Arthropathies Associated with Basic Calcium Phosphate Crystals

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Abstract

Basic calcium phosphate (BCP) crystals refer to a family of crystals including partially carbonate substituted hydroxyapatite, octacalcium phosphate, and tricalcium phosphate. These crystals have been found in and around joints and have been associated with several forms of arthritis and periarthritis. Identification of BCP crystals remains problematic because of the lack of a simple, reliable analytic procedure. Methods currently in use include alizarin red S staining, labelled diphosphonate binding, scanning and transmission electron microscopy with energy dispersive X-ray microanalysis, X-ray diffraction, and atomic force microscopy. Periarthropathies associated with BCP crystals include calcific tendinitis and bursitis. Intra-articular BCP crystal deposition is common in osteoarthritis, often found together with calcium pyrophosphate dihydrate crystals. Uncommon conditions in which BCP crystals are found include destructive shoulder arthropathies, acute inflammatory attacks of arthritis, and erosive arthritis. Secondary deposition of BCP crystals has been observed in chronic renal failure, in patients with "collagen vascular" diseases, following neurologic injury and after local corticosteroid injection.

Introduction

Basic calcium phosphate (BCP) crystals, usually referred to as "apatite" or hydroxyapatite, actually consist of mixtures of partially carbonate-substituted hydroxyapatite, octacalcium phosphate, and tricalcium phosphate [39]. The latter two species may represent transition states leading to the final formation of hydroxyapatite. These crystals have now been associated with a variety of arthropathies yet their significance for the most part remains unclear. This review will discuss methods of BCP crystal identification and the periarthropathies and arthropathies associated with BCP deposition (Table 1).

Table 1. Periarthropathies and arthropathies associated with basic calcium phosphate (BCP) crystal deposition

Periarticular BCP arthropathies

Calcific periarthritis
Calcific tendinitis
Calcific bursitis

Intra-articular BCP arthropathies

Osteoarthritis with BCP crystals
Mixed crystal deposition (BCP and CPPD)
Milwaukee shoulder/knee syndrome (Idiopathic
   destructive arthritis, cuff tear arthropathy)
Acute inflammatory attacks
Erosive polyarticular disease

Secondary BCP crystal arthropathy/periarthropathy

Chronic renal failure
Calcinosis in connective tissue diseases
Post neurologic injury calcification
Post corticosteroid injection
Miscellaneous

Methods of Detection of BCP Crystals

BCP crystals are not birefringent. Thus, polarized light microscopy, which is quite sensitive and specific for identifying the other two most common "pathological" crystals, monosodium urate monohydrate and calcium pyrophosphate dihydrate (CPPD), is not useful for detecting BCP crystals. This is because individual BCP crystals, which are usually needle-shaped and less than 0.1 μm long, cannot be resolved by light microscopy and are randomly oriented in the much larger aggregates (2-19 μm) in which they are usually found. Occasionally, light microscopy will reveal laminated bodies referred to as "shiny coins" [37] but the usual appearance of these crystals is nondescript.

The calcium dye, Alizarin red S, has been utilized by some investigators to identify BCP crystals [43]. Alizarin is highly sensitive but frequent false positive results suggest that it lacks sufficient specificity to qualify for routine identification of BCP crystals without some other type of confirmatory testing [2, 21].

We developed a semiquantitative binding assay for BCP crystals which utilizes [^{14}C] ethane-1-hydroxy-1,1-diphosphonate (EHDP) [27]. This method is performed on a synovial fluid pellet resuspended in phosphate buffered saline and the results are expressed as μg/ml of hydroxyapatite standard. The limit of sensitivity is approximately 2 μg/ml standard hydroxyapatite.

Both scanning and transmission electron microscopy (SEM & TEM) with energy dispersive X-ray (EDX) microanalysis have been utilized to identify BCP crystals in synovial fluid pellets [29]. TEM with electron diffraction is also effective [45]. For SEM the pellet is air dried on a carbon planchet and sputter coated with carbon. Crystals may be released from tissue specimens by collagenase digestion [25]. Specimens to be examined by TEM must be fixed in glutaraldehyde and processed by routine methods. Calcium:phosphorus molar ratios vary from 1.3-1.7:1 [29]. This is consistent with Fourier transform infrared spectroscopy (FTIR) which has shown that BCP crystal deposits consist of mixtures of hydroxyapatite, octacalcium phosphate, and rarely tricalcium phosphate crystals [39]. Amorphous calcium phosphates may be detected by EHDP binding and possibly FTIR.

