Basic calcium phosphate crystals activate human osteoarthritic synovial fibroblasts and induce matrix metalloproteinase-13 (collagenase-3) in adult porcine articular chondrocytes

G M McCarthy, P R Westfall, I Masuda, P A Christopherson, H S Cheung, P G Mitchell

Abstract

Objective—To determine the ability of basic calcium phosphate (BCP) crystals to induce (a) mitogenesis, matrix metalloproteinase (MMP)-1, and MMP-13 in human osteoarthritic synovial fibroblasts (HOAS) and (b) MMP-13 in cultured porcine articular chondrocytes.

Methods—Mitogenesis of HOAS was measured by [\(^{3}H\) thymidine incorporation assay and counts of cells in monolayer culture. MMP messenger RNA (mRNA) accumulation was determined either by northern blot analysis or reverse transcriptase-polymerase chain reaction (RT-PCR) of RNA from chondrocytes or HOAS treated with BCP crystals. MMP-13 secretion was identified by immunoprecipitation and MMP-1 secretion by western blot of conditioned media.

Results—BCP crystals caused a 4.5-fold increase in [\(^{3}H\) thymidine incorporation by HOAS within 28 hours compared with untreated control cultures (p < 0.05). BCP crystals induced MMP-13 mRNA accumulation and MMP-13 protein secretion by articular chondrocytes. In contrast, in HOAS, MMP-13 mRNA induced by BCP crystals was detectable only by RT-PCR, and MMP-13 protein was undetectable. BCP crystals induced MMP-1 mRNA accumulation and MMP-1 protein secretion by HOAS. MMP-1 expression was further augmented when HOAS were co-incubated with either BCP and tumour necrosis factor α (TNF-α; threefold) or BCP and interleukin 1α (IL1α; twofold).

Conclusion—These data confirm the ability of BCP crystals to activate HOAS, leading to the induction of mitogenesis and MMP-1 production. MMP-13 production in response to BCP crystals is substantially more detectable in porcine articular chondrocytes than in HOAS. These data support the active role of BCP crystals in osteoarthritis and suggest that BCP crystals act synergistically with IL1α and TNF-α to promote MMP production and subsequent joint degeneration.

Intra-articular basic calcium phosphate (BCP) (hydroxyapatite, octacalcium phosphate, and tricalcium phosphate) crystal deposition is found in the joint fluid of up to 70% of osteoarthritic (OA) knees and is associated with severe degenerative arthritis. Periarticular synovial lining hyperplasia and more severe radiographic degeneration and larger joint effusions than OA knees without crystals. BCP crystals are also associated with severe destructive arthropathies, such as Milwaukee shoulder syndrome. This condition is characterised by marked synovial proliferation and non-inflammatory degradation of articular structures, such as cartilage, tendons, ligaments, and adjacent bone.

Matrix metalloproteinases (MMP) play a major part in cartilage degradation and BCP crystals cause production of MMP (collagenase, stromelysin, and 92 kDa gelatinase) from human fibroblasts in vitro. They also induce collagenase (MMP-1) production from adult porcine articular chondrocytes. BCP crystal induction of these MMP from intra-articular cells probably promotes the severe joint degeneration associated with their presence. In addition, BCP crystals themselves are at least partly responsible for the associated synovial lining proliferation, and increased cell numbers in the synovial lining enhance the capacity for secretion of proteolytic enzymes.

Tumour necrosis factor α (TNF-α) and interleukin 1 (IL1) have both been implicated in the pathogenesis of OA. TNF-α and IL1 promote the release of MMP and suppress the synthesis of proteoglycans and collagen by human chondrocytes and synovial cells. Also, TNF-α induces MMP-1 and MMP-3 production from adult porcine chondrocytes. A more recently isolated MMP, MMP-13, has a 10-fold greater catalytic activity towards type II collagen than MMP-1. It has been identified in human OA cartilage and is inducible in human and porcine articular chondrocytes. Reports of the expression of MMP-13 in human osteoarthritic synoviocytes (HOAS) have varied. Furthermore, although BCP crystals induce MMP-1 in chondrocytes, the ability of BCP crystals to induce MMP-13 in chondrocytes has not been reported.

