Calcium Pyrophosphate Dihydrate and Basic Calcium Phosphate Crystal-induced Arthropathies: Update on Pathogenesis, Clinical Features, and Therapy

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Calcium-containing crystals are the most common class for the osteoarthritic joint. They are responsible for acute periarthritis and destructive arthropathies, and for tissue deposits mimicking tumor-like masses. These crystals encompassed mainly calcium pyrophosphate dihydrate and basic calcium phosphate crystals, with the latter being related to hydroxyapatite, carbonate-substituted apatite, and octacalcium phosphate. Calcification deposit mechanisms will be reviewed with respect to extracellular inorganic pyrophosphate dysregulation mainly caused by modulation of specific membrane channel disorders. Genetic defects have been extensively studied and identified mutation of specific genes such as *ANKH* and *COL*. Pathogenesis of crystal-induced inflammation is related to synovial tissue and direct cartilage activation. Besides classical knee or wrist pseudogout attacks or Milwaukee shoulder arthropathies, clinicians should be aware of other specific common presentations, such as erosive calcifications, spinal cord compression by intraspinal masses, ligamentum flavum calcification, or atypical calcified tophus. Promising clinical results for preventing calcium crystal deposits and cartilage degradation are lacking. Practical imaging tools are needed to monitor reduction of calcification of fibrocartilage and articular cartilage as markers of drug efficacy.

Introduction

Articular calcification frequently occurs in aging and osteoarthritis (OA). The most common calcium-containing crystals involved in joint disorders are calcium pyrophosphate dihydrate (CPPD) and basic calcium phosphate (BCP) crystals, which include carbonated-substituted hydroxyapatite (HA), tricalcium phosphate, and octacalcium phosphate (OCP). The latter are heterogeneous in terms of structure, chemical composition, and biologic properties. Often asymptomatic, calcium-crystal deposition can cause acute attacks of inflammatory arthritis, such as pseudogout, erosive arthritis, or periarthritis, and is associated with an exaggerated form of OA [1••]. Although specific treatments to reduce calcification are not well developed, recent studies have further contributed to the understanding of calcification mechanism and the pathogenesis of calcium-containing crystal deposition diseases.

Calcification Mechanism: Role of Extracellular Inorganic Pyrophosphate

This subject was recently reviewed comprehensively [2,3]. Articular calcification results from an imbalance between physiologic calcification inhibitors and mediators. Extracellular inorganic pyrophosphate (ePPi) is now recognized as an important factor for controlling calcium crystal formation [4,5]. Specifically, sporadic CPPD crystal deposition in aging is linked with excess ePPi generation via the chondrocytes [6], and ePPi deficiency leads to HA crystal deposition. Production of ePPi is the result of PPi-generating nucleoside triphosphate pyrophosphohydrolase (NTPPPH) activity or anion transport of intracellular PPi across the cell membrane by ANK protein, which is a multipass transmembrane PPi transporter.

The linkage between HA crystal deposition and deficiency of ePPi level has been described in several animal models and human diseases. Experiments by Ho *et al.* [7••] described the linkage of homozygosity for a truncation mutation of ank with the hyperostotic, hypercalcifying phenotype of murine progressive ankylosis in the ank/ank mouse. The defective protein of mutated

