WHY BASIC CALCIUM PHOSPHATE CRYSTALS SHOULD BE TARGETED IN THE TREATMENT OF OSTEOARTHRITIS

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ABSTRACT

Osteoarthritis (OA) is the most common form of arthritis and results in significant social, psychological, and economic costs. It is characterised by progressive cartilage loss, bone remodelling, osteophyte formation, and synovial inflammation with resultant joint pain and disability. Since OA affects the entire joint, it is not surprising that there has been difficulty developing an effective targeted treatment. Treatments available for structural disease modification are limited. Current options appear to mostly reduce symptoms. Basic calcium phosphate (BCP) crystals represent a potential therapeutic target in OA; they have been found in 100% of knee and hip cartilages removed at joint replacement. Intra-articular BCP crystals are associated with large joint effusions and dissolution of intra-articular structures, synovial proliferation, and marked degeneration as assessed by diagnostic imaging. While BCP deposition has been considered by many to be simply a consequence of advanced OA, there is substantial evidence to support BCP crystal deposition as an active pathogenic mediator of OA. BCP crystals exhibit a multiplicity of biologic effects *in vitro* including the ability to stimulate mitogenesis and prostaglandin, cytokine, and matrix metalloproteinase (MMP) synthesis in a number of cell types including macrophages, synovial fibroblasts, and chondrocytes. BCP crystals also contribute to inflammation in OA through direct interaction with the innate immune system. Intra-articular BCP crystals can elicit synovial inflammation and cartilage degradation in mice *in vivo*. Although intra-articular BCP crystals are difficult to detect at the bedside, advances in modern technology should allow improved identification and quantitation of BCP crystals. Our article focuses on why basic calcium crystals are important in the pathogenesis of OA. There is ample evidence that BCP crystals should be explored as a therapeutic target in OA.

Keywords: Osteoarthritis, basic calcium phosphate, calcium crystals, degenerative disease.

INTRODUCTION

Osteoarthritis (OA) is the most common form of joint disease and is one of the leading causes of pain and disability worldwide as it affects up to 13% of the world's population. The lifetime prevalence of symptomatic hip OA is estimated at 25.3%.[1] Knee OA is even higher at 44.7%.[2] OA results in significant social, psychological, and economic costs.[3] Billions of euros are spent on the management of OA especially surgical interventions, mainly joint replacement, which is still the gold standard of treatment for advanced OA. Piscitelli et al.[4] reviewed the socioeconomic burden of total joint arthroplasty for hip and knee OA in the Italian population and showed that hospital costs increased from €741 million to €1 billion over a 5-year period. A US study revealed that OA raised aggregate annual medical care expenditures by $185.5 billion.[5]

OA is a complicated disease as it affects all structures of the joint. Not only does it affect articular cartilage, but it also affects the subchondral bone, synovium, ligaments, tendons, and menisci. The multifaceted nature of the disease poses understandable difficulty in developing targeted therapies.

The multifactorial nature of OA is well-recognised. These factors can work independently or in combination to lead to joint degeneration. While

the clinical and structural characteristics of OA are well-recognised, the aetiopathogenesis remains poorly understood. Non-modifiable risk factors for OA include advanced age and genetics. However, aging appears to be insufficient for the development of OA as bone and cartilage changes in OA are different from those of normal aging. Genetic defects can give rise to premature OA but in the majority of those with OA, no such genetic defects have been identified.[6] Risk factors such as obesity and joint injury are potentially modifiable. But we are aware that OA occurs in those with normal body mass index (BMI) and in those who have never experienced joint trauma.

