Immobilization increases bone prostaglandin E

Effect of acetylsalicylic acid on disuse osteoporosis studied in dogs

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The effect of acetylsalicylic acid (aspirin) on bone mass and bone prostaglandin E (PGE) in immobilization osteoporosis was studied in 12 growing dogs using a unilateral hind limb cast-fixation model. Osteoporosis was induced by fiberglass-cast immobilization of the right hind limb for 4 weeks, with the left hind limb as a control. Six dogs received buffered aspirin at 25 mg/kg body weight per os every 8 hours; 6 dogs received no treatment. All the dogs were killed after 4 weeks, and bone samples were collected. Bone mineral content of the distal tibial metaphysis was measured by single-photon absorptiometry. In vitro release of PGE from the calcaneus, tibial cortical bone, tibial cancellous bone, and ilium were measured using a specific radioimmunoassay for PGE.

Compared with the controls, the casted limb of untreated dogs had half the bone mass and a twofold increase in bone PGE. Aspirin treatment was associated with a 65 percent reduction in bone PGE and a 13 percent bone mass sparing effect. These results provide indirect evidence that PGE plays a role in immobilization osteoporosis.

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Increasing evidence suggests that prostaglandins play an important role in bone resorption. The E series prostaglandins are known to be potent bone resorption agents (Dietrich et al. 1975), and large quantities of prostaglandin E_2 are produced by bone in culture (Raisz et al. 1979). Furthermore, treatment with inhibitors of prostaglandin synthesis has been associated with bone mass sparing in certain states of pathologic bone resorption, including cancer metastases (Powles et al. 1973, Galasko et al. 1979), osteomyelitis (Dekel and Francis 1981, Rissing and Buxton 1986), and periodontal disease (Williams et al. 1985). Aspirin (acetylsalicylic acid) inhibits prostaglandin synthesis via irreversible acetylation of cyclooxygenase, the enzyme that converts arachidonic acid to prostaglandin endoperoxides (Higgs et al. 1984, Clissold 1986).

Bone prostaglandin E (PGE) has been measured in cultured bone (Raisz et al. 1979, Somjen et al. 1980, Voelkel et al. 1980, Katz et al. 1983, Feyen and Raisz 1987), and experimentally in osteomyelitis (Corbett et al. 1979, Dekel and Francis 1981, Rissing and Buxton 1986) and fractures (Dekel et al. 1981) in rabbits. To our knowledge, PGE has not been previously measured in canine bone or in immobilized limbs.

The objectives of this study were (1) to assess the effect of immobilization on bone mass and bone PGE, (2) to determine the effect of aspirin treatment on bone PGE, and (3) to evaluate the possible sparing effect of aspirin on the bone mass loss associated with immobilization.

Material and methods

Experimental animals

Twelve random source 10 to 14-week-old female mixed-breed dogs with a mean body weight of 6.7 (5.3–8.9) kg were used. The dogs were housed in individual cages and had access to water and commercial dog ration ad libitum. Right hind limb osteoporosis was induced by fiberglass-cast immobilization (mid-femur to digits) for 4 weeks,

while the left hind limb served as a control. Evaluation prior to cast fixation included physical examination, anterior-posterior and lateral radiographs of both hind limbs from the stifle to the digits, complete blood count, serum biochemical profile, and plasma immunoreactive parathyroid hormone (iPTH) determination (PTH-MM Radioimmunoassay Kit, INCSTAR Corp., Stillwater, MN, U.S.A.). The dogs were matched for body weight and then randomly assigned to one of two groups: aspirin-treated (n 6) or untreated (n 6). Aspirin-treated dogs received buffered aspirin (Ascriptin®, William H. Rorer, Inc., Fort Washington, PA, USA) at a dosage of 25 mg/kg of body weight per os every 8 hours for the duration of the experiment. In a previous study, dogs receiving this dosage of buffered aspirin maintained therapeutic serum salicylate concentrations (Lipowitz et al. 1986). Assessment during the experimental period consisted of a daily physical examination, and anterior-posterior and lateral radiographs of both hind limbs on Days 14 and 28. A complete blood count, serum biochemical profile, and plasma iPTH determination were repeated on Day 28, at which time, all the dogs were killed.