BCP crystals have been shown to induce mitogenesis and MMP-1 production in a number of cell lines, neovascularisation, and matrix metalloproteinase activity in fibroblasts, porcine articular chondrocytes, and canine synovial fibroblasts, but their effect on
human synovial cells has not previously been shown. Furthermore, in OA joints BCP crystals probably exert their effects in association with cytokines such as IL1 and TNFα, allowing an opportunity for synergy.

To elucidate further the biological effects of BCP crystals we suggested that BCP crystals induce MMP-13 in adult porcine articular chondrocytes and HOAS. We also suggested that BCP crystals activate HOAS leading to mitogenesis and MMP-1 production, thereby enhancing TNFα and IL1 induced MMP-1 production.

**Materials and methods**

**PROBES AND REAGENTS**

The MMP-13 probe was a full length human cDNA. The MMP-1 probe was a 2.05 kb HindIII/Sou31 insert from the pC11ase 1 clone, obtained from the repository of human DNA probes of the American Type Culture Collection (Rockville, MD). The pHcGAP plasmid containing glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA was also from the American Type Culture Collection. TRizol reagent (a monophasic solution of phenol and guanidine isothiocyanate) was from Gibco BRL Life Technologies (Grand Island, NY). FORMAoxol (formamide) and BCP phase separation reagent (1-bromo-3-chloropropane) were from Molecular Research, Inc. 45Ca was from DuPont NEN Research Products (Boston, MA). Two MMP-1 antibodies were used: a polyclonal antibody which was raised in rabbit against full length recombinant human MMP-13 and a monoclonal antibody (No F17 against full length recombinant human MMP-13) were obtained from Genzyme Corporation. Biotin labelled, goat antirabbit IgG and peroxidase labelled streptavidin were from Kirkegaard and Perry Laboratories, Inc (Gaithersburg, MD). Enhanced chemiluminescence was performed using a kit from Amersham Life Sciences and UK. Tritiated thymidine (50 Ci/mmol) was from Amersham Life Sciences (Buckinghamshire, UK). SUPERSCRIPT II, oligo(dT)15 primer and enzymatically dissociated with 1 mg/ml trypsin/DMEM (50 ml) and 1% phenylmethylsulphonyl fluoride was added for an additional 30 minutes' incubation. Liberated cells were spun at 400 g for 10 minutes and washed twice with 50% FBS, 0.5% FBS, and subsequently incubating in this medium, washing with DMEM containing 1% PMSF and 0.2% gentamycin. They were rendered quiescent by removing the medium, washing with DMEM containing 0.25% trypsin/DMEM (50 ml) and 1% PMSF was added for an additional 30 minutes' incubation. Liberated cells were spun at 400 g for 10 minutes and washed twice with 50% phosphate buffered saline (PBS)-50% DMEM. Cells were resuspended, filtered through nylon, and then grown and maintained in DMEM supplemented with 10% FBS containing 1% PMSF and 0.2% gentamycin. They were incubated at 37°C in a humidified atmosphere containing 10% CO2. Cultures were routinely re-fed with 10% FBS in DMEM on days 2 and 4 after plating. These levels are equivalent to concentrations of BCP crystals found in pathological joint fluids.

**CELL CULTURE**

Adult porcine chondrocytes were used as a model for human chondrocytes because they have been shown to have numerous biological responses to treatment with BCP crystals and to produce collagenase and stromelysin when treated with TNFα or epidermal growth factor.11 45 Chondrocytes were prepared by digestion of cartilage from adult porcine knees as described previously. The cells were plated at 4 × 10^5/cm^2 in DMEM supplemented with 10% FBS, allowed to attach, and incubated at 37°C in a humidified atmosphere containing 10% CO2. Cultures were routinely re-fed with 10% FBS in DMEM on days 2 and 4 after plating. These levels are equivalent to concentrations of BCP crystals found in pathological joint fluids.

