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Alendronate inhibits osteopontin expression enhanced by parathyroid hormone-related peptide (PTHrP) in the rat kidney

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Abstract It has been reported that osteopontin (OPN) plays an important role during urolithiasis as well as bone formation. Generation of stones in the urinary tract may be associated with osteoporosis and bisphosphonates are potent inhibitors of bone resorption, being used with effect in the management of bone disease. We therefore investigated the relationship between alendronate, a bisphosphonate derivative, and OPN expression in the kidney. Alendronate was administered to rats made hypercalcemic by treatment with parathyroid hormone-related peptide (PTHrP). The renal expression of OPN was then evaluated at both protein and mRNA levels. OPN expression was enhanced in the distal tubular cells of hypercalcemic rats and was decreased by alendronate. The observed inhibition of OPN expression suggests an ability of alendronate and other bisphosphonates to act as inhibitors of stone formation in the urinary tract.

Key words Osteopontin · PTHrP · Bisphosphonate · Alendronate · Urolithiasis · Kidney

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Introduction

Urinary stones contain 1%–5% protein, and many reports have suggested the importance of proteins in stone formation [4]. Recently, we cloned and sequenced the cDNA encoding osteopontin (OPN), an important soluble stone protein component of calcium oxalate stone proteins extracted with 0.1 M EDTA [16]. Thus we found strong expression of OPN mRNA by distal tubular cells in the kidneys of stone-forming rats [17]. OPN is a highly acidic, 44-kDa glycosylated phosphoprotein, which is one of a group of noncollagenous bone matrix components, with the amino acid sequenced Arg-Gly-Asp (RGD) and high affinity to hydroxyapatite [3, 12, 22, 23]. Localization of OPN mRNA and protein in mouse and rat embryos indicates that the gene is expressed on some preosteoblasts and osteoblasts in the early stages of bone formation [22]. It has also been reported that certain nonbone tissues, specifically calcification tissues, such as the kidney and inner ear express OPN mRNA [22]. These reports suggested that the protein plays a role in modulating mineralization, cell attachment and pathological calcium deposition such as in urinary tract stones [12, 16, 17, 22]. We therefore suppose that by controlling OPN it might be possible to inhibit the propensity for stone formation.

Bisphosphonate is one of the carbon-substituted pyrophosphate analogues that include potent inhibitors of bone resorption [10], which have been effectively used in the management of bone disorders such as Paget's disease [15], malignant hypercalcemia [11], osteopaenia of immobilization [20], and postmenopausal osteoporosis [18]. While almost all factors and conditions studied to date have produced a stimulation of OPN synthesis, a marked suppression of OPN expression is observed with bisphosphonates in bone [32]. This selective effect thus blocks OPN stimulation of bone resorption [32]. Bisphosphonates might therefore also be expected to inhibit stone formation. In an in vitro model some complexes of metal ions with bisphosphonates

indeed exerted strong inhibitory effects in comparison with citrate complexes which had minimal influence [33]. However, the assay used cores of matrix components present in human calculi granules and limited the effects of the inhibitors to the surface of calculi.

Alendronate (4-amino-1-hydroxybutylidene-1,1-bisphosphonic acid) is a bisphosphonate causing marked inhibition of bone resorption [1, 28]. In the present study we focused on a stone matrix protein by investigating the relationship between alendronate and OPN expression in rat kidney. The experiment was designed to determine the effect of alendronate on the enhanced expression of OPN in kidneys of hypercalcemic rats induced by parathyroid hormone-related peptide (PTHrP).

Materials and methods

Hypercalcemia model (PTHrP-induced hypercalcemic rats)

All experimental procedures were performed in accordance with protocols approved by the Animal Care Committee of the Faculty of Medicine, Nagoya City University. We used 12-week-old male Sprague-Dawley (SD) rats, weighing approximately 410 g (Charles River Japan, Yokohama, Japan). Each animal underwent thyroparathyroidectomy (extirpation of the thyroid and parathyroid) or a sham operation under ketamine hydrochloride anesthesia (60 mg/kg body weight) injected intraperitoneally. The animals were observed for their postoperative condition, and blood samples were drawn from the venous plexus of the ocular fundus of surviving animals at the end of the second week after surgery to measure plasma calcium concentration. Thyroparathyroidectomy was considered to be successful in those rats with values of 7.0 mg/dl or less. The animals fulfilling this criterion were used in the study. A miniosmotic pump (Model 2001, Alzet, Palo Alto, Calif.) filled with bovine-PTHrP (20 pmol/μl, Bachem, Torrance, Calif.) was implanted into the neck region of each thyroparathyroidectomy rat (Fig. 1).

