

Phosphocitrate Blocks Calcification-Induced Articular Joint Degeneration in a Guinea Pig Model

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Objective. Calcium deposition occurs frequently in osteoarthritic (OA) joints. However, evidence for a causal role of calcification in cartilage degeneration is inferential. The present study was undertaken to examine the role of calcification in OA disease progression and to evaluate a formulation of phosphocitrate (PC) as a potential therapeutic agent.

Methods. We have identified a guinea pig OA model in which meniscal calcification appears to correlate with aging and disease progression. We synthesized a new formulation of PC, $[\text{CaNa}(\text{PC})_2(\text{H}_2\text{O})]_n$ (CaNaPC), which is a potent antiminerization agent and a specific inhibitor of crystal-induced biologic effects. After weekly treatment of guinea pigs with experimental OA with CaNaPC for 3 months, we examined calcification in menisci and cartilage degeneration. As a control, we examined whether similar CaNaPC treatment had any therapeutic effect in a hemi-menisectomy model in which there is no known crystal involvement.

Results. Meniscal calcification correlated with cartilage degeneration in this animal model. PC treatment led to significant reduction of calcium deposits and arrested OA disease progression. Similar treatment had no effect in the hemi-menisectomy model.

Conclusion. CaNaPC diminishes mineralization in a cutaneous calcergy model and a model of OA in which intraarticular mineralization is a prominent feature. In the OA guinea pig model, inhibition of calcifi-

cation is accompanied by diminished cartilage degeneration. CaNaPC has no therapeutic effect in the hemi-menisectomy model. We conclude that pathologic calcification may initiate or amplify processes leading to cartilage degeneration and that CaNaPC may interrupt such a pathway.

Osteoarthritis (OA) in humans can result from a number of factors, including genetic predisposition, joint trauma, or joint disuse. A potentially important, but still inadequately investigated, feature of articular cartilage and other articular tissue is its tendency to form calcium-containing crystals. These crystals precipitate in the extracellular matrix of the cartilage and can be released into the joint space (1–3), where they can interact with synovial cells and neutrophils and initiate cellular responses such as synthesis of matrix metalloproteinases (MMPs) and prostaglandin E_2 , leading to cartilage degeneration (4). Calcium deposition in cartilage is a widely recognized feature of OA (3). Articular calcification may lead to altered loading of the joint, causing injury to the cartilage matrix, which then fails under normal loading, causing chondrocytes to respond by elaborating MMPs and developing inappropriate repair responses (5). A general treatment strategy should therefore include the formulating of compounds that block new crystal formation and crystal-stimulated intracellular responses (4,6).

Our present understanding of the mechanisms of pathologic calcification is limited; therefore, there is no reliable method to prevent calcific deposition (7). If crystals promote chondrolysis, then removal of crystals from the joint would be therapeutic. This can be accomplished in gout, where a systemic accumulation of urate can be targeted and eliminated with hypouricemic therapy. Unfortunately, no systemic defect underlying most cases of calcium-containing crystal deposition has been recognized.

Once crystals form, they may be solubilized by

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enzymatic or chemical methods, e.g., magnesium (Mg^{2+}) promotes dissolution of calcium pyrophosphate dihydrate (CPPD) and basic calcium phosphate (BCP) crystals. Oral Mg^{2+} has been reported to lessen symptoms in CPPD crystal deposition disease, although no proof of decreased deposits was identified (8). Alkaline phosphatase has significant pyrophosphatase activity, dissolving CPPD crystals *in vitro*; the same effect would be expected if alkaline phosphatase were inducible *in vivo* (9). However, with treatments directed at dissolution of crystals there is a risk that dissolution will be only partial, with shedding of crystals from the cartilage into the synovial fluid. Predictably, this resulted in acute attacks of pseudogout when Mg^{2+} and EDTA lavage of joints containing crystals was attempted (10).

Phosphocitrate (PC) is a naturally occurring compound found in mammalian mitochondria (11). Tew et al speculated that PC prevents $CaPO_4$ precipitation in cells or cellular compartments maintaining high concentrations of Ca^{2+} and PO_4 (11). Moro and colleagues suggested that PC arises from the cytosolic phosphorylation of citric acid (12), which explains why it is nontoxic and not harmful to the environment. Results of *in vitro* studies have indicated that PC in concentrations of up to 1.5 mM (4.5 mg/ml) does not affect normal basal cellular functions, including DNA and protein synthesis (13–15). PC specifically inhibits crystal-induced MMP synthesis and mitogenesis in cells, while it has no effect on similar processes induced by growth factors or serum (7,13,16–18). This blocking effect is likely explained by the influence of PC on the interaction of calcium crystals with biomembranes (19–22). PC is a potent *in vitro* inhibitor of hydroxyapatite crystal formation (23). It prevents soft tissue calcification *in vivo* and does not produce any significant toxic side effects in rats or mice when given in dosages of up to 150 mmoles/kg/day (24,25).

