Calcium Pyrophosphate Dihydrate and Hydroxyapatite Crystal Deposition in the Joint: New Developments Relevant to the Clinician

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The major types of crystals containing calcium, which causes arthropathy and periarticular disease, are calcium pyrophosphate dihydrate and basic calcium phosphates, including hydroxyapatite. Exciting advances include the identification of mutations in the gene *ANKH* associated with disordered inorganic pyrophosphate (PPi) transport in some kindred with familial chondrocalcinosis linked to chromosome 5p. In addition, central basic mechanisms governing cartilage calcification and their relationship to aging and osteoarthritis have now been elucidated. These include the role of plasma cell glycoprotein-1, the PPi-generating ecto-enzyme, in chondrocalcinosis and the linkage of low-grade inflammation to expression and activation of two cartilage-expressed transglutaminase isoenzymes with direct calcification-stimulating activity. This review discusses clinically pertinent new information on pathogenesis. The authors also address, in detail, current diagnostic and therapeutic issues pertaining to calcium pyrophosphate dihydrate and hydroxyapatite crystal deposition in the joint, as well as possible therapeutic directions for the future.

Introduction

Crystals of calcium pyrophosphate dihydrate (CPPD) and of basic calcium phosphate (BCP), such as hydroxyapatite (HA), are the most common calcium-containing crystals associated with joint and periarticular disorders. Deposition of these crystals is frequently asymptomatic or can be intermittently symptomatic. However, common clinical manifestations of the calcium-containing crystal deposits can include primary manifestations of acute or chronic inflammatory and degenerative arthritides, symptomatic flares, and a contribution to the worsening of cartilage degeneration in osteoarthritis (OA) [1,2], as well as rotator cuff inflammation and certain forms of periarthritis. In addition, focal or multifocal tumoral deposits of calcium-containing crystals may become symptomatic.

Advances in Pathogenesis of Cartilage Calcification in Aging and Osteoarthritis

Role of plasma cell glycoprotein-1 and altered chondrocyte differentiation and viability

Recent studies have furthered understanding of the mechanisms responsible for CPPD and HA crystal deposition in OA and cartilage aging. Specifically, articular cartilage matrix calcification can reflect deficiencies of certain physiologic calcification inhibitors or upregulation of mediators that actively drive stereotypical patterns of tissue injury culminating in calcification within degenerating cartilage [1]. A special circumstance promoting chondrocalcinosis is the relatively unique capacity of chondrocytes to produce copious amounts of extracellular inorganic pyrophosphate (PPi) [1,3•,4•,5••]. The linkage with sporadic CPPD crystal deposition in aging of excess chondrocyte PPi–generating nucleoside triphosphate pyrophosphohydrolase (NTPPPH) activity, excess PPi generation by the chondrocytes, and cartilage supersaturation with PPi is well established [3•,4•,5••,6].

The molecular mechanisms transducing excess chondrocyte PPi generation and the key role of the chondrocyte growth factor transforming growth factor-beta (TGFβ) in elevating chondrocyte extracellular PPi are now increasingly understood [3•,4•,5••]. Specifically, the NTPPPH isoenzyme plasma cell glycoprotein-1 (PC-1; also known as NPP1) plays a critical role in sustaining and augmenting extracellular PPi in chondrocytes and certain other cells [7••]. The isoenzyme PC-1 plays a larger role than the closely related NTPPPH isoenzymes autotaxin/NPP2 and B10/NPP3 in augmenting extracellular PPi in chondrocytes [3•,4•]. Cartilage intermediate layer protein, a secreted matrix molecule unrelated to the NTPPPH isoenzymes, has been claimed NTPPPH [8], but this claim has not been adequately documented or substantiated elsewhere.

Expression of PC-1 is upregulated by TGFβ, and PC-1 expression, and subcellular movement toward the plasma membrane plays a major role in the capacity of TGFβ to elevate chondrocyte extracellular PPi [4•,9]. Interleukin-1 suppresses PC-1 expression and extracellular PPi in chondrocytes and blocks the effects of TGFβ on PPi [9]. The capacity of TGFβ to raise chondrocyte PPi rises in association with aging [10], as does TGFβ-stimulated NTPPPH activity [11••]. At the same, the beneficial growth-promoting effects of TGFβ decrease with aging in articular chondrocytes [10]. These effects likely reflect altered signal transduction with chondrocyte aging, and, in this context, protein kinase C and protein kinase A signaling differentially affect PPi levels of chondrocytes [12].

