Piroxicam-induced reduction in osteopenia after external fixation of rabbit tibia

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Twelve rabbits were treated with a unilateral external fixator in one tibia for 12 weeks, while the other tibia served as an intact control. Half of the animals were also treated with 10 mg/kg/day of piroxicam, given in two daily oral doses. Changes in bone mineral content were determined using single photon absorptiometry. After 12 weeks, we found a 3 percent decrease in the bone mineral content in the tibia of the animals treated with piroxicam versus 9 percent in the nonpiroxicam group (P = 0.04). In the femurs, there was an insignificant decrease in bone mineral, 2 percent (piroxicam) and 1 percent (nonpiroxicam) respectively. The results indicate that piroxicam may reduce the osteopenia caused by external fixation.

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Osteopenia in the rabbit hindleg after external fixation of diaphyseal bones was described by Terjesen and Benum (1983), and in an earlier study we found a decrease in bone mineral content in the rabbit tibia of 7 percent after 6 weeks (Adolphson et al. 1990). However, other studies have shown that osteopenia can be moderated by inhibitors of prostaglandin synthesis (Powles et al. 1973, Sudmann and Bang 1979).

We report the influence of piroxicam on osteopenia after external fixation with a unilateral frame.

Material and methods

Twelve New Zealand white rabbits of both sexes, weighing 4–5.4 kg, were randomized into two groups. The animals were anesthetized with intramuscular injections of Hypnorm® Vet (Leo). One of the tibiae was chosen at random, while the other served as a control. All the operations were performed under strictly sterile conditions. The anteromedial aspect of the tibia was exposed by a longitudinal anterior incision. Three proximal and three distal threaded pins of 1.5 mm diameter were used (Jaquet Orthopédie S.A., Geneva). They were inserted after predrilling, with a pin group separation of 68 mm between the middle pins in each pin group. The distance between the pins in each group was 10 mm, and thus the free bone segment between the inner pins was 48 mm. A unilateral external stainless-steel fixator frame was mounted as described by Adolphson et al. (1990). None of the rabbits lost any body mass during the experiment. There was no difference between the two groups regarding mean weights. To obtain therapeutic plasma levels of piroxicam, much higher oral doses must be used for rabbits than for humans because of the much more rapid metabolization: viz., the plasma half-life in humans is approximately 45 h versus only 4.5 h in the rabbit (Wiseman 1982).

Half of the animals were given piroxicam orally at a dose of 10 mg/kg/day. Piroxicam was mixed in a vehicle consisting of spiritus fortis 30 g, carboxymethyl-cellulose 4 g, syrupus ribris nigri (a palatable flavor) 100 mL, and aqua pura to 400 mL. The treatment started 48 h before the operation, and it was given during the entire experimental period. The solution was squirted into the mouths of the animals twice a day on all the days. The rabbits were kept in separate cages, where they could move freely. They received a standard laboratory diet and water ad libitum, and were observed daily. The postoperative course was uneventful in all the cases. The animals resumed full weight bearing after a short period of time. After 1 week all of their bandages were changed, and no infection or abnormal swelling was seen. All the animals were killed after 12 weeks. The fixation equipment was removed, and both femurs and tibiae were dissected free from all the soft tissue. The bones were wrapped in towels soaked with Ringer's solution and stored at –18 °C until testing.

Table 1. Plasma levels of piroxicam (μg/mL) at the operation and on the day of killing

Rabbit no.	Start	After 12 weeks
1	4.8	3.2
2	2.0	2.3
3	3.8	3.2
4	4.3	0.9
5	0.5	1.7
6	0.7	1.3

Analyzes of piroxicam

Venous blood samples were taken from an ear vein with EDTA as an anticoagulant at the time of the operation and on the day of killing. After centrifugation the plasma was frozen and stored at –70 °C until testing. The plasma analyzes were performed with a high performance liquid chromatography (HPLC) method (Twomey et al. 1980). The plasma levels of piroxicam varied considerably among the animals (Table 1).

Bone mineral content

All the bones were thawed to room temperature before testing. There was a visible periosteal thickening of the tibiae in the pin regions of all the bones. The mineral content of the bones was measured by a modified computerized photon absorptiometer (ND 1100A Bone Density Scanner, Christiansen and Rødbro 1977). The scanner was monitored by a EPSON® PC AX2 computer equipped with specially developed software. The entire length of the bones was scanned at intervals of 1 mm. Typical scans of the tibiae are shown in Figure 1. From these data a region of interest was chosen for statistical evaluation in the same free diaphyseal segment of all the bones.

Statistical analysis

The Wilcoxon signed-rank test for paired observations was used to calculate the differences between the bone pair—both on the femurs and the tibiae. The percentage difference in relative bone mineral content in the bone pair was calculated, and thereafter the Mann-Whitney *U*-test (unpaired observations) was used to compare the differences between the two different animal groups. Differences were considered significant at *P*-values < 0.05.

