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Leaders

Understanding inorganic pyrophosphate metabolism: toward prevention of calcium pyrophosphate dihydrate crystal deposition

Monosodium urate monohydrate (MSU) crystals form from their constituent ions extracellularly: sodium concentrations within cells are too small to permit MSU nucleation and growth. Calcium pyrophosphate dihydrate (CPPD) crystals must form extracellularly for the same reason—the intracellular concentration of calcium is orders of magnitude smaller than that of extracellular calcium. We have generally attributed the rare examples of CPPD crystals within chondrocytes to endocytosis. An ultrastructural study of chondrocytes from a temporomandibular joint containing CPPD crystals was interpreted as showing intracellular crystals, perhaps formed within mitochondria,¹ but even if seed crystals form within cells, the very bulk of crystals present in cartilage strongly suggests an extracellular mechanism of crystal formation.

The facts that most inorganic pyrophosphate (PPi) is produced within cells during the synthesis of macromolecules and, like other phosphorylated compounds, does not diffuse across cell membranes, posed a conundrum in 1962 when CPPD crystals were discovered and characterised.² What is the source of the PPi driving extracellular CPPD crystal deposition? The figure summarises potential mechanisms.

Studies of intermediary PPi metabolism over the past 30 years by ourselves and others now permit formulation of a general hypothesis of the pathogenesis of CPPD crystal deposition based on the following observations:

- Plasma and urinary PPi are not increased in patients with CPPD crystal deposits, except in cases associated with hypophosphatasia. Plasma levels ($\approx 2 \mu\text{mol/l}$) are under tight homeostatic control.³ Thus the problem of CPPD crystal deposition must relate chiefly to local, articular factors. In contrast, gout is arguably a disease of the kidney (defective tubular urate secretion) and liver (which contains the bulk of the body's xanthine oxidase). MSU crystal deposition is driven by extracellular urate supersaturated with respect to sodium at the temperature of the affected local tissue,⁴ and by the presence of poorly understood nucleation factors.⁵

- Synovial fluid PPi concentrations exceed those in plasma and are increased in proportion to the degree of degeneration assessed radiographically.⁶ Even normal synovial fluid PPi levels are greater than plasma levels^{7–8}—supporting the thesis of extracellular PPi elaboration by articular tissue(s).

Schematic diagram of potential mechanisms of formation of extracellular PPi for formation of CPPD crystals: generation of PPi from ATP by an ectoenzyme (NTPPPHase) on a chondrocyte (A) or a vesicle (G); ATPase (D) competes for ATP substrate; ectoenzymes 5'nucleotidase (C) and PPiase (B) remove the products of NTPPPHase; compartmentalised intracellular PPi cotransported to the extracellular milieu with a matrix-destined macromolecule, in this example type II collagen (F); and passage of PPi through an anion channel (E).

- Hyaline articular and fibrocartilage, tendon, and ligament in organ culture release PPi into the ambient medium.^{9–10} Basal PPi release is stimulated twofold or more by transforming growth factor β (TGF β) or ascorbate, and this increase is inhibited by probenecid, insulin-like growth factor, or inhibitors of protein synthesis.^{11–14} In organ culture, normal human cartilage produced on average 2.5 pmol of PPi/mg/h.⁹ Measurements of joint surfaces in

an anatomical skeleton showed that normal human knee contains about 37 g of cartilage (hyaline plus fibrous: unpublished data). This mass of tissue should generate approximately 0.08 μmol of PPI per hour if the in vitro production rate is assumed.

- Injection of radiolabelled PPI and inorganic phosphate into human knee joints permitted calculation of the intra-synovial PPI pool and its rate of turnover.¹⁵ Clearance of both nuclides was linear for three hours, implying a single kinetic compartment the mean volume of which exceeded that of the joint fluid by about 30 ml. Approximately 0.1 μmol of PPI was produced per hour by knees with normal radiographs, increasing to about 0.8 $\mu\text{mol/h}$ in severely degenerated knees. The demonstration of a single PPI pool that included synovial fluid should have suggested to us that this compartment might contain both enzyme and substrate for PPI generation. PPI production calculated from kinetic studies (0.1 $\mu\text{mol/h}$) closely approximates that extrapolated from in vitro release of PPI (0.08 $\mu\text{mol/h}$).