PC specifically inhibits crystal-induced protooncogenes, MMP synthesis, mitogenesis, signal transduction, and cyclooxygenase synthesis (4,15,26,27), but exerts no effect on similar processes induced by growth factors or serum in cultured cells. Although PC does not have any effect on basal or transforming growth factor β -induced inorganic pyrophosphate elaboration and NTPPPH activity, it blocks calcification in matrix vesicles and cartilage in an *in vitro* model of chondrocalcinosis (14), nitric oxide-induced calcification of cartilage, and apoptotic bodies (28). In short, PC is the only agent examined to date that blocks the deleterious biologic effects of crystals and also prevents calcification (7).

Recently, a new chemical formulation of PC, $[CaNa(PC)_2(H_2O)]_n$ (CaNaPC), a mixed salt of calcium

and sodium of PC, has been synthesized (29). Like its precursor, NaPC, CaNaPC is a potent and specific inhibitor of the biologic effects of calcium-containing crystals, but CaNaPC is a significantly more potent antimineralization agent. This increased potency to block biomineralization and the biologic effects of calcium crystals makes PC a potential therapeutic agent for crystal deposition diseases.

Hartley strain guinea pigs develop an arthropathy that histologically mimics human OA. The joints in this animal model have been characterized both histologically (30,31) and radiographically (32). OA begins in the knee joints of the Hartley guinea pig at ~3 months of age, reaching an advanced stage by age 12 months. Histologically, the OA is characterized by chondrocyte and proteoglycan loss, fibrillation, chondrocyte cloning, osteophyte formation, and subchondral sclerosis (31). By 12 months of age, extensive degeneration of the articular cartilage of the central medial tibial plateau and femoral condylar and meniscal cartilage has occurred. Huebner et al reported that both MMP-1 and MMP-13 play an active role in the cartilage degeneration in this animal model (33). Significant calcification of medial menisci appears to correlate with disease progression and aging (5).

In addition to being a model of OA, the Hartley guinea pig model may reflect, in some aspects, human chondrocalcinosis and BCP crystal deposition diseases. In both diseases, there is mineralization of the articular cartilage and menisci, synovial hypertrophy, and cartilage thinning. Most patients also have OA (1,2). It is possible that the increased mineralization observed in humans with chondrocalcinosis and BCP crystal deposition disease could result in altered joint biomechanics, ultimately resulting in cartilage destruction.

After examination of 215 human cadavers, McCarty et al (34) reported that calcification of menisci was quite common. They found BCP deposits in the outer two-thirds and CPPD in the inner one-third of menisci. Since there is no perfect animal model for calcium crystal deposition disease, we selected the guinea pig model to examine the role of calcium crystals in OA and the use of PC as a potential therapeutic agent. If CaNaPC can block calcification, promote dissolution, and interfere with the untoward biologic consequences of crystal-cell interactions, a salutary effect on disease would be anticipated.

MATERIALS AND METHODS

All animals (male Hartley guinea pigs and New Zealand white rabbits) were housed and cared for in accordance

with the approved protocols for use of laboratory animals at the Miami Veterans Administration Medical Center. Guinea pigs were killed by inhalation of 100% CO₂. After CO₂ exposure, the chest cavity was surgically opened to ensure no recovery. Rabbits were killed with sodium pentobarbital solution (0.5 ml/kg) administered via heart puncture.

Gross anatomic assessment. Upon removal, all cartilage surfaces of the knees were examined grossly after coating of the surfaces with carbon black, to determine the extent of degeneration, pitting, and ulcer formation as previously described (35). The menisci, tibial plateaus, and femoral condyles with cartilage and subchondral bone were harvested.

Microscopic anatomic assessment. The specimens were immediately placed in 10% neutral buffered formalin before decalcification in Cal-Ex II (Fisher Scientific, Fair Lawn, NJ). After decalcification, the specimens were placed in paraffin blocks and sectioned. Slides were stained with Safranin O, counterstained with fast green, and graded according to the 14-point Mankin scale (36). Slides were labeled in a coded manner. Two separate and independent readers without knowledge of the treatment categories recorded histologic findings. Results from the 2 slides per animal and 2 readers were averaged.

Modified model of calcergy in the guinea pig. The efficacy of the PC formulations to inhibit pathologic mineralization *in vivo* were first tested in a modified animal model of subcutaneous calcergy (37). The modification substituted 3-month-old guinea pigs for rats (24). Briefly, the guinea pigs were injected at 2 dorsal subcutaneous sites with 0.2 ml of 0.1% (weight/volume) potassium permanganate solution to induce calcergy. Following injection, treatment groups (3 guinea pigs per group) were given various formulations of PC intraperitoneally (IP), as described in Results. The control group received phosphate buffered saline (PBS). After 7 days, the animals were killed and their calcific plaques were removed and weighed. Plaque samples were digested for 5 hours at 75°C with a perchloric acid/hydrogen peroxide mixture, and calcium and phosphate content was measured (38).

