sparse mineralized callus (C) on the ulnar side 3 weeks after the operation. The saw-cut (S) is barely visible. The distance between the osteotomy and the saw-cut measured 12 mm.

fluorescence microscopy with a standard Leitz Orthoplan fluorescence microscope. Blue excitation radiation and Leitz suppression filter 530 nm were used. The area of the cortex, excluding any periosteal and endosteal callus, was measured in mm² by mixed-image planimetry (Sudmann 1975 a) and fluorochrome-labelled haversian systems were counted. The number of OTC-labelled haversian

systems in specimens from the right side were expressed as a percentage of the calcein-labelled ones in the same sections.

Statistics. Differences in measurements were tested by the Wilcoxon two-sample rank sum test and the Wilcoxon-Van Elteren block test for grouped data (Høyland & Walløe 1977). The *P* values given refer to two-tailed tests. Differences were considered significant when $2P < 0.05$, except for differences in weight gain, which were considered significant when $2P < 0.10$. The results are given as medians and ranges.

RESULTS

The animals tolerated the osteotomies well. One rabbit died after 37 days of indomethacin treatment and was excluded from the results of OTC fluorochrome labelling. At autopsy (by the State Veterinary Laboratory for Northern Norway, Harstad, Norway) recent gastrointestinal bleeding was observed. There was no significant difference in weight

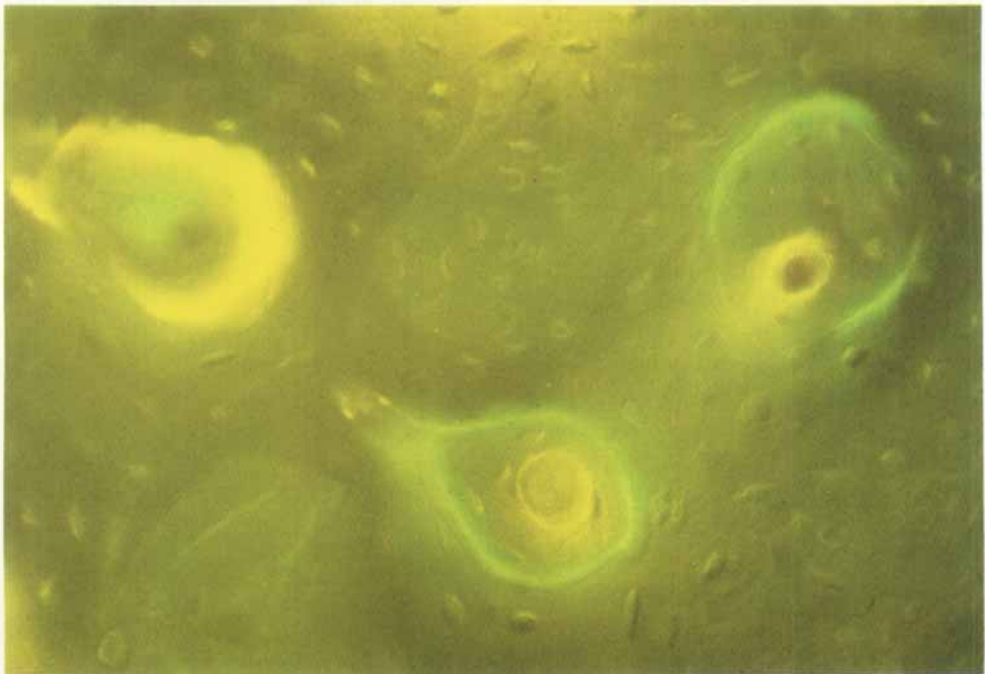


Figure 3. Fluorescence photomicrograph of three fluorochrome-labelled haversian systems in different phases of remodelling. Left and middle: outer label is green (calcein) and inner yellow (OTC). Right: yellow label (OTC) only. Undecalcified cross section from right radius of placebo-treated rabbit. Objective 25x/0.65. Field width, 0.4 mm.

gain between the indomethacin-treated and placebo-treated groups. At sacrifice the osteotomies and the saw-cuts were sheathed in a cuff of newly formed bone in all specimens.