Recently, it was demonstrated that the natural antagonist of PC-1-mediated PPi generation was tissue-nonspecific alkaline phosphatase (TNAP) [13,14••]. Specifically, PPi levels and mineralization disturbances in tissues of PC-1 knockout and TNAP knockout mice were mutually corrected by crossbreeding to generate double knockout mice [14••]. The TNAP knockout mouse is a model for infantile hypophosphatasia [14••]. Significantly, articular cartilage PPi excess and chondrocalcinosis are associated with hypophosphatasia, which further suggests the potential use of PC-1 antagonism as a therapeutic strategy for certain forms of chondrocalcinosis. An interesting finding in the aforementioned study was that disordered growth plate chondrocyte organization in TNAP knockout mice was also corrected, in large part, by breeding onto the PC-1 null background [14••]. Hence, PC-1 plays a major role in PPi metabolism, and PPi metabolism appears in the regulation of chondrocyte differentiation.

Regulated changes in chondrocyte differentiation and viability appear to mediate chondrocalcinosis. Such changes include the development of chondrocyte hypertrophy associated with expression of stereotypic bone matrix proteins, and the presence of heightened hypertrophy and apoptosis of chondrocytes adjacent to articular cartilage calcifications [15]. Chondrocyte hypertrophy is associated with heightened PPi generation [16] and increased production of calcifying cell fragments (matrix vesicles) [17]. Upregulation of local parathyroid hormone–related protein expression also may be one of the shared features driving sequential chondrocyte proliferation and altered differentiation in growth plate chondrocytes and articular chondrocytes [18,19].

Chondrocyte apoptosis is enhanced in OA cartilage and is directly associated with HA crystal deposits [15]. In this context, nitric oxide (NO), a mediator of OA pathogenesis, also stimulates apoptosis in chondrocytes [20] and NO donor treatment of cultured chondrocytes stimulates calcification in vitro. Furthermore, upregulated expression of the PPi-generating ecto-enzyme PC-1 directly promotes chondrocyte extracellular PPi elevation and matrix calcification and also apoptosis in vitro [3•,21•,22]. Hence, the conjoint excesses in NTPPPH activity and extracellular PPi generation characteristic of sporadic CPPD crystal deposition disease of the elderly could be one of the factors compromising chondrocyte viability in aging and OA.

Role of inflammation and transglutaminase activity

Inflammatory mediators that promote degradation of the articular cartilage matrix may also help prepare the matrix for calcification in OA. In this context, interleukin-1-beta stimulates expression of other cytokines and matrix metalloproteinases, inducible NO synthase expression, and increased NO generation, as well as factor XIIIA transglutaminase (TGase) and tissue TGase (tTGase) expression in cartilage [11••]. Interleukin-1-beta (as well as tumor necrosis factor-alpha and donors of NO and the potent oxidant peroxynitrite) induces increased chondrocyte TGase activity, and may be modulated via NO and oxidant-mediated TGase post-translational modifications [11••]. The authors discovered marked upregulation of tTGase and factor XIIIA expression in hypertrophic cells in the superficial and deep zones of knee OA articular cartilage and the central (chondrocytic) zone of OA menisci [11••]. Moreover, increased factor XIIIA and tTGase activities directly stimulated calcification by chondrocytic cells [11••]. Transglutaminase activity also promotes activation from a latency state of TGFβ-1 [23], a factor that, as cited, upregulates PC-1 expression and extracellular PPi levels.

Transglutaminase activity and extracellular PPi levels are concurrently elevated in association with articular cartilage aging [10,11••,24–26]. Furthermore, OA severity-related, donor age-dependent, and marked age-dependent interleukin-1–induced increases in TGase activity also were observed in chondrocytes from human knee menisci, which is a major site for CPPD deposition disease in aging and OA [11••]. Taken together, inflammation-induced TGase activity appears to be a substantial factor driving cartilage calcification in OA.

Homologue *ank* gene mutations in chondrocalcinosis

Chondrocytes and other cells regulate extracellular PPi levels by the generation of PPi and by PPi transport [5••]. Recently, Ho *et al.* [27••] cloned the multiple-pass transmembrane protein *ank* and described the PPi channeling function of *ank*. Furthermore, the same study 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 [27••]. Deficient channeling of PPi from the cytosol to the extracellular space was implicated [27••].