The animals were divided into five groups: (1) intact rats receiving sham operation and no drug (intact group, $n=6$); (2) rats undergoing thyroparathyroidectomy (TPTX group, $n=6$); (3) hypercalcemic rats receiving 20 pmol/hr infusion of PTHrP with an osmotic pump, 2 weeks after thyroparathyroidectomy (PTHrP group, $n=6$); (4) rats given daily (two times/day) intramuscular injections of elcatonin (16.0 U/kg per day) starting one day after PTHrP treatment (ELC group, $n=6$); and (5) rats receiving a single intravenous injection of alendronate (6.25 ng/kg) one day after PTHrP treatment (ALN group, $n=6$).

After blood samples had been collected from the ocular fundus all animals were killed under ether anesthesia on day 7, when both kidneys were excised. Samples of urine were obtained from each rat 2 days before death by individually housing the animals in metabolic cages for 24 h. Bone mineral density (BMD) was calculated at the time of death, using dual energy X-ray absorptiometry (QDR2000; Hologic). Measurements were made for lumbar vertebra (L2, L3, L4, and L5).

Alendronate was synthesized by Merck Research Laboratories (West Point, Pa.). Elcatonin, synthetic eel calcitonin, was commercially purchased from Asahi Chemical Industries (Tokyo, Japan).

Probe preparation

Digoxigenin-uridine triphosphate (UTP)-labeled single-strand sense and antisense mouse OPN RNA probes were prepared for hybridization using a DIG RNA labeling Kit (Boehringer Mannheim Biochemica, Mannheim, Germany) according to the manufacturer's instructions. For this purpose a 984-bp fragment of mouse 2ar (osteopontin) cDNA was subcloned into Bluescript pKS(−) plasmid [22]. The plasmid was then linearized with EcoRI and transcribed with T3 RNA polymerase to generate an antisense cRNA probe. Then, the plasmid was linearized with Xho1 and transcribed with T7 RNA polymerase to generate a sense cRNA probe.

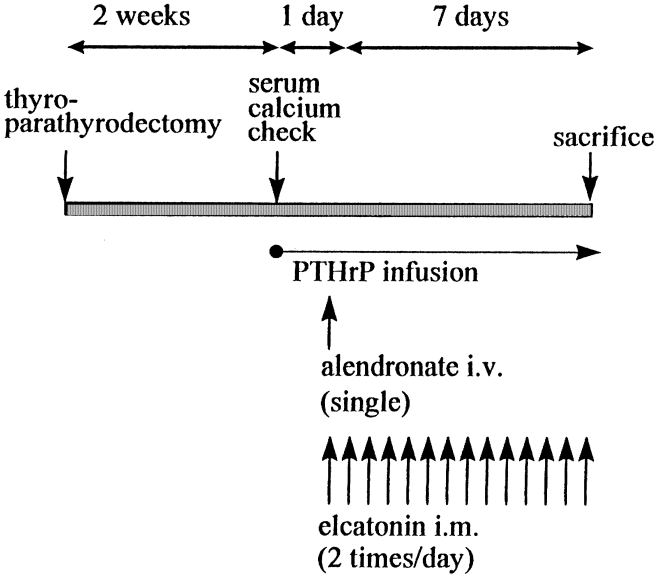

Fig. 1 Experimental animal protocol

In situ hybridization

Kidney tissue samples for in situ hybridization were fixed with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.0), dehydrated in ethanol and embedded in paraffin under RNase-free conditions. Serial sections, 4 μm thick, were cut. Details of the in situ hybridization technique used here have been described previously [22]. Hybridization solution containing approximately 0.5 μg/ml of cRNA probes was placed on each section, and hybridization was performed at 50°C for 16 h. Signals were detected with a Nucleic Acid Detection Kit (Boehringer). Controls included: (1) hybridization with sense (mRNA) probes; (2) RNase treatment before hybridization; and (3) use of antisense RNA or removal of anti-digoxigenin antibody. No positive signals were observed under any of these three conditions.