X-rays

Sparse mineralized callus covered the ulnar side of the osteotomy 3 weeks after the operation (Figure 2). In the osteotomy a dislocation *ad latus* of about the width of one cortex was found in three rabbits in the indomethacin group and in four rabbits in the placebo group.

Fluorochrome labels

Seven histological sections in the indomethacin group and seven in the placebo group were technically unsuitable for microscopy and were excluded. In four sections the exact area of the cortex could not be measured, but these sections were not excluded except from the results given in Figure 4. The green fluorescent calcein labels were easily distinguished from the yellow fluorescent OTC labels (Figure 3). A few haversian systems were both calcein- and OTC-labelled.

Calcein labels. In three animals in each group there were no calcein-labelled haversian systems at all in the non-operated left radius and the remaining 10 rabbits had only 0.17 to 2.6 labelled haversian systems per mm² (median 0.13/mm²) (Figure 4).

On the operated right radius calcein-labelled haversian systems were found in all the animals. The placebo group had a median of 2.65/mm², range 0–8/mm², and the indomethacin group had a median of 2.04/mm², range 0.52–6.03/mm². There was no significant difference in the number of calcein-labelled haversian systems between the placebo group and the indomethacin group.

There seemed to be fewer calcein-labelled haversian systems midway between the osteotomy and the saw-cut (median 1.8/mm², range 0.59–6.5/mm²) than in the osteotomy (median 2.43/mm², range 0–6.3/mm²) or in

the saw-cut (median 2.96/mm², range 0.52–8/mm²), but this trend was not significant (Figure 4).

OTC labels. There were no OTC-labelled haversian systems in the non-operated left radius in one indomethacin-treated and in two placebo-treated animals and there were only 0.17 to 2.5/mm² in 12 out of 15 animals.

In the operated right radius we found OTC-labelled haversian systems in all animals. When expressed as the percentage of calcein-labelled systems in the same sections there were significantly ($2P < 0.04$) fewer OTC-labelled haversian systems in the indomethacin-treated (median 313 per cent) than in the placebo-treated animals (median 367 per cent) when the results from all sections were pooled (Figure 5). Likewise, when the results from sections 2, 3, 4 and 5 were pooled there were significantly ($2P < 0.019$) fewer OTC-labelled haversian systems in the indomethacin group (median 271 per cent) than in the placebo group (median 365 per cent) (Figure 5). When the proximal, middle and distal sections were compared according to location, fewer OTC-labelled haversian systems were found in the indomethacin group, but this trend was significant only in sections 1 and 2 ($2P < 0.05$) (Figure 5).

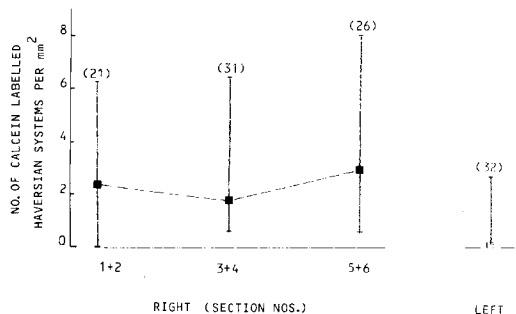


Figure 4. Number of calcein-labelled haversian systems per mm² in the operated right radius and non-operated left radius. The number of sections examined at each location is given in parentheses.

