Calcif Tissue Int (2002) 71:356–363
DOI: 10.1007/s00000-002-1006-9

Calcified Tissue International

The Amino Bisphosphonate Ibandronate Prevents Calciphylaxis in the Rat at Doses that Inhibit Bone Resorption

P. A. Price, N. Omid, T. N. Than, M. K. Williamson

Division of Biology, 0368, University of California, San Diego, La Jolla, Ca, 92093-0368, USA

Received: 18 January 2002 / Accepted: 27 March 2002 / Online publication: 19 August 2002

Abstract. The present experiments were carried out to test the hypothesis that there is a common underlying biochemical mechanism that accounts for the different kinds of soft tissue calcification observed in animals that are treated with toxic doses of vitamin D. In previous studies we showed that lethal doses of vitamin D cause extensive calcification of arteries, lungs, kidneys, and cartilage, and that doses of the amino bisphosphonate ibandronate that inhibit bone resorption completely inhibit each of these soft tissue calcifications and prevent death. In the present experiments we have examined the effect of ibandronate on an entirely different type of calcification, the calciphylaxis induced by administration of a challenger to rats previously treated with sub-lethal doses of vitamin D. These studies show that ibandronate doses that inhibit bone resorption completely inhibit artery calcification as well as, in the same rat, the calciphylactic responses to either subcutaneous injection of 300 μg $FeCl_3$ or intrascapular epilation. Since the vitamin D-treated animals had dramatically increased levels of bone resorption, and concurrent treatment with ibandronate normalized resorption, these results support the hypothesis that soft tissue calcifications in the vitamin D-treated rat may be linked to bone resorption. The ability of ibandronate to inhibit all vitamin D-associated calcifications in the rat cannot be explained by an effect of ibandronate on serum calcium, since serum calcium remained 30% above control levels in the vitamin D-treated animals that also received ibandronate.

Key words: Vitamin D — Calciphylaxis — Ectopic calcification — Artery calcification — Ibandronate — Bisphosphonates.

The present experiments were carried out to test the hypothesis that there could be a common underlying biochemical mechanism that accounts for the different kinds of soft tissue calcification observed in animals that are treated with toxic doses of vitamin D. In previous studies we showed that lethal doses of vitamin D cause extensive calcification of arteries, lungs, kidneys, and cartilage, and that doses of ibandronate that inhibit bone resorption completely inhibit each of these soft tissue calcifications and prevent death [1, 2]. In the present experiments we have examined the effect of ibandronate on an entirely different type of calcification, the calciphylaxis induced by administration of a challenger to rats previously treated with sub-lethal doses of vitamin D.

The calciphylaxis response in the rat requires two treatments, an initial treatment with a sensitizer, such as a high dose of vitamin D or PTH, followed after an interval of a day or more by treatment with a calciphylactic challenger [3]. In the present study we have employed the same 3-day vitamin D injection schedule used in the prior ibandronate studies [1, 2], but have reduced the dosage of vitamin D to nonlethal levels. Two completely different kinds of calciphylactic challengers were used, subcutaneous injection of $FeCl_3$ and intrascapular epilation, and both challengers were administered 24 hours after the last of the three vitamin D doses. In previous studies of these calciphylactic responses in rats sensitized by prior treatment with toxic doses of vitamin D, calcification induced by epilation (hair plucking) started in the fibrous tissue surrounding the hair follicles and subsequently spread throughout the dermis, and calcification induced by $FeCl_3$ was accompanied by an inflammatory response in the ventral fascia and culminated in the formation of a highly calcified tissue button [3, 4].

Materials and Methods

Materials

Vitamin D_3 (cholecalciferol) was purchased from ICN (Costa Mesa, CA) and Ibandronate (Bondronat, Boehringer Mannheim) was purchased from Idis World Medicines (Surrey, UK). Ibandronate was diluted with 0.15 mol/l NaCl and stored at 4°C. Stock solutions of vitamin D were prepared fresh for each 3 day subcutaneous injection cycle at a concentration of 4.3 mmol/l in 7% emulphor (alkamuls EL-620, Rodia, Inc.) and then placed in foil-wrapped containers and stored at 4°C, as described previously [5]. Simonsen albino rats