ank did not support PPi transport, resulting in ePPi level diminution and unrestrained HA formation in joint capsules and ligaments. Mice deficient for PC-1, the NTPPPH isoenzyme plasma cell glycoprotein-1 (tiptoe walking [ttw/ttw] mice and PC-1 knockout mice) [8,9], have a similar phenotype to that of ank/ank mice, including spontaneous HA crystal deposition in articular cartilage, which is likely a consequence of decrease ePPi. The genetic abnormality in ttw mice is a change of a codon for Gly568 to a stop codon in an NTPPPH. PC-1 is the specific form of the NTPPPH that is truncated in ttw mice. Mutations of the human ortholog of the murine ank gene have been described in craniometaphyseal dysplasia [10,11]. Excess bone formation is observed in affected patients and is characterized by progressive thickening and increased mineral density of craniofacial bones and hyperostotic flaring at metaphyses in long bones. Another rare disease is idiopathic infantile arterial calcification (IIAC; Online Mendelian Inheritance in Man 208000) because of PC-1 deficiency [12,13]. Terkeltaub [3,4] and Nurnberg *et al.* [10] analyzed affected individuals from 11 unrelated kindreds and demonstrated that IIAC was associated with mutations that inactivated ecto-nucleotide pyrophosphatase/phosphodiesterase-1 [14••]. This cell surface enzyme generates PPi, and PPi regulates cell differentiation and serves as an essential physiologic inhibitor of matrix calcification with HA. Affected individuals had low levels of ePPi, diminished PC-1 protein, and presented with periarticular and arterial HA formation early in life.

Excess ePPi has long been recognized as a likely cause of CPPD crystal deposition disease, and recently ANKH mutations were identified in UK, French, and Argentinean families with chondrocalcinosis [15,16]. Pendleton *et al.* [15] showed that intracellular PPi levels significantly diminished in COS cells transfected with one of the mutant ANKH protein. This suggested that gain of function of ANKH PPi channeling activity, over a long period of time, can lead to increased ePPi and CPPD deposition. Another example of the effect of elevated ePPi is seen in patients with hypophophastasia, a deficiency of the tissue-nonspecific form of alkaline phosphatase, which hydrolysis PPi to inorganic phosphate. This results in ePPi accumulation and CPPD deposition in cartilage. Recently, Hessle *et al.* [9] and Johnson *et al.* [17] demonstrated a coregulation and antagonistic relation between alkaline phosphatase and PC-1 in controlling ePPi and HA formation in bone.

Numerous soluble factors modulate ePPi production, as does aging. The primary stimulus of ePPi production is transforming growth factor (TGF) [18]. The effect of TGF-β to raise chondrocyte PPi rises in association with aging [19], as does TGF-β–stimulated NTPPPH activity [20]. TGF-β enhances expression of cartilage intermediate layer protein/extracellular NTPPPH [21], PC-1 [22], and ANK [23], and the latter two directly increase ePPi levels. Transglutaminase activates latent TGF-β to increase chondrocyte ePPi production. Two dominant forms of transglutaminase have been identified in articular cartilage—type II transglutaminase and factor XIIIA [24]. Interleukin-1–beta (as well as tumor necrosis factor–alpha, donors of nitric oxide, and the potent oxidant peroxynitrite) induces increased chondrocyte transglutaminase activity [20]. The authors discovered marked upregulation of transglutaminase and factor XIIIA expression in hypertrophic cells in the superficial and deep zones of knee OA menisci [20]. Increased factor XIIIA and transglutaminase activities directly stimulated calcification by chondrocyte cells. Other stimuli for ePPi production include ascorbate, retinoic acide, and thyroid hormone [5]. Negative regulators include interleukin-1–beta, tumor necrosis factor–alpha, some isoforms of parathyroid hormone–related peptide, and insulin-like growth factor–1 [4,5].

New data on genetics of *ANKH* gene mutations have been recently reported by Williams *et al.* [25] on two US families with CPPD disease whose disease phenotypes have been linked to chromosome 5p15.1. These US families displayed unique haplotypes as distinct from that observed in the Argentinean kindred. An amino acid change in exon 1 was observed in both families, generating a point mutation in the P5 codon to change a proline to a threonine. All three of these mutations arose independently, suggesting that this position in *ANKH* may be a specific site for mutation. A newer large family kindred from Tunisia has been recently reported [26], with possible relationships with the previous described Tunisian kindred [27], and is awaiting genetic study. However, no relationship has been found with human leukocyte antigen genes.

Pathogenesis of Calcium-containing Crystal Deposition Disease

Clinical observations support the hypothesis that crystal deposition causes cartilage degeneration and differs from primary OA by the distribution of involved joints and the severity of the disease. Unusual sites of OA, such as the elbow, the shoulder, or the ankle joint, should lead to further investigation for evidence of CPPD or BCP crystal disease.