Rabbit partial meniscectomy model of OA. Twelve 6-month-old New Zealand white rabbits underwent right medial hemi-meniscectomy as described by Moskowitz et al (39,40). Briefly, OA of the right stifle joint was induced in rabbits weighing ~3 kg each, by partial medial meniscectomy. Animals were anesthetized with a mixture of xylazine HCl-ketalar-acepromazine 1 (3:3:1; 0.1 ml/kg) given intramuscularly. A proximal-distal vertical incision through the skin over the medial right knee was made, followed by dissection and exposure of the medial meniscus from the anterior lateral insertion to the medial collateral ligament. The exposed meniscus was freed of its capsular attachment and the lateral attachment of the medial meniscus; a small portion of the lateral anterior horn of the medial meniscus was removed and discarded. The remaining freed meniscus was reinserted into the medial compartment of the stifle; in layers, the soft tissues were returned to their original position and held in place by sutures. The skin was closed with hidden resorbable sutures.

After surgery, the rabbits were divided into 2 groups ($n = 6$ each). The OA group received no treatment, while the PC treatment group received a weekly IP injection of CaNaPC (40 mg/kg). A third group of animals (negative controls; $n = 6$) had no surgery and no PC treatment. The animals were killed

at 12 weeks postsurgery, and joints were removed for gross and microscopic examination.

Calcium content on menisci. Excised menisci were stored in chloroform/methanol (1:1 [volume/volume]) and, prior to analysis, were dried at 80°C. Calcium content was analyzed by the chloroanilate method as previously described (38), but modified for microscopic determination of calcium in tissue samples. The calcified head of each meniscus was dissected under a microscope. The sample was then subjected to acid hydrolysis (500 μ l of 1N HCl) in a Pyrex tube under vacuum for 6 hours at 100°C. An aliquot of the hydrolysate (50 μ l) was removed to an Eppendorf vial for processing. The acid was neutralized with 1N NaOH (50 μ l), and a final volume of 600 μ l obtained by additions of 100 μ l 1M sodium acetate (pH 5.0) and 400 μ l water. Chloroanilic acid solution (250 μ l of reagent prepared as a 1% solution in alkali and filtered before use) was added with vigorous mixing, and the reaction was allowed to continue for 1 hour. Thereafter, the calcium chloroanilate was collected by centrifugation (10,000g for 5 minutes), and the precipitate was washed twice in 1.0 ml isopropanol/water (1:1 [v/v]), with recentrifugation before dissolving in 2 drops of 5% EDTA. The solubilized material was then added to 5.0 ml aqueous 0.6% FeCl₃, and the developed chromophore assessed spectrophotometrically at 490 nm. The calcium concentration in the samples was obtained by reference to a standard calcium solution in the range of 0–100 μ g.

Synthesis of NaPC and CaPC. CaNaPC and NaPC were synthesized according to previously described procedures (29,41).

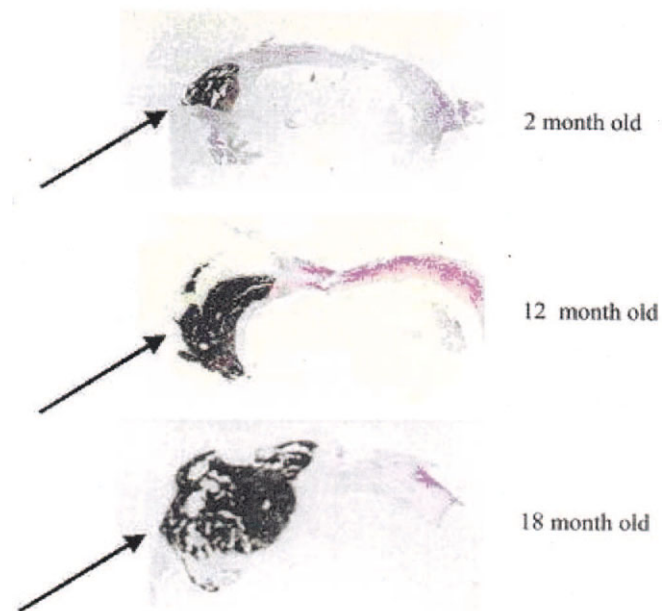


Figure 1. Histologic findings in cross-sections of menisci from 2-month-old, 12-month-old, and 18-month-old guinea pigs. Mineral deposits are indicated as dark brown staining (von Kossa's specific staining for calcium) (arrows). Mineralization appears to correlate with the progression of age and disease in this guinea pig model of osteoarthritis (original magnification $\times 50$).