Mice deficient for PC-1 (*ttw/ttw* mice and PC-1 knockout mice) [14••,28] have a remarkably similar phenotype to that of *ank/ank* mice, including spontaneous HA crystal deposition in articular cartilage, which is a likely consequence of decreased extracellular PPi and loss of the physiologic function of PPi to suppress HA crystal deposition [5••].

The PPi channeling function of *ank* (and the human homologue of *ank*, whose gene is termed *ANKH* by some) has recently been modeled. There are 10 or 12 membrane-spanning domains with an alternating inside-out orientation and with a central channel to accommodate the passage of PPi (Fig. 1) [27••,29]. Significantly, *ank* mutations at different locations in the molecule can affect the skeleton in different ways, and this is schematized in Figure 1. For example, there is an association of distinct regional *ank* mutations with the *ank/ank* mouse (with truncation of C-terminal putative cytosolic domain) compared with more N-terminal human *ANKH* mutations in craniometaphyseal dysplasia (CMD; widely known as "mask disease") [29,30]. Craniometaphyseal dysplasia is a pediatric disorder characterized by progressive thickening and increased mineral density of craniofacial bones and hyperostotic flaring at metaphyses in long bones, as well as the classic "mask facies" [29,30]. The effects of the *ANKH* CMD mutations on PPi transport and other potential functions of *ANKH* are not currently understood, but may involve dysregulated intramembranous bone formation.

The *ANKH* locus resides in human chromosome 5p. Reports linking familial CPPD deposition disease to human chromosome 5p (CCAL2) have been detailed in studies of kindred from the UK, Argentina, France, and the US [31–34]. Phenotypes of all affected individuals with chondrocalcinosis linked to chromosome 5p are not completely identical. For instance, members of the UK kindred with primary chondrocalcinosis also presented with infantile recurrent febrile seizures [31]. Kindred from Argentina and the Alsace region of France did not manifest a seizure disorder, but had similar phenotypic features of chondrocalcinosis, including early age at onset (third decade of life), common but not universal premature OA, some cases of pseudorheumatoid arthritic peripheral joint disease, and radiographic evidence of fibrocartilage and hyaline cartilage calcifications typical of CPPD deposition (and without evidence that the crystal deposition was secondary to chondrodysplasia) [32,33]. The most commonly affected joints in these kindred were the knees and wrists, and involvement of the pubic symphysis and intervertebral discs also occurred [32,33].

Initial studies linked the chondrocalcinosis in the British kindred to a 5.6-cM locus of chromosome 5p15.1-15.2 between D5S810 and D5S416 [31]. The unrelated French and Argentinean families had linkage to a 0.8-cM locus of chromosome 5p between the polymorphic markers D5S416 and D5S2114 [32,33]. A family of UK and German ancestry from the US linked to chromosome 5p was identified; they displayed similar phenotypic features [34].

ANKH is nearly identical to mouse *ank* over its entire length, with only 9 amino acid substitutions, and the *ANKH* gene mapping on human chromosome 5 is closely linked to D5S1954. Recently, mutations in *ANKH* were identified in UK, French, and Argentinean families with chondrocalcinosis (Fig. 1) [35••,36••]. Specifically, Williams *et al.* [36••] reported linkage of a P5L mutation in *ANKH* to autosomal dominant chondrocalcinosis in an Argentine family of Northern Italian descent. Pendleton *et al.* [35••] identified autosomal dominant *ANKH* mutations in previously reported UK and French families in whom CPPD crystal deposition had been clinically characterized. They also tested 95 subjects with sporadic chondrocalcinosis in whom they found one subject, a 79-year-old man, with an *ANKH* mutation [35••]. This group found that all affected members of the French family were heterozygous for a T-C nucleotide base change