A recent study by Derfus *et al.* [28] demonstrated that calcium-containing crystals were found in synovial fluids of up to 60% of patients at the time of knee arthroplasty. The authors reported that presence of calcium crystals was correlated with severe radiographic scores. Similar results were observed by Nalbant *et al.* [29], who found that aside from CPPD and BCP crystals, the presence of fibrils also was correlated with higher radiographic grades of OA. Fibrils were identified in 60% of synovial fluid samples. Moreover, the authors reported that the apatite crystals and fibrils appeared with disease progression. Apatite crystals bind avidly to collagen fibrils [30].

The pathogenesis of inflammation in calcium-containing crystal deposition disease remains incompletely resolved (*see* review by Molloy and McCarthy elsewhere in this issue), and may be secondary to synovial lining cell stimulation by calcium-containing crystals, resulting in synovial cell proliferation, matrix-degrading molecule release, and secretion of inflammatory mediators and cytokines that, in turn, stimulate chondrocytes to generate matrix-degrading molecules [31]. Because crystals were rarely seen in immediate contact with chondrocytes in pathologic specimens, most studies have not yet considered chondrocytes as a passive bystander in the pathogenesis of BCP crystal-associated OA and CPPD disease. However, Cheung *et al.* [32] demonstrated, via electronic microscopy, that porcine chondrocytes could cause endocytosis BCP crystals. The authors also reported that BCP crystals induced prostaglandin E2 and collagenase secretion by chondrocytes. McCarty *et al.* [33] demonstrated that BCP crystals stimulated production of matrix metalloproteinase (MMP)-1 and MMP-13 by articular porcine chondrocytes.

The authors of this paper showed that BCP crystals directly induced non-adherent bovine chondrocytes inducible nitric oxide (NO) synthase messenger RNA expression and NO production. According to BCP crystal type, the response was different. OCP crystals induced two times more NO production than carbonated-substituted HA crystals, whereas HA crystals had no effect on NO stimulation (Ea *et al.*, Unpublished data). Using OCP crystals, the authors demonstrated that NO production was independent of interleukin-1–beta induction and involved p38 and c-Jun-N-terminal kinase mitogen-activated protein (MAP) kinase pathways, while the p42/44 MAP kinase pathway was not concerned (Ea *et al.*, Unpublished data). Crystals such as monosodium urate crystals have been recently shown to directly activate NO and MMP-3 production via articular chondrocyte through such a specific signaling pathway, including Pyk-2, Src, and p38 MAP kinase [34]. These studies demonstrate that chondrocytes may play a direct and active role in cartilage destruction via specific microcrystals.

The molecular mechanism of BCP crystal effect is complex. It can be secondary to intracellular calcium increase, leading to the activation of calcium-dependent pathways, the activation of the MAP kinase pathways by a yet unknown receptor, or a cell membrane modification induced by crystal contact. Halverson *et al.* [35] demonstrated that BCP crystals induced a biphasic calcium increase in human fibroblasts. An initial rapid rise in intracellular calcium derived from extracellular calcium influx; a later sustained rise in intracellular calcium resulted from BCP crystal dissolution in lysosomes. The initial transient rise probably served as a second messenger, leading to activation of early cellular response, such as *c-fos* expression. It has been demonstrated that endocytosis and dissolution of BCP crystals were required for mitogenic effect [36,37]. In contrast, intralysosomal dissolution was not necessary to induce MMP synthesis, although endocytosis was required [38]. BCP crystals, at least as represented by a complex association, are able to stimulate the endocytic activity of cells, small molecules, such as DNA fragments, and also possibly peptides [39]. Phosphocitrate, as discussed by Cheung [40], also was able to inhibit BCP crystal–stimulated endocytosis [39], further supporting its potential role as a disease-modifying drug for BCP crystal–induced or associated arthropathies.

