Acta orthop. scand. 53, 43–49, 1982

LACK OF EFFECT OF INDOMETHACIN ON ORDERED GROWTH OF THE FEMUR IN RATS

EINAR SUDMANN, TORKJEL TVEITA & JOHN HALD, JR.

Departments of Orthopaedic Surgery and Radiology, University of Tromsø, Norway

The effect of indomethacin on ordered growth in length and width of the femur was studied in 48 adolescent rats given 2 mg/kg/day of the drug, a regimen previously shown to inhibit fracture healing. The animals were given either indomethacin suspension, or the vehicle alone, orally for 6 weeks. All the animals tolerated the treatment well. During drug treatment the femur distal to the intertrochanteric crest grew about 7 mm in length. Indomethacin plasma levels were about 1 μg/ml in the indomethacin-treated animals. Indomethacin did not inhibit ordered growth in either length or width. This indicates that indomethacin does not inhibit the normal homeostasis of the skeletal system in adolescent rats.

Key words: anti-inflammatory agents; bone; bone formation; bone resorption; fractures; indomethacin; rats

Accepted 16.iii.81

Previous studies have shown that indomethacin inhibits bone resorption and bone formation in both heterotopic and orthotopic bone in rabbits (Sudmann 1975b, Sudmann & Bang 1979). In rats indomethacin was observed to inhibit the healing of femur fractures (Rø et al. 1976, Sudmann et al. 1979). We therefore wished to test whether doses of indomethacin large enough to inhibit fracture healing also inhibit ordered bone growth and bone remodelling in the rat.

METHODS

Animals

Forty-eight male adolescent Wistar rats (Møllegaards Avlslaboratorium, Eiby, Denmark) were divided into two weight-matched groups. At the start of the experiment they were about 4 weeks old and weighed from 86 to 125 g (median 102 g). They were given an ordinary laboratory rat diet (Felleskjøpet, Oslo, Norway) and water *ad libitum*. They were housed in plastic cages in a room with a temperature of about 23°C, 60 per cent humidity, and a 12 hours light/12 hours darkness cycle. During the first 11 days there were 6 rats per cage, later 5.

Indomethacin treatment

One ml of indomethacin suspension (Confortid, Dumex, Copenhagen, Denmark) was given orally once a day for 6 weeks through a plastic stomach tube. The control group was given the vehicle alone. The rats were weighed once a week and the dilution of the suspension was adjusted to maintain the daily dose of 2 mg/kg/day (Rø et al. 1976, Sudmann et al. 1979). The first dose was given immediately after the first radiograph had been taken (day zero) (Table 1).

Evaluation

Indomethacin analysis. Four randomly selected rats from each group were killed on day 11, and the remaining rats were killed when the experiment was ended on day 42. Blood (5 ml) for indomethacin analysis was drawn from the aorta using heparinized syringes under ether anaesthesia 24 hours after the last dose (Table 1). The samples were centrifuged, and

0001-6470/82/010043-07 $02.50/0

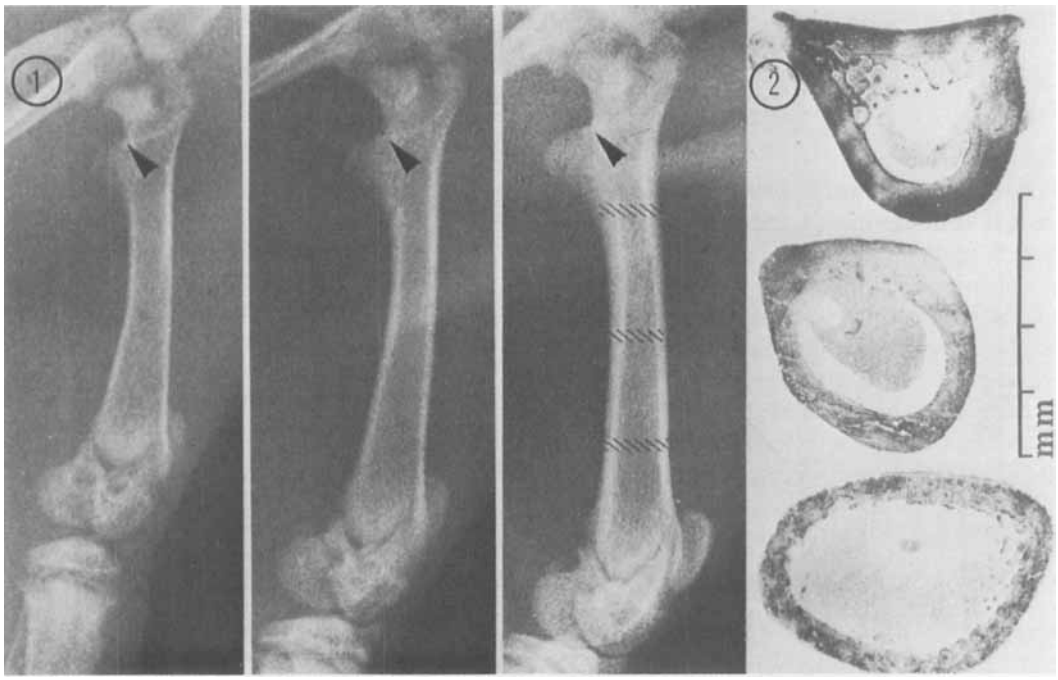
Table 1. Design of indomethacin experiment