Correspondence to: P.A. Price, e-mail: pprice@ucsd.edu

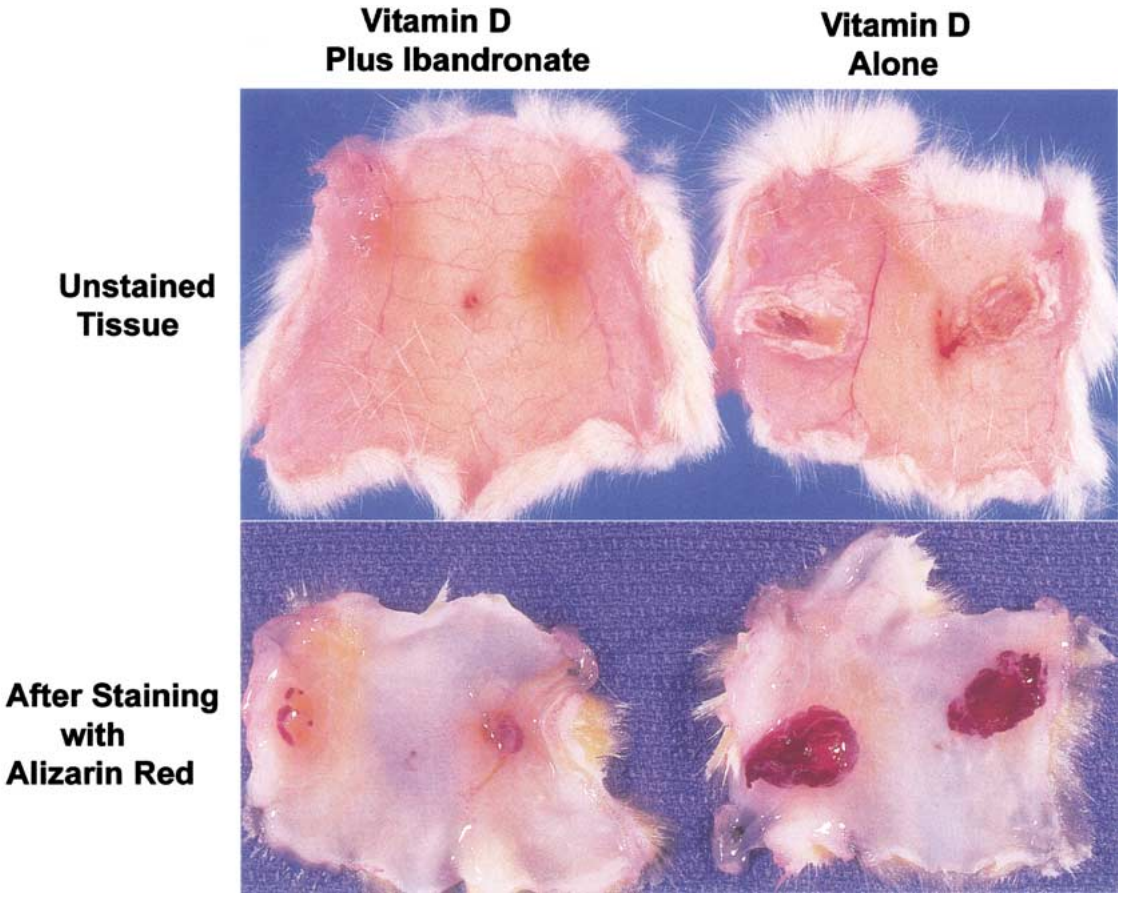

Fig. 1. Effect of ibandronate on the subcutaneous calciphylaxis induced by $FeCl_3$ injection. Six 7-week-old male Sprague Dawley rats received subcutaneous injections of 400,000 IU of vitamin D_3/kg body weight at t = 0, 1 and 2 days. Three of these rats were also injected subcutaneously with ibandronate at a dose of 0.25 mg/kg/day beginning 4 days prior to the first vitamin D injection, and the remaining 3 rats received no ibandronate. Calciphylaxis was induced by the subcutaneous injection of the 300 μg $FeCl_3$ challenger at two ventral sites 3 days after the first vitamin D injection, and rats were killed 7 days after the $FeCl_3$ injection. The typical gross appearance of the unstained, $FeCl_3$-induced calciphylactic buttons in each group is shown on the top, and the appearance of the same specimen after staining for calcification with Alizarin red S is shown on the bottom. Left, treatment with vitamin D plus ibandonate; right, treatment with vitamin D alone.

(Sprague-Dawley derived) were purchased from Simonsen labs (Gilroy, Ca).

Treatment of Rats

Male Sprague Dawley rats were fed *ad libitum* with rodent diet 5001 (Purina Mills Inc., St. Louis, MO), a diet that is 0.67% phosphorus and 0.95% calcium by weight. In all experiments, rats were killed by exsanguination while under ether anesthetic. In the calciphylaxis experiments, 7-week-old male rats were first sensitized by 3 subcutaneous injections of 400,000 IU of vitamin D_3/kg body weight made at t = 0, 24, and 48 hours. Calciphylaxis was then initiated at t = 72 hours either by subcutaneous injection with 300 μg of $FeCl_3$ at each of two ventral sites in the thorax (see Fig. 1) or by mechanically removing all of the hair in a 9 cm^2 area of skin in the intrascapular region of the anesthesized rat (epilation; see Fig. 4). To assess the effect of ibandronate on ectopic calcifications, a subset of the rats also received subcutaneous injections of ibandronate at a dose of 0.25 mg/kg/day beginning 4 days prior to the first vitamin D injection and continuing until the animals were killed. Animals were killed by exsanguination 7 days after administration of the calciphylactic challenger and the appropriate tissues were removed within 30 min of death and either immediately frozen at −20°C until chemical analysis or fixed in formalin. The effect of vitamin D dose on the level of calcification in the aorta and at the site of calciphylactic challenge was determined in rats that received subcutaneous injections of 100,000, 200,000 or 300,000 IU of vitamin D_3/kg body weight at t = 0, 24, and 48 hours and of 300 μg of $FeCl_3$ at t = 72 hours. The UCSD Animal Subjects Committee approved all animal experiments.