OA involves dynamic biochemical, biomechanical, and cellular processes. Synovial inflammation is frequently observed and can occasionally mimic RA synovium.[7] Furthermore, inflamed synovium is an important source of pain in OA. Inflammation in OA is now well recognised and this is reflected in ongoing research. Abou-Raya et al.[8] recently demonstrated in a randomised placebo-controlled trial that methotrexate significantly reduced pain and improved synovitis and physical function in patients with OA. They suggested that methotrexate may be a therapeutic option in the treatment of pain and inflammation related to knee OA.[8] A recent systematic review has shown that serum high-sensitivity-C-reactive protein (hs-CRP) levels were modestly but statistically significantly higher in OA than in controls.[9]

OA remains the focus of many academic and, to a lesser extent, industry research programmes. These studies are largely focused on molecular genetics, imaging, biomarkers, and novel pain targets in OA. However, there is paucity in the literature regarding the influence of BCP crystals in the pathogenesis of this disease. This is despite the fact that BCP crystals have been found in 100% of knee and hip cartilages removed during joint replacement, and calcium pyrophosphate dihydrate (CPPD) crystals were found in 20%.[10]

Current treatments for OA include non-pharmacological therapies such as exercise, weight loss, and orthotics to alter joint biomechanics. Pharmacological therapies include analgesics, nonsteroidal anti-inflammatory drugs (NSAIDs), viscosupplementation, and intra-articular corticosteroids.

Our article focuses on why basic calcium phosphate (BCP) crystals are important in the pathogenesis of OA, and why these crystals should be further explored as a target for the treatment of OA.

BCP CRYSTALS

'BCP crystals' is an umbrella term to describe a few types of calcium phosphates. These include partially carbonate-substituted hydroxyapatite (HA), octacalcium phosphate (OCP), tricalcium phosphate, and magnesium whitlockite.[11] HA crystals are the most prevalent. BCP crystals deposit synovium in the cartilage, the joint capsule, tendons, and even in intervertebral discs. BCP crystal deposits increase with age and the crystals frequently co-exist with CPPD crystals. The origin of BCP crystals is not fully understood; however, both CPPD and HA crystals may be generated in matrix vesicles (MV) derived from articular cartilage.[12] There is histologic evidence of MV near BCP crystal deposits in the articular cartilage. Substances within the extracellular matrix (ECM) strongly influence the mineralising activity of MV *in vivo*.[13] Another likely source of BCP crystals in advanced OA is the bony shards embedded in damaged cartilage, and bony debris resulting from the exposure of subchondral bone due to cartilage erosion.[14]

A prime illustration of the potent and destructive nature of BCP crystals is Milwaukee shoulder syndrome wherein abundant intra-articular BCP crystal deposits are found. This syndrome typically occurs in elderly females and is associated with large, and sometimes massive, joint effusions, complete rotator cuff tears, dissolution of the intra-articular portion of the long head of biceps, gross cartilage degeneration, and eburnation of subchondral bone. Rupture of the joint effusion can lead to a massive extravasation of blood and synovial fluid into the surrounding tissues.[15]

Detection of BCP Crystals

Some progress has been made in the past few years in the detection of BCP crystals. However, unfortunately unlike monosodium urate (MSU) and CPPD crystals that are easily detectable using polarised light microscopy, BCP crystals are too small (20–100 nm) to be identified by conventional techniques. Under a light microscope, clumps of BCP crystals are not birefringent and they can be mistaken for artefacts or debris. BCP deposits are rarely detectable using plain radiography, unlike CPPD that is more often visible on knee and wrist radiographs. Larger BCP aggregates have been detected by Alizarin Red S (ARS) staining, but

this method is difficult to interpret and also stains other calcium containing particulates. Rosenthal et al.[16] showed that BCP crystals could be identified using oxytetracycline staining in conjunction with ultraviolet light. There were fewer false-positive test results than with ARS staining and oxytetracyline did not bind to other particulates in joint fluid. Estimates of the quantities of synthetic BCP crystals were also possible.

More advanced microscopic techniques for detecting BCP crystals include electron microscopy, atomic force microscopy, electron microprobe, Raman spectroscopy, radiograph diffraction, scanning, a binding assay using 14C-labeled ethane-1-hydroxy-1,1-diphosphonate, and bisphosphonate-modified superparamagnetic beads. These techniques are expensive and unfortunately not readily available. BCP crystals must first be isolated from the synovial fluid prior to analysis. Therefore, progress in appreciating the role of BCP crystals in OA has been hampered by difficulties in bedside identification.[17]

