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INHIBITION OF PARTIAL CLOSURE OF EPIPHYSEAL PLATE IN RABBITS BY INDOMETHACIN

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The efficiency of indomethacin (10 mg/kg/day) in inhibiting recurrent partial closure of the epiphyseal plate was tested in rabbits. An epiphysiodesis was done laterally in the distal left femoral epiphyseal plate in 19 adolescent rabbits. This produced a valgus deformity in 14 of them. The bone bridge was then removed operatively and the rabbits were treated with either indomethacin or vehicle for 21 days postoperatively. Indomethacin plasma levels were about 180 ng/ml. The valgus deformity improved in indomethacin-treated rabbits, whereas it became worse in the controls ($P = 0.004$).

Key words: bone; epiphysis; indomethacin

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Partial closure of an epiphyseal plate can reduce growth in length and cause angular deformation of the bone. The bone bridge between the epiphysis and the metaphysis, which inhibits growth, can be removed operatively, leaving a cavity, and improvement of the deformity by normal growth is then possible (Langenskiöld 1967 and 1975, Österman 1972). However, during adolescence the cavity is usually rapidly filled in by new bone causing reformation of the bone bridge.

Previous studies have shown that both heterotopic and orthotopic bone formation can be inhibited by indomethacin (Sudmann 1975, Rø et al. 1976, Sudmann & Hagen 1976, Almåsbakk & Røysland 1977, Sudmann et al. 1979, Sudmann & Bang 1979). In the present study we wanted to test whether indomethacin could prevent the recurrence of partial closure of an epiphyseal plate in adolescent rabbits, permitting gradual correction of angular deformities by growth.

METHODS

Animals

A total of 19 adolescent rabbits of either sex were used. At the start of the experiments they were 4–6 weeks old and weighed from 0.4 to 0.9 kg (median 0.5 kg). They were given an ordinary rabbit laboratory diet and water *ad libitum*. They were housed in metal cages at 20°C, with a 12-h light, 12-h dark cycle. At the end of the experiments they were about 20 weeks old and weighed from 3.0 to 3.5 kg (median 3.2 kg).

Experimental design

Two consecutive experiments were carried out in exactly the same way except for the indomethacin analysis, which was omitted in the first experiment (Table 1). To create a valgus deformity at the knee, a lateral distal femoral epiphysiodesis was performed on the left leg in all the rabbits. The animals in which a valgus deformity developed were operated on once more and the bone bridge created at the first operation was removed. The reoperated animals were divided into two groups so that the valgus angle of the femurs matched as closely as possible. One group was treated with indomethacin and the other with vehicle. At the start of indomethacin treatment the valgus angle varied from

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Table 1. Design of two consecutive experiments

No. of rabbits	Valgus deformation		Drug treatment		Indomethacin analysis
	Yes	No	Indomethacin	Vehicle	
3	3	0	2	1	0
16	11	5[1]	6	5	10[2]
Total number 19	14	5	8	6	10

The figures in the table refer to the number of rabbits.
1. These animals were not operated on a second time and were excluded from the rest of the study.
2. One rabbit died during indomethacin treatment.

12 to 42 degrees (median 26 degrees) in the indomethacin-treated group, and from 7 to 56 degrees (median 27 degrees) in the controls.

Operative procedure

Under neurolept anaesthesia (Hypnorm Vet. 0.3 ml/kg i.m., Mecos, Helsingborg, Sweden) and sterile conditions a rectangular piece of bone (measuring about 2 × 6 × 2 mm) lying transversely across the epiphyseal plate was cut out, rotated 180 degrees, and replaced in its bed (Figure 1). In a second operation, performed 14 to 21 days later when a valgus deformity had formed, the bone bridge between the epiphysis and the metaphysis was removed. The bone defect was deliberately not filled with any interposition material.