Northern blot analysis

Tissue samples for Northern hybridization were frozen with liquid nitrogen, and total RNAs were extracted from kidney tissues by the method of Chirgwin et al. [8] from the above group of rats. For Northern hybridization, total RNAs were fractionated on formaldehyde/agarose gel system, transferred to Hybond N^+ nylon membranes (Amersham, UK) and fixed to the filters by baking at 80°C for 3 h. The membranes were prehybridized for 3 h in prehybridization buffer and hybridized for 16 h in hybridization buffer at 65°C with digoxigenin (DIG)-UTP-labeled mouse OPN cRNA probe. Immunodetection of hybrized DIG-UTP-labeled RNA probe was performed using a DIG luminescent detection kit (Boehringer). After hybridization, the filters were washed and exposed to X-ray film to detect the chemiluminescence of 3-(2′-spiroadamanatate)-4-methoxy-4-(3″-phosphoryloxy)-phenyl-1,2-dioxetane (0.1 mg/ml, Tropix, USA) for 10 min at 37°C [14]. The same membrane was used for all probes. Equal loading of RNA was confirmed by staining of 28S and 18S RNA bands with ethidium bromide. The relative mobilities of 18S (2.2 kb) and 28S (4.7 kb) ribosomal RNAs are shown as size markers.

Immunohistochemical staining

Immunohistochemical staining was carried out on sections which were blocked with 3% H_2O_2 in methanol for 30 min, followed by washing in 0.01 M phosphate-buffered saline. To block nonspecific binding, all sections were treated with 1% normal rabbit serum for 30 min at room temperature. The slides were then incubated with the primary mouse monoclonal antibody MPIIIB10(1) (from Developmental Studies Hybridoma Bank, Iowa.) recognizing rat OPN protein for 30 min at room temperature. Antibody binding was demonstrated with an LSAB2 Kit, AP, Rat (DAKO, Calif.) for paraffin sections, following the manufacturer's instructions. Signals were respectively detected with the New Fuchsin Substrate System (DAKO) and a DAB chromogen. As negative controls, sections were incubated with nonimmune mouse serum instead of the primary antibodies, or with PBS, instead of the secondary antibody.

Statistical analysis

The data are expressed as mean ± standard deviation (SD), and Student's *t*-test for paired values was applied to assess the significance of differences.

Results

The serum calcium level of PTHrP infused animals (mean ± SD. 12.43 ± 0.76 mg/dl) was significantly increased as compared with the value for intact rats (10.23 ± 0.32 mg/dl) ($P < 0.05$) and alendronate, but not elcatonin, slightly decreased the increment (11.23 ± 0.71 mg/dl; $P = 0.058$) (Table 1). The 24-h urinary calcium excretion did not differ between the PTHrP and intact groups, and elcatonin and alendronate had no significant effect on urinary calcium excretion. Serum phosphorus did not vary significantly with any of the treatments and neither alendronate nor elcatonin altered the serum creatinine level. It could be concluded therefore that renal function was not affected. The BMD in the PTHrP group was significantly decreased, compared with the intact group and alendronate administration inhibited this BMD loss. BMD was not measured in ELC group.

The sites of OPN protein expression were found to be both the distal and proximal convoluted tubule, loops of Henle and collecting duct (mainly the distal tubular cells of the renal medulla) in intact kidneys (Fig. 2A). But glomeruli in the renal cortex were negative (Fig. 2A). In TPTX rats, OPN staining was only weak (Fig. 2B) but in hypercalcemic rats given PTHrP, there was a marked enhancement of expression (Fig. 2C). Although elcatonin administration did not affect the OPN staining (Fig. 2D), alendronate was decreased as compared with the PTHrP injection (Fig. 2E).

The expression sites of OPN mRNA demonstrated by in situ hybridization was the same as those for OPN protein revealed by immunohistochemistry (Fig. 3). In intact rat kidneys, OPN mRNA was detected in a small proportion of the distal tubular cells and proximal tubular cells of the renal medulla whereas glomeruli were negative (Fig. 3A). TPTX decreased the OPN mRNA expression (Fig. 3B). In contrast, rats with hypercalcemia induced by PTHrP demonstrated a marked increase (Fig. 3C). This was not altered by elcatonin (Fig. 3D) but was no longer as strong in rats given alendronate (Fig. 3E).