X-ray diffraction definitively identifies BCP crystals although the quantity of crystals necessary for analysis exceeds that required for the other techniques.

Atomic force microscopy has been recently applied to the identification of synovial fluid microcrystals [4]. This technique is capable of achieving subnanometer resolution of crystal surface topology and measurement of lattice unit cell dimensions.

These techniques have not been rigorously compared and none has yet been shown to be clearly superior to the others. They are also unable to identify the site of origin of BCP crystals. Unfortunately, there is still no simple, readily available method to detect BCP crystals comparable to polarized light microscopy for monosodium urate monohydrate and CPPD.

Periarthropathies associated with BCP crystals

Pathologic calcifications have been identified in a wide variety of periarticular sites, most commonly in bursae and tendons, especially near the sites of tendon insertions (entheses). For the most part, these appear to be isolated local dystrophic calcifications which are often asymptomatic. Mechanisms causing dystrophic calcification remain obscure. Local tissue damage, age related changes and perhaps other factors may predispose to calcification. In the rotator cuff, Uthoff and Sarkar [51] found that "primary" calcifications occurred in areas of metaplastic fibrocartilage accompanied by matrix vesicles and crystals being phagocytosed by macrophages and giant cells. Others have not found chondrometaplasia and matrix vesicles but instead found calcifications in psammoma bodies and necrotic cells [22]. Dystrophic calcification occurs with normal calcium and phosphorus concentrations in contrast to metastatic calcification which occurs with disordered calcium or phosphorus metabolism. A few individuals have been described with multiple areas of dystrophic calcification suggesting the presence of a systemic disorder [37, 44]. Several families in which multiple members have had articular and peri-articular calcifications provide evidence that a heritable component may be present in some instances [7, 13, 23].

Many periarticular calcifications probably remain asymptomatic and may only be discovered fortuitously [5]. In some, though, dramatic inflammatory episodes may occur with severe pain, swelling and erythema resembling gout. Asymptomatic deposits are usually gritty and hard, whereas some symptomatic deposits may be aspirable and appear as a whitish paste. Acute calcific tendinitis refers to inflammation of the tendon sheath along with BCP crystal deposition. The most common site of tendon calcification is the supraspinatus of the shoulder. Other common sites include the wrist, hip and knee but no site should be considered immune. This entity differs from non-calcific tendinitis in that it is less likely to be related to trauma and inflammation tends to be more severe. Calcific bursitis refers to calcification and inflammation in one of the many cell lined bursal sacs which facilitate movement of tissues usually over bony prominences. One type, subacromial calcific bursitis, is thought to occur when preformed, usually asymptomatic deposits of BCP crystals, rupture into the

bursa.

Mechanisms of calcification remain obscure. Local tissue damage, age related changes, or hypoxia may predispose to calcification. These may somehow stimulate chondrocyte metaplasia in tendons with subsequent production of calcifying matrix vesicles [51].

Arthropathies Associated with Intra-articular BCP Deposition

Although extra-articular BCP crystal deposition has long been recognized, the presence of BCP crystals within joints and synovial fluid was established more recently by Dieppe *et al.* [10] and Schumacher *et al.* [48] using electron microscopy with EDX microanalysis.

Several types of arthritis have now been associated with BCP crystals including osteoarthritis, destructive shoulder arthropathy, acute gout-like attacks, and erosive polyarthritis. BCP crystal deposition also occurs secondarily in chronic renal failure, in several of the connective tissue diseases, rarely following severe neurologic injury and intra-articular injection of crystalline corticosteroid preparations, and in other miscellaneous conditions.

Several studies have suggested that the incidence of BCP crystals in synovial fluids of patients with knee osteoarthritis is between 30-60% [11, 18, 28]. The radiographic appearance of these joints does not differ from osteoarthritis although the amount of BCP crystals does correlate with the radiologic grade of degeneration [27, 43].

BCP crystals are frequently observed together with CPPD crystals and have been referred to as "mixed crystal deposition disease" [12] (Figure 1). In two series of patients with osteoarthritis of the knee, CPPD and BCP were found together in 29% and 33% of synovial fluids whereas CPPD was found alone in 13% and 12% and BCP was found alone in 18% and 26% [18, 28]. Thus, BCP and CPPD seem to occur more often together than alone. This is surprising in light of the likelihood that BCP and CPPD form in different sites. BCP may emanate from exposed bone, metaplastic synovium and cartilage [14] whereas CPPD crystals form within cartilage. Perhaps factors which favor crystal deposition in general affect both BCP and CPPD. Although many studies have found an association between CPPD deposition and joint degeneration, most of these have not employed methodology which would have detected BCP crystals. Whether or not CPPD crystal deposition alone is associated with joint degeneration is still open to question. One study did find that CPPD crystals, while associated with age, were not associated with joint degeneration [28].