For measurement of mineral accumulation in the aorta, the abdominal aorta section beginning 1 cm above the renal branch and ending at the femoral bifurcation was demineralized in 150 mmol/l HCl overnight at room temperature. For analysis of mineral accumulation at sites of calciphylaxis, the well-defined tissue button that formed at the subcutaneous sites of $FeCl_3$ injection (see Fig. 1) and the region of skin that underwent epilation (see Fig. 4) were removed and demineralized in 10% formic acid. Calcium levels in serum and in the acid extract of tissues were determined colorimetrically using cresolphthalein complexone (Sigma) and phosphate levels in serum and in the acid extract of tissues were determined colorimetrically as described [6]. Serum samples were analyzed to determine the level of cross-linked N-teleopeptides (OSTEOMARK NTx) by Ostex, Inc. (Seattle, WA) using a specific ELISA assay [7].

For histological analysis of mineral accumulation, the appropriate tissues were fixed in formalin for at least 24 hours at room temperature. Sectioning and histological staining (He-

matoxylin & Eosin and von Kossa) of formalin-fixed tissues were carried out by San Diego Pathology (San Diego, Ca). Alizarin red staining of formalin-fixed tissues was carried out as described [8, 9]. Photographs of freshly dissected and Alizarin red-stained tissues were made by the OLR Photo Lab at UCSD.

Statistical Analysis

All data are presented as means ± standard deviation. Differences between groups were analyzed by the Student's *t*-test. Differences of $P < 0.05$ were accepted as significant.

Results

Effect of Ibandronate on Calciphylaxis Induced by Subcutaneous Injection with $FeCl_3$

The first experiments were carried out to determine the effects of ibandronate on the subcutaneous calciphylaxis induced by the injection of the $FeCl_3$ challenger into rats sensitized by the prior injection of vitamin D. As seen in Figure 1, the calciphylactic response at the two sites of $FeCl_3$ injection forms a whitish button about 0.8 cm in diameter that is intensely stained for calcification with Alizarin red. This calciphylactic response is identical to that previously reported for calciphylaxis produced by $FeCl_3$ injection into rats sensitized by prior vitamin D administration [3]. In agreement with these earlier studies, no calciphylactic button was found at the site of $FeCl_3$ injection in animals that had not been sensitized by prior vitamin D treatment (data not shown).

The effect of ibandronate on $FeCl_3$-induced calciphylaxis was examined using the ibandronate dose and injection schedule previously shown to completely inhibit artery calcification in rats treated with vitamin D [1, 2]. As seen in Figure 1, ibandronate treatment markedly reduced the size of the button formed at the site of $FeCl_3$ injection and largely eliminated the Alizarin red staining of the button. Figure 2 shows that the buttons contained high levels of calcium and phosphate and that ibandronate treatment dramatically lowered the concentration of both mineral components. Arteries were removed from each experimental animal at the end of the calciphylaxis experiment and stained for calcification with Alizarin red in order to confirm the effects of vitamin D and ibandronate on artery calcification. As seen in Figure 3, vitamin D treatment caused extensive calcification of the artery similar to that reported previously, and ibandronate treatment completely inhibited this effect.

Previous studies have shown that the timing of the calciphylactic challenge with agents such as $FeCl_3$ has a critical bearing on the strength of the calcification response [3]. Additional measurements were therefore undertaken to establish serum chemistry at the time of