**Northern blot analysis**

Northern blot analysis of total cellular RNA was used to study the expression of MMP-13...
Induction of matrix metalloproteinase in porcine articular chondrocytes

Figure 1 Basic calcium phosphate (BCP) crystal induced accumulation of matrix metalloproteinase-13 (MMP-13) messenger RNA (mRNA) in adult articular porcine chondrocytes. Time course. Confluent cultures of chondrocytes, in 60 mm plates, were incubated in serum-free media for 24 hours before being stimulated with BCP crystals (24 µg/cm²). At various times after treatment, cultures were harvested and total RNA was isolated. Northern blot analysis was performed using a specific probe for MMP-13, followed by autoradiography. The probe was then removed by washing and the blot was re-probed with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA as a control. The positions of the 18S and 28S ribosomes are shown. B = BCP crystals; C = unstimulated control cultures.

Table 1:

<table>
<thead>
<tr>
<th>Hours after treatment</th>
<th>12</th>
<th>24</th>
<th>48</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-13</td>
<td>28S</td>
<td>28S</td>
<td>28S</td>
</tr>
<tr>
<td>GAPDH</td>
<td>18S</td>
<td>18S</td>
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mRNA in chondrocytes or HOAS after stimulation with BCP crystals or TNFα. Control cultures were treated with 12-O-tetradecanoylphorbol 13-acetate or IL1 or left untreated. Confluent, quiescent monolayer cultures of chondrocytes in 60 mm plates, or HOAS in 100 mm plates, were washed twice with cold PBS 24 hours after treatment. Total RNA was recovered with TRizol reagent based on the methods of Chomczynski and Sacchi30 but using 1-bromo-3-chloropropane reagent instead of chloroform. RNA was solubilised in FORMAsox. Ten micrograms of RNA were fractionated on a 1.2% agarose-formaldehyde gel, ribosomal RNA was visualised with ethidium bromide, and the fractionated RNA was transferred to supported nitrocellulose membrane (Micron Separations Inc, Westborough, MA) by the capillary method.33 The RNA was cross linked to the membrane after transfer by UV cross linking in a Fisher brand UV cross linker (Fisher Pittsburg, PA). The blot was pre-hybridised for four hours at 42°C and hybridisation of the filters with DNA probes was performed overnight at 42°C. Probes were labelled using the random primer method to a specific activity of >5 × 10⁶ cpm/µg.34 The filters were washed at a maximal stringency of 0.25 × standard saline citrate (SSC), 1 × SSC = 0.15 M sodium chloride, 0.015 M sodium citrate, pH 7.0, at 60°C for 30 minutes.

 Autoradiography was performed with Kodak XAR-5 film (Eastman Kodak, Rochester, NY) and signal intensity was quantified by scanning laser densitometry (LKB Instruments, Stockholm, Sweden).

DETERMINATION OF MITOGENESIS BY UPTAKE OF [3H]THYMIDINE

HOAS were grown to confluence in 24 well plates and rendered quiescent by incubation in 0.5% FBS for 24 hours. [3H]Thymidine (1 µCi/ml) was added to the wells 23 hours after the addition of 10% FBS or BCP crystals (18 µg/cm²) and pulse labelled for one hour. Control cultures were in 0.5% FBS. The cells were then washed three times with PBS, and macro molecules were precipitated with 5% trichloroacetic acid (TCA) solution. The precipitate was washed again with PBS and dissolved in 1 ml 0.1 N NaOH/1% sodium dodecyl sulphate (SDS). Levels of TCA precipitable H were determined in quadruplicate, using a liquid scintillation counter (Packard Instruments, Downers Grove, IL). In separate experiments, mitogenic stimulation of monolayers was followed by trypsinisation after 48 hours and cell counts were determined with a Coulter counter (Coulter Electronics, Hialeah, FL).