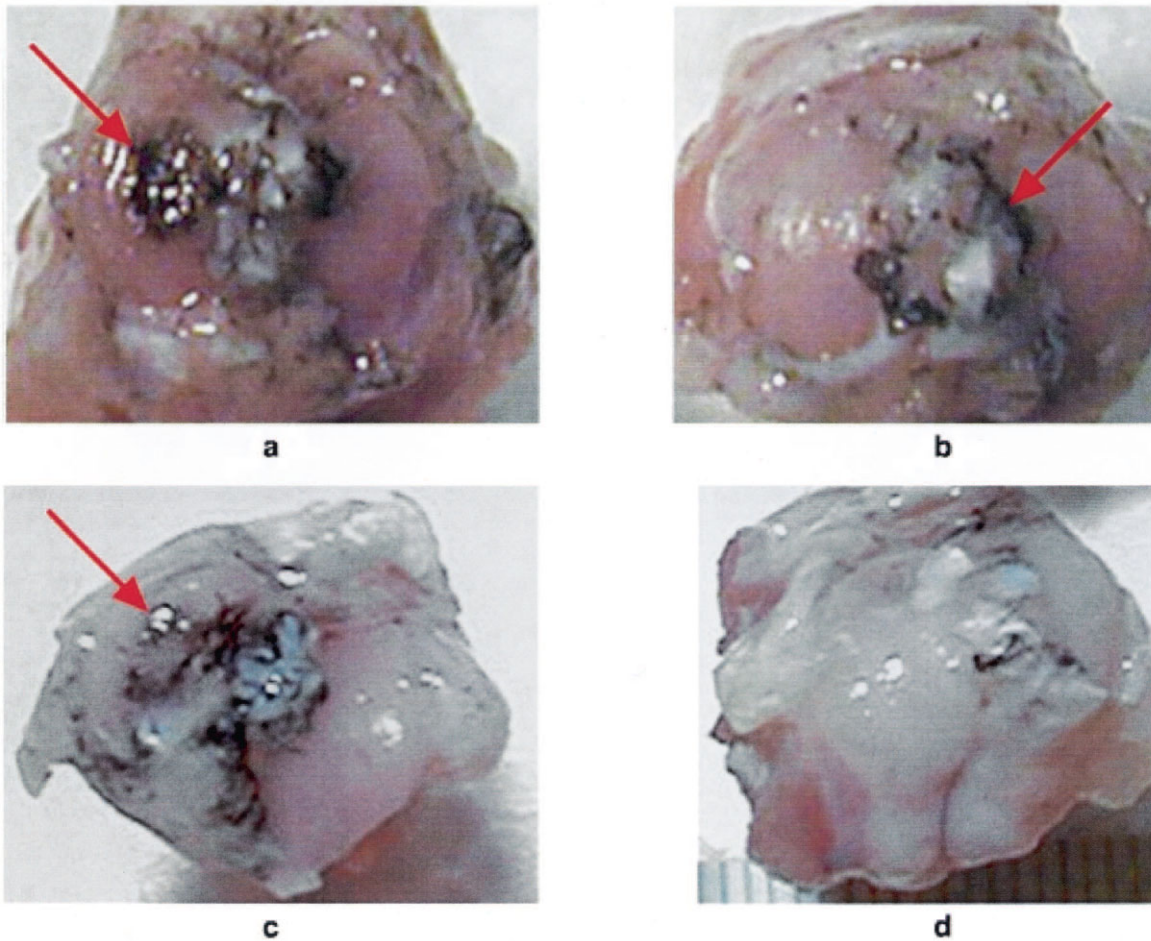


Figure 2. Gross findings at the cartilage surface after coating with carbon black. **a**, Control femoral condyle. **b**, CaNaPC-treated femoral condyle. **c**, Control tibial plateau. **d**, CaNaPC-treated tibial plateau. The cartilage surface in CaNaPC-treated animals is white and glistening, with few erosions, little carbon black retention, and little synovial thickening. Control cartilage demonstrates a discolored surface, surface ulcerations, pitting lesions, retention of carbon black staining, and erythematous, thickened synovium. **Arrows** indicate damaged areas coated with carbon black on the surfaces.

Statistical analysis. All experiments were run in quadruplicate and repeated at least twice. Values are expressed as the mean \pm SEM.

RESULTS

While there was no evidence of calcification in hyaline articular cartilage, substantial calcium deposits were found in the medial menisci of 2-month-old, 12-month-old, and 18-month-old guinea pigs. Based on Ca^{2+} and PO_4 analysis of the calcium deposits in menisci, the ratio of Ca^{2+} to PO_4 was 1.45, suggesting that the deposits were likely to be BCP crystals. Calcification and degeneration of menisci appeared to increase significantly with age. More than 60% of the medial

menisci of the 18-month-old animals were covered with calcification (Figure 1), consistent with earlier observations (5).

To establish a possible efficacious dosage of CaNaPC in guinea pigs and to use the minimum number of animals necessary, the calcergy assay (37) was used. This assay is a well-accepted model used to test the effect of antimineralization agents, including PC, on calcification (24,29,42), with reasonable correlation with findings in *in vivo* studies (42,43). The calcergy assay has a 7-day turnover time rather than months, as in the guinea pig OA model. Weekly IP injections of 10 mg, 20 mg, and 40 mg CaNaPC per kg body weight resulted in ~30%, 60%, and >80% inhibition, respectively, of

calcified plaque size and weight, as compared with untreated controls, while similar weekly injections of 80 mg NaPC per kg body weight resulted in <50% inhibition. These results confirmed the observation that CaNaPC is a more potent antiminerallization agent than its precursor, NaPC (29). The 40 mg CaNaPC per kg body weight was established as the dosing regimen to be used in the present study.

Two groups of 3-month-old guinea pigs ($n = 16$ animals per group) were used in the study. One group of

animals received weekly IP injections of CaNaPC (40 mg/kg), and the other (controls) received injections of PBS. Animals were killed after 3 months of treatment with either CaNaPC or PBS. The hind legs were removed, and the joints opened for gross examination. Cartilage surfaces of the knees were examined grossly after being coated with carbon black, to determine the extent of degeneration, pitting, and ulcer formation, as previously described (35).