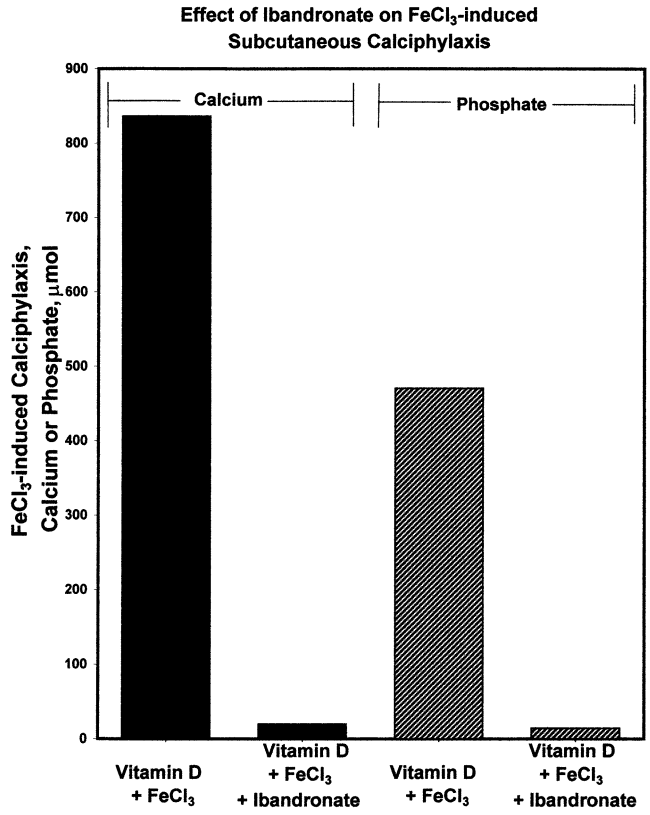

Fig. 2. Effect of ibandronate on the accumulation of calcium and phosphate at sites of $FeCl_3$-induced calciphylaxis. Eight 7-week-old male Sprague Dawley rats received subcutaneous injections of 400,000 IU of vitamin D_3/kg body weight at t = 0, 1, and 2 days. Four of these rats were also injected subcutaneously with ibandronate at a dose of 0.25 mg/kg/day beginning 4 days prior to the first vitamin D injection. Calciphylaxis was induced by the subcutaneous injection of the 300 μg $FeCl_3$ challenger at two ventral sites 3 days after the first vitamin D, and rats were killed 7 days after the $FeCl_3$ injection. The 8 well-defined tissue buttons from each treatment group were removed by dissection and then extracted with acid to dissolve mineral. Acid extracts were analyzed for calcium and phosphate and the means for each group are shown. The mean and SD for each group of 8 buttons were; vitamin D only, calcium 836.4 ± 233.4 and phosphate 470.7 ± 158.7; vitamin D plus ibandronate, calcium 19.8 ± 11.7, and phosphate 14.4 ± 9.6.

$FeCl_3$ injection, which was 72 hours after the first vitamin D injection. Table 1 shows that treatment with vitamin D alone increased serum levels of cross-linked N-teleopeptides, a marker for bone resorption activity, by 82%. Serum levels of cross-linked N-teleopeptides were slightly below control values in the rats treated with vitamin D plus ibandronate, which shows that ibandronate treatment completely inhibited the increased level of bone resorption activity produced by vitamin D treatment. Serum calcium levels were elevated by 30% in all animals treated with vitamin D, but there was no significant difference between the elevated serum calcium levels seen in animals treated with vitamin D alone and those with vitamin D plus ibandronate ($P > 0.15$) (Table 1).

Fig. 3. Effect of ibandronate on the calcification of arteries in rats subjected to calciphylactic challenge with $FeCl_3$. In the experiment described in the Figure 2 legend, the carotid arteries, aorta, and portions of the pulmonary, mesenteric, hepatic, renal, and femoral arteries were dissected as a unit, fixed in formalin, and stained with Alizarin red S (see Materials and Methods). Left, arteries from 4 rats treated with vitamin D plus ibandronate (carotid arteries on left); right, arteries from 4 rats treated with vitamin D alone. (Untreated control rats have no Alizarin red staining for calcification in their arteries; see [10] for example).

Table 1. Effect of ibandronate on bone resorption activity and serum calcium and phosphate in rats subjected to calciphylactic challenge with $FeCl_3$

	Vitamin D only ($\bar{X} \pm$ SD, n = 4)	Vitamin D + ibandronate ($\bar{X} \pm$ SD, n = 4)	Age-matched control ($\bar{X} \pm$ SD, n = 4)
Calcium, mg/dl	14.4 ± 0.8[a]	13.6 ± 0.4[a]	10.8 ± 0.4
Phosphate, mg/dl	10.9 ± 0.3	9.3 ± 0.3[b,c]	10.9 ± 0.9
Cross-linked N-teleopeptides, ng/ml	90.1 ± 8.8[a]	49.6 ± 6.0[b,d]	58.2 ± 3.6

Serum samples were obtained at 72 hours after the first vitamin D injection in the experiment described in Figure 2 and analyzed to determine the level of calcium, phosphate, and cross-linked N-teleopeptides (OSTEOMARK NTx, Ostex), a specific marker of bone resorption activity. See Materials and Methods

