Osteoarthritis and Cartilage (2007) **15,** 414–420

doi:10.1016/j.joca.2006.10.003

Osteoarthritis and Cartilage

International Cartilage Repair Society

OARSI OSTEOARTHRITIS RESEARCH SOCIETY INTERNATIONAL

BCP crystals increase prostacyclin production and upregulate the prostacyclin receptor in OA synovial fibroblasts: potential effects on mPGES1 and MMP-13[1]

E. S. Molloy M.D., M.R.C.P.I.†*[a], M. P. Morgan Ph.D.‡[a], B. McDonnell B.A. (Mod.)§[a], J. O'Byrne M.D., F.R.C.S.I.‡[a] and G. M. McCarthy M.D., F.R.C.P.I.†‡§[a]

† *Department of Molecular and Cellular Therapeutics, Royal College of Surgeons of Ireland, 123 St Stephen's Green, Dublin 2, Ireland*

† *National Orthopaedic Hospital, Cappagh, Dublin 15, Ireland*

§ *Division of Rheumatology, Mater Misericordiae University Hospital, Eccles St, Dublin 7, Ireland*

Summary

Objective: To investigate the potential involvement of prostacyclin in basic calcium phosphate (BCP) crystal-induced responses in osteoarthritic synovial fibroblasts (OASF).

Methods: OASF grown in culture were stimulated with BCP crystals. Prostacyclin production was measured by enzyme immunoassay. Expression of messenger RNA (mRNA) transcripts was assessed by real-time polymerase chain reaction (PCR). Expression of prostacyclin synthase (PGIS) and the prostacyclin (IP) receptor was measured. The effects of iloprost, a prostacyclin analogue, on expression of genes implicated in osteoarthritis such as microsomal prostaglandin E_2 synthase 1 (mPGES1) and matrix metalloproteinases (MMPs) were also studied. FPT inhibitor II, a farnesyl transferase inhibitor, was used to antagonize iloprost-induced responses.

Results: BCP crystal stimulation led to a five-fold increase in prostacyclin production in OASF compared to untreated cells. This induction was attenuated by cyclooxygenase (COX)-2 and COX-1 inhibition at 4 and 32 h, respectively. PGIS and IP receptor transcripts were constitutively expressed in OASF. BCP crystals upregulated IP receptor expression two-fold. While iloprost diminished BCP crystal-stimulated IP receptor upregulation, the inhibitory effect of iloprost was blocked by the farnesyl transferase inhibitor. In addition, iloprost upregulated mPGES1 and downregulated MMP-13 expression in BCP crystal-stimulated OASF, effects that were not influenced by the farnesyl transferase inhibitor.

Conclusions: These data showed for the first time that BCP crystals can increase prostacyclin production and upregulate expression of the IP receptor in OASF. The potential of prostacyclin to influence BCP crystal-stimulated responses was supported by the effects of iloprost on the expression of the IP receptor, mPGES1 and MMP-13. These data demonstrate the potential involvement of prostacyclin in BCP crystal-associated osteoarthritis (OA) and suggest that inhibition of PG synthesis with non-steroidal anti-inflammatory drugs may have both deleterious and beneficial effects in BCP crystal-associated OA.

Key words: Prostacyclin, Basic calcium phosphate crystals, Osteoarthritis, Synovial fibroblasts, Prostacyclin receptor, Microsomal prostaglandin E_2 synthase 1, Matrix metalloproteinases.

Abbreviations: BCP basic calcium phosphate, COX cyclooxygenase, DMEM Dulbecco's modified Eagles medium, FBS fetal bovine serum, HFF human foreskin fibroblasts, IL-1β interleukin-1β, IP prostacyclin receptor, MMP matrix metalloproteinase, mPGES1 microsomal prostaglandin E_2 synthase 1, NSAIDs non-steroidal anti-inflammatory drugs, OA osteoarthritis, OASF osteoarthritic synovial fibroblasts, PGE_2 prostaglandin E_2, PGI_2 prostaglandin I_2 (prostacyclin), PGIS prostacyclin synthase, S.E.M. standard error of the mean.