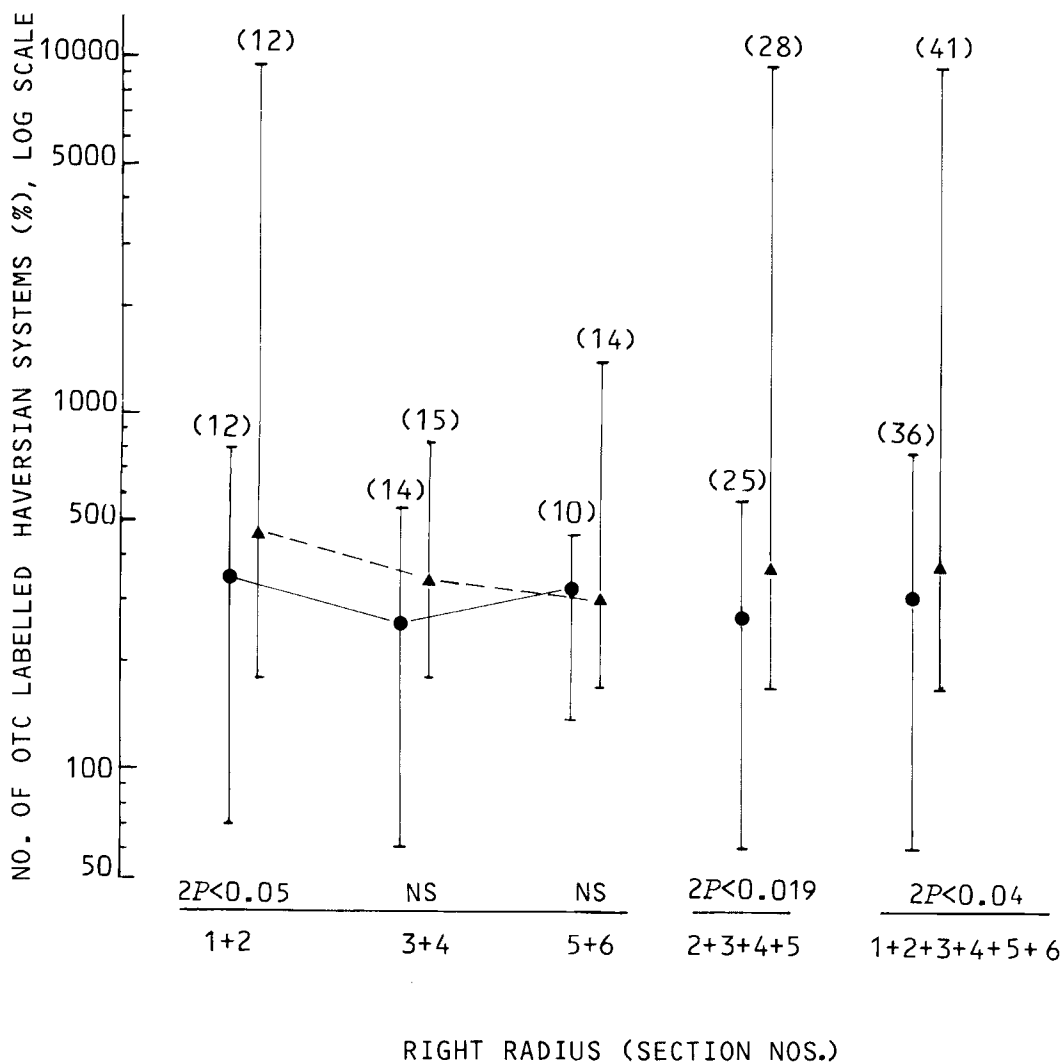


Figure 5. Number of OTC-labelled haversian systems expressed as a percentage (log scale) of calcein-labelled systems in the same sections. The P values are based on the Wilcoxon-Van Elteren block test for grouped data. When the sections are grouped in pairs, according to location, the indomethacin group (●) is not significantly (NS) different from the placebo group (▲) except for sections 1 and 2 ($2P < 0.05$). The two groups are significantly different when the results from sections 2–5, obtained from the 12-mm long bone fragment, are pooled ($2P < 0.019$) and when the results from all sections are pooled ($2P < 0.04$). The number of sections examined is given in parentheses.

Indomethacin analysis

No indomethacin was found in any placebo plasma sample. Twenty-four hours after the previous indomethacin dose, 12 days after the start of treatment (10 mg/kg/day per os), the median plasma level was 0.18 µg/ml (range

0.11–1.35 µg/ml). After a further 4 weeks of indomethacin treatment (5 mg/kg/day per os) the corresponding value was 0.09 µg/ml; no indomethacin was found in two samples and in the remaining three samples the level varied from 0.07 to 0.55 µg/ml.