[a] Significantly different from age-matched control value, $P < 0.001$

[b] Significantly different from vitamin D only value, $P < 0.001$

[c] Significantly different from age-matched control value, $P < 0.01$

[d] Significantly different from age-matched control value, $P < 0.05$

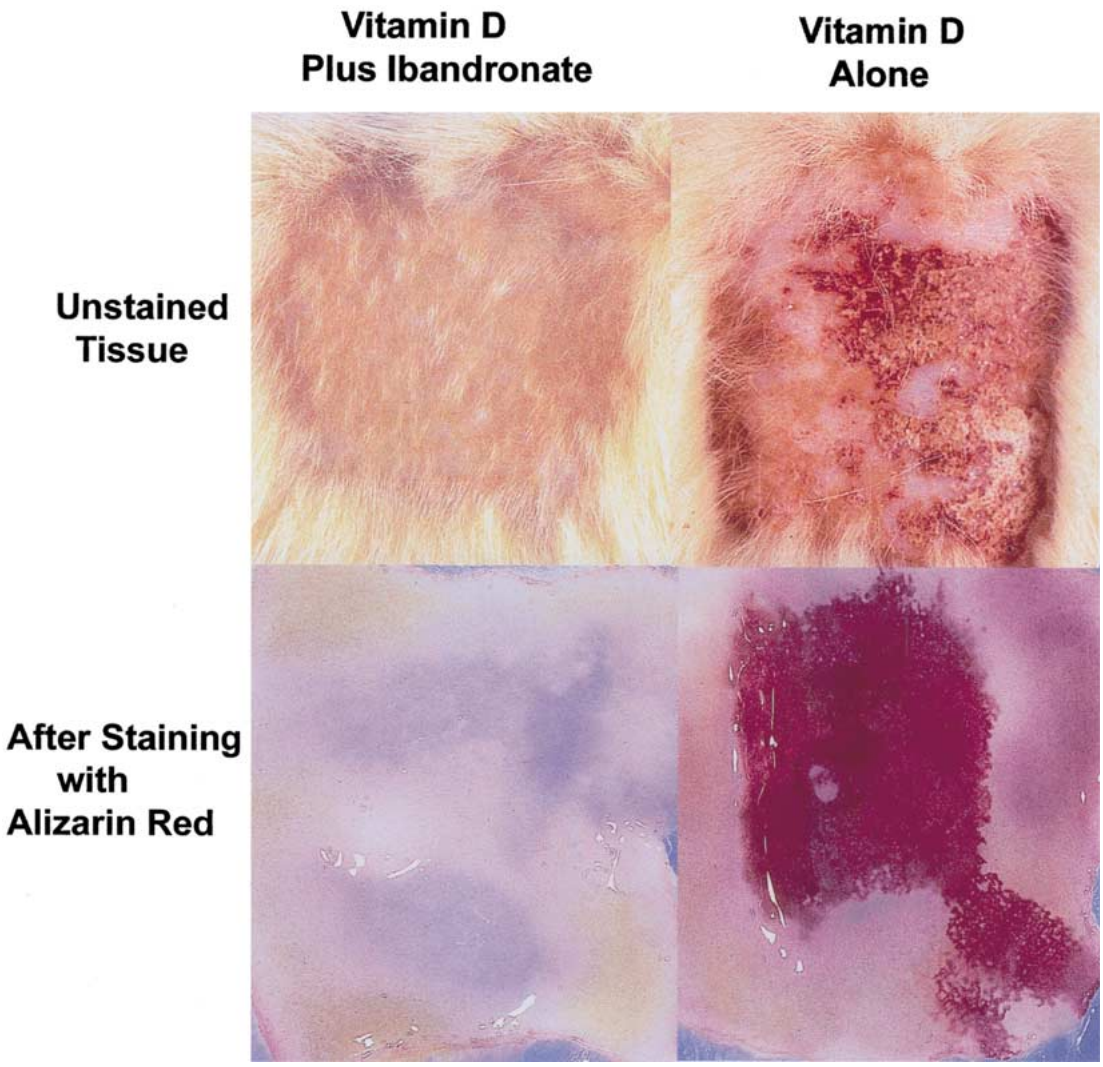

Fig. 4. Effect of ibandronate on skin calciphylaxis induced by epilation. Six 7-week-old male Sprague Dawley rats received subcutaneous injections of 400,000 IU of vitamin D_3/kg body weight at t = 0, 1, and 2 days. Three of these rats were also injected subcutaneously with ibandronate at a dose of 0.25 mg/kg/day beginning 4 days prior to the first vitamin D injection, and the remaining 3 rats received no ibandronate. Calciphylaxis was induced by plucking the hair from a 9 cm^2 area of skin in the intrascapular region of the anesthesized rat at 3 days after the first vitamin D injection. Rats were killed 7 days after epilation. The typical gross appearance of the epilation-induced skin calciphylaxis in each group is shown on the top, and the appearance of the same specimen after staining for calcification with Alizarin red S is shown on the bottom. Left, treatment with vitamin D plus ibandonate; right, treatment with vitamin D alone. Note the instance of skin calcification that has metastasized beyond the area of epilation in the specimen from the vitamin D-treated animal.

Effect of Ibandronate on Calciphylaxis Induced by Epilation

The second experiments were carried out to determine the effects of ibandronate on the skin calciphylaxis induced by epilation in rats sensitized by the prior injection of vitamin D. The vitamin D injection schedule used to sensitize rats for the calciphylaxis response in these experiments is identical to the injection schedule used in the $FeCl_3$-induced calciphylaxis studies above, and to that used in previous studies to induce artery calcification [2, 10]. The timing of calciphylactic challenge by epilation was again 24 hours after the last vitamin D injection, and the epilation was achieved by mechanically plucking the hair from a 9 cm^2 area of skin above the shoulders of the anesthetized rat. As seen in Figure 4, the calciphylactic response at the site of the epilation challenge is seen as raised areas of skin discoloration that are intensely stained for calcification with Alizarin red. These raised areas of skin are palpably hard. This calciphylactic response to epilation is identical to that previously reported in rats sensitized by prior vitamin D administration [3, 4]. In agreement with these earlier studies, no skin calcification was found at the site of epilation in animals that had not been sensitized by prior vitamin D treatment, and no skin calcification was seen in animals treated with vitamin D at