Recently, Sun *et al.* [41] also showed that the induction of MMP-1 expression by BCP crystals in canine fibroblast-like synoviocytes is p42/44 MAP kinase–dependent and uses the Ras/MAPK/*c-fos*/AP-1/MMP-1 signaling pathway. Nair *et al.* [42] demonstrated that BCP and CPPD crystals activated MAP kinase p42/44, but not p38 protein kinase cascade pathway in fibroblasts [42]; in contrast, the authors showed that OCP crystal–induced inducible NO synthase expression in bovine articular chondrocytes involved p38 and c-Jun-N-terminal kinase MAP kinase pathways and not p42/44 MAP kinase (Ea *et al.*, Unpublished data). These suggested a cell-specific response triggered by calcium-containing crystals. Prudhommeaux *et al.* [43] demonstrated the variation in the inflammatory properties of basic calcium phosphate crystals according to crystal type. The inflammatory potential increased with the specific surface area of the BCP crystals. The authors of this paper thought that a large crystal surface allowed a greater amount of proteins to bound [44–46], leading to an increased crystal-cell membrane contact. This could trigger intracellular signaling via a cell surface receptor–like receptor, or could modify the cell membrane properties, as was demonstrated by Sun *et al.* [39]. Other studies have recently confirmed this biologic variability of BCP crystals on monocyte activation on cytokine release, such as tumor necrosis factor–alpha, interleukin-6, and interleukin-10 [47], or MMP stimulation [48].

Clinical Manifestations Related to Basic Calcium Phosphate Crystal Deposition

Basic calcium phosphate crystals are associated with a number of clinical syndromes, including calcific tendinitis, Milwaukee shoulder syndrome, and a severe form of OA. BCP crystals are mainly responsible for acute periarthritis that involves all possible tendon or capsular sites. The clinician should be aware of this critical point when facing an acute inflammatory articular or periarticular attack mimicking septic arthritic or abscess, and should consider acute HA attack as a differential diagnosis [49]. Unusual sites, such as toes (pseudo-pseudo-podagra), wrists, elbows, and ankles, can be involved. One patient even presented with a spontaneous coccygeal pain (precoccygeal deposit). Diagnostic clues are the knowledge of para-articular tendons, capsulae, and bursae, with the typical aspects of plain radiographs showing calcifications of varying size. These calcifications can be unfragmented

and dense, with a round shape, at the very beginning or in asymptomatic joints; conversely, they start to be fragmented, with fluffy edges, when inflammation has started. They can vanish within days or weeks, justifying repetition of radiographs. This is very characteristic of apatite calcification. It is often asymptomatic, but can be associated with chronic pain, and provokes self-limited episodes of acute periarticular inflammation that corresponded to the resorption phase of the crystals with migration in the subacromial bursa and acute bursitis. Persistent calcification is rare and can lead to local tenderness, which becomes the priority of the treatment. However, the natural course of the calcification is to disappear spontaneously. Recently Lemaire *et al.* [50] reported another possible outcome of the calcification. Using computerized tomography (CT), they described apatite crystal penetration within the trochiter into the femoral head in two patients who presented with an acute periarticular inflammation. This outcome is rather rare, but the authors thought that it is under-recognized because CT scan was generally not used in calcific tendinitis diagnosis.

Destructive arthropathy is not unusual, especially at the shoulder joint, and is called *Milwaukee shoulder syndrom* in the English literature and *l'épaule destructrice rapide* in the French literature [51]. Clinical presentation nowadays is the following classical description: elderly patients presenting with long-standing shoulder pain complicated by a sudden joint effusion along with hematoma. Bloody synovial fluid contains a large amount of apatite crystals. This also could be associated with large rupture of the rotator cuff. A large Italo-Argentinean kindred with OA, chondrocalcinosis, and Milwaukee shoulder syndrome was described [52]. Milwaukee shoulder syndrome was seen in one member of the first generation and six members of the second generation, while eight members of the third generation showed an incomplete form of Milwaukee shoulder syndrome. OA of the spine and peripheral joints was seen in 31 affected members, while chondrocalcinosis was only observed in six members of the first generation. A search for linkage to some potential candidate genes was inconclusive in this peculiar family [52]. On rare occasions, diagnosis can be uneasy when a pseudo-tumoral mass is present, alone or associated with bone erosions, with respect to paradiaphyseal calcification (Fig. 1) [53].