Examination of gene expression in rat kidneys by Northern blot analysis using OPN cRNA as a probe revealed a faint signal of approximately 1.6 kb in the intact rats (Fig. 4, lane 1). TPTX slightly decreased OPN expression (Fig. 4, lane 2). In PTHrP rats the level of OPN mRNA signals was clearly enhanced (Fig. 4, lane 3), and with no effect of elcatonin (Fig. 4, lane 4), and inhibition of the enhancement by alendronate (Fig. 4, lane 5).

Discussion

Certain noncollagenous and plasmaproteins, including OPN, sialoproteins, albumin and α_2HS-glycoprotein [3, 9], known to accumulate in bone and other mineralized tissues are also found in human kidney stones. Of these, OPN is the most prominent constituent of the calcium oxalate-associated crystal "ghosts" observed in the nuclei, lamellae, and striations of organic matrix material in deposits within epithelial cells [19]. This suggests that during stone formation, secretion of OPN and its subsequent incorporation into stone matrices may influence their nucleation and aggregative growth [19].

Table 1 Summary of serum values for calcium, phosphorus and creatinine, as well as daily urinary calcium excretion and bone mineral density (BMD) after 14 days of parathyroid hormone-related peptide (PTHrP) treatment. Data were expressed as mean ± standard deviation (SD) and the two-tailed Student's *t*-test for paired values was applied to assess the significance of differences. The thyroparathyroidectomy (TPTX) and PTHrP groups were compared with the intact group and the elcatonin-treated (ELC) and alendronate-treated (ALN) groups with the PTHrP group

	Serum calcium (mg/dl)	Serum phosphorus (mg/dl)	Serum creatinine (mg/dl)	Urinary calcium excretion (mg/24 hr)	Lumbar BMD (g/cm^2)
Intact	10.23 ± 0.32	7.03 ± 1.79	0.67 ± 0.12	0.82 ± 0.34	0.2654 ± 0.0180
TPTX	4.7 ± 0.92*	11.10 ± 2.62*	0.87 ± 0.21*	1.12 ± 0.98^{NS}	0.2484 ± 0.0072*
PTHrP	12.43 ± 0.76*	4.67 ± 0.31^{NS}	0.65 ± 0.21^{NS}	0.64 ± 0.54^{NS}	0.2244 ± 0.0117*
ELC	14.30 ± 3.42^{NS}	5.73 ± 1.04^{NS}	0.83 ± 0.15^{NS}	1.85 ± 0.69^{NS}	–
ALN	11.23 ± 0.71^{NS}	4.57 ± 0.15^{NS}	0.73 ± 0.06^{NS}	1.60 ± 0.71^{NS}	0.2510 ± 0.0205**

*$P < 0.05$ compared with intact rats, **$P < 0.05$ compared with PTHrP rats, ^{NS}not significant