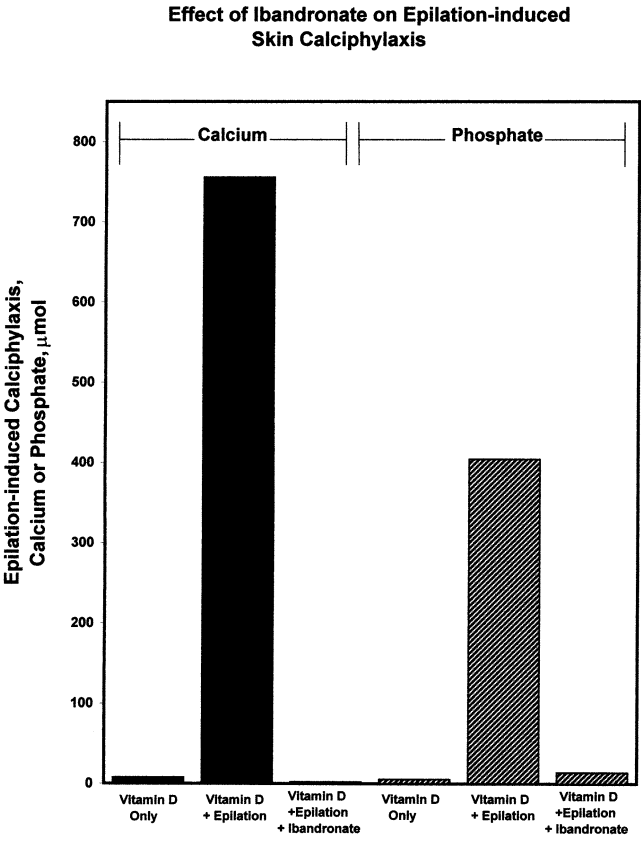

Fig. 5. Effect of ibandronate on the accumulation of calcium and phosphate at sites of epilation-induced skin calciphylaxis. Eighteen 7-week-old male Sprague Dawley rats received subcutaneous injections of 400,000 IU of vitamin D_3/kg body weight at t = 0, 1, and 2 days. Six of these rats were also injected subcutaneously with ibandronate at a dose of 0.25 mg/kg/day beginning 4 days prior to the first vitamin D injection. Calciphylaxis was induced by epilation 3 days after the first vitamin D injection, except for 4 vitamin D-treated rats that served as epilation-negative controls. Rats were killed 10 days after the first vitamin D injection and the region previously freed of hair was removed by dissection and extracted with acid to dissolve mineral. Acid extracts were analyzed for calcium and phosphate, and the means for each group are shown. The mean and SD for each group were: vitamin D only (n = 4), calcium 7.7 ± 2.4, and phosphate 5.5 ± 1.7; vitamin D plus epilation (n = 8), calcium 755.6 ± 292.9, and phosphate 404.5 ± 156.5; vitamin D plus epilation plus ibandronate (n = 6), calcium 2.2 ± 3.9, and phosphate 14.0 ± 3.4.

skin sites where hair had not been removed (data not shown).

The effect of ibandronate on epilation-induced skin calciphylaxis was examined with the ibandronate dose and injection schedule used in the $FeCl_3$-induced calciphylaxis experiments and in previous studies on the effect of ibandronate on vitamin D-induced artery calcification. As seen in Figure 4, ibandronate treatment eliminated all visible signs of calciphylaxis at the site of epilation, and completely eliminated the Alizarin red staining of the skin. Figure 5 shows that skin at the epilation site contained high levels of calcium and phosphate and that ibandronate treatment reduced the level of each mineral component to that seen in rats that were not subject to epilation. The level of calcium and phosphate in the skin from untreated control rats was not significantly different from that seen in vitamin D-treated rats that were not subjected to epilation (data not shown).

Dependence of Calciphylaxis, Artery Calcification, and Bone Resorption on Vitamin D Dose

Additional experiments were carried out to determine whether the dose of vitamin D needed to sensitize rats for calciphylaxis is comparable to the dose needed to induce artery calcification and to increase bone resorption activity. Animals were first treated with different vitamin D doses according to the same 3-day injection protocol used in the above experiments, and the calciphylaxis response to the $FeCl_3$ challenge and the level of artery calcification were evaluated in each animal. As can be seen in Table 2, the 100,000 IU/kg dose of vitamin D produced barely detectable calcification in the artery and at the $FeCl_3$ calciphylaxis site and no significant increase in bone resorption activity, whereas higher doses of vitamin D dramatically increased bone resorption activity and calcification at both sites. Serum calcium was elevated at all vitamin D doses and did not correlate with bone resorption activity or with the extent of either type of ectopic calcification (Table 2). The dissociation of serum calcium and bone resorption activity is further supported by the observation that serum calcium levels remained elevated at 10 days after the first vitamin D injection, whereas the serum NTx measure of bone resorption activity had returned to normal by this time (data not shown).