Results

In the animals treated with piroxicam, the relative mineral content in the middle section of the externally fixated tibia decreased on an average of 3 percent as compared with the controls (P = 0.028, Table 2). There was a 2 percent decrease in the femurs on the same side, which was not significant. In the nonpiroxicam group the tibiae had a 9 percent decrease in bone mineral content (P = 0.028). The corresponding femurs had a 1 percent decrease in mineral content, which was not significant. The difference in bone mineral loss in the operated on tibiae caused by piroxicam was 6 percent (P = 0.037). The difference in bone

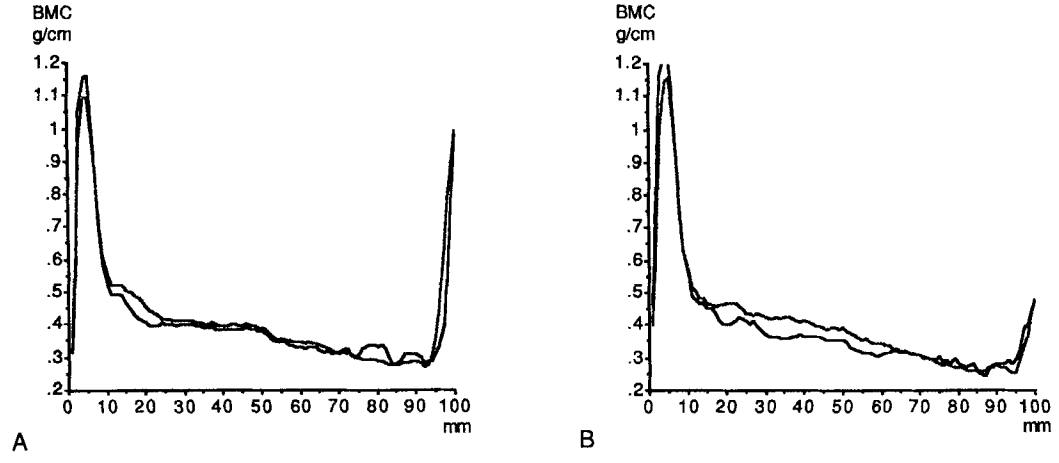

Figure 1. Bone mineral content along the tibiae. Knee region (left), ankle (right). ——operated on tibia. ---- control.
A Rabbit treated with and **B** without piroxicam.

Table 2. Bone mineral content in the externally fixated tibiae and femoral diaphyseal segments after 12 weeks, expressed as the percentage of the contralateral control bone. Median (range)

	Piroxicam (n 6)	Nonpiroxicam (n 6)
Femur	98 (88–98)	99 (95–103)
Tibia		
diaphyseal segment	97 (89–99)	91 (87–94)
segment with pins	106 (100–114)	107 (98–119)

mineral loss in the femurs on the operated on side between the groups (1 percent) was not significant. In all the externally fixated tibiae, there was an increase, both proximally and distally, in the bone mineral content of the bone segment around the pins (Table 2). However, between the two groups of tibiae, there was no difference as regards an increase in bone mineral content around the pins.

Discussion

The 9 percent decrease in bone mineral in the middle section of the externally fixated tibia in the nonpiroxicam group accords with the observations of Terjesen and Benum (1983), who found an average 10 percent decrease in the mineral content after 12 weeks with a bilateral frame. In an earlier study (Adolphson et al. 1990), with the same experimental design but treatment for 6 weeks, a slightly smaller, but significant, osteopenia (7 percent) was found; thus, it seems reasonable to assume that a longer fixation time causes more pronounced stress-shielding. A question of interest is whether the bone loss found was caused by true stress-shielding or just disuse. In our earlier investigation (Adolphson et al. 1990), half of the animals received a complete external fixator and the other half were operated on in the same way, but without mounting of the external bar. We found a 7 percent bone loss in animals with a complete fixator and no bone loss in the animals that were operated on with pins, but without the bar, after 6 weeks. Thus, we interpreted the bone loss as a stress-shielding effect, and not as an effect of disuse or merely a posttraumatic osteopenia.

The prostaglandin compounds are extremely short-lived, locally produced, unsaturated fatty acids, and especially types E_2 and I_2 (prostacyclin) are very potent stimulators of bone resorption (Klein and Raisz 1970, Katz et al. 1981, 1983, Raisz and Martin 1984). One of the main actions of PGE_2 is the induction of hyperplasia of the osteoclasts (Schelling et al. 1980). Prostaglandin production is stimulated by trauma (Staszewska-Barczak and Vane 1975); further, Floman et al. (1977) and Dekel et al. (1981) found high concentrations early in inflamed regions, i.e., in arthritis and in fractures. Harris et al. (1973) and Goodson et al. (1974) had also found high concentrations of prostaglandins in periodontal conditions and in dental cysts.

Nonsteroid anti-inflammatory drugs (NSAIDs) are a family of drugs commonly used in rheumatoid arthritis, arthrosis, and ankylosing spondyloarthritis. The inhibitory capacity of nonsteroid anti-inflammatory drugs on prostaglandin synthesis in vivo and in vitro is generally accepted (Vane 1974). They interfere with cyclo-oxygenase and inhibit the transformation of arachidonic acid to prostaglandins. Inhibition of bone formation and resorption of both heterotopic and orthotopic bone in rabbits treated with indomethacin has been reported (Sudmann 1975, Sudmann and Bang 1979). Several studies have demonstrated that NSAIDs, which inhibit the synthesis of prostaglandins, can reduce the number of osteoclasts and reduce the bone resorption caused by experimentally induced peridontitis (Nyman et al. 1979, Williams et al. 1985). There are also experimental studies that have proved that NSAIDs will arrest bone resorption in rabbits after induced osteomyelitis (Dekel and Francis 1981) and after osteotomy (Sudmann and Bang 1979). Therefore, one aim of the study was to investigate if a NSAID would reduce the osteopenia caused by stress-shielding.

The bone mineral loss due to the external fixator in the tibiae of the animals in our piroxicam group was 6 percent lower than in the nonpiroxicam group. Thus, this study supports the hypothesis that an inhibitor of prostaglandin synthesis could reduce the osteopenia caused by stress-shielding.

Törnkvist et al. (1983) found no difference in the mineral content in the rat femur of ordered bone, but they found a decreased heterotopic new bone formation after treatment with the anti-inflammatory drugs ibuprofen and flubiprofen. However, in the present study the amount of periosteal new bone formation around the pins was not influenced by piroxicam.

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