Body weight, serum calcium (Ca), phosphate (P), alkaline phosphatase (Alk Phos) and plasma iPTH at Day 0 and Day 28 were compared. Differences between Day 0 and Day 28 values within treatment groups were analyzed using a paired Student's *t*-test; differences between treatment groups were compared using an unpaired Student's *t*-test. In each instance, differences were considered significant if $P < 0.05$.

Bone sampling

Immediately after death, bone samples were collected for determination of bone mass and bone PGE. After the disarticulated tibiae were cleansed of soft tissue, measured for length, and weighed, a transverse cut was made and the distal 60 percent of the tibia was placed in 10 percent neutral buffered formalin for subsequent bone mass determination. The proximal piece was then cut longitudinally, and cancellous bone was harvested using a bone currette. The cancellous bone was vigorously rinsed with saline, placed in plastic bags, and frozen to minus 20 °C for subsequent PGE analysis. Following removal of all the cancellous bone and marrow contents, a second transverse cut was made to obtain a periosteum-free cortical sample, which was frozen for subsequent prostaglandin analysis. A periosteum-free sample from each calcaneus and a trephine biopsy (8-mm internal diameter) specimen from the left ilium were also collected and frozen for prostaglandin analysis.

Bone mass determination

Bone mass of the distal tibial metaphysis was measured using a single-photon absorptiometric system capable of whole-bone and region-specific measurements of bone mineral content. The system employs an ^{125}I gamma-ray source of sufficient specific activity to provide greater than 2,000 counts per second through a 1.0-mm collimator. The system software establishes baseline activity, converts count attenuation to ash weight from a standard curve (Aro et al. 1989), and produces high resolution visual images that permit identification and analysis of specific regions of interest. Bone samples are held stationary in a lucite box containing 2.0 cm 70 percent ethanol, and rectilinear scanning is performed with a scan line width of 0.5 mm.

The accuracy of the instrument, expressed as the correlation coefficient between count attenuation and ash weight of incinerated samples, is 0.999 ($P < 0.0001$). Precision, assessed by repeated measurements (n 6) on different days for 4 samples and expressed as the coefficient of variation, is 0.8 percent. The distal tibial metaphysis was scanned for a length of 1.0 cm (twenty 0.5-mm-wide scan lines). Bone mineral content was normalized for bone width at the scanning site, and was expressed as mg/mm^2. The effect of immobilization on bone mass was assessed by comparing normalized bone mineral content (NBMC) of the casted limb with NBMC of the uncasted limb in untreated dogs. The effect of aspirin treatment on bone mass was assessed by comparing the fractional bone loss of the casted limb in aspirin-treated with untreated dogs.

Bone PGE analysis

Measurement of in vitro release of PGE from bone was performed using a modification of a previously reported method (Corbett et al. 1979). Calcaneus, tibia cortical bone, tibia cancellous bone, and ilium were individually assayed for PGE release. Bone samples weighing 0.3–1.5 g were incubated in 20 cc Krebs-bicarbonate buffer at 37 °C for 20 minutes. Following incubation, the unextracted samples were

Table 1. Serum calcium, phosphate, alkaline phosphatase, and plasma immunoreactive parathyroid hormone (iPTH) in aspirin-treated and untreated dogs before and following 4 weeks of unilateral hind limb immobilization

	Serum calcium (mg/dL)		Serum phosphate (mg/dL)		Serum alkaline phosphatase (U/L)		Plasma iPTH (pmol/L)	
	Day 0	Day 28	Day 0	Day 28	Day 0	Day 28	Day 0	Day 28
Untreated (n 6)								
mean	10.1	10.1	8.62	7.85[a]	146	163	53	54
SEM	*0.15*	*0.21*	*0.22*	*0.33*	*22*	*31*	*11.4*	*5.2*
Aspirin-treated (n 6)								
mean	10.3	10.0[a]	7.93	8.33	112	136	48	72
SEM	*0.13*	*0.12*	*0.34*	*0.46*	*12*	*21*	*3.2*	*13.8*

[a]Differs ($P < 0.05$) from Day 0 value within treatment group.