DISCUSSION

General considerations

Relatively high doses of anti-inflammatory drugs are often given to reduce post-traumatic inflammation for a period immediately after the trauma, followed by reduced doses later. The same treatment plan was used in this study, but the reduced dose, 5 mg/kg/day orally, gave either very low or not measurable plasma levels of indomethacin (median 0.09 µg/ml). To obtain indomethacin plasma levels comparable to those seen in clinical practice (about 1 µg/ml) (Alván et al. 1975), relatively high oral doses of indomethacin must be used in rodents, i.e., 10 mg/kg/day in rabbits (Sudmann 1975 b) and 2 mg/kg/day in rats (Rø et al. 1976, Sudmann et al. 1979).

One indomethacin-treated rabbit died of gastrointestinal bleeding. Since anti-inflammatory doses of indomethacin are known to cause gastrointestinal bleeding, this adverse drug effect should not invalidate the results of the present study.

In a rigidly plated osteotomy in dogs the osteotomy heals by longitudinal haversian remodelling and not by the formation of a sheath of callus (Schenk & Willenegger 1964). Thus, the stability in the osteotomy necessary for such primary bone healing is provided by the plate. However, in adolescent rabbits treated with placebo a rigidly plated osteotomy of the radius will also be covered by a sheath of newly formed bone, whereas indomethacin-treated ones (10 mg/kg/day) do not have a bone sheath (Sudmann 1977, unpublished data). This inhibitory effect of indomethacin is in accord with previous studies reporting indomethacin-induced inhibition of periosteal fracture healing (Rø et al. 1976, Sudmann & Hagen 1976, Sudmann et al. 1979). Since haversian remodelling across a fracture or an osteotomy seems to depend above all on stability, osteotomies bridged by both a plate and a bone sheath cannot serve as controls for osteotomies bridged only by a plate. Furthermore, in rigidly plated fractures

or osteotomies the plate in itself is supposed to induce marked remodelling of the cortical bone underneath it (Matter et al. 1974). To study the effect of indomethacin on haversian remodelling in an osteotomized bone we, accordingly, avoided plate osteosynthesis and used a biological osteosynthesis formed by a sheath of newly formed bone.

Haversian remodelling

Since we found many more calcein-labelled haversian systems per mm² in the right operated radius than in the contralateral non-operated one, the operation induced a marked increase in the rate of haversian remodelling (Figure 4). As evaluated by the OTC labelling, this increase was significantly ($2P < 0.019$) inhibited by 3 weeks of oral indomethacin treatment (10 mg/kg/day for 2 weeks and 5 mg/kg/day for 1 week).

Indomethacin may inhibit fracture healing by reducing inflammation and pain and by increasing the fracture haematoma (Sudmann et al. 1979). In this study indomethacin treatment was started when the osteotomy was immobilized by a sheath of mineralized callus. There are no observations indicating that pain and fracture haematoma have any significance for the rate of fracture healing when a biological osteosynthesis has already been achieved. Thus, this study supports the contention that the anti-inflammatory effect of indomethacin is the essential one.

Remodelling of bone tissue, including the haversian systems, depends fundamentally on osteoclastic and osteoblastic activity. The inhibition of haversian remodelling observed here may be caused by inhibition of either osteoclastic activity or osteoblastic activity or both. Since very low plasma levels of indomethacin or none at all were found at sacrifice, no attempt to differentiate between these factors was made here.

Clinical implications

Skeletal homeostasis depends on ordered osteoclastic and osteoblastic activity. Sudmann

& Hagen (1976) found subnormal osteoclastic and osteoblastic activity indices in the iliac crest of a patient with indomethacin-induced inhibition of periosteal fracture healing. The findings of the present study and of this previous study seem to support the contention that indomethacin inhibits normal skeletal remodelling. However, in a fractured or osteotomized bone the rate of haversian remodelling is markedly increased by the trauma inflicted on the tissues, whereas post-traumatic inflammation is absent in normal skeletal homeostasis. Thus our findings cannot throw light on the question of whether indomethacin also affects the normal rates of skeletal remodelling.

Recent studies indicate that indomethacin may inhibit fracture healing in patients treated by plaster casts (Sudmann 1975 b, Rø et al. 1976, Sudmann & Hagen 1976, Almåsbygg & Røysland 1977, Sudmann et al. 1979). This study suggests that the same applies to fractures and osteotomies treated by rigid internal osteosynthesis. Thus, indomethacin should preferably not be given to any patients with recent fractures or osteotomies.

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