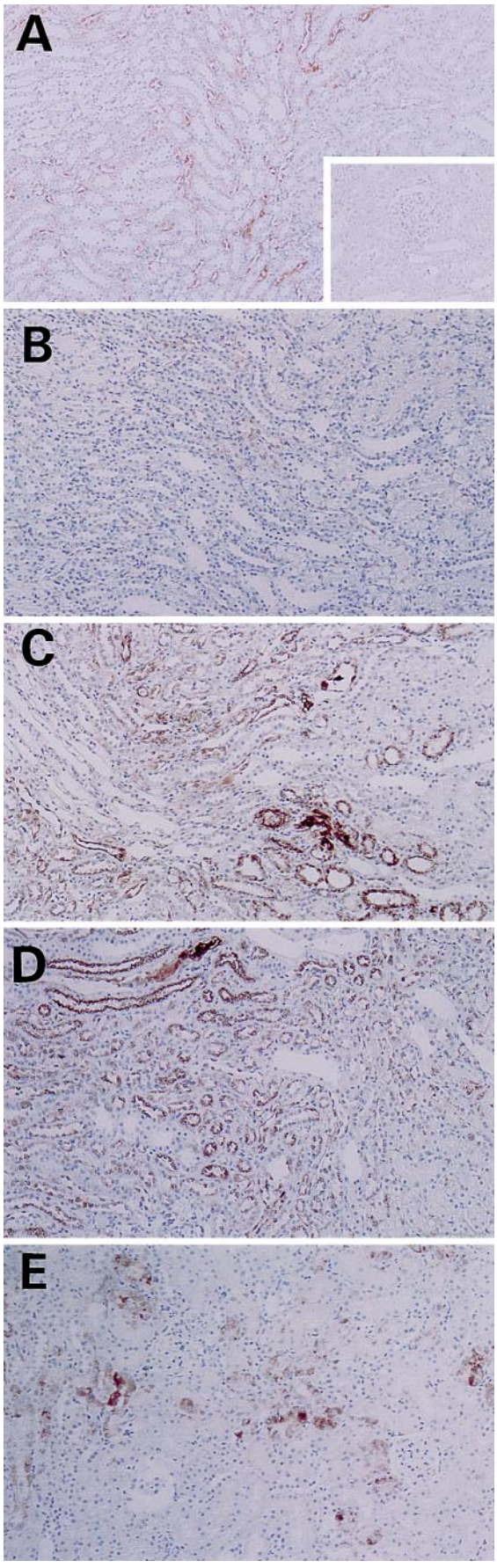

Fig. 2 A–E Immunohistochemical staining analysis to determine the cell types of ostepontin (OPN)-positive cells in rat kidney. OPN protein is especially present in distal tubular cells of the renal medulla, but not in glomeruli in the renal cortex. **A** intact rat, insert in **A** shows negative staining of a glomerulus, **B** TPTX rat, **C**, PTHrP rat, **D** ELC rat, **E** ALN rat. Original magnification × 100

In the present study alendronate decreased the serum calcium level in animals treated with PTHrP (Table 1) in line with the established use of bisphosphonates for the treatment of malignant hypercalcemia [11]. It also blocked the significant enhancement of OPN expression caused by PTHrP, as evident from investigation of both protein (Fig. 2) and mRNA (Fig. 3, Fig. 4) levels. This was in clear contrast to the lack of influence of elcatonin administration and suggests that it might act as an inhibitor of stone formation.

Bisphosphonates are potent inhibitors of bone resorption. The underlying mechanisms remain unclear, although several mechanisms have been proposed including cytoxic or metabolic injury to mature osteoclasts [6], inhibition of osteoclast attachment in bone [6], inhibition of osteoclast differentiation or recruitment [5], interface with osteoclast structural features necessary for bone resorption [29] or inhibition of osteoclast activity mediated by decreasing the stimulating activity of osteoblasts [27].

Wolf et al. [33] earlier showed that aggregation and growth of both calcium oxalate and calcium phosphate crystals are slowed by bisphosphonates. They suggested that the effects were due to adsorption of bisphosphonates to calcium at active growth sites on crystal surfaces.

The first bisphosphonate marketed for therapeutic use, etidronate, was found to decrease calcium oxalate crystalluria in individuals at high risk of stone formation [24]. Subsequent human trials were, however, disappointing due to incomplete dissolution of stones and/or excessive musculoskeletal side effects [2]. It was concluded that, as the high doses of etidronate required to constantly inhibit urine crystallization had adverse effects on bone, such therapy was not advisable for urolithiasis. On the other hand, alendronate was recently found to avert hypercalciuria and increase the solubility of stone-forming calcium salts during prolonged bedrest [25]. Guilland and Fleish [13] suggested that bisphosphonates act on calcium ion transport at cell membranes. Owing to the similarities in structure between several bisphosphonates and nucleoside phosphates, their putative targets could include kinases, such as phosphatidyl inositol-3-kinase, phosphatase or GTP-binding protein [21, 34]. Cells from osteopetrotic mice homozygous for c-*src* disruption have been shown, both in vivo and in vitro, to suppress the synthesis of OPN [7]. This provided the first evidence for a role of protooncogene c-*src* in regulation of OPN gene expression. Schmidt et al. [30] subsequently indicated that protein tyrosine phosphatases are involved in c-Src activation