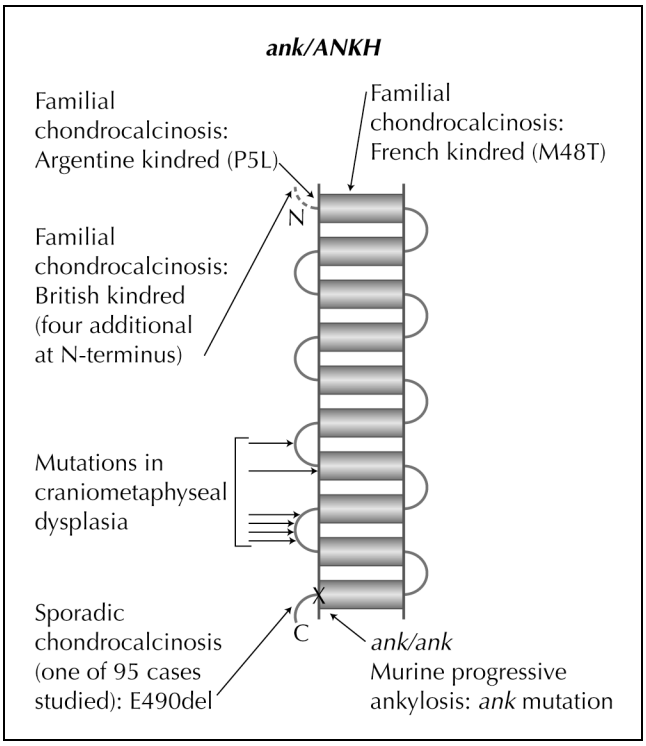

Figure 1. Model for *ank/ANKH* and for associations of distinct mouse *ank* and human *ANKH* mutations with different phenotypes of skeletal disease. The figure schematizes the putative structure of *ank/ANKH* and the multiple-pass transmembrane protein that appears to function in inorganic pyrophosphate (PPi) channeling from the cytosol to the extracellular space. As illustrated, distinct mutations in *ank* or *ANKH* promote distinct phenotypes. Impairment of *ank* function via homozygosity for a C-terminal cytosolic domain truncation mutation in murine progressive ankylosis mice leads to a decrease in extracellular PPi. Because PPi is a potent natural inhibitor of hydroxyapatite crystal deposition, this results in deposition of hydroxyapatite crystals in articular cartilage and other sites, and also results in peripheral synovial and intervertebral bony ankyloses. The figure summarizes sites of known *ANKH* mutations associated with autosomal dominant familial chondrocalcinosis (calcium pyrophosphate dihydrate crystal deposition disease) and with sporadic chondrocalcinosis in one subject (as discussed in the text). It is believed that most if not all of the autosomal dominant human chondrocalcinosis mutations lead to excess extracellular PPi, promoting calcium pyrophosphate dihydrate crystal formation. The sites of autosomal dominant *ANKH* mutations implicated in human craniometaphyseal dysplasia (CMD) also are depicted, and CMD is discussed in the text. The effects of the CMD mutations on PPi transport and other potential functions of *ANKH* are not currently understood.

in exon 2, which causes the substitution of threonine for methionine in a predicted transmembrane domain (M48T). In a UK family (that also manifested febrile seizures), all affected individuals were heterozygous for a –11 C-T base change capable of giving rise to an alternative ATG initiation codon, thereby adding four amino acids to the N-terminus of *ANKH*. The sporadic chondrocalcinosis subject displayed heterozygosity for a 3-bp deletion in exon 12, which deletes a glutamate residue (E490del) three amino acids from the C-terminus of *ANKH*.

Reconstruction of the –11CT, M48T, and E490del mutations and preliminary evaluation of their effects on intracellular PPi levels in transfected COS cells were performed [35••]. The authors determined that each of the three mutant *ANKH* proteins lowered intracellular PPi [35••]; however, only the –11CT *ANKH* mutant appeared significantly more potent at promoting intracellular PPi lowering than wild-type *ANKH* in their cell system [35••]. Cell lines from the affected subjects in the UK kindred were not tested for PPi levels.

The Pendleton *et al.* [35••] and Williams *et al.* [36••] papers do lend credence to the possibility that subtle "gain of function" of intrinsic *ANKH* PPi channeling activity may, over long periods of time, lead to chondrocyte "PPi leakiness" and matrix-saturating increases of extracellular PPi that result in CPPD crystal deposition and degenerative joint disease in 5p familial chondrocalcinosis. Limitations in the Pendleton *et al.* [35••] and Williams *et al.* [36••] studies also included the reliance on transfection of cells other than chondrocytes, and the absence of results for extracellular PPi (and, therefore, incomplete evidence of altered PPi channeling). The Pendleton *et al.* [35••] paper addressed intracellular PPi, but there was a lack of demonstration of significant functional effects for the M48T and E490del mutations. Significantly, nonchondrocytic cell lines from the French family had previously been determined to have markedly elevated intracellular PPi [37,38]. Thus, *ANKH* PPi channeling function may have be deficient in some of these subjects rather than overactive ("leaky") for PPi transport to the cell exterior, and elevated intracellular PPi could provide substrate for intracellular and extracellular CPPD crystal formation. Alternatively, transport of another solute modulating CPPD deposition may be affected by the M48T mutation, or the effects of *ank* on PPi channeling may be different in chondrocytes than in other cells.