Introduction

Basic calcium phosphate (BCP) crystals are predominantly composed of partially carbonate-substituted hydroxyapatite, but also include octacalcium phosphate and tricalcium phosphate. BCP crystal deposition is associated with a number of rheumatic syndromes including acute calcific periarthritis, calcific tendonitis, Milwaukee shoulder syndrome and osteoarthritis (OA) as well as non-rheumatic syndromes such as breast cancer and atherosclerosis[1,2]. The prevalence of BCP crystals in synovial fluid from patients with knee OA is between 30 and 60%[3–5], in contrast to other destructive arthropathies such as rheumatoid arthritis where they are rarely found. Ample data support the role of BCP crystals in cartilage degeneration as their presence correlates strongly with radiographic severity of OA[6,7] and larger joint effusions are seen in affected knee joints when compared with joint fluid from OA knees without crystals[8]. *In vitro* properties of BCP crystals that may contribute to OA pathogenesis include the induction of mitogenesis[9,10], increased nitric oxide production[11,12], upregulation of tumor necrosis factor α (TNFα) and interleukin-1β (IL-1β)[13,14] and stimulation of matrix metalloproteinase

[1]Funded by the Health Research Board, Wellcome Trust and the PRTLI Cycle III.

[a]The authors have no conflict of interest to declare.

*Address correspondence and reprint requests to: E. S. Molloy, Department of Rheumatic and Immunologic Diseases, Desk A50, Cleveland Clinic Foundation, Cleveland, OH 44195, USA. Tel: 1-216-444-0646; Fax: 1-216-445-7569; E-mail: eamonn.molloy@ireland.com

Received 17 February 2006; revision accepted 4 October 2006.

(MMP) -1, -3, -8, -9, and -13 secretion[10,15–18], which can promote cartilage degradation.

It has been known for some time that BCP crystals cause an increase in prostaglandin E_2 (PGE_2) production[16,19,20]. We have recently shown that this PGE_2 induction is mediated by upregulation of cyclooxygenase (COX)-2 and COX-1[14,21]. COX-2 expression and COX-2 derived PGE_2 production were maximal at 4 h after stimulation with BCP crystals, whereas COX-1 expression and COX-1 derived PGE_2 production was maximal at 32 h. Microsomal prostaglandin E_2 synthase 1 (mPGES1) is generally upregulated by inflammatory stimuli, is preferentially coupled with COX-2 and is thought to be the key terminal PGE_2 synthase in inflammatory responses[22]. We have noted upregulation of mPGES1 in human foreskin fibroblasts (HFF) in response to BCP crystal stimulation[23], but not in osteoarthritic synovial fibroblasts (OASF)[24]. This discordance was attributable to downregulation of the EP4 receptor in OASF in response to BCP crystals and was overcome by the addition of exogenous PGE_2. Therefore, although HFF have long been used in the investigation of the *in vitro* effects of BCP crystals and were considered comparable to synovial fibroblasts, we have noted differences in biological responses between HFF and OASF. As the latter are more relevant from a pathophysiologic viewpoint, OASF were used in this investigation.

Prostacyclin can contribute to hyperalgesia and vasodilatation in acute inflammation, modulate MMP expression[25–27], promote angiogenesis[28] and influence cell proliferation in other settings[29]. Angiogenesis has been observed in OA synovium[30], with the synovium implicated as the primary source of pro-angiogenic mediators. Angiogenesis has been proposed as a mechanism of cartilage loss in OA[31], and also plays a key role in the process of endochondral ossification that is involved in osteophyte formation[31,32]. Increased prostacyclin production has been demonstrated in adjuvant-induced arthritis[33,34]. Prostacyclin has also been implicated in inflammatory and nociceptive responses in animal models of inflammatory arthritis[35,36] and in exudate formation in a carrageenin-induced pleurisy model in mice[37]. However, synovial fibroblasts from patients with rheumatoid arthritis can produce prostacyclin spontaneously[38]. In addition, rheumatoid and non-rheumatoid synovial fibroblast-like synoviocytes release prostacyclin (and PGE_2) when incubated in conditioned media from human peripheral blood mononuclear cells[39]. The observed PG production was suppressed by small doses of dexamethasone[39].