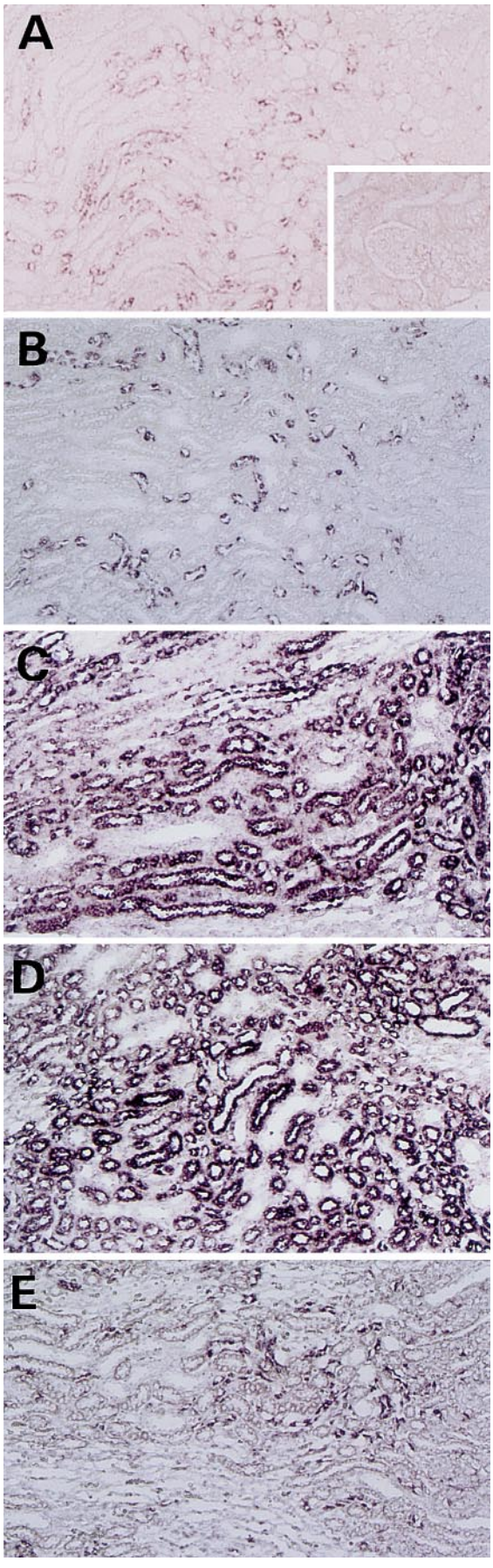

Fig. 3 In situ hybridization analysis of OPN mRNA in rat kidney. In situ hybridization analysis to determine the cell types of OPN mRNA-positive cells in rat kidney. Distal tubular cells and collecting duct cells of the renal medulla are sporadically positive, and proximal tubular cells and glomerula in the renal cortex are negative for OPN mRNA. **A** intact rat, insert in **A** showing no signals in a glomerulus, **B** TPTX rat, **C** PTHrP rat, enhancement of OPN mRNA expression is observed in distal tubular cells and collecting duct cells **D** ELC rat **E** ALN rat. Original magnification × 100

and suggested that specific forms may be the molecular targets of alendronate action. The possibility that similar molecular mechanisms could be occuring in distal tubular cells.

Shiraga et al. [31] isolated an inhibitor of calcium oxalate crystal growth from human urine by monoclonal antibody immunoaffinity chromatography and named it uropontin. The N-terminal sequence from residues 1–44 of uropontin showed complete homology with human OPN. Uropontin has an inhibitory effect on calcium oxalate crystal growth in vitro. OPN is also speculated to have an inhibitory effect on calcium oxalate crystallization in vitro, because it is abundant in acidic amino acids and binds firmly to hydroxyapatite. We extracted and identified OPN from renal stone proteins, and it will be important to determine what roles OPN plays in renal stone formation in vivo. Although uropontin is an inhibitor of calcium oxalate crystal growth in vitro, it may not play the same role in vivo. It has thus been hypothesized that in the specific matrix environment, it

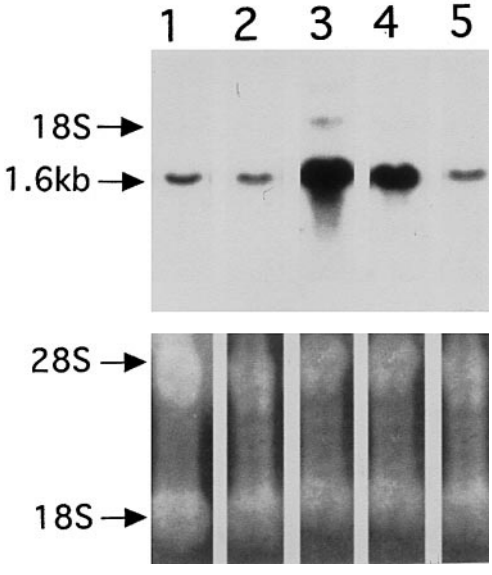

Fig. 4 Northern blot analysis of the level of OPN mRNA in intact rats (*lane 1*), in TPTX rats (*lane 2*), in PTHrP rats (*lane 3*), in ELC rats (*lane 4*), and in ALN rats (*lane 5*). Transcription of an approximately 1.6 kb mRNA was detected. Equal amounts of RNA were analyzed as is evident from the ethidium bromide-stained nitrocellulose blot. The mRNA probed for OPN (top) in relation to the 28S and 18S ribosomal RNA bands (bottom) are shown. *Arrows* indicate 18S, 1.6 kb and 28S and 18S

undergoes a configurational change in its hairpin structure whereby it gives the characteristic of enhancing crystallization, possibly by apposition of ions [26].