Expression of *ank* and PC-1 is highly regulated. Thus, researchers speculate that secondary alterations in chondrocyte expression of wild-type *ank* (or PC-1) in OA and aging are significant factors in promoting a significant fraction of sporadic CPPD crystal deposition disease.

The heterogeneity of familial chondrocalcinosis is noteworthy [39]. This observation is illustrated partly by linkage of early onset osteoarthritis and chondrocalcinosis in a New England family with chromosome 8q (CCAL1) [40], but this study is limited by absent crystallographic studies. Better definition of whether the crystal type deposited in cartilage in specific familial chondrocalcinosis kindred is CPPD or HA. Thorough assessment for extracellular PPi deficiency (as in the *ank/ank* and PC-1 knockout mice) compared with extracellular PPi excess may help in future definitions of candidate genes other than *ANKH* and PC-1 in individual forms of familial chondrocalcinosis.

Update on Other Clinical Issues in Calcium Pyrophosphate Dihydrate Deposition Disease

Clinical features and diagnosis

The clinical manifestations of CPPD deposition disease vary widely. The disease can be asymptomatic or can mimic OA, gout, acute-onset or insidious rheumatoid arthritis, or neuropathic joint disease [41]. The contributions of the forms of CPPD deposited and of host factors to these wide differences in clinical manifestations are not clear, but they potentially include variations in deposition of more inflammatory monoclinic CPPD crystals compared with less inflammatory triclinic CPPD crystals. It remains unexplained why cartilage degenerative changes in CPPD deposition disease can be observed in typical joints for primary OA, such as the knee and hip, and atypical primary OA joints, such as the shoulders, elbows, wrists, and metacarpophalangeal joints. Systemic disturbances in PPi metabolism clinically manifested primarily in the joint may help account for such findings.

Degenerative cartilage disease associated with sporadic CPPD crystal deposition disease may be less or more destructive than that observed in primary OA. Prospective analysis of CPPD deposition disease that principally involved the knee has suggested that radiographic worsening of degenerative changes may be slow [42]. The disease also may not appear to be clinically progressive in the involved knee after substantial periods of follow-up in a subset of patients, though clinical involvement may spread to other joints in the same time frame [42]. Most patients develop changes in radiographic extent of chondrocalcinosis over time [42], but there is no clear correlation between the extent of calcification and progression of CPPD deposition arthropathy. Patients with initial presentation of CPPD deposition disease in the knee as acute pseudogout attacks alone may do particularly well [42].

In primary OA, the presence of CPPD crystals has been reported as an adverse prognostic factor. For example, Reuge *et al.* [43•] reported that patients with primary OA and CPPD crystals needed more knee replacement surgery compared with primary OA without crystals. In addition, Derfus *et al.* [44•] found that 60% of their patients undergoing joint replacement had pathologic calcium crystals (CPPD or basic calcium phosphates, such as HA) in their knee synovial fluids. The authors reported that higher mean radiographic scores correlated with the presence of calcium-containing crystals [44•].

Calcium pyrophosphate dihydrate crystals affect periarticular structures more frequently than generally appreciated. Recently, Canhae *et al.* [45•] reported in a cross-sectional study that 52% of subjects had periarticular involvement. The most common forms of periarticular involvement were carpal tunnel syndrome (24%) and periarthritis of shoulder (20%); less commonly seen were anserine bursitis and epicondylitis [45•]. Calcium pyrophosphate dihydrate crystal deposition may also involve the sacroiliac joints and spine, where radiographic findings, such as linear calcification and bony ankylosis, occasionally appear [46]. Clinical manifestations resembling ankylosing spondylitis, as well as acute pseudogout of lumbar facet joints, were reported recently [46,47].