Indomethacin treatment

When the bone bridge had been removed, indomethacin suspension (Confortid, Dumex Ltd., Copenhagen, Denmark) or vehicle (vehicle, Confortid suspension) was squirted into the rabbits' mouths once a day, 6 days a week, for 3 weeks. The doses were 10 mg/kg/day. The rabbits were weighed twice a week, and the dosage adjusted accordingly.

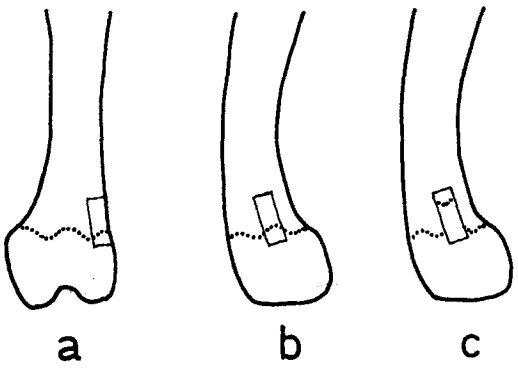


Figure 1. Distal left femur seen from the front (a) and from the lateral side (b–c). Epiphysiodesis: A rectangular piece of bone, crossing the epiphyseal line, was cut (a–b), turned 180 degrees, and replaced in its bed (c).

Indomethacin analysis

Heparinized blood samples for indomethacin analysis were obtained from anaesthetized animals 10 and 21 days after the start of indomethacin treatment. They were taken 24 hours after the daily dose of indomethacin or vehicle. The blood samples were centrifuged and plasma samples were collected, frozen and stored at 21°C until analysed (Jensen 1978). The analysis was carried out as a blind test by Dumex Ltd., Copenhagen, Denmark.

Radiographs

During drug treatment anteroposterior radiographs were taken of all the femurs at 4-week intervals. In addition, radiographs of the dissected femurs were made at the end of the experiments. The valgus angle of the femur (a) was defined as the angle between the tangent to the femoral condyles and a perpendicular to the longitudinal axis of the femur. The longitudinal axis of the femur was drawn from the midpoint of the shaft at the proximal junction of the diaphysis and the metaphysis to the midpoint of the distal epiphyseal line/metaphysis (Figure 2). The length of the femur was defined as the distance from the trochanter major to the midpoint of the tangent to the femoral condyles.

Statistics

Differences between the two groups were tested by the Wilcoxon rank sum test for unpaired data (the two-sample test) and the Wilcoxon-van Elteren test. Four-fold tables were tested by the exact probability test (Fischer-Irwin-Yates test) (Swinscow 1976, Høyland & Walløe 1977). The *P* values given were found by one-

Table 2. Plasma levels of indomethacin in 5 indomethacin-treated rabbits 24 hours after last dose

Day of experiment	Plasma level (ng/ml)
10	147 (96–500) $n=5$
21	224 (155–452) $n=5$

The plasma levels are median values with ranges in parentheses.

tailed tests and differences were considered significant when $P < 0.05$.

RESULTS

The animals tolerated the operations well. No postoperative complications were noted. After the first operation no valgus deformity was observed in 5 rabbits (Table 1). These animals were not operated on a second time and were excluded from the rest of the study.

One rabbit died after 14 days of indomethacin treatment, autopsy revealing aspiration pneumonia. This animal was not included in the results (Table 1). There was no significant difference ($P > 0.2$) in weight gain between the indomethacin-treated and the vehicle-treated animals.

Indomethacin analysis

No indomethacin was found in any vehicle plasma sample. During indomethacin treatment the median steady state level on day 10 was 147 ng/ml and at the end of the treatment it was 224 ng/ml (Table 2).

Radiographs

The valgus angle of the femur decreased through growth from 2 to 32 degrees in all the indomethacin-treated animals except one, in which the angle increased 19 degrees (Figures 2 and 4), while it increased from 5 to 39 degrees in the vehicle-treated animals (Figures 3–4). Thus, indomethacin treatment resulted in a significant correction of the deformity ($P = 0.004$) (Table 3). At the end of the experiments all the epiphyseal plates were closed. The difference in length between the nonoperated right femur and the contralateral operated one was significantly smaller ($P = 0.01$) in the indomethacin-treated rabbits than in the controls (Table 4).