Acute attacks of arthritis have been observed occasionally in radiographically normal joints and in joints affected by osteoarthritis [17, 48]. Synovial fluid demonstrated hydroxyapatite crystals and elevated polymorphonuclear leucocyte counts. "Hydroxyapatite pseudopodagra" refers to gout-like attacks occurring in the first metatarsal phalangeal joint usually in young women associated with periarticular calcifications [16].

A polyarticular, erosive form of arthritis associated with BCP crystals has also been described [47]. Pain and swelling were most prominent in the wrists, metacarpophalangeal joints and proximal interphalangeal joints. Massive articular calcification was observed radiographically and joint damage was progressive.

"Milwaukee shoulder/knee syndrome" [38], also called "idiopathic destructive arthritis of the shoulder" [6] and "cuff tear arthropathy" [41], refers to a severe destructive arthropathy with large rotator cuff defects that is found mostly in elderly women. In a report of 30 cases, the average age was 73 years (range 53-90) [24]. The dominant side was the presenting joint in all except one case, but 60% had bilateral involvement. Radiographic evaluation showed rotator cuff defects in 46 of 55 shoulders examined, marked destructive changes of the glenohumeral joint, sclerosis, peri-articular calcifications in 55%, and partial collapse of the humeral head in some. Synovial fluids show "non-inflammatory" leucocyte counts, usually less than 1000/cmm with few polymorphonuclear cells. EHDP binding (>1 μg/ml hydroxyapatite standard) was found in 73 of 78 fluids. SEM demonstrated microspherioidal crystal masses with calcium to phosphorus molar ratios from 1.3-1.7 suggesting BCP crystals (Figure 2). Symptomatic knee involvement was also found in 16 patients. Unexpectedly, lateral compartment disease (39%) exceeded medial compartment involvement (31%).

A number of secondary forms of BCP crystal-associated arthropathies have been described. Chronic renal failure predisposes to BCP crystal deposition but also to MSU, CPPD, calcium oxalate [30], aluminum phosphate [42], and β_2 microglobulin amyloid deposition as well [3, 40]. Symptomatic joints with BCP crystal deposition have had both normal and elevated synovial fluid leucocyte counts [20, 53].

Calcinosis occurs in several connective tissue disorders including myositis, scleroderma, CREST syndrome, systemic lupus erythematosus, and overlap syndromes [32, 34]. Usually calcifications are found in skin and muscles but rarely bursal and intra-articular calcifications have been found.

Occasionally, severe neurologic damage has been followed by heterotopic ossification in periarticular tissues that may mimic arthritis [19, 46].

Intra-articular injection of the long lasting steroid triamcinolone hexacetonide has rarely been followed by periarticular calcification [33, 36].
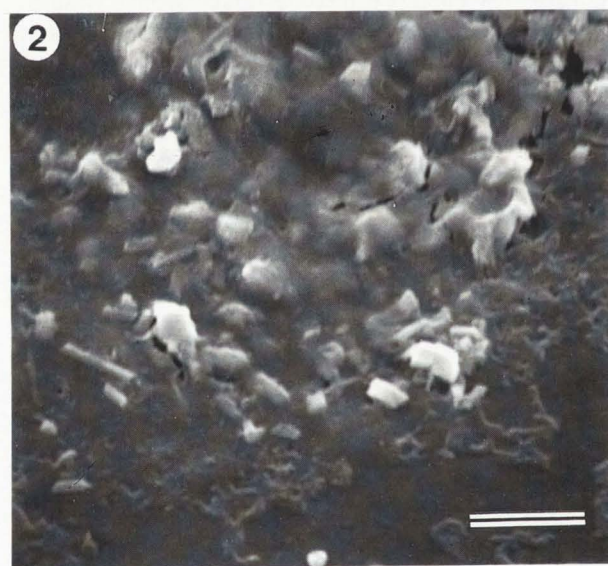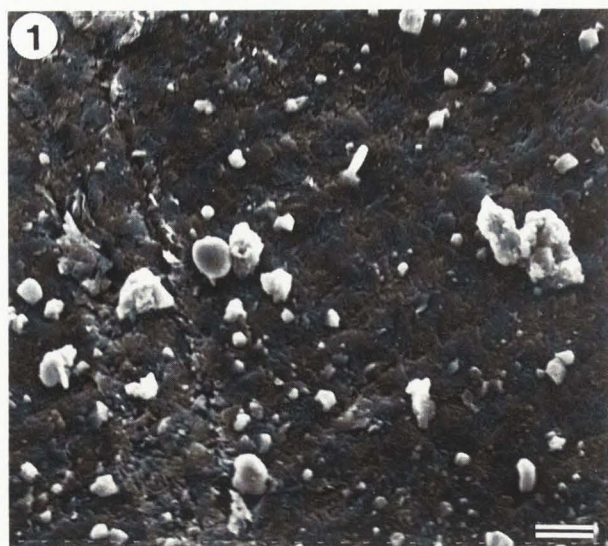
Figure 1 (above). Scanning electron micrograph showing mixed crystal deposition. Rhomboidal-shaped crystals represent calcium pyrophosphate dihydrate and the irregular spheroids represent aggregates of basic calcium phosphate crystals. Bar = 10 μm.