The ability of CPPD crystals to cause tumoral deposits was recently reviewed by Yamakawa *et al.* [48••], who also reported clinicopathologic analysis of five of their own cases. They divided the reported cases into two main categories according to the anatomic distribution of tumoral CPPD crystals—61% central (head and neck) and 39% distal (in an extremity). The authors pointed out that the most common anatomic locations involved with tumoral CPPD crystals were the temporomandibular joint (37%), cervical spine (22%), and hand (18%) [48••]. Less common locations included the toe, hip, wrist, shoulder, elbow, and parotid gland. Painful mass and neurologic disturbances were the most common signs observed in patients with the central type, whereas painless mass or swelling without neurologic findings, or acute arthritic attacks similar to tophaceous gout, were more characteristic of the distal type.

Several recent papers have reminded clinicians of the potential development in CPPD deposition disease of cervical myelopathy, foramen magnum syndrome, and odontoid fractures caused by the calcification of cervical ligamentum flavum, the transverse ligament of atlas, and the atlantoaxial joint, respectively [49–51,52•]. Thus, CPPD deposition disease can factor in the differential diagnosis of patients with neurologic disturbances, especially in the elderly.

Conventional radiography is usually the first method to evaluate patients with suspected chondrocalcinosis. Radiograph findings may not correlate with pathologic and clinical manifestations. For example, the correlation between radiographic and pathologic findings was only 39.2% in a study of patients using knee arthroscopy [53]. Radiographic diagnostic approaches to CPPD deposition disease other than conventional radiograph have the potential to improve sensitivity [53]. For example, computed tomography, magnetic resonance imaging, and ultrasonography are useful in determining presence of CPPD crystals, particularly in the knee [53,54•,55,56].

Synovial fluid analysis for CPPD crystal deposition has been further evaluated and refined [57•]. It has been suspected that there is a decrease in the number of CPPD crystals seen if synovial fluid wet preparations are not analyzed. Recently, however, Galvez *et al.* [57•] reported that CPPD crystals were detected equally well at 24 and 72 hours after arthrocentesis when samples were stored at 4°C, whether or not anticoagulant was used. It has been suggested that there is potential usefulness for Gram stain and Diff Quick staining methods for crystal analysis in synovial fluids under conditions where the specimens are not fresh [58,59].

Demonstration of CPPD crystals in articular tissues is generally difficult in specimens routinely stained with hematoxylin-eosin, because the strong acidity of hematoxylin solutions promotes decalcification. Ohira and Ishikawa [60] recently showed that the decalcifying effect of hematoxylin could be lessened by limiting the staining period with Mayer's hematoxylin to 3 minutes.

Current and Future Therapies of Calcium Pyrophosphate Dihydrate Deposition Disease

As in gout, therapeutic approaches to patients with CPPD deposition disease involve treatment and prophylaxis of acute arthritic attacks, and therapy of chronic and anatomically progressive sequelae of crystal deposition. For acute attacks of pseudogout, Roane *et al.* [61] reported the efficacy of one-two doses of 60-mg triamcinolone acetonide by intramuscular injection. This approach is a potential alternative in patients with polyarticular involvement in whom nonsteroidal anti-inflammatory drugs are contraindicated. In a 6-month, double blind trial, Robertson *et al.* [62] reported that hydroxychloroquine, which is being investigated for treatment of erosive OA [63,64], was effective in patients with chronic polyarticular CPPD deposition disease [65].

Oral low-dose colchicine is well recognized as effective prophylactic treatment for gout and pseudogout attacks. Recently, Das *et al.* [66,67] suggested some efficacy of colchicine in chronic pain of primary knee OA (with and without evidence of inflammation) in two different randomized, double-blind, placebo-controlled 20-week duration studies. The prevalence of CPPD crystals in these knee joints was relatively high in these studies [66,67]. However, the study designs were complex and involved significant concurrent nonsteroidal anti-inflammatory drug and intra-articular steroid therapies. As such, the potential therapeutic benefit of colchicine in knee OA is not clarified adequately by these studies. Furthermore, it is not clear in these studies that any effects of colchicine are attributable to suppression of subclinical crystal-induced inflammation in these knees.

Strikingly reduced meniscal calcification was reported over a 10-year period in association with administration of oral magnesium to a patient with secondary CPPD deposition disease caused by hypomagnesemia [68]. In addition, pseudorheumatoid CPPD crystal deposition disease is potentially responsive to methotrexate. However, effective cartilage-preserving therapy is still lacking in idiopathic

and metabolic disease–associated forms of chronic progressive CPPD deposition disease [69]. It has been suggested that clinical trials of the anion transport inhibitor, probenecid, which suppresses *ank*-induced and TGFβ-induced increases in extracellular PPi [27••,35••,70], may compel further clinical investigation. Prevention of CPPD deposition by polyphosphates could provide another therapeutic approach [71].