BCP Crystals: Cause or Effect

Deposition of BCP crystals is a common finding in advanced OA. There is controversy in the literature as to whether these crystals cause OA, are a consequence of the degenerative process, or merely exacerbate the disease. Current evidence suggests that calcium crystal deposition contributes directly to joint degeneration and causes inflammation within the joint. Despite this, reviews of OA, written or presented, rarely include BCP crystals as a potential pathogenic factor in OA.[18]

Even if intra-articular BCP crystals are present as a consequence of joint damage, they can still play a role in perpetuating and aggravating the symptoms and signs of OA, especially by their effects on the synovium. Supportive evidence includes the fact that larger joint effusions are seen in knee joints containing BCP crystals when compared to joint fluid from knees without crystals.[19] BCP crystals correlate strongly with the rapid progression of arthritis and the severity of radiographic OA.[20] Furthermore, BCP crystals have been found not only

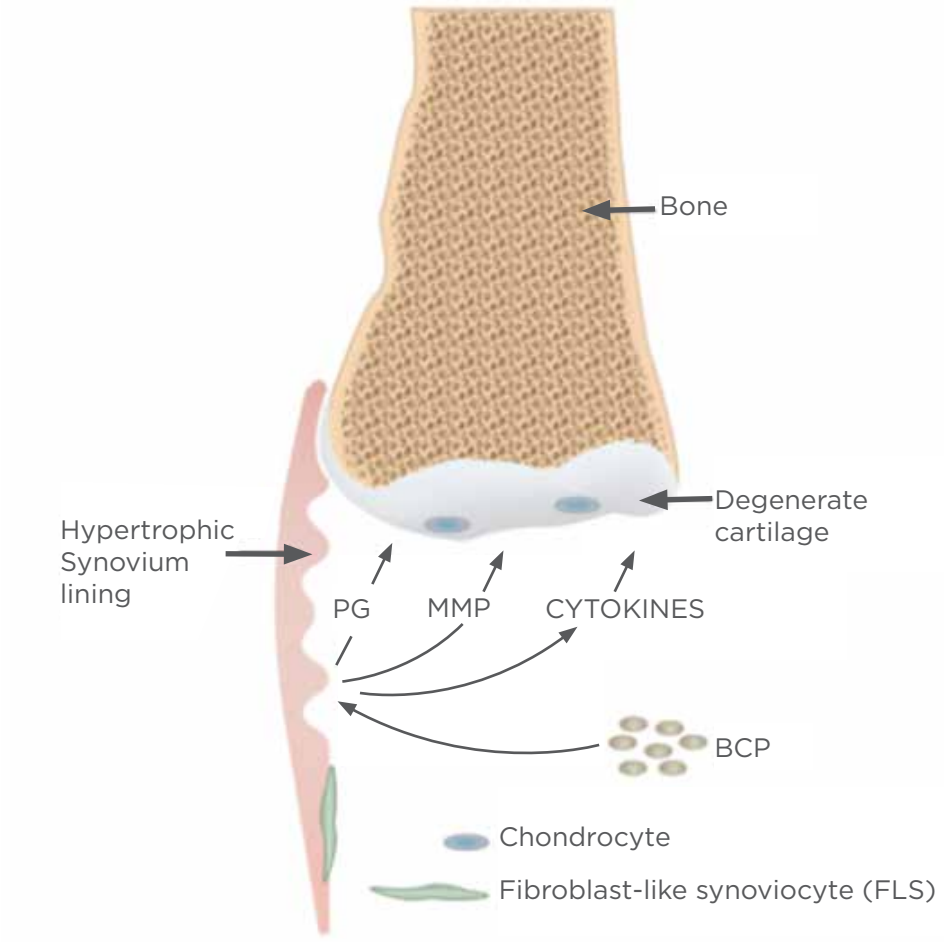

Figure 1: Proposed pathogenic effects of BCP crystals.
PG: prostaglandin; MMP: matrix metalloproteinase; BCP: basic calcium phosphate.

in advanced, but also in mild and moderate OA.[21] If BCP deposition was merely a consequence of bone exposure resulting from cartilage wear, how could the relative lack of BCP deposition in inflammatory, potentially destructive arthritis such as rheumatoid arthritis be explained?[22] All evidence points to a unique association between OA and BCP deposition.