CaNaPC-treated cartilage surface was white and

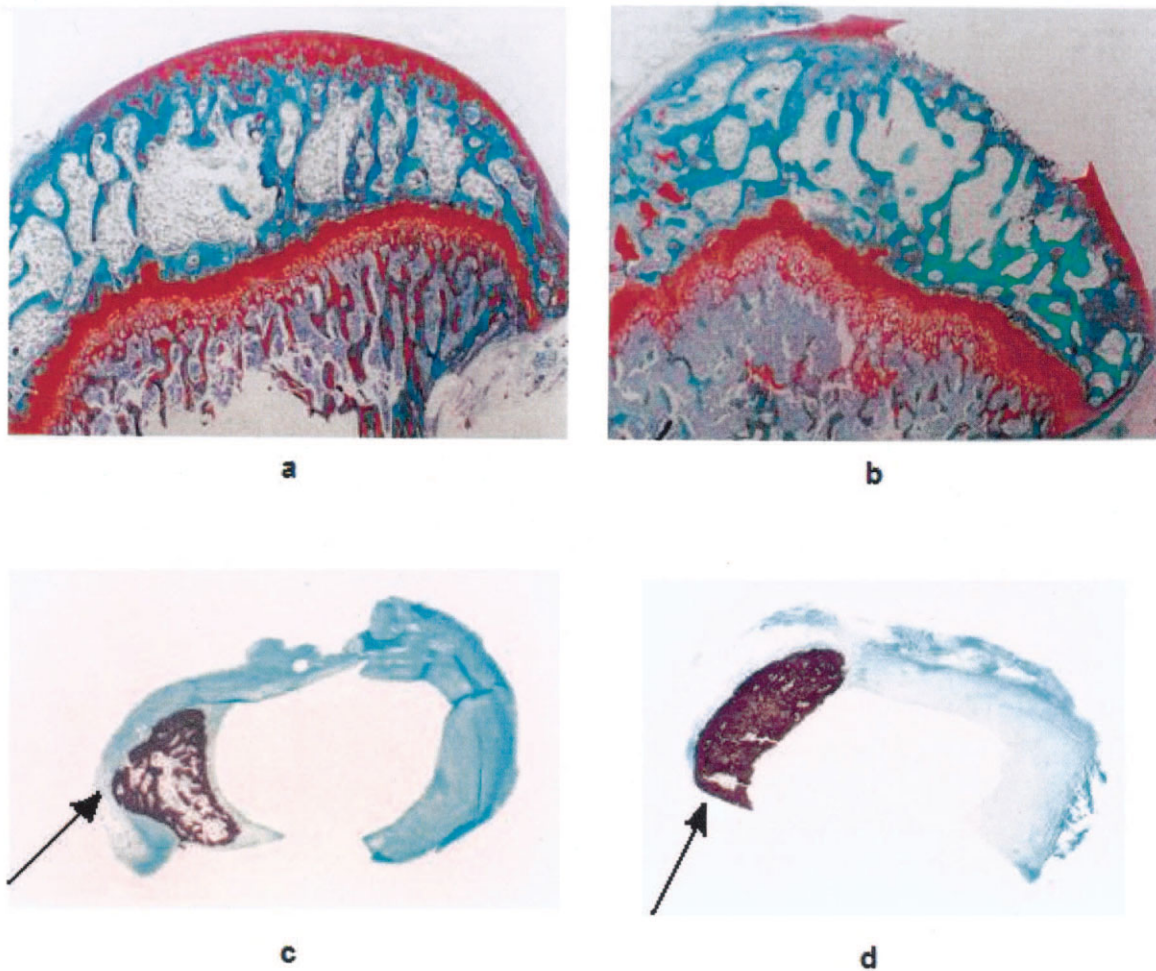


Figure 3. Histologic findings demonstrating the efficacy of CaNaPC treatment in the guinea pig model of osteoarthritis. Animals were killed after 3 months of saline treatment (control) or treatment with CaNaPC (40 mg/kg/week intraperitoneally). Calcified deposits were significantly decreased in the menisci of treated animals compared with controls, and the femoral condyle cartilage appeared normal in treated animals, while the femoral condyle cartilage of controls was eroded or badly fibrillated. **a**, Tibial plateau of a 6-month-old guinea pig treated with CaNaPC. **b**, Tibial plateau of a 6-month-old untreated control guinea pig. **c**, Cross-section of the meniscus of a 6-month-old guinea pig treated with CaNaPC. Note the significant reduction of the calcified deposits (dark brown color). **d**, Cross-section of the meniscus of a 6-month-old control guinea pig. **Arrows** indicate calcification in the meniscus, which is massive in the control animal as compared with the treated animal. (Original magnification $\times 50$.)