Treatment	Duration of treatment (days)	Indomethacin analysis		*In vivo* X-ray examination		Postmortem examination	
		On day	No. of animals	On day	No. of animals	On day	No. of femurs
Indomethacin	42	11	4	0	24[1]		
				21	20		
		42	20	42[2]	20	42	40
Vehicle (control)	42			0	24[1]		
		11	4	21	20		
		42	20	42[2]	20	42	40

1. The femurs from 4 rats killed on day 11 were not measured.
2. Radiographs used only for calculating location of distal histological section.

plasma samples were frozen and kept at –20°C until analysis (Jensen 1978). The metabolite desmethylindomethacin is not measured by this method (Jensen 1978). The samples were analysed by Dumex Ltd., Copenhagen, Denmark.

X-rays and length measurements. Standardized radiographs were made of the right and left femur under ether anaesthesia *in vivo* on days zero, 21 and 42 (Table 1, Figure 1). The distances from the intersection of the intertrochanteric crest and the neck of the femur to the distal epiphyseal plate, and from the same intersection to the lateral femoral condyle were measured in the radiographs using an engineer's micrometer (Figure 1). The dissected right and left femurs were measured from the head of the femur to the intercondylar notch with



Figures 1 and 2. Radiographs (Figure 1) and photomicrographs (Figure 2). Consecutive A.P. radiographs of a typical femur during drug-treatment taken in vivo (left to right) on days zero, 21 and 42, respectively; arrowheads indicate proximal point of reference for length measurements in radiographs (see text) (Figure 1). Hatched areas in Figure 1 indicate location of cross-sections shown in Figure 2. Distal hatched area indicates approximate location of epiphyseal plate on day zero.
Objective 2.5/0.08, decalcified, toluidine blue (Figure 2).

the same technique (Table 1). When comparing groups of animals a length measurement in a given rat was expressed as the arithmetic mean of the measurements of the right and left femurs.

Histological examination. Eighty dissected femurs were fixed in 4 per cent buffered formaldehyde and marked by a small saw cut 2 mm distal to where we assumed the distal epiphyseal plate had been on day zero (Table 1, Figure 1). The location of the saw cut was calculated as follows: The length of the diaphysis including the distal metaphysis was measured in radiographs taken on day zero as above. To this measurement we added the length of the femur measured proximally to the intertrochanteric crest in radiographs taken *in vivo* on day 42. In the dissected specimens (day 42) the sum of these two measurements was assumed to be the approximate distance from the head of the femur to where the epiphyseal plate had been located on day zero (Figure 1).

The femurs were decalcified and sectioned transversely at three places: halfway between the head of the femur and the intercondylar notch, just distal to the lesser trochanter, and just distal to where the epiphyseal plate was assumed to have been located on day zero. (The third place had already been marked by the saw cut.) They were then embedded in Epon. From each location 6 μm thick Epon cross-sections were cut and stained with 1 per cent toluidine blue (Figures 1–2).

In the sections the cross-sectional area of the whole bone, and of the medullary cavity and the cortex separately were measured by mixed-image planimetry, a method that gives a precision better than 1 per cent (Sudmann 1975a). The ratio between length and width at each point on the femur was calculated by dividing the length of the dissected femur by the cross-sectional area of the cortex.