IMMUNOBLOTTING

MMP-1 secretion by HOAS was confirmed by western blot.35 Briefly, samples of conditioned media from cultures treated with BCP crystals, TNFα, IL1α, or unstimulated control cultures were electrophoresed through 10% polyacrylamide gels under reducing conditions and then transferred onto nitrocellulose membranes. After transfer, the membranes were incubated in 2.5% non-fat dry milk in Tris buffered saline.
before being stimulated with BCP crystals (18 µg/cm²) incubated in 0.5% fetal bovine serum–Dulbecco’s modified Eagle’s medium for 24 hours. Confluent cultures of synovial fibroblasts, in 100 mm plates, were treated with tumour necrosis factor α (TNF) or interleukin 1 (IL1) = interleukin 1 (10 ng/ml) or interleukin 1 (0.5% HEPES buffer and 1% PMSF. Cultures were then incubated in methionine-free media. One million samples of methionine-free DMEM, conditioned in the presence of 50 µCi/ml of [35S]methionine, from cultures stimulated with BCP crystals or TNPu were collected after 24 or 48 hours. Control cultures were in 0.5% FBS with or without IL1α. Fifty microlitres of 10 × immunoprecipitation buffer (100 mM Tris, pH 7.5, 5% triton x-100, 1% SDS, 5% deoxycholate) and 40 µl of 50% suspension of protein A-Sepharose CL-4B, and 10 µl of normal rabbit serum were added and samples mixed at 4°C for one hour. The supernatant was removed after brief centrifugation, and 40 µl of fresh protein A-Sepharose added. The sample was mixed at 4°C for 30 minutes. The supernatant was again removed after brief centrifugation. Ten microlitres of MMP-13 antiserum, 10 µl immunoprecipitation buffer, and 40 µl 50% protein A-Sepharose were added to each sample. The samples were mixed overnight at 4°C and the immunoprecipitates washed three times in 1 ml × immunoprecipitation buffer (10 mM Tris, pH 7.4, 0.5% Triton X-100, 0.1% SDS, and 0.5% deoxycholate) and once in PBS. After the final spin the control cultures were harvested and total RNA was isolated. Northern blot analysis was performed using a specific probe for MMP-13, followed by autoradiography. The bands were then normalised and quantified using Scanalytics (Ambis, San Diego, CA).

IMMUNOPRECIPITATION

MMP-13 secretion was confirmed by immunoprecipitation of the conditioned media by a polyclonal antibody to MMP-13. Chondrocyte cultures were incubated overnight in DMEM with 20 mM HEPES buffer and 1% PMSF. Cultures were then incubated in methionine-free media. One million stimulated samples of methionine-free DMEM, conditioned in the presence of 50 µCi/ml of [35S]methionine, from cultures stimulated with BCP crystals or TNPu were collected after 24 or 48 hours. Control cultures were in 0.5% FBS with or without IL1α. Fifty microlitres of 10 × immunoprecipitation buffer (100 mM Tris, pH 7.5, 5% triton x-100, 1% SDS, 5% deoxycholate) and 40 µl of 50% suspension of protein A-Sepharose CL-4B, and 10 µl of normal rabbit serum were added and samples mixed at 4°C for one hour. The supernatant was removed after brief centrifugation, and 40 µl of fresh protein A-Sepharose added. The sample was mixed at 4°C for 30 minutes. The supernatant was again removed after brief centrifugation. Ten microlitres of MMP-13 antiserum, 10 µl immunoprecipitation buffer, and 40 µl 50% protein A-Sepharose were added to each sample. The samples were mixed overnight at 4°C and the immunoprecipitates washed three times in 1 ml × immunoprecipitation buffer (10 mM Tris, pH 7.4, 0.5% Triton X-100, 0.1% SDS, and 0.5% deoxycholate) and once in PBS. After the final spin the supernatant was discarded. Samples were placed in SDS reducing sample buffer and separated on a 10% polyacrylamide gel according to Laemmli. The gel was then dried and autoradiography performed.

REVERSE TRANSCRIPTASE-POLYMERASE CHAIN REACTION (RT-PCR)