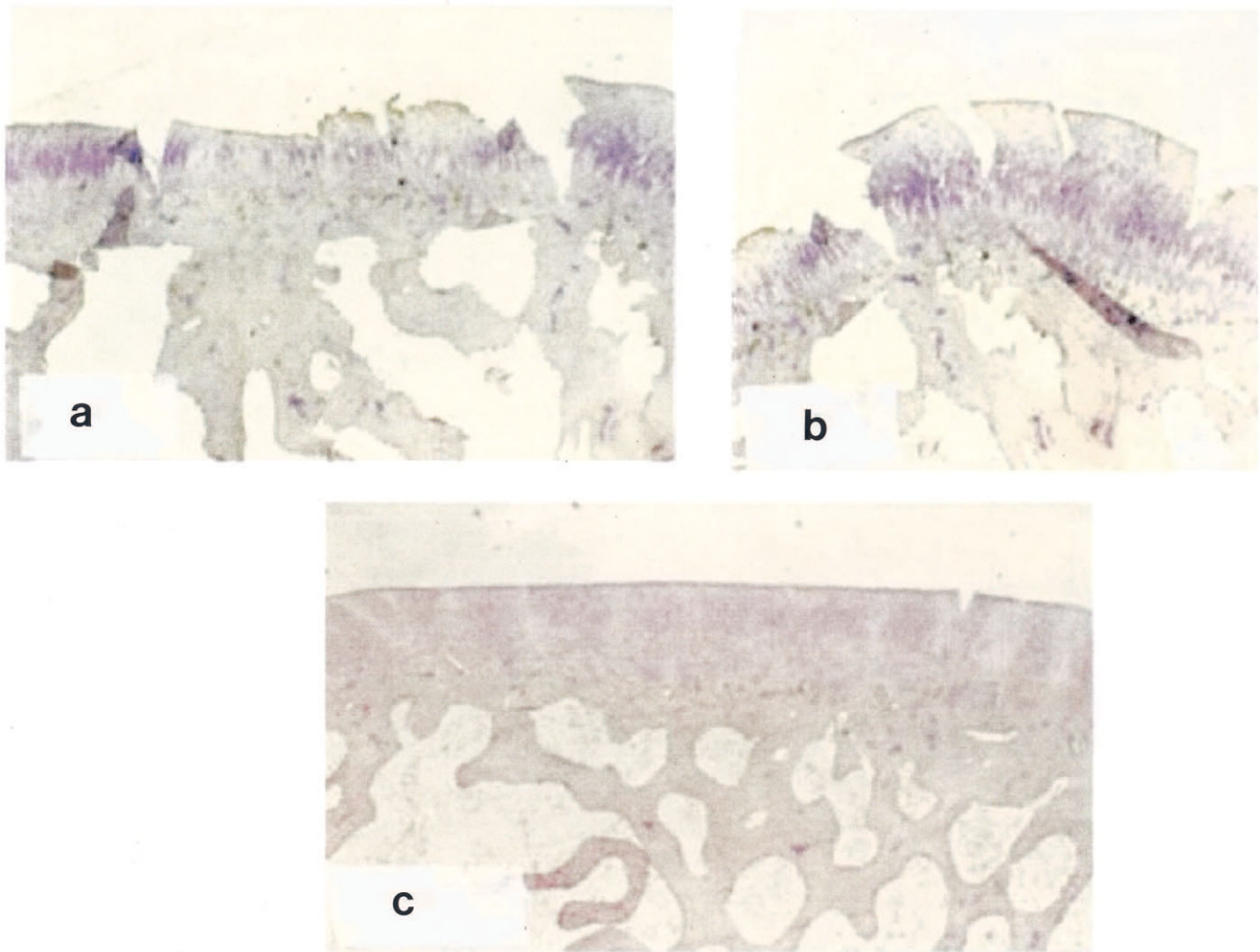


Figure 4. Histologic findings demonstrating the lack of therapeutic effect of CaNaPC in the rabbit partial meniscectomy osteoarthritis model, in which there is no known association with articular calcification. **a**, Tibial plateau of a saline-treated control rabbit. **b**, Tibial plateau of a CaNaPC-treated rabbit. **c**, Tibial plateau of an untreated control rabbit that did not undergo surgery. (Original magnification $\times 50$.)

glistening, with few erosions, little carbon black retention, and little synovial thickening (Figures 2b and d). Cartilage from all of the PBS-treated animals exhibited discolored surface, surface ulcerations, pitting lesions, retention of carbon black staining, and an erythematous, thickened synovium (Figures 2a and c).

The Mankin 14-point grading system was used to evaluate cartilage degeneration (36). By histochemical examination, CaNaPC-treated cartilage appeared normal (Figure 3a), with a mean \pm SEM Mankin score of 1.6 ± 0.8 . In contrast, control cartilage was either eroded or badly fibrillated (Figure 3b), as demonstrated by a Mankin score of 6.3 ± 1.4 ($n = 4$ animals per group) ($P < 0.01$).

A significant decrease ($P < 0.01$) in calcific deposits was found in CaNaPC-treated animals compared with control animals. Based on the calcium content of the menisci, CaNaPC treatment resulted in an $\sim 50\%$ reduction of calcific deposits. The mean \pm SEM calcium content of menisci isolated from treated animals was $498 \pm 133 \mu\text{g}$, while that of menisci from control animals was $970 \pm 221 \mu\text{g}$ ($n = 6$ animals per group) ($P < 0.01$). Histochemical examination with the calcium-specific von Kossa's stain confirmed these observations (Figures 3c and d). In treated animals the horn of the menisci appeared to be intact, while in PBS-treated animals it was badly fibrillated (Figures 2c and d).