Figure 2 (below). Scanning electron micrograph showing microspheroidal particles consisting of basic calcium phosphate crystals. Bar = 10 μm.

Miscellaneous causes of BCP crystal deposition include vitamin D intoxication [35] and injection of olive oil into a knee [31]. BCP crystals have been identified in a few cases of rheumatoid arthritis with advanced joint destruction quite likely representing fragmentation

of bone. It is possible that very low levels of BCP crystals may occasionally be found in a variety of arthropathies.

Significance of BCP Crystal Deposition

The pathogenetic significance of BCP crystal deposition remains uncertain. Synthetic BCP crystals clearly possess phlogistic potential [48], but in synovial fluid containing BCP crystals, leucocyte counts are usually not elevated [11]. Thus, BCP crystals do not generally induce an inflammatory response of the type seen in attacks of gout and pseudogout. The exceptions are the unusual gout-like episodes, "hydroxyapatite pseudopodagra" and calcific bursitis. Most likely, adsorbed proteins such as α_2-HS glycoprotein play an important role in modulating crystal-cell interactions and resultant inflammation [50].

It is possible that BCP crystals may represent only an epiphenomenon. On the other hand, the association of crystals with advanced joint damage suggests that BCP crystals could amplify destructive processes within a joint. In osteoarthritis of the knee, severity of joint symptoms has been correlated with hydroxyapatite crystals, other particles and the synovial fluid leucocyte count [49]. Biopsies of the synovium from joints with BCP deposition have shown patchy synovial lining cell proliferation suggesting low grade inflammation [26]. *In vitro* studies have shown that BCP crystals increase production of prostaglandin E_2, collagenase and other proteases, and also increase mitogenesis in cultured synovial cells [8, 9]. Cartilaginous wear particles also increase neutral protease production [15]. Thus, low grade inflammatory changes could be mediated by BCP crystals, cartilage fragments, or possibly other factors. BCP crystals caused release of PGE_2 but not Il-1 from cultured mouse macrophages [1]. Tumor necrosis factor but not Il-1 was increased in synovial fluids of osteoarthritic patients relative to rheumatoid arthritis [52]. This information is confusing because even higher levels of tumor necrosis factor were found in two other non-inflammatory joint fluids from patients without osteoarthritis. Thus, the presence and role of cytokines in BCP related arthritis remains uncertain. Further studies will be necessary to elucidate the exact role of BCP crystals in arthritis.

Treatment of BCP arthropathies is hampered by the poor understanding of mechanisms of calcification and the inability to interfere with that process in a selective fashion. Thus, treatment approaches have been largely symptomatic including the use of nonsteroidal anti-inflammatory agents, physical therapy, and joint aspiration with corticosteroid therapy. If severe joint damage has occurred, joint replacement surgery may be considered.

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Discussion with Reviewers

M. Doherty: In rapidly destructive joint disease associated with plentiful BCP crystals, what is the likely site of origin of the crystals? Are they merely being shed from preformed deposits in capsule or tendon, are they predominantly coming from damaged subchondral bone, or is it possible that they form *de novo* in the articular space?

Author: All the sites mentioned above are possible sources of BCP crystals. Unfortunately, there is very little data with which to answer the question. One of our early patients with Milwaukee Shoulder Syndrome had an extensive synovectomy performed on her shoulder. Although an effusion returned postoperatively, the synovial contained no detectable BCP crystals suggesting that the synovium was the source of crystals in this case.