The reverse transcriptase reaction was performed with 1 μg total RNA using the SUPERSCIPT II (moloney-murine leukaemia virus reverse transcriptase) and an oligo(dT)₁₅ primer. Aliquots of cDNA were amplified by PCR using Taq DNA polymerase up to a cycle number of 40. The PCR products were electrophoresed in 2.0% agarose gels containing ethidium bromide. The cycle number was determined as the amplification in the linear range. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA levels were used as an internal control. The following primers were used in the amplification reactions: GAPDH, 5'-AATCCCTCAGGAT GTCAGCA-3', 5'-TCCACACCCCTGTGTTG CTGTAT-3', resulting in a 553 bp product; and MMP-1, 5'-GTCAGCA-3', 5'-TCCACACCCCTGTGTTG CTGTAT-3', resulting in a 553 bp product.
either BCP crystals (18 µg/cm²) or interleukin 1α (TNFα) were stimulated with modified Eagle’s medium containing 0.5% fetal bovine serum (FBS) were stimulated with BCP crystals (18 µg/cm²). Interleukin-1α (100 U/ml), tumor necrosis factor α (10 ng/ml) and BCP crystals (48 µg/cm²) were added concurrently, BCP crystal induced MMP-13 mRNA was markedly inhibited. This inhibition persisted when cycloheximide was added up to four hours after treatment with BCP crystals (data not shown). When chondrocytes were incubated for 24 or 48 hours with various concentrations of BCP crystals the secretion of a protein of approximately 63 kDa, immunorecognizable with a polyclonal antibody to MMP-13, was induced at all concentrations tested. MMP-13 protein was not evident in unstimulated control cultures.

![MMP-1 Induction](image)

**Figure 5** Basic calcium phosphate (BCP) crystal induced stimulation of matrix metalloproteinase-1 (MMP-1) protein synthesis and secretion in human osteoarthritic synovial fibroblasts. Confluent, quiescent human osteoarthritic synovial fibroblasts incubated in Dulbecco’s modified Eagle’s medium containing 0.5% fetal bovine serum (FBS) were stimulated with either BCP crystals (18 µg/cm²), interleukin-1α (100 U/ml) or tumor necrosis factor α (10 ng/ml). Unstimulated cultures were in 0.5% FBS. The culture medium was analysed by western blotting using a specific monoclonal antibody to MMP-1. Numbers refer to hours after treatment. Molecular weight markers are shown to the left. C = unstimulated control cultures; B = BCP crystals; IL1 = interleukin 1α; TNF = tumor necrosis factor α; CB

BCP CRYSTAL INDUCTION OF MITOGENESIS IN HOAS

The addition of BCP crystals (25 µg/cm²) in 0.5% FBS-DMEM stimulated an increase in [³H]thymidine incorporation, an index of mitogenesis, at all time points tested (fig 3). The increase in thymidine incorporation reached statistical significance at 22.25, and 23 hours compared with control (p<0.05). Maximal [³H]thymidine incorporation was noted in cultures harvested 20 hours after treatment, when there was a fourfold increase in [³H]thymidine incorporation compared with unstimulated control cultures. We also performed cell counts four days after treatment to confirm that the increased [³H]thymidine incorporation was accompanied by an increase in cell number. The mean (SD) number of cells (×10⁵/cm²) in control cultures incubated with 0.5% FBS was 1.65 (0.3). The number of cells (×10⁵/cm²) in cultures incubated with BCP crystals was 2.92 (0.51). The number of cells in cultures incubated with 10% FBS was 4.09 (0.3). The increase in cell number in response to both BCP crystals and 10% FBS was significant (p<0.05).

**Table 2**

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean ± SD</th>
<th>p Value</th>
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<tbody>
<tr>
<td>Control</td>
<td>1.65 ± 0.3</td>
<td>0.001</td>
</tr>
<tr>
<td>BCP crystals</td>
<td>2.92 ± 0.51</td>
<td>0.001</td>
</tr>
<tr>
<td>10% FBS</td>
<td>4.09 ± 0.3</td>
<td>0.001</td>
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**Figure 6** Basic calcium phosphate (BCP) crystal induced stimulation of matrix metalloproteinase-13 (MMP-13) protein synthesis and secretion in human osteoarthritic synovial fibroblasts (HOAS). Effect of co-incubation with tumour necrosis factor α (TNFα) or interleukin-1α (IL1α) on MMP-13 mRNA accumulation in HOAS. Confluent, quiescent HOAS incubated in Dulbecco’s modified Eagle’s medium containing 0.5% fetal bovine serum (FBS) were stimulated with either BCP crystals (18 µg/cm²) and IL1α (10 µU/ml) or TNFα (10 ng/ml). For comparison, some cultures were treated with both IL1α and TNFα. Unstimulated cultures were in 0.5% FBS. The culture medium was analysed by western blotting using a specific monoclonal antibody to MMP-13. Numbers refer to hours after treatment. Molecular weight markers are shown to the left. C = unstimulated control cultures; B = BCP crystals.