In Vitro Findings

An understanding of the molecular mechanisms involved in the pathological effects of BCP crystals, although incomplete, has been significantly advanced in recent years. Recent studies have emphasised the important role of the innate immune system in the pathogenesis of OA. The NALP3 (NACHT, LRR, and PYD domains-containing protein 3) inflammasome complex has been implicated in MSU and CPPD crystal induced inflammatory disease. Activation of Toll-like receptor (TLR) pathways may play an essential role in progression of OA, and BCP crystals appear to be inherently involved in this process. BCP crystals have been shown in numerous studies to have multiple biological effects on articular cells such as chondrocytes and synovial fibroblasts (Figure 1).

In vitro BCP crystals induce cellular proliferation and stimulate matrix MMP expression. MMPs accelerate the degradation of cartilage matrix components such as Type 2 collagen, fibronectin, laminin, and proteoglycan. BCP crystals can activate synovial fibroblasts through numerous pathways, including extracellular signal-related kinases (ERK) 1 and 2, nuclear factor κB (NFκB), and protein kinase C (PKC). This in turn leads to upregulation of various inflammatory cytokines including tumour necrosis factor alpha (TNF-α), interleukin-6 (IL-6), and IL-1β.[23-25] These cytokines target articular chondrocytes and synovial cells, inducing expression of cartilage degrading enzymes that ultimately lead to joint destruction.

Nitric oxide (NO) is a known central mediator in OA. NO is generated by the oxidation of arginine, catalysed by the nitric oxide synthases (NOSs). BCP crystals increase NO production. BCP crystal deposition in OA cartilage enhances chondrocyte hypertrophy and apoptosis. Cheung et al.[26] demonstrated that treatment of cultured chondrocytes with the NO donor sodium nitroprusside stimulated calcification.

BCP crystals are unusual in that they upregulate both cyclooxygenase-1 and cyclooxygenase-2, followed by increased prostaglandin E2 in human fibroblasts.[27] They induce apoptosis in synovial fibroblasts and articular chondrocytes. These combined processes lead to an imbalance in anabolic versus catabolic mediators of cartilage turnover, which ultimately leads to ECM degradation.

In vivo studies have shown that accumulation of crystals in the joint leads to upregulated calcification or friction which activates NALP3 in synovial macrophages, leading to the production of IL-1β and IL-18. Jin et al.[28] showed that HA crystals lead to release of inflammatory cytokines in an NLRP3-dependent manner via reactive oxygen species (ROS) production, potassium efflux, and lysosomal damage.

IL-1β in particular has been identified as a key driver of destructive and inflammatory responses in OA as a result of its ability to upregulate aggrecanases and MMPs while also suppressing the biosynthesis of ECM. In keeping with this, IL-1β has been shown to be increased in the articular cartilage and synovial fluid of patients with OA. BCP crystals initiate IL-1β–mediated inflammatory processes through NALP3 inflammasome-dependent as well as inflammasome-independent pathways. Non–lipopolysaccharide (LPS)-primed murine macrophages incubated with BCP crystals produce high levels of IL-1β as well as IL-18, also an important cytokine in propagating joint damage. More importantly, longer incubation of LPS-primed macrophages with BCP crystals resulted in production of S100A8, a well-described damage-associated molecule that may further activate the macrophages through TLRs leading to production of IL-1β. Therefore, BCP crystals may cause the production of IL-1β both directly and indirectly through the autocrine effect of S100A8.[29] Also, the spleen tyrosine kinase (SyK) and PI3 kinase appear necessary for the induction of IL-1β following macrophage activation by BCP crystals.