In conclusion, the present study demonstrated a significant increase OPN protein in distal tubular cells of hypercalcemic rats receiving PTHrP treatment. Alendronate administration inhibited the elevation at both protein and mRNA levels, suggesting that it might have the ability to act as a stone-forming inhibitor of urolithiasis.

References

1. Azuma Y, Sato H, Oue Y, Okabe K, Ohta T, Tsuchimoto M, Kiyoki M (1995) Alendronate distributed on bone surface inhibits osteoclastic bone resorption in vitro and in experimental hypercalcemia models. Bone 16: 235
2. Bone HG, Zerwekh JE, Britton F, Pak CYC (1979) Treatment of calcium urolithiasis with diphosphonate: efficacy and hazards. J Urol 121: 568
3. Boskey AL, Maresca M, Ullrich W, Doty SB, Butler WT, Prince CW (1993) Osteopontin–hydroxyapatite interaction in vitro: inhibition of hydroxyapatite formation and growth in a gelatin-gel. Bone Miner 22: 147
4. Boyce WH (1968) Organic matrix of human urinary concretions. Am J Med 45: 673
5. Cacchini MG, Felix R, Freisch H, Cooper PH (1987) Effect of bisphosphonates on proliferation and viability of mouse bone marrow-derived macrophages. J Bone Min Res 2: 135
6. Carano A, Teitelbaum SL, Konsek JD, Schlesinger PH, Blair HC (1990) Bisphosphonates directly inhibit the bone resorption activity of isolated avian osteoclasts in vitro. J Clin Invest 85: 456
7. Chackalaparampil I, Peri A, Nemir M, Mckee MD, Lin PH, Mukherjee BB, Mukherjee AB (1996) Cells in vivo and in vitro from osteopetrotic mice homozygous for c-src disruption show supression of synthesis of osteopontin, a multifunctional extracellular protein. Oncogene 12: 1457
8. Chirgwin JM, Przybyla AE, MacDonald RJ, Rutter WJ (1979) Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry 18: 5294
9. Dussol B, Geider S, Lilova A, Leonetti F, Dupuy P, Daudon M, Berland Y, Dagorn JC, Verdier JM (1995) Analysis of the soluble organic matrix of five morphologically different kidney stones. Urol Res 23: 45
10. Fleisch H (1987) Bisphosphonates: history and experimental basis. Bone 8: S23
11. Fleisch H (1991) Bisphosphonates: pharmacology and use in the treatment of tumor-induced hypercalcemia and metastatic bone disease. Drugs 42: 919
12. Franzen A, Heinegard D (1985) Isolation and characterization of two sialoproteins present only in bone calcified matrix. Biochem J 232**:** 715
13. Guilland DF, Fleish H (1974) The effect of in vivo treatment with EHDP and/or 1,25-DHCC on calcium uptake and release in isolated kidney mitochondria. Biochem Biophys Res Commun 61: 906
14. Hirota S, Itoh A, Nagoshi J, Takeda M, Kurata A, Takatsuka Y, Kohri K, Nomura S, Kitamura Y (1995) Expression of bone matrix protein messenger ribonucleic acids in human breast cancers: possible involvement of osteopontin in development of calcifying foci. Lab Invest 72: 64
15. Hosking DJ (1990) Advances in the management of Paget's disease of bone. Drugs 40: 829
16. Kohri K, Suzuki Y, Yoshida K, Yamamoto K, Amasaki N, Yamate T, Umekawa T, Iguchi M, Shinohara H, Kurita T (1992) Molecular cloning and sequencing of cDNA encoding urinary stone protein, which is identical to osteopontin. Biochem Biophys Res Commun 184: 859
17. Kohri K, Nomura S, Kitamura Y, Nagata T, Yoshioka K, Iguchi M, Yamate T, Umekawa T, Suzuki Y, Shinohara H, Kurita T (1993) Structure and expression of the mRNA encoding urinary stone protein (Osteopontin). J Biol Chem 268: 15180