DISCUSSION

In partial closure of an epiphyseal plate a bone bridge is formed between the epiphysis and the metaphysis. When operatively removed it reforms rapidly in an adolescent (Österman 1972).

Table 3. Effect of indomethacin treatment on angular deformity of the femur

Treatment		Angular deformity Correction	Increase
Indomethacin	(7)	6	1
Vehicle	(6)	0	6

The figures in the table refer to the number of rabbits. There was a significant difference between the two groups ($P = 0.004$) (exact probability test).

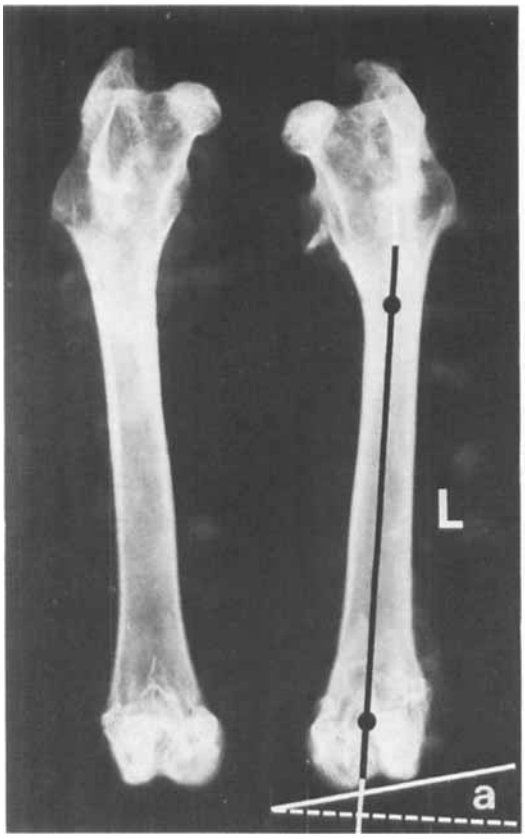
Table 4. Difference in final length between right and left femurs

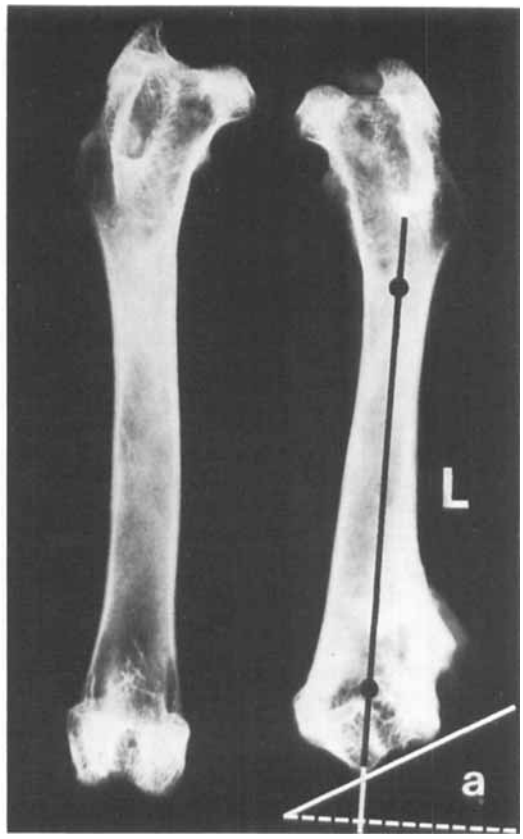
Treatment (No. of rabbits)		Difference (mm)[1]
Indomethacin	(7)	0.2 (0–1.2)
Vehicle	(6)	0.9 (0.3–2.2)

The differences in the table are median values with ranges in parentheses.

1. In all indomethacin-treated rabbits the difference was ≦ 0.3 mm except in one with a difference of 1.2 mm. This particular rabbit was the only one in which the valgus angle increased (see Table 3).