Statistics

Differences in measurements were tested by the Wilcoxon rank sum test for paired data (the signed rank test) and the Wilcoxon rank sum test for unpaired data (the two-sample test) (Swinscow 1976). The P values given were found by two-tailed tests and differences were considered significant when $P < 0.05$, except for differences in weight gain, which were considered significant when $P < 0.10$. The results are given as medians and ranges.

RESULTS

All the animals tolerated the treatment well. There were no significant differences in weight gain between those treated with indomethacin and those treated with the vehicle (Table 2).

Indomethacin analysis

On day 11 the median plasma level of indomethacin was 0.64 μg/ml (0.40–1.24 μg/ml) 24 hours after the last dose. On day 42 the corresponding level was 0.69 μg/ml (0.24–2.31 μg/ml)

Table 3. Plasma levels of indomethacin at death in indomethacin-treated rats

Hours after last dose	Day of experiment	Plasma level (μg/ml)
24	11	0.64 (0.40–1.24) $n=4$
24	42	0.69 (0.24–2.31) $n=20$

The plasma levels are median values with ranges in parentheses.

Table 2. Body weight (g) during the study

Treatment	Day of experiment						
	zero	7	14	21	28	35	42
Indomethacin	102 (86–113) $n=24$	141 (123–154) $n=24$	176 (153–200) $n=20$	214 (182–250) $n=20$	240 (198–291) $n=20$	254 (213–308) $n=20$	267 (203–334) $n=20$
Vehicle	100 (87–125) $n=24$	137 (120–174) $n=24$	177 (154–221) $n=20$	211 (181–255) $n=20$	239 (199–278) $n=20$	259 (212–291) $n=20$	272 (226–310) $n=20$

The weights are given as median values with ranges in parentheses.
There was no significant difference ($P > 0.4$) between the two groups.

Table 4. Length measurements of the femur

Treatment	Day of experiment: zero	21		42	
	Length[1] (mm)	Length (mm)[1]	Increase in length (%)[2]	Length[3] (mm)	Increase in length (%)[2]
Indomethacin	21.1 (20.5–21.7) $n=20$	26.6 (25.8–27.4) $n=20$	26.0 (22.8–30.8)	32.5 (31.2–33.8) $n=20$	54.3 (48.9–59.4)
Vehicle	21.1 (20.3–21.9) $n=20$	26.4 (25.3–27.4) $n=20$	25.1 (21.4–32.6)	32.0 (30.2–33.3) $n=20$	52.3 (47.0–61.6)

The length measurements are median values with ranges in parentheses.
There was no significant difference ($P > 0.1$) between the two groups.
1. Length of femur distal to the intertrochanteric crest (see text).
2. Increase in length is shown as percentage of length of femur distal to the intertrochanteric crest in X-rays taken on day zero.
3. Length of dissected femur (see text).

(Table 3). No indomethacin was found in the samples from the control group.

Growth of the femur in length and width

Nine radiographs of the indomethacin group and ten of the control group were lost during development, but a radiograph of either the right or the left femur was available for all the rats. The femurs of the eight rats used for indomethacin analysis on day 11 were not measured.

During the 6 weeks of treatment with indomethacin or vehicle the part of the femur distal to the intertrochanteric crest grew about 7 mm in length. Measurements in radiographs taken *in vivo* on day 21 showed no significant differences ($P > 0.2$) in either absolute or relative growth in length between the indomethacin-treated rats and the controls (Table 4).

In the dissected specimens there was no significant difference ($P > 0.1$) between the length of the femurs in indomethacin-treated rats (me-

Table 6. Cross-sectional

Treatment	Location: Proximal				Location
	Total (mm²)	Cortex (mm²)	Medullary cavity (mm²)	Ratio F/C	Total (mm²)
Indomethacin	9.7 (8.1–11.5)	7.0 (5.6–8.4) [32]	2.6 (1.6–3.2)	4.6 (3.8–5.5)	7.6 (6.6–9.5)
Vehicle	9.5 (7.5–11.1)	6.7 (5.4–8.0) [35]	2.6 (1.9–3.6)	4.8 (4.0–5.9)	7.5 (6.4–8.9)

The values in the table are median values with ranges in parentheses.
Ratio F/C: Length of dissected femur (F) divided by cross-sectional area of cortex (C).
The total numbers of sections examined from right and left femurs are given in square brackets.
There were no significant differences between the two groups.