Discussion

The present studies show that the calcification of arteries and calcification at two different kinds of calciphylaxis sites are all comparably inhibited by doses of the amino bisphosphonate ibandronate that inhibit bone resorption, and that calcification at the calciphylactic site and in the artery have a similar dependence on vitamin D dose. These observations strongly support the hypothesis that there is a common underlying biochemical mechanism in the vitamin D-treated rat that is responsible for the calcification of arteries and other soft tissues as well as the calcification at calciphylaxis sites.

We speculate that the underlying biochemical mechanism that is responsible for the diverse array of soft tissue calcifications and calciphylactic responses in rats treated with toxic doses of vitamin D arises from bone resorption activity. This hypothesis is supported by the observation that doses of vitamin D that promote these types of ectopic calcification also potently stimulate

Table 2. Effect of vitamin D dose on artery calcification, calciphylaxis, bone resorption activity, and serum calcium

	Age-matched control ($\bar{X} \pm$ SD, n = 4)	100,000 IU D_3/kg ($\bar{X} \pm$ SD, n = 4)	200,000 IU D_3/kg ($\bar{X} \pm$ SD, n = 4)	300,000 IU D_3/kg ($\bar{X} \pm$ SD, n = 4)
Abdominal aorta				
Calcium, μmol	0.20 ± 0.01	0.28 ± 0.03[a]	2.01 ± 0.96[a]	4.83 ± 0.74[b]
Phosphate, μmol	0.03 ± 0.01	0.08 ± 0.04	1.23 ± 0.60[a]	3.16 ± 0.55[b]
Calciphylactic button				
Calcium, μmol	None present	46.2 ± 21.6	240.9 ± 155.7[c]	965.9 ± 398.3[d]
Phosphate, μmol	None present	30.9 ± 14.2	167.2 ± 103.8[c]	563.1 ± 203.0[d]
Serum				
Calcium, mg/dl	9.9 ± 0.3	12.8 ± 0.9[e]	15.1 ± 0.8[b]	14.3 ± 0.6[b]
Serum cross-linked teleopeptides, ng/ml	56.8 ± 6.4	58.0 ± 1.3	82.1 ± 14.5[f]	98.9 ± 13.8[e]

Twelve 7-week-old male Sprague Dawley rats received subcutaneous injections of the indicated vitamin D dose at t = 0, 1, and 2 days. Calciphylaxis was induced by subcutaneous injection of the 300 μg $FeCl_3$ challenger at two ventral sites 3 days after the first vitamin D injection. Blood samples were obtained 4 days after the first vitamin D injection and analyzed to determine serum levels of calcium, phosphate, and cross-linked N-teleopeptides (OSTEOMARK NTx, Ostex), a specific marker of bone resorption activity. Rats were killed 10 days after the first vitamin D injection and the abdominal aorta and calciphylactic buttons were removed by dissection and extracted with acid to dissolve mineral. Acid extracts were analyzed for calcium and phosphate. See Materials and Methods

[a] $P < 0.01$ vs age-matched control
[b] $P < 0.0001$ vs age-matched control
[c] $P < 0.005$ vs 100,000 IU D_3/kg
[d] $P < 0.0001$ vs 100,000 IU D_3/kg
[e] $P < 0.001$ vs age-matched control
[f] $P < 0.025$ vs age-matched control

bone resorption activity (Tables 1 and 2), and by the observation that treatment with the bone resorption inhibitor ibandronate normalizes bone resorption activity and prevents all vitamin D-induced calcifications [1, 2]. This hypothesis is also consistent with the finding that vitamin D-induced calcification of arteries, lungs, kidneys, tracheal cartilage, stomach, and intestine are each prevented by the inhibition of bone resorption using the cytokine osteoprotegerin [10].

The nature of the biochemical mechanism that is responsible for the putative linkage between bone resorption and soft tissue calcification in the vitamin D-treated rat is presently unclear. One possibility is that soft tissue calcification could be a direct physicochemical consequence of the effect of the observed vitamin D-induced hypercalcemia on the nucleation and growth of calcium phosphate mineral in soft tissues. This hypothesis is not, however, supported by the observations that serum calcium levels in animals treated with vitamin D plus ibandronate are not significantly different from those seen in animals treated with vitamin D alone in this (Table 1) or in previous experiments [1, 2]. This hypothesis is also not consistent with the present observation that increasing the vitamin D dose from 200,000 IU to 300,000 IU/kg caused a 2.5-fold increase in the accumulation of calcium and phosphate in the abdominal aorta but did not further increase the level of hypercalcemia (Table 2). Another possibility is that soft tissue calcification is promoted by crystal nuclei generated at sites of bone resorption which travel in blood and occasionally lodge in soft tissue structures [1]. This hypothesis is supported by the observation that, under some circumstances, a complex of a calcium phosphate mineral phase and the proteins fetuin and matrix Gla protein is released from bone and can be detected in blood [11], and by the observation that the release of this complex from bone is inhibited by inhibitors of bone resorption [12].