There was a significant difference between the two groups ($P = 0.01$).





Figures 2–3. Anteroposterior radiographs of typical dissected femurs in indomethacin-treated (Figure 2) and in vehicle-treated rabbits (Figure 3) 20 weeks after removal of a bone bridge in the left (L) femur. Figure 2: In the left femur the valgus angle (a) was corrected 11 degrees through growth. The femurs are almost equal in size. Figure 3: In the left femur the valgus angle increased 9 degrees through growth and the left femur is shorter than the right femur.

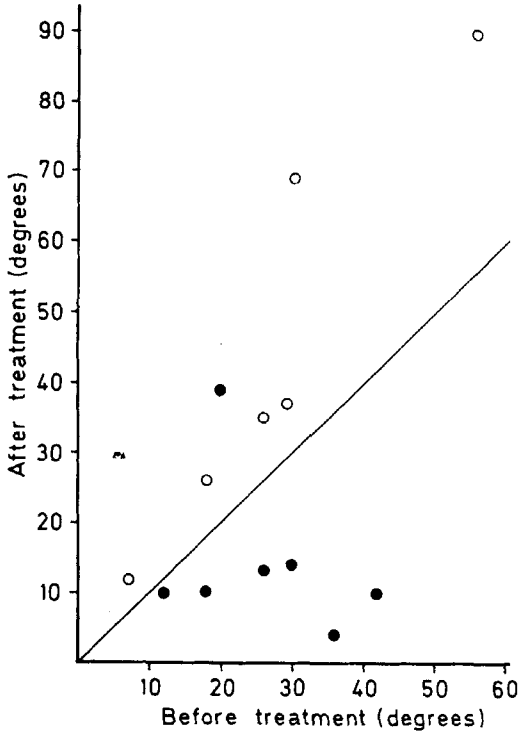


Figure 4. Scatter diagram of 13 pairs of radiographic measurements in 13 rabbits. The valgus angles of the left femurs before drug treatment are plotted against the corresponding angles after treatment. In indomethacin-treated animals the valgus angle was corrected by growth in all but one animal. In the vehicle-treated animals the valgus angle increased through growth.
● = *indomethacin-treated*, ○ = *vehicle-treated.*

The therapeutic problem is to find a consistent method of preventing such bone formation. In principle, it can be inhibited by an interposition material or by inhibiting osteoblastic activity or both.

Several different interposition materials have been tried, e.g. bone wax and fat grafts. Free fat grafts have given encouraging results in a few patients (Langenskiöld 1967 and 1975). Good results were also obtained in an experimental study in rabbits, but in about one-fifth of the animals the fat grafts apparently failed to correct the angular deformity (Österman 1972). Thus, it appears that free fat grafts, at least in rabbits, cannot consistently inhibit osteoblastic activity enough to prevent reformation of the bone bridge.

Previous studies have shown that indomethacin treatment results in a non-specific inhibition of the osteoblastic activity triggered by postfracture and / or postoperative inflammation (Sudmann 1975, Almåsbakk & Røysland 1977, Sudmann et al. 1979). These studies suggest that if the bone bridge between the epiphysis and the metaphysis is *operatively* removed, administration of indomethacin can inhibit the osteoblastic activity that causes its reformation. We found this to be true, since the angular deformities in indomethacin-treated rabbits were gradually corrected by growth in all but one animal, whereas a worsening of the deformity was observed in the controls. But this raises the question of whether a dose that prevents reforming of the bone bridge will also inhibit normal growth in length and width. A recent study reports that indomethacin has no effect on ordered growth in length and width (Sudmann et al. 1982). The present findings indicate that indomethacin can prevent reformation of a bone bridge between epiphysis and metaphysis without inhibiting normal growth and remodelling of the bones. In our limited clinical experience along the lines described (unpublished data) we have in addition to postoperative indomethacin treatment for 3–4 weeks used bone wax to achieve haemostasis after removing the bone bridge.

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