The nature of the calcification also can play a role in the crystal evolution fashion, because it has been demonstrated that BCP crystals possessed variable inflammatory potential [43]. Recently, Hamada *et al.* [54] reported that calcium deposits in 34 patients with calcific periarthrtis were composed of carbonated apatite.

Basic calcium phosphate crystals can cause acute synovitis of small or large joints. This is difficult to ascertain because BCP crystals are not detectable by light polarized microscopy. Alizarin red S staining can provide can disclosed small aggregates, coin-like objects of 1 to 5 μm in length. It is quite difficult or even impossible to detect these crystals under polarized light, especially at small digit joint [55]. These crystals have been most closely associated with OA. Using Alizarin red S or electron microscopy, they can be seen in up to 30% to 60% of synovial fluids from patients with OA. Such an association also has been observed between the presence of crystals and the radiographic severity of OA [56]. As discussed before, these BCP crystals could contribute to the disease process because they can interact with articular cells or shed from the subchondral bone. Furthermore, Nalbant *et al.* [29] found that BCP crystals appeared with joint degeneration. Some of the HA deposits seen in cartilage in OA are caused by subchondral bone shards, but many are perichondrocytic. These HA deposits are likely attributable to mechanisms such as chondrocyte hypertrophy, apoptosis, and also PPi excess, that help provide PPi for HA crystal formation [57,58].

The management of crystal-associated OA remains identical to primary OA because the prevention of calcification is yet unavailable. However, recent research to develop specific therapy to prevent BCP crystal deposition disease is advancing. Specifically, the PPi analogue phosphocitrate, a natural compound in mammalian mitochondria and in the urinary tract, is a potent inhibitor of HA crystal formation [40].

Treatment of calcific tendinitis includes symptomatic treatment (nonsteroidal anti-inflammatory drugs, local corticosteroid injection, physical modalities including heat application, and range of movement exercises) and specific treatment (needle aspiration, surgical or arthroscopic removal, shock-wave, and ultrasound). The capacity of ultrasound to promote resorption of calcification is noteworthy. Ebenbischler *et al.* [59] reported the results of a randomized, double-blind comparison of ultrasound therapy and sham treatment in 63 consecutive patients with symptomatic shoulder chronic calcific tendinitis. Ultrasound treatment was associated with increased rate of resorption and greater reduction pain. There was, however, no significant difference between the two groups at 9 months.

Clinical Expression of Calcium Pyrophosphate Dihydrate Deposition

Calcium pyrophosphate dihydrate disease often presents as 1) pseudogout attacks (25% of patients with CPPD deposition exhibit this pattern), 2) pseudo-rheumatoid presentation (5% of cases) with multiple joint involvement, usually along an additive pattern, 3) progressive OA-like joint degradation of numerous joints, especially at unusual sites, 4) asymptomatic but radiologic disease; and 5) chronic destructive arthropathies presenting with geodes of various size. CPPD disease rarely affects the spine. Two sites, the periodontoid region and the