Table 2. Distal tibia metaphyseal bone mass for aspirin-treated and untreated dogs following 4 weeks of unilateral hind limb immobilization (mean, *SEM*)

	Normalized bone mineral content (mg/mm^2)				Fractional bone loss of	
	Uncasted limb		Casted limb		Casted limb (%)	
Untreated (n 6)	4.07	*0.20*	1.84[b]	*0.15*	55	*1.97*
Aspirin-treated (n 6)	3.96	*0.25*	2.04[b]	*0.09*	48[a]	*2.51*

[a]Differs ($P < 0.025$) from value for untreated dogs.
[b]Differs ($P < 0.005$) from value for uncasted limb within treatment group.

immediately frozen to minus 20 °C for subsequent PGE determination by specific radioimmunoassay. The radioimmunoassay utilized a specific antibody against PGE, with less than 0.1 percent cross-reaction with 6 Keto PGF_1, PGF_2, PGD_2, and thromboxane B_2 (Trachte et al. 1987). The antibody was obtained from Dr. B. Zimmerman (Minneapolis, MN) and cross reacted equally with PGE_1 and PGE; therefore, results were expressed as PGE. Limits of detectability were 2 to 100 picograms. Assays were performed in duplicate and mean values recorded. The amount of PGE released from bone was normalized for sample weight and expressed as ng PGE per g bone. The ratio of PGE casted/uncasted limb was calculated in each dog for calcaneus, tibia cortical bone, and tibia cancellous bone.

Results

There was no difference between mean body weight of aspirin-treated and untreated dogs on Day 0; mean weight gain over the experimental period did not differ between the two groups. There were no differences between treatment groups at Day O or Day 28 with respect to serum Ca, P, Alk Phos, and plasma iPTH (Table 1). Although serum P in untreated dogs and serum Ca in aspirin-treated dogs decreased over the experimental period, all the values were within the normal range. Plasma iPTH concentrations did not change during the experiment.

Effect of immobilization in untreated dogs

In untreated dogs, the mean NBMC of the uncasted tibial metaphysis was 4.07 mg/mm^2, whereas the mean NBMC of the casted tibial metaphysis was 1.84 mg/mm^2 ($P < 0.005$). Mean (± SEM) fractional bone loss of the casted limb was 55.4 ± 1.97 percent (Table 2). Immobilization was associated with a twofold increase in bone PGE in the casted limb (Table 3).

Effect of aspirin treatment

Bone PGE in aspirin-treated dogs was less than in untreated dogs (Table 4). Aspirin treatment was

Table 3. The effect of immobilization on bone prostaglandin E (PGE) in untreated dogs (n 6). Mean *SEM*

	Calcaneus	Tibia cortical	Tibia cancellous
^{PGE}casted/^{PGE}uncasted	2.2 *0.7*	2.7[a] *0.6*	1.2 *0.3*

[a]Differs ($P < 0.05$) from 1.0.

associated with a 64.7 ± 5.3 percent reduction in mean bone PGE. Aspirin treatment was associated with a significant reduction in bone loss in the casted limb (Table 2). Aspirin-treated dogs had a 48 percent mean fractional bone loss of the casted limb. The calculated bone mass sparing effect of aspirin treatment was 13.4 percent.

Discussion

Immobilization osteoporosis has been studied experimentally following unilateral motor denervation (Kharmosh and Saville 1965, Turner and Bell 1986, Wakley et al. 1988), patellar or calcanean tenotomy (Thompson and Rodan 1988), and cast fixation (Burkhart and Jowsey 1967, Mattsson 1972, Uhthoff and Jaworski 1978, Caywood et al. 1979, Uhthoff et al. 1985). Long-term (16 weeks) unilateral forelimb cast immobilization resulted in a 45 percent decrease in bone mass in the distal radial metaphysis of adult dogs (Uhthoff et al. 1985). In the present study using growing dogs, unilateral hind limb cast immobilization for 4 weeks resulted in a 55 percent decrease in bone mass in the distal tibial metaphysis. Thus, profound osteopenia may be achieved in growing dogs following a relatively brief period of immobilization, making this a useful model in the investigation of potential therapeutic strategies to prevent immobilization-induced bone loss.