Table 5. Length of dissected right and left femurs

Treatment	Length (mm) Right	Left	No. of pairs	Level of significance (P)[1]
Indomethacin	32.5 (31.3–33.9)	32.5 (31.2–33.6)	20	⩾0.1
Vehicle	31.9 (30.6–33.5)	32.1 (30.8–33.2)	18[2]	⩾0.1

Length is given as median values with ranges in parentheses.
1. Wilcoxon rank sum test for paired data.
2. Two femurs could not be measured exactly due to unintended epiphysiolysis of the head of the femur during dissection.

dian 32.5 mm) and in vehicle-treated rats (median 32.0 mm) on day 42 (Table 4). There was also no significant difference when the length of the dissected femurs was expressed as a percentage of the length of the femur distal to the intertrochanteric crest in radiographs taken on day zero ($P > 0.1$) (Table 4). Nor was there any significant difference ($P \geqslant 0.1$) in the length of the dissected right femurs as compared to the contralateral left femurs in either indomethacin-treated or in vehicle-treated animals (Table 5).

Seventeen sections in the indomethacin group and 14 sections in the control group out of a total of 240 sections were technically unsuitable for analysis. These 31 sections were excluded.

There were no significant differences ($P \geqq 0.1$) between indomethacin-treated rats and controls as regards the cross-sectional areas of the whole femur (cortex and medullary cavity), of the cortex alone, or of the medullary cavity alone at any corresponding points on the femur (Table 6). There was no significant difference ($P \geqq 0.2$) between the ratio of the length of the dissected femurs to the area of the cortex in the indomethacin-treated rats as compared to the controls (Table 6).

areas of the femur

Middle Cortex (mm²)	Medullary cavity (mm²)	Ratio F/C	Total (mm²)	Distal Cortex (mm²)	Medullary cavity (mm²)	Ratio F/C
4.8 (3.8–6.0) [36]	2.9 (2.0–4.4)	6.8 (5.4–8.6)	9.9 (7.9–12.3)	5.0 (4.0–6.1) [35]	4.9 (3.6–6.9)	6.5 (5.4–7.9)
4.6 (3.8–5.3) [35]	3.0 (2.0–3.9)	6.8 (6.1–8.3)	10.0 (7.4–12.0)	5.0 (4.0–6.9) [36]	4.6 (2.8–6.3)	6.5 (5.2–10.0)

DISCUSSION

General considerations

In rodents relatively high weight-related doses of indomethacin are needed to obtain a plasma level of about 1 μg/ml, which is the therapeutic level in man (Alván et al. 1975, Sudmann et al. 1979). Our results show that adolescent rats tolerate well a daily dose of 2 mg/kg/day (Table 2). The median plasma levels found here (0.64–0.69 μg/ml) (Table 3) were slightly higher than those found in a previous study in which inhibited fracture healing and pseudarthrosis development were shown to be due to indomethacin (Sudmann et al. 1979).

Bone growth and remodelling

During the period of growth the length and shape of the femur depend fundamentally on ordered growth in length and consecutive ordered remodelling of bone tissue. The length of the femur depends mainly on the growth in the distal epiphyseal plate. The size and shape of the distal metaphysis and the shaft depend on ordered osteoclastic activity and ordered osteoblastic activity. During growth the distal metaphysis is reduced considerably in size, being gradually remodelled to the size of the diaphysis by external osteoclastic activity (Figure 1). The medullary cavity of the diaphysis increases in size by ordered osteoclastic activity on the internal endosteal side, while the diaphysis grows in width by ordered, external periosteal osteoblastic activity. Inhibition of such ordered osteoclastic activity or osteoblastic activity or both – e.g. by indomethacin – would thus lead to abnormal growth and abnormal bone remodelling.

Doses of indomethacin of the order of 2 mg/kg/day inhibit the healing of femoral fractures in adolescent rats (Rø et al. 1976, Sudmann et al. 1979). This effect is directly proportional to the dose (Allen et al. 1980). But the results of the present study show that the same dose of indomethacin (2 mg/kg/day) did not significantly inhibit the growth in length and width of the femur in adolescent rats (Tables 4–6). This indicates that neither the cellular activity in the epiphyseal plate nor the delicate balance between bone resorption and bone formation in the remodelling of the bone was affected by indomethacin. Thus, the study supports the view that the inhibitory effect of indomethacin on fracture healing is non-specific (Sudmann et al. 1979); that is, indomethacin probably inhibits fracture healing by reducing the post-fracture inflammation which is supposed to activate fracture repair. This is supported by a recent study reporting inhibition of fracture healing in rats not only by indomethacin, but also by aspirin when given in equivalent anti-inflammatory doses (Allén et al. 1980).