In view of cartilage degeneration in CPPD crystal deposition disease, the use of intra-articular hyaluronan presents a potential treatment option. Though one study of glycosaminoglycan polysulphate was interesting in chondrocalcinosis [72], there are numerous case reports of acute arthritis in patients with chondrocalcinosis and of pseudogout after intra-articular hyaluronan injections [73–75]. Thus, the risk-to-benefit ratio for use of intra-articular hyaluronan in joints with detectable chondrocalcinosis is currently under question.

Kalunian *et al.* [76] demonstrated the efficacy of arthroscopic irrigation with 3000 mL of saline in a multicenter randomized controlled trial of patients with early OA. In this work, in which patients were evaluated at 12 months, patients with synovial fluid crystals had statistically greater improvements in pain [76]. Taken together, it is possible that the subset of OA patients with chondrocalcinosis may respond differently to some intra-articular treatments.

Articular and Periarticular Hydroxyapatite Crystal Deposition

Articular cartilage HA deposition is often concurrent with CPPD deposition in OA and aging cartilage, and vice versa, as has been recently re-emphasized [44•]. 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 including chondrocyte hypertrophy, apoptosis, and also PPi excess that helps provide inorganic phosphate for HA crystal formation [15,22,77]. It is believed that HA and CPPD crystal-induced chondrocyte matrix metalloproteinase expression promotes OA progression, as does the traffic of HA or CPPD crystals from articular cartilage to synovium [78–80]. The calcific crystal-synovial interactions likely contribute to synovial proliferation and inflammation and cartilage matrix–degrading matrix metalloproteinase expression by synovium in a significant fraction of subjects with OA [2,44•,78,81].

Hydroxyapatite crystal deposition in articular cartilage, as well as synovitis and OA, are seen in association with extracellular PPi deficiency in *ank*/*ank* and PC-1 knockout and PC-1-deficient *ttw*/*ttw* mice [14••,27••,28]. These findings illustrate that chronic extracellular PPi excess and also extracellular PPi deficiency are deleterious for chondrocytes and promote calcification [1,5••]. Deficiency of PC-1 in humans also has been associated with calcification [7••]. Specifically, a PC-1-deficient male infant was recently identified with idiopathic infantile arterial calcification, which is characterized by large artery media HA crystal deposition and smooth muscle cell proliferation and by periarticular calcifications [7••].

Recently, Pons-Estel *et al.* [82] reported an Italo-Argentinean kindred with familial OA and apparent Milwaukee shoulder-knee syndrome associated with BCP crystals. This family had an unusual type of degenerative joint disease with secondary intra-articular and periarticular calcifications and Milwaukee shoulder-knee syndrome. Genetic linkage was undefined in this kindred.

Hydroxyapatite and related BCP crystal deposits in periarticular soft tissues can be asymptomatic or promote clinical manifestations, including acute calcific periarthritis, tendonitis and bursitis. Women tend to be affected more commonly than men, and young people are affected more often than the elderly. Hydroxyapatite crystal–associated inflammation of the rotator cuff and subacromial bursa of the shoulder can be successfully treated using needle aspiration, irrigation, and steroid injections, and ultrasound-guided techniques can enhance the success of such approaches [83–90]. The capacity of ultrasound to promote resorption of rotator cuff and bursal calcifications is particularly noteworthy [89].

Conclusions

Fundamental research to develop specific therapy to prevent BCP crystal deposition disease is advancing. In this context, the PPi analogue phosphocitrate, a natural compound in mammalian mitochondria and in the urinary tract, is a potent inhibitor of HA crystal formation [91••]. Systemic phosphocitrate treatment also inhibits HA and CPPD crystal–associated cell stimulation, including induction of matrix metalloproteinase-3 in fibroblasts that promote degradation of the cartilage matrix [80]. Phosphocitrate suppresses ankylosing ossification in murine progressive ankylosis of *ank/ank* mice [92]. In addition, phosphocitrate inhibits NO-induced calcification of cartilage [21•]. Thus, phosphocitrate and molecular therapeutics targeted to *ANKH* and PC-1 are prime examples potential rational molecular therapeutic approaches for calcific crystal deposition diseases.

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