The term calciphylaxis has also been used to refer to the syndrome of ischemic ulceration of skin due to metastatic calcification of subcutaneous tissue and small arteries that occurs in uremic patients [13–15]. While there is as yet no biochemical evidence that the ectopic calcifications seen in uremic patients are caused by the same basic biochemical mechanisms that appear to be responsible for the diverse types of soft tissue calcification seen in rats treated with toxic doses of vitamin D, there are similarities that should be noted. Uremic calciphylaxis in human subjects and vitamin D-induced calciphylaxis in the rat are both characterized by massive calcification of the elastic lamellae in the media of arteries. Rats treated with toxic doses of vitamin D and human patients with end-stage renal disease are also both prone to extra-vascular calcification at a number of ectopic sites, suggesting that both could involve a systemic increase in the tendency of soft tissues to calcify. Finally, early studies by Selye [3] showed that uremia acts as a calciphylactic sensitizer in the rat with an activity similar to that of exogenous vitamin D or PTH, and that parathyroidectomy abolishes this ability of uremia to promote calciphylaxis [3]. We speculate that the calcification of arteries and other soft tissues found in uremic patients is linked to the increase in bone resorption that arises as a consequence of the secondary hyperparathy-

roidism often found in these patients. This observation is supported by the observation that uremic calciphylaxis is, in some patients, ameliorated by parathyroidectomy [16, 17]. An important future test of the hypothesis that soft tissue calcification is linked to bone resorption in the uremic patient will be to determine whether inhibitors of bone resorption will prevent soft tissue calcification induced by uremia.

Acknowledgments. This work was supported in part by Grant HL58090 from the National Heart, Lung, and Blood Institute of the National Institutes of Health.

References

1. Price PA, Faus SA, Williamson MK (2001) The bisphosphonates alendronate and ibandronate inhibit artery calcification at doses comparable to those which inhibit bone resorption. Arterioscler Thromb Vasc Biol 21:817–824
2. Price PA, Buckley JR, Williamson MK (2001) The amino bisphosphonate ibandronate prevents vitamin D toxicity and inhibits vitamin D-induced calcification of arteries, cartilage, lungs, and kidneys in rats. J Nutr 131:2910–2915
3. Selye H (1962) Calciphylaxis. University of Chicago Press, Chicago
4. Schibler D, Fleisch H (1966) Inhibition of skin calcification (calciphylaxis) by polyphosphates. Experientia 22:367–369
5. Price PA, Faus SA, Williamson MK (2000) Warfarin-induced artery calcification is accelerated by growth and by vitamin D. Arterioscler Thromb Vasc Biol 20:317–327
6. Chen PS, Toribara TY, Warner H (1956) Microdetermination of phosphorus. Anal Chem 28:1756–1758
7. Gorski JP, Apone S, Shaffer KA, Batchelder A, Jean W, Williams JA, Shacter E, Eyre DR (2000) Hypercalcemia during the osteogenic phase after rat marrow ablation coincides with increased bone resorption assessed by the NTx marker. Bone 27:103–110
8. Hanken J, Wassersug R (1981) A new double-stain technique reveals the nature of the hard tissues. Func Photog 16:22–26, 44
9. Rosa-Molinar E, Proskocil BJ, Ettel M, Fritzsch B (1999) Whole-mount procedures for simultaneous visualization of nerves, neurons, cartilage, and bone. Brain Res Protoc 4:115–123
10. Price PA, June HH, Buckley JR, Williamson MK (2001) Osteoprotegerin inhibits artery calcification induced by warfarin and by vitamin D. Arterioscler Thromb Vasc Biol 21:1610–1616
11. Price PA, Thomas GT, Pardini AW, Figueira WF, Caputo J, Williamson MK (2002) Discovery of a high molecular weight complex of calcium, phosphate, fetuin, and matrix Gla protein in the serum of etidronate-treated rats. J Biol Chem 277:3926–3934
12. Price PA, Caputo JM, Williamson MK (2002) Bone origin of the serum complex of calcium, phosphate, fetuin, and matrix gla protein: biochemical evidence for the cancellous bone remodeling compartment. J Bone Miner Res 17:1171–1179
13. Anderson DC, Stewart WK, Piercy DM (1968) Calcifying panniculitis with fat and skin necrosis in a case of uraemia with autonomous hyperparathyroidism. Lancet 2:323–325
14. Rees JKH, Coles GA (1969) Calciphylaxis in man. Bri Med J 2:670–672
15. Mathur RV, Shortland JR, El Nahas AM (2001) Calciphylaxis. Postgrad Med J 77:557–561
16. Hafner J, Keusch G, Wahl C, Sauter B, Hurlimann A, von Weizsacker F, Krayenbuhl M, Biedermann K, Brunner U, Helfenstein U (1995) Uremic small-artery disease with medial calcification and intimal hyperplasia (calciphylaxis): a complication of chronic renal failure and benefit from parathyroidectomy. J Am Acad Derm 33:954–962
17. Kriskovich MD, Holman JM, Haller JR (2000) Calciphylaxis: Is there a role for parathyroidectomy? Laryngoscope 110:603–607