Clinical implications

Using mixed-image longitudimetry, a precise and accurate new method of quantitative microscopy (Sudmann 1979), Sudmann & Hagen (1976) found indications of subnormal osteoclastic and osteoblastic activity in a biopsy taken from the iliac crest of a patient with indomethacin-induced pseudarthrosis. In a prospective clinical study Almåsbakk & Røysland (1977) were able to reduce periarticular heterotopic ossifications in patients given indomethacin prophylactically after hip surgery. The reduction was significant as compared to the controls. In another study, in which radiographs also were used, the osteoarthritis in indomethacin-treated patients progressed significantly more rapidly than in the controls (Rønningen & Langeland 1979). The ordered remodelling of haversian systems after osteotomies in rabbits has also been found to be inhibited by indomethacin (Sudmann & Bang 1979). However, in fractures, in osteotomies, and after hip surgery there will always be aseptic inflammation, and in osteoarthritic hip joints there will be pathological bone resorption and bone formation. The inhibitory effect of indomethacin observed in such conditions cannot therefore throw any light on the question of whether indomethacin also affects the normal homeostasis of the skeletal system.

On the other hand, the present study indicates that it is unlikely that indomethacin affects the normal homeostasis of the skeletal system during growth.

ACKNOWLEDGEMENT

The indomethacin suspension and the vehicle suspension were supplied by Dumex Ltd., Copenhagen, Denmark, who also performed the indomethacin analysis. We would like to express our gratitude to O. Bakke M.D. for helpful discussions.

REFERENCES

Allen, H. L., Wase, A. & Bear, W. T. (1980) Indomethacin and aspirin: Effect of nonsteroidal anti-inflammatory agents on the rate of fracture repair in the rat. *Acta Orthop. Scand.* **51**, 595–600.

Almåsbakk, K. & Røysland, P. (1977) Does indomethacin (IMC) prevent postoperative ectopic ossification in total hip replacement? (Abstract). *Acta Orthop. Scand.* **48**, 556.

Alván, G., Orme, M., Bertilsson, L., Ekstrand, R. & Palmér, L. (1975) Pharmacokinetics of indomethacin. *Clin. Pharmacol. Ther.* **18**, 364–373.

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

Rø, J., Sudmann, E. & Marton, P. F. (1976) Effect of indomethacin on fracture healing in rats. *Acta Orthop. Scand.* **47**, 588–599.

Rønningen, H. & Langeland, N. (1979) Indomethacin treatment in osteoarthritis of the hip joint. Does the treatment interfere with the natural course of the disease? *Acta Orthop. Scand.* **50**, 169–174.

Sudmann, E. (1975a) Mixed-image planimetry. Assessment of a new method for stereometric analysis in microscopy. *Acta Orthop. Scand.*, Suppl. 160, 45–64.

Sudmann, E. (1975b) Effect of indomethacin on bone remodelling in rabbit ear chambers. *Acta Orthop. Scand.*, Suppl. 160, 91–115.

Sudmann, E. & Hagen, T. (1976) Indomethacin-induced delayed fracture healing. *Arch. Orthop. Unfallchir.* **85**, 151–154.

Sudmann, E. (1979) Mixed-image longitudimetry: A new method of length measurement in microscopy. Calif. Tissue Res. **27**, 27–32.

Sudmann, E., Dregelid, E., Bessesen, A. & Mørland, J. (1979) Inhibition of fracture healing by indomethacin in rats. *Eur. J. Clin. Invest.* **9**, 333–339.

Sudmann, E. & Bang, G. (1979) Indomethacin-induced inhibition of haversian remodelling in rabbits. *Acta Orthop. Scand.* **50**, 621–627.

Swinscow, T. D. V. (1976) *Statistics at square one.* British Medical Association, London.

Correspondence to: E. Sudmann, M.D., N-5220 Hagavik, Norway