MMP-13, 5'-GTGCCCCTCTCTTACAGCAG AC-3', 5'-CAGAATTCAAGGCCACATCCACACAG-3', resulting in a 100 bp product. Sequences for MMP-13 to design primer sets were found from Genbank accession number X75308.26

**MMP-13 mRNA and Protein Secretion in HOAS**

BCP crystals induced significant accumulation of MMP-1 mRNA in HOAS, first evident at eight hours and continuing to at least 48 hours, the longest time point tested (fig 4). At 24 and 48 hours, MMP-1 mRNA accumulation induced by BCP crystals was comparable with that induced by IL1α and TNFα (fig 4). MMP-1 mRNA accumulation was followed by significant MMP-1 protein secretion in conditioned media which was maximal when the conditioned media was harvested at 48 hours (fig 5). Similarly, when HOAS were treated with IL1α or TNFα, there was significant MMP-1 protein secretion, which was maximal at 48 hours. MMP-1 protein expression was further increased when HOAS were co-incubated with

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amplification was performed for 40 cycles as described in “Materials and methods.”

Total RNA was isolated from synovial fibroblasts and subjected to RT-PCR

to measure matrix metalloproteinase-13 (MMP-13) mRNA in human osteoarthritic synovial fibroblasts. Total RNA was isolated from synovial fibroblasts and subjected to RT-PCR to measure matrix metalloproteinase-13 (MMP-13) mRNA in human osteoarthritic synovial fibroblasts. No MMP-13 protein secretion was identified, in response to BCP crystals, TNF-α alone (fig 6).

The time course of induction of MMP-13 in response to BCP crystals was similar to that of crystal induction of MMP-1, MMP-3, and MMP-9, suggesting the coordinate production of all four MMP in response to BCP crystals.27 In HOAS we found that MMP-13 mRNA accumulation was detectable only by RT-PCR in response to BCP crystals, TNF-α, or IL-1β. No MMP-13 protein secretion was identified, presumably because quantities secreted were below the level of detection of our methods.

BCP crystal induction of MMP-13 mRNA was inhibited by the protein synthesis inhibitor cycloheximide, suggesting that new protein synthesis is required for the transcription activation of MMP-13 by BCP. Structural analysis of the 5′ flanking region of the MMP-13 gene has shown the presence of an AP1 motif.28 AP1 is a heterodimer composed of the protein products of Fos and Jun. BCP crystals induce c-jun and AP1 in human fibroblasts.29 It is therefore likely that the inhibition of BCP crystal induction of MMP-13 mRNA by cycloheximide, at least in part, reflects inhibition of the synthesis of an AP1 complex, normally induced in response to BCP crystals.

Although this is the first report of MMP-13 production in chondrocytes of any species treated with BCP crystals, MMP-13 expression has been consistently described in human OA chondrocytes and in chondrocytes treated with various inflammatory cytokines, including TNF-α, IL-1β, and IL-1α.30 MMP-13 expression determined by northern blot was reported in the synovial membranes of one patient with rheumatoid arthritis (RA) and one patient with OA.30 MMP-13 mRNA, identified by northern blot, was reported in the synovial membranes of six patients with RA and seven patients with OA. The overall expression of MMP-13 in the synovial stroma was substantially higher in RA than in OA.30 MMP-13 was identified by RT-PCR and by immunohistochemistry in the synovial lining of patients with aseptic loosening of a total hip replacement and with OA. MMP-13 was found in the synovial fluid of these patients by western blotting.30 MMP-13 mRNA expression was detected in the synovial membranes of 21 of 36 patients with RA, and increased synovioctye MMP-13 expression correlated with increases in the clinical measurements of erythrocyte sedimentation rate and C reactive protein.30 Four of 10 primary RA synovial fibroblast cell cultures manifested basal expression of MMP-13 mRNA, which was stimulated two- to four-fold in response to IL1β or TNF-α.30 Finally, Vincenti et al have shown that MMP-13 mRNA and protein is inducible in rabbit synovial fibroblasts by IL1β, TNF-α, or phorbol esters.30 In contrast, however, Reboul et al showed MMP-13 expression by RT-PCR in OA chondrocytes but not HOAS.31 Borden et al studied patients with RA and found MMP-13 gene expression in RA chondrocytes but not RA synovial fibroblasts.32 We compared healthy adult porcine chondrocytes with OA fibroblasts, thus introducing the variables of species differences and healthy versus diseased states. None the less, current data, including ours, suggest that MMP-13 is more consistently inducible and expressed in greater quantities in chondrocytes than in synoviocytes. Therefore, MMP-13 derived from chondrocytes probably has a more important role in OA, RA, and in BCP crystal associated joint degeneration than MMP-13 derived from synoviocytes.