- An enzyme, nucleoside triphosphate pyrophosphohydrolase (NTPPPHase), found in articular cartilage detergent extracts, was shown to hydrolyse ATP and other nucleotides to PPI.¹⁶

The reaction may be driven to the right by removal of the PPI coproduct (AMP) by 5'nucleotidase. The activity of the enzyme was greatest in cartilage containing CPPD crystals, and virtually all the activity was outside the chondrocyte (that is, it was an ectoenzyme).¹⁷ ATP added to chondrocytes in monolayer culture or cartilage slices in organ culture was preferentially hydrolysed to extracellular PPI.¹⁸ Chondrocytes depleted of NTPPPHase elaborated little measurable PPI.^{17, 19}

There is now evidence for three ectoNTPPPHases. One, termed PC-1, was originally isolated from a plasmacytoma cell line, and has been cloned and sequenced.²⁰ It is situated in the plasma membrane of many cells including osteoblasts, skin fibroblasts, and chondrocytes.²¹ A soluble form of PC-1 has been described. A second NTPPPHase, a 127 kDa molecule,²² appears to be specific for cartilage, ligament, and tendon.²³ It is pelletable and associated with 100 nm vesicles released from these tissues. The 127 kDa molecule and enzymatically active soluble split products have been found in cartilage organ culture conditioned medium. A third, 58 kDa, enzyme is being characterised.²⁴ All three enzymes have been identified in synovial fluids.²⁵

- ATP substrate for NTPPPHase was found in both platelet poor plasma (100–800 nmol/l) and synovial fluid.²⁶ Concentrations of extracellular ATP were significantly greater in fluids from joints containing CPPD crystals (approximately 200 nmol/l, compared with 100 nmol/l in osteoarthritis (OA)).

- The rate of hydrolysis of ATP in cell free synovial fluid incubated under physiological conditions was rapid; the half life averaged 72 seconds in OA fluids, 30 seconds in CPPD fluids, and 86 seconds in one normal fluid.²⁷ When ATP was infused into such fluids at rates intended to mimic steady state levels, it was converted preferentially to PPI (60–83%).²⁷ Synovial fluid was as effective as whole cartilage in generating ATP from extracellular ATP, as would be predicted from the kinetic in vivo studies that indicated a single kinetic compartment for PPI production. Using the previously determined mean in vivo PPI pool size (approximately 50 ml),¹⁵ we estimated that total PPI production by degenerated human knees would be 0.3–1.75 $\mu\text{mol/h}$. The greatest estimated production rates were found in cases of severely degenerated joints containing CPPD crystals. These estimates, although

admittedly crude, are consistent with the estimates of PPI production based on in vitro PPI generation by human cartilage organ cultures, and with rates calculated directly from in vivo radiolabelled PPI pool and turnover studies. Collectively, the data suggest that the measured PPI production in joint tissues compares quantitatively with estimates of PPI generation from extracellular ATP via ectoNTPPPHases. As it is likely that this process is substrate driven, we are actively seeking the source(s) of extracellular, intra-articular ATP.

- Vesicles derived from chondrocytes are rich in NTPPPHase and are able to nucleate and grow monoclinic CPPD crystals in the presence of ATP.²⁸ Moreover, ATP added to cartilage explants likewise induced the formation of perichondral CPPD-like crystals.²⁹

Clearly, both normal and abnormal joint tissues generate extracellular PPI. As this molecule is a potent inhibitor of apatite crystal nucleation and growth, we speculate that non-bony joint tissues require PPI to prevent ossification. Excess production of this inhibitor may result in the sporadic variety of CPPD crystal formation.

Unlike gout, a disease generally associated with a systemic abnormality of excess anion (hyperuricaemia), CPPD crystal deposition is driven by a local abnormality of excess articular anion production. In either condition, local factors determine the foci of crystal deposition. Local factors favouring CPPD crystal deposition could include bursts of increased PPI generation by selected chondrocytes, leading to a pericellular increase in anion; or chondrocytes may synthesise molecules or particulates capable of heterologous CPPD crystal nucleation.

CPPD crystals, in common with other crystals containing calcium, have potent, predictable, dose dependent, biological effects on synovial cells, macrophages, and chondrocytes.³⁰ These effects include stimulation of neutral protease synthesis, arachidonic acid metabolism, inosinic acid metabolism, and growth factor activity, and occur in the presence of concentrations of crystals known to exist in synovial fluid. Pharmacological control of excess PPI levels may prevent CPPD crystal deposition much as control of excess urate prevents MSU crystal deposition in gout. Such control is our goal.

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