We next addressed whether the therapeutic effect

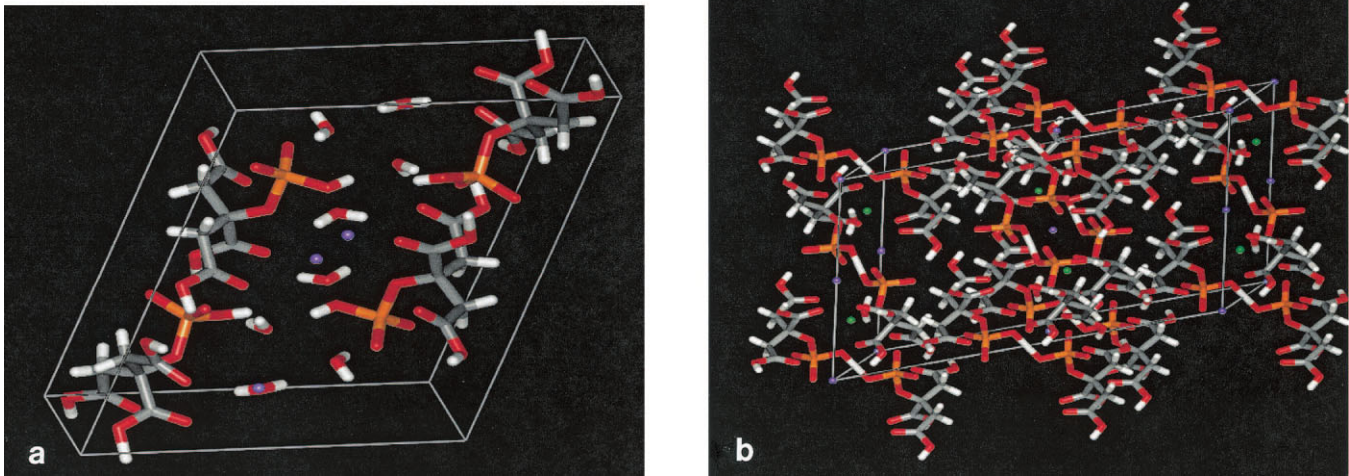


Figure 5. Structure of NaPC and CaNaPC. **a**, Unit cell of NaPC, as determined by single crystal-derived x-ray analysis (21). Na⁺ ions are shown as purple spheres. **b**, Unit cell of CaNaPC, as determined from data derived from ref. 29. Ca²⁺ and Na⁺ ions are shown as green and purple spheres. Polyhedrons delineate the unit cell of the crystal.

of CaNaPC on cartilage degeneration may be due to other, as-yet-unidentified, effects on chondrocytes, independent of the antimineralization and specific inhibitory effect on calcium crystal-induced cellular responses. We examined the effect of PC in an animal model of OA that has no known crystal involvement (44). Briefly, 2 groups ($n = 6$ each) of 6-month-old New Zealand white rabbits were subjected to right medial hemimiscectomy. One group received no treatment, and the other received weekly IP injections of CaNaPC (40 mg/kg). A third group (negative controls; $n = 6$) had no surgery and no PC treatment. Tissues from the articular joints were examined for anatomic and histologic changes at 12 weeks. The mean \pm SEM Mankin scores were 9.8 ± 1.7 in the untreated hemi-miscectomized group, 8.1 ± 2.2 in the CaNaPC-treated hemi-miscectomized group, and 1.0 ± 0.2 in the negative control group. Histologic analysis confirmed that there was no difference between the PC-treated and untreated OA groups (Figures 4a–c). Based on these findings, we concluded that PC has no therapeutic effect on cartilage degeneration in OA that is not associated with calcium-containing crystals.

DISCUSSION

The currently held concept of osteoarthritis is that there are 2 general pathways leading to development of the disease. The first involves cartilage with fundamentally defective biomaterial properties that cause the cartilage matrix to fail under normal loading of the joint, directly or indirectly leading to OA. The

second is based on the concept that physical forces have a major role in causing damage to normal articular cartilage matrix, with 2 subpathways involved. First, physical forces cause direct injury to matrix. Second, the same forces cause injury to chondrocytes embedded in the matrix. Over the course of time, these chondrocytes react to injury by elaborating degradative enzymes and developing inappropriate responses (45).

In the guinea pig model, the age-related increase in calcification of the medial meniscus could result from dramatically different loading forces in the medial versus the lateral compartment. This mineralization within the meniscus could then contribute to a further load imbalance within the joint and possibly stimulate significant metabolic changes in the cartilage (5). Altered loading of cartilage has been shown to cause matrix degradation as well as cell death (46).