18. Liberman UA, Weiss SR, Broll J, Minne HW, Quan H, Bell NH, Rodriguez-Portales J, Downs RW Jr, Dequeker J, Favus M, Seeman E, Recker RR, Capizzi T, Santora AC, Lombardi A, Shah RV, Hirsch LJ, Karpf DB (1995) Effect of oral alendoronate on bone mineral density and the incidence of fractures in postmenopausal osteoporosis. N Engl J Med 333: 1437
19. Mckee MD, Nanci A, Khan SR (1995) Ultrastructual immunodetection of osteopontin and osteocalcin as major matrix components of renal calculi. J Bone Miner Res 12: 1913
20. Minaire P, Depassio J, Bernard E, Meunier PJ, Edouard C, Pilonchery G, Goedert G (1987) Effects of clodronate on immobilization bone loss. Bone 8: S63
21. Nakamura I, Takahashi N, Sasaki T, Fukui Y, Udagawa N, Murakami H, Tanaka S, Kurokawa T, Suda T (1994) Wortmannin, an inhibitor of phosphatidyl inositol 3-kinase blocks the formation of ruffled border membrane in osteoclast. J Bone Miner Res 9: S155
22. Nomura S, Wills AJ, Edwards DR, Heath JK, Hogan BLH (1988) Developmental expression of 2ar (osteopontin) and SPARC (osteonectin) RNA as revealed by in situ hybridization. J Cell Biol 106: 441
23. Oldberg A, Franzen A, Heinegard D (1986) Cloning and sequence analysis of rat bone sialoprotein (osteopontin) cDNA reveals an Arg-Gry-Asp cell binding sequence. Proc Natl Acad Sci USA 83: 8819
24. Robertson WG, Peacock M, Marshall RW, Knowles F (1974) The effect of ethane 1-hydroxy-1,1-biphosphonate (EHDP) on calcium oxalate crystalluria in recent renal stone fomers. Clin Sci Mol Med 47: 13
25. Ruml LA, Dubois SK, Roberts ML, Pak CYC (1995) Prevention of hypercalciuria and stone-forming prolonged bedrest by alendoronate. J Bone Miner Res 10: 655
26. Saavedra RA (1994) The roles of autophosphorylation and phosphorylation in the life of osteopontin. Bioassays 16: 913
27. Sahni M, Guenther HL, Fleisch H, Collin P, Martin TJ (1993) Bisphosphonates act on rat bone resorption through the mediation of osteoblasts. J Clin Invest 91: 2004
28. Sato M, Grasser W (1990) Effect of bisphosphonates on isolated rat osteoclasts as examined by reflected light microscopy. J Bone Miner Res 5: 31
29. Sato M, Grasser W, Endo N, Akins R, Simmons H, Thompson DD, Golub E, Rodan GA (1991) Bisphosphonate action, alendronate localization in rat bone and effects on osteoclast ultrastracture. J Clin Invest 88: 2059
30. Schmidt A, Rutledge SJ, Endo N, Opas EE, Tanaka H, Wesolowski G, Leu CT, Huang Z, Ramachandaran C, Rodan SB, Rodan GA (1996) Protein-tyrosine phosphatase activity regulates osteoclast formation and function: inhibition by alendronate. Proc Natl Acad Sci USA 93: 3068
31. Shiraga H, Min W, VanDusen WJ, Clayman MD, Miner D, Terrell CH, Sherbotie JR, Foreman JW, Przysiecki C, Neilson EG, Hoyer JR (1992) Inhibition of calcium oxalate crystal growth in vitro by uropontin: another member of the aspartic acid-rich protein superfamily. Proc Natl Acad Sci USA 89: 426
32. Sodek J, Chen J, Nagata T, Kasugai S, Todescan Jr R, Li IWS, Kim RH (1995) Regulation of osteopontin expression in osteoblast. Ann N Y Acad Sci 760: 223
33. Wolf JS Jr, Stoller ML (1994) Inhibition of calculi fragment growth by metal-bisphosphonate complexes demonstrated with a new assay measuring the surface activity of urolithiasis. J Urol 152: 1609
34. Zeuzem S, Zimmermann P, Schulz I (1992) Association of 19- and a 21-kDa GTP-binding protein to pancreatic microsomal vesicles is regulated by the intravesicular pH established by a vacuolar-type H + -ATPase. J Membrane Biol 125: 231