Furthermore, enhanced and coordinated expression of IL1β, TNF-α, and inducible nitric oxide synthase by chondrocytes compared with HOAS has previously been seen in OA.29 We have shown here that the production of MMP-13 in response to BCP crystals is enhanced in chondrocytes compared with synovial cells, thus following a similar pattern.

Figure 7 Reverse transcriptase-polymerase chain reaction (RT-PCR) determination of matrix metalloproteinase-13 (MMP-13) mRNA in human osteoarthritic synovial fibroblasts. Total RNA was isolated from synovial fibroblasts and subjected to RT-PCR analysis for MMP-13 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). PCR amplification was performed for 40 cycles as described in “Materials and methods.” Numbers refer to time in hours after treatment with BCP crystals (10 μg/cm²), tumour necrosis factor α (TNF-α, 10 μg/ml; 24 and 48 hours).
Taken together, these data support the hypothesis that chondrocytes are a major site of production of mediators of inflammation in human OA. Previous studies of BCP crystal induced cell activation have been performed using model systems of human skin and foreskin fibroblasts, canine synovial and murine 3T3 fibroblasts, and porcine chondrocytes. Data presented here confirm that BCP crystals also activate human (OA) synovial fibroblasts, leading to mitogen- and MMP-1 production. These observations confirm the relevance of previous work using other model systems, including human, non-articular fibroblasts to study BCP crystal mediated events.

BCP crystals occur commonly in OA joints. None the less, whether BCP crystals are simply an epiphenomena of cartilage damage or whether BCP crystals cause cartilage damage has long been contested. If the crystals were present simply as a consequence of cartilage damage and joint destruction, we would expect them to be present in other arthropathies characterized by cartilage dissolution and synovial lining proliferation, such as RA. However, BCP crystals are rarely found in RA joint fluids. Thus current data support the active role of BCP crystals in the exacerbation of OA.

We have shown that BCP crystals induce MMP-1 in HOAS as in vitro. Furthermore, BCP crystals, IL1α, and TNFα appear to act in synergy to increase MMP-1 production by HOAS in vivo, because BCP crystals and cytokines such as TNFα and IL1α co-exist, they probably act in concert to augment joint degeneration. Historically, the role of cytokines in the pathogenesis of OA was also considered to be speculative. Furthermore, as with BCP crystals, levels of pro-inflammatory cytokines such as IL1 or TNFα are not routinely measured in joint fluid from patients with arthritis. After considerable further investigation, however, the roles of IL1 and TNFα in mediating joint destruction in OA are now well accepted. As a consequence of such recognition, Pellegrini and Smith have written extensively on the effects of IL1 and TNFα in mediating joint destruction in OA. In vivo, BCP crystals induce synovial and cartilage inflammation in OA in experimental model by means of a variety of mechanisms.

Inhibition of matrix metalloproteinase in porcine articular chondrocytes is of considerable further investigation, however, the roles of IL1 and TNFα in mediating joint destruction in OA are now well accepted. As a consequence of such recognition, Pellegrini and Smith have written extensively on the effects of IL1 and TNFα in mediating joint destruction in OA. In vivo, BCP crystals induce synovial and cartilage inflammation in OA in experimental model by means of a variety of mechanisms.


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