M. Doherty: In addition to arthropathies where BCP crystals are found in high concentration, BCP can be detected in many other joint conditions (e.g., rheumatoid, psoriatic arthropathy, etc.) and even in normal joint fluid. What comments can be made concerning the

specificity of BCP for any particular joint disease or arthritic process?

Author: Trace amounts of BCP crystals found in a variety of arthropathies probably represent non-specific findings. Most likely, some threshold concentration of crystals needs to be exceeded in order to observe crystal-induced effects.

K.P.H. Pritzker: Is the binding assay specific? Is it too sensitive, i.e., does it pick up bound calcium and phosphorous, rather than mineralization?

Author: The binding assay does not detect calcium pyrophosphate crystals. Amorphous calcium phosphates would potentially be detected by the binding assay except that they are dissolved out from the synovial fluid pellet by washing and resuspending in phosphate buffered saline.

P.A. Dieppe: Given the difficulty in identification and quantification, where do we go from here in trying to unravel the problem?

Author: Unfortunately, there is no satisfactory animal model which reproduces the BCP associated arthropathies that have been observed. Animal models such as the *ank-ank* mouse or the rat air pouch model may provide some useful information. Tissue/organ culture of synovium and cartilage from affected patients would also be interesting. In our laboratory, we are examining crystal cell interactions at a molecular biological level.

P.A. Dieppe: Why is the shoulder so vulnerable to these disorders?

Author: The rotator cuff is unique to the shoulder. This structure is susceptible to disorders which are not found in other joints including partial and complete tears, and probably ischemia of the supraspinatus tendon.

P.A. Dieppe: Could you speculate on the role of BCP in joint destruction? Three positions have been taken: one that BCP has a major role in disease pathogenesis (McCarty and others), one that it is an irrelevant secondary phenomenon, and one that BCP may act as an amplification loop (Doherty, Dieppe and others). What do you think, and why?

Author: All three scenarios may in fact have some validity. BCP crystals may be doing different things which may depend on the amount of crystals present, the size of the crystal masses, and probably other factors. BCP crystals have been detected at very low levels in some unrelated arthropathies in which they are most likely irrelevant. The difference between BCP crystals playing a "major role" versus "acting as an amplification loop" is seen as a semantic question representing degree

of affect. In acuted calcific bursitus and "hydroxyapatite pseudopodagra", BCP seems to be playing a major role. In chronic conditions where several processes may be in play, there is no way to answer how much BCP contributes. Thus, BCP could be amplifying another process which is primary.

K.P.H. Pritzker: It would be desirable to have a table outlining the sensitivity and specificity of each of the methods of detection of BCP crystals.

Author: A direct comparison of the methods of detection of BCP crystals has not been completed. Thus, a meaningful table showing sensitivity and specificity of the methods is not possible.

K.P.H. Pritzker: Regarding deposition of CPPD and basic calcium phosphate deposits: There is some contrary information in the literature [e.g., Pyrophosphate, phosphate ion interaction: Effects on calcium pyrophosphate and hydroxyapatite crystal formation in aqueous solutions. Pritzker *et al*. J. Rheumatology 10(5):769-777, 1983]. As well, the same group has found that the ionic conditions under which CPPD and BCP crystals can form are mutually exclusive [e.g., Calcium pyrophosphate crystal deposition in hyaline cartilage: Ultrastructural analysis and implications for pathogenesis. Pritzker KPH, *et al*. J. Rheumatology 15:828-835, 1988]. This would support the author's contention that the BCP is derived from fragmented bone or other oxygenous sources.

Author: The gist of this comment appears to be that BCP and CPPD crystals do not form under the same ionic conditions. The finding of "mixed" crystals, simultaneous occurrence of BCP and CPPD in synovial fluid, does not require that they formed in the same locale. It seems quite possible that BCP and CPPD form in different sites and merely escape from those sites to be found together in synovial fluid. The fact that these crystals are found often together may suggest the presence of a nucleator or some other promoter of crystallization or the absence of an inhibitor of crystallization.

C.R. Brown, Jr.: In clinical practice Alizarin Red is useful as a screening technique during synovial fluid analysis, followed by electron microscopy. The clinical use of Alizarin Red should be discussed.

Author: Unless additional studies establish the utility of the Alizarin Red test alone, I believe it is dangerous to recommend it for routine screening of synovial fluids.