Few attempts have been made to characterize the loading status of the contralateral limb during unilateral limb immobilization. If supranormal loading of the contralateral limb occurs, the resultant increase in bone density in that limb would contribute to the difference between casted and uncasted limbs. We have found that the contralateral limb in this model is supranormally loaded; approximately 84 percent of the calculated fractional bone loss of the casted limb may be attributed to actual diminished bone mass, whereas 16 percent is attributable to increased bone mass in the supranormally loaded control limb (unpublished data).

It is likely that prostaglandins play an important role in bone metabolism. Prostaglandins, particularly PGE, are potent bone resorption agents in vitro (Dietrich et al. 1975). The role of prostaglandins in certain states of pathologic bone resorption is supported by the bone mass sparing effect of nonsteroidal anti-inflammatory drug (NSAID) treatment of cancer metastases (Powles et al. 1973, Galasko et al. 1979), osteomyelitis (Dekel and Francis 1981, Rissing and Buxton 1986), and periodontal disease (Williams et al. 1985). In experimental osteomyelitis, reduction in bone PGE was associated with decreased bone destruction in rabbits treated with sodium salicylate (Dekel and Francis 1981) or ibuprofen (Rissing and Buxton 1986). PGE-mediated bone resorption has also been implicated in the loosening of cemented total hip prostheses (Goldring et al. 1986, Herman et al. 1989).

However, the role of arachidonic acid metabolites in the regulation of bone turnover is complex, because prostaglandins demonstrate the unique capacity to stimulate bone resorption and bone formation (Chyun and Raisz 1984, Nefussi and Baron 1985, Shih and Norridin 1986). Prostaglandin

Table 4. The effect of aspirin treatment on bone prostaglandin E (ng/g) following 4 weeks of unilateral hind limb immobilization. Mean *SEM*

	Calcaneus		Tibia cortical		Tibia cancellous		Ilium	
Uncasted Limb (n 6)								
Untreated	1.6	*0.5*	1.7	*0.5*	5.2	*0.9*	9.5	*1.6*
Aspirin-treated	0.5[b]	*0.1*	0.9	*0.3*	1.4[b]	*0.7*	1.5[c]	*0.4*
Casted Limb (n 6)								
Untreated	2.8	*0.8*	3.2	*0.5*	6.0	*1.4*	–	–
Aspirin-treated	0.7[b]	*0.3*	1.6[b]	*0.2*	2.7[a]	*0.6*	–	–

Differs [a]($P < 0.05$), [b]($P < 0.025$), [c]($P < 0.005$) from value for untreated dogs.

production by resting or senescent osteoblasts may stimulate the replication and differentiation of primitive osteoblasts (Raisz and Martin 1983). Further, it is believed that the osteoblast may be the initial target cell for prostaglandin-mediated bone resorption (Rodan and Martin 1981).

The role of prostaglandins in immobilization osteoporosis is supported by results of a previous study using a unilateral knee tenotomy model in the rat (Thompson and Rodan 1988). In that study, histomorphometric analysis of the proximal tibial metaphysis of indomethacin-treated and untreated rats were compared. Indomethacin treatment was associated with an 80 percent sparing effect on bone mass as measured by trabecular bone volume following 10 days of immobilization. The increase in osteoclast number and percentage of resorption surface seen in the immobilized tibia of untreated rats was not observed in indomethacin-treated rats. However, physiologic evidence of cyclo-oxygenase inhibition was not demonstrated, and bone PGE concentrations were not determined.

Although aspirin-treated dogs in the present study had decreased bone PGE, immobilization osteoporosis was only partially prevented by aspirin treatment. This observation may reflect (1) an absence of an effect on decreased bone formation and/or (2) the role of prostaglandin-independent bone resorption in immobilization osteoporosis. The bone mass sparing effect of indomethacin on immobilized rat tibiae was associated with decreased bone resorption without an effect on the decreased bone appositional rate (Thompson and Rodan 1988). Under certain circumstances, prostaglandin inhibitors may have the capacity to inhibit bone formation by decreasing prostaglandin-mediated differentiation of primitive osteoblasts (Raisz and Martin 1983). Certain cells (e.g., monocytes) and hormones (e.g., osteoclast activating factor) demonstrate both prostaglandin-dependent and prostaglandin-independent mechanisms of bone resorption (Raisz and Martin 1983). This duality of resorption mechanism may contribute to continued bone loss despite NSAID treatment.

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