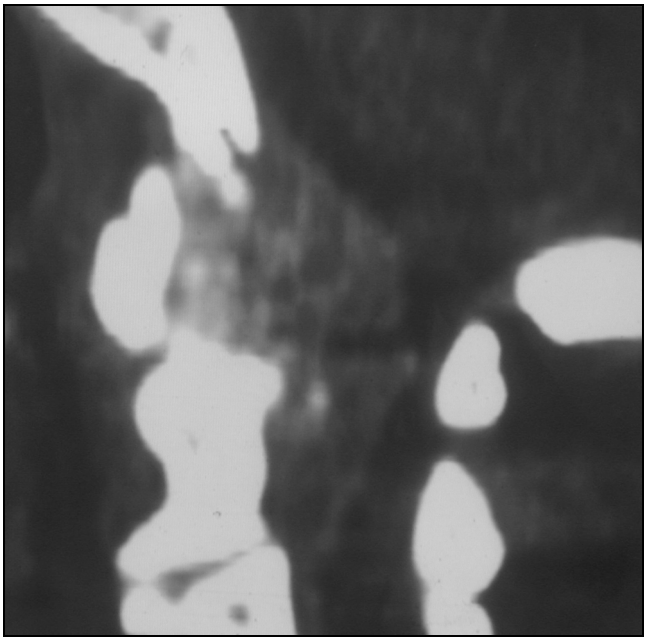

Figure 1. Pseudotumor of the foramen magnum and the upper cervical spine. Patient presented with dizziness, vertigo, and sudden faintness. Pathologic study showed macrophagic reaction to calcium crystals that content was related to apatite and deposits by electron microscopy. (*Courtesy of* George and Lioté.)

cervicothoracic spine, are prone to CPPD deposits. This involvement has been increasingly recognized over the past decade [60••]. Muthukumar and Karuppaswamy [60••] reported six cases presenting with insidious myelopathy caused by CPPD mass deposits involving the ligamentum flavum of the cervical and thoracic spine. From the review of the literature, it appears that spinal involvement in CPPD is more common in Japanese than other populations, although scattered cases have been reported in the French Caribbean [61]. The cervical spine is more frequently involved, followed by the thoracic and lumbar regions. At the cervical spine, the presentation could be a periodontoidal mass leading to foramen magnum syndrome or calcification of the ligamentum flavum. This tumoral CPPD deposit of the ligamentum flavum occurs more commonly in middle-aged or elderly women and presents with progressive myelopathy.

Imaging studies include magnetic resonance imaging (MRI) and CT scan. A low-signal mass is displayed at MRI, related to calcified tissues, and CT scan showed the origin of the CPPD tophus, namely the ligamentum flavum, the facet joint, or even the disk. Pathologic examination, including giant cell granuloma, rarely demonstrated foreign body reaction or, rather, a compact collagen-rich tissue containing calcium deposits, and no inflammatory infiltrates. Microscopic examination of the nodules with the polarized light can revealed extensive deposition of CPPD crystals. Occasionally, via radiograph diffraction study, the crystal was determined to be CPPD [62]

Although this condition is rare, rheumatologists and neurosurgeons should be aware of these complications, because only a surgical procedure, including cervical or thoracolumbar posterior decompressive laminectomy with removal of the calcified nodule, can relieve the symptoms and signs. Because the foramen magnum is involved, a transoral decompression of the cervical region can be necessary.

Imaging

Because treatment aimed to control calcium deposits are still under investigation [40], clinicians should keep in mind any method to assess changes in calcification size. Radiographs are not precise enough for assessment, but sonography could represent an alternative technique. Sonography is useful in evaluating the patellofemoral joint, including the trochlear cartilage, which is often difficult to image adequately on conventional radiographs, because true tangential views of the patellofemoral joint may be difficult to obtain. Several cases of sonographic detection of cartilage calcification in the trochlea of the knee, which was radiographically occult or even confirmed radiographically, have been described. One can speculate that CT scan could be another imaging procedure to evaluate calcifications.

Conclusions

Calcium-containing crystals are an important mediator of joint inflammation and cartilage degradation. Recent studies have highlighted the role of extracellular inorganic pyrophosphates in the mechanism of pathologic articular cartilage calcifications, as well as the place of the transmembrane PPi transporter ANK protein and the ePPi generating NTPPPH isoenzyme PC-1. The knowledge of the molecular mechanism involved in articular damage triggered by microcrystals has been expanded [65]. Specifically, chondrocytes seem to play an active role and can be directly stimulated by BCP crystals. Clinical presentation is ascertained by identification of crystals under polarized light and Alizarin red S staining, which should be more widely used. A challenge for the upcoming years will be the development of drugs targeted to modulate the calcification processes and the development of radiologic procedures and techniques to evaluate this effect.

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