Since PC is a powerful antimineralization agent, one concern regarding its therapeutic use is whether it would interfere with normal bone mineralization. PC does not produce any toxic side effects in rodents when given in dosages of up to 150 mmole/kg/day (24,43,47,48). In a study using the murine progressive ankylosis model, PC significantly inhibited calcification and disease progression in peripheral joints that were not involved at the start of treatment (43). There was no significant difference in body weight between the PC-treated group (mice with murine progressive ankylosis and normal heterozygous littermates) and untreated normal heterozygous animals. PC treatment of mice with murine progressive ankylosis and of normal control

mice did not alter the histologic or gross appearance of the subchondral bone compared with that of mice with untreated murine progressive ankylosis. Similarly, in the present study we did not detect any difference in the degree of bone mineralization between CaNaPC-treated and saline-treated guinea pigs with OA. Moreover, bone may be protected from the effects of PC because bone cells have high levels of alkaline phosphatase, which can hydrolyze PC readily (7). However, a definitive answer to the question of whether CaNaPC has any adverse effects on bone mineralization would require measurement of the femoral bone mineral density of the hind limbs by densitometry. According to earlier studies (49), this is the region in the guinea pig knee that yields the highest-precision measurement.

Our results confirm the earlier observation (29) that the antiminerization activity of CaNaPC is significantly more potent than that of NaPC. The increased potency of CaNaPC could be due to its stability in an aqueous environment. The asymmetric unit cell of NaPC (Figure 5a) consists of a monovalent ion of PC, the single ion of sodium, and 2 hydration waters. The sodium ion has 6-fold coordination with 3 waters and 3 PC ions. The NaPC is very soluble, and at neutral pH it remains essentially uncomplexed, leaving the PC ion prone to hydrolysis. This can be inferred by inspecting the complex shown in Figure 5a. Weak coordination of the sodium ion by only 3 PC ions can be easily replaced by water, leaving PC ions exposed to solvent. Citrate, resulting from decomposition of PC, is a much weaker inhibitor. The inhibitory effectiveness of NaPC is therefore significantly restricted due to its instability in aqueous solutions, especially when its delivery to the crystallization site requires time (i.e., in vivo systems). In contrast, examination of the unit cell of CaNaPC (Figure 5b) reveals that this complex is more stable in aqueous solutions, due to very tight coordination of phosphate groups, which remain protected from the solvent in this complex. The calcium ion is coordinated by 4 phosphate ions, 4 carbonyl ions, and 1 water oxygen atom, defining an irregular polyhedron (Figure 5b). This formulation also includes a 6-fold coordinated sodium ion that bridges 2 calcium-PC complexes.

Based on the present findings, we propose that there are 2 potential mechanisms by which articular calcification can cause cartilage degeneration. The first involves changes in joint biomechanics. Articular calcification may lead to altered loading of the joint, causing injury to the cartilage matrix, which then fails under normal loading, causing chondrocytes to respond by elaborating MMPs and developing inappropriate repair

responses. Supporting this notion is the finding that the equilibrium modulus of calcified menisci from the right knee joint of 6-month-old guinea pigs with untreated experimental OA is more than 300-fold greater than that of calcified menisci from CaNaPC-treated animals (Cheung HS, et al: unpublished observations). The second potential mechanism involves the biologic effect of crystals on articular cells (4,6). In the advanced stages of disease, crystals shedding from the meniscus or cartilage into synovium induce synovioocyte proliferation and MMP synthesis, which amplify the disease progression in the guinea pig OA model (10).

PC treatment has no therapeutic effect in the hemi-menisectomy model of OA (39), in which there is no known crystal involvement (Figure 4). Based on this finding, together with our previous observation of a therapeutic effect of PC in murine progressive ankylosis (43) and the known in vitro specific inhibitory effect of PC on the biologic action of calcium-containing crystals (4,6), we conclude that PC has no therapeutic effect on cartilage degeneration in OA that is not associated with calcium-containing crystals. We propose that CaNaPC blocks calcification-induced cartilage degeneration and arrests OA disease progression via inhibition of new calcification of the menisci, thus preventing abnormal joint loading, and via specific inhibition of crystal-induced cellular response damage. However, it is still possible that the therapeutic effect of PC may be due to other, as-yet-unidentified, mechanisms.

In summary, our data confirm that meniscal calcification correlates with the cartilage degeneration in this guinea pig model of OA, suggesting that this is a useful model for examining the role of calcification in OA (5). Treatment with CaNaPC, a new formulation of PC, led to significant reduction of calcium deposits in menisci and arrested the OA disease progress. Similar treatment had no therapeutic effect in a model in which articular calcification does not occur. These results support the hypotheses that calcification plays an important role in OA disease progression and that CaNaPC is a potential therapeutic agent for human chondrocalcinosis and BCP deposition disease.

The present observations may have broader implications as well. Tissue trauma or abnormal fluctuations in intracellular calcium ion concentrations can trigger calcific deposits. Initially, calcium salts may accumulate in an amorphous state, but under continuing favorable environmental conditions, nucleation and transformation to an insoluble, crystalline salt may occur, which can activate cellular responses leading to the development of specific pathologic conditions. This sce-

nario prevails in diseases such as renal calcinosis, urinary lithiasis, arteriosclerosis, heart valve calcification, and soft tissue and tumor calcification. Whether PC has any potential therapeutic effect in any of these diseases remains to be investigated.

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