The ability of BCP crystals to induce mitogenesis in many cell types including synoviocytes and macrophages may explain the macroscopic synovial proliferation found in OA. For example, BCP crystals activate human OA synovial fibroblasts (HOAS), leading to the induction of mitogenesis and MMP-1 production. Also, BCP crystals can act synergistically with IL1-α and TNF-α to promote MMP production and likely subsequent joint degeneration.[30] BCP crystals also induce the secretion of several other MMPs including MMP-1, 3, 8, and 9, and can also downregulate tissue inhibitor

of metalloproteinases (TIMP).[31-33] BCP crystals can also induce proto-oncogenes, c-fos, and c-myc.[34]

Sun et al.[35] showed that BCP crystals may stimulate the endocytosis of various extracellular molecules, such as DNA fragments, nucleotides, and small peptides that might contribute to the pathogenesis of BCP crystal-associated diseases.

Animal Studies

Narayan et al.[36] demonstrated that OCP crystals induce inflammation *in vivo* through IL-1-dependent peritoneal inflammation without requiring the NALP3 inflammasome. BCP crystals injected into the peritoneal cavity of mice led to neutrophil recruitment and up-modulation of IL-1α, IL-1β, and myeloid-related protein (MRP)-8-MRP-14 complex, to levels comparable with those induced by MSU crystals. This OCP crystal-induced inflammation was both IL-1α and IL-1β-dependent, as shown by inhibitory effects of anakinra and anti-IL-1β antibody treatment. This study[36] indicated that macrophages, rather than mast cells, are important for initiating and driving OCP crystal-induced inflammation.

Hang-Korng Ea et al.[37] showed that intra-articular BCP crystals have a direct pathogenic role in OA. BCP crystals injected into mouse knees induced synovial inflammation, cartilage degradation, and chondrocyte apoptosis. The effects observed were independent of the inflammasome-IL-1 pathway.

Two studies to date have looked at the effects of preventing BCP crystal deposition using pharmacological agents. Krug et al.[38] evaluated phosphocitrate (PC), a potential therapy for BCP crystal deposition. PC is the only agent to date that blocks the effects of BCP crystals and prevents calcification; the murine progressive anklyosis (MPA) model was used. This is a manifestation of an autosomal recessive mutation that produces an inflammatory joint disorder, associated with BCP crystal deposition, and results in fusion of the joints. Mice with MPA were treated with PC *in vivo* and there was a significant difference in disease progression and severity between the treated and the control group. Unfortunately, this model was somewhat inadequate as it resembled inflammatory arthritis more than OA.[38,39]

Cheung et al.[40] examined a guinea pig OA model with meniscal calcification, consistent with BCP crystal deposition. After weekly treatment of this animal model for 3 months with a new, more potent formulation of PC containing salt and calcium (CaNaPC), the content of calcification in menisci and cartilage degeneration was examined. As a control they evaluated whether similar CaNaPC treatment had a therapeutic effect in a hemi-meniscectomy model with no known crystal involvement. Meniscal calcification correlated with the cartilage degeneration in this animal model. CaNaPC treatment led to significant reduction of calcium deposits and arrested OA disease progression. Similar CaNaPC treatment had no effect in the hemi-meniscectomy model in which articular calcification does not occur. These results support the hypothesis that calcification in the form of BCP crystals plays an important role in OA disease progression, and that CaNaPC is a potential therapeutic agent for CPPD and BCP crystal deposition disease.[40] Unfortunately, no version of PC has been studied in humans nor is any available for clinical use.

CONCLUSION

No disease modifying osteoarthritis drug (DMOAD) has been approved by a regulatory body for OA as no DMOAD has clearly shown definite efficacy in patients with OA. With OA being the most prevalent rheumatic disease, affecting approximately 40 million patients in Europe, it is essential that we develop an effective treatment.[41] Enhanced efforts should be made to pursue BCP crystals as a potential target for OA. Advances have been made in the understanding of BCP crystals in the pathogenesis of OA.

We unfortunately have no therapy that prevents BCP crystal formation or removes BCP crystals. Drugs that could dissolve BCP crystals or prevent their formation would be of great interest. Modern technology should allow improved identification and quantitation of BCP crystals. Similar BCP crystal deposits are found in atherosclerotic vessels so there is now an increasing interest amongst cardiologists in exploring the biological effects of calcification in vascular disease. We are in an urgent need of an effective safe disease modifier for OA. There is ample evidence in the literature to show that BCP crystals should be explored as a therapeutic target in OA.

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