Prostacyclin production has not previously been reported in OA synovial tissues. Consequently, the expression of prostacyclin synthase (PGIS) or that of the prostacyclin (IP) receptor therein has not been reported. We hypothesized that BCP crystals increase prostacyclin production in OASF and aimed to characterise the expression profile of PGIS and the IP receptor in OASF and to ascertain if their expression is influenced by BCP crystal stimulation. We also examined whether, in BCP crystal-stimulated OASF, prostacyclin could influence expression of mPGES1 and MMP-1, -3 and -13, all of which have been implicated in OA pathogenesis.

Materials and methods

BCP CRYSTAL PREPARATION

BCP crystals were synthesized by alkaline hydrolysis of brushite, as previously described[40]. Mineral prepared by this method has a calcium/phosphate molar ratio of 1.59 and contains partially carbonate-substituted hydroxyapatite as determined by Fourier transform infrared spectroscopy. The crystals were weighed into vials and rendered pyrogen-free by heating at 200°C for 90 min. The sterile crystals were resuspended by brief sonication in Dulbecco's modified Eagles medium (DMEM) prior to use. Crystals were used at a final concentration of 18 $\mu g/cm^2$ for all experiments.

CELL CULTURE

Synovial tissue was obtained from primary OA patients undergoing joint replacement surgery. Informed consent was obtained from all patients. Ethical approval was granted by the National Orthopaedic Hospital Ethics Committee. The synovial tissue was minced and enzymatically digested by 1.5 mg/ml collagenase in DMEM for 90 min at 37°C. Then 0.5% trypsin in DMEM was added for a further 30 min incubation. Cells were centrifuged at 1000*g* for 5 min and washed with phosphate-buffered saline/DMEM four times. Cells were resuspended, grown and maintained in DMEM containing 10% fetal bovine serum (FBS), 1% penicillin/streptomycin/fungizone and 1% sodium pyruvate, in a humidified incubator at 37°C with 5% carbon dioxide/95% air. All cultures used were fourth or fifth passage cells. Cells were rendered quiescent by incubation in media containing 0.5% FBS for 24 h. Fresh 0.5% FBS containing media was then added and the cells treated with BCP crystals or left untreated. All experiments were repeated at least three times.

Table I
Primer sequences for real-time PCR

Gene of interest	Primer sequence (5′ → 3′)	Tm (°C)	Amplicon (bp)
IP	Forward: CAC GAG GAG CAA AGC AAG TG	54	74
	Reverse: AGG TCT GGG CTC TCC AGT CTT	56	
PGIS	Forward: AAA AGG CCA GGA TGA AAC TGA CT	53	102
	Reverse: TCT GTA GCA TCG CCC AAC AG	54	
mPGES1	Forward: CCT AAC CCT TTT GTC GCC TG	54	74
	Reverse: CAG GTA GGC CAC GGT GTG T	55	
MMP-1	Forward: GGG AGA TCA TCG GGA CAA CTC	56	79
	Reverse: CAA TAC CTG GGC CTG GTT GA	54	
MMP-3	Forward: AAGGATACAACAGGGACCAATTTATT	53	84
	Reverse: CAGTGTTGGCTGAGTGAAAGAGA	55	
MMP-13	Forward: CCA AAG GCT ACA ACT TGT TTC TTG	54	76
	Reverse: AGG GTC CTT GGA GTG GTC AA	54	

IP = prostacyclin receptor, PGIS = prostacyclin synthase, MMP = matrix metalloproteinase, Tm = annealing temperature, bp = base pairs (length of amplicon).

PROSTAGLANDIN IMMUNOASSAYS

Prostacyclin is unstable and rapidly converted to an inactive, stable metabolite, 6-keto prostaglandin $F_{1\alpha}$ (6-keto $PGF_{1\alpha}$). Prostacyclin synthesis was assessed by measuring 6-keto $PGF_{1\alpha}$ using a commercially available immunoassay (R&D Systems; sensitivity 1.4 pg/ml). Quiescent OASF were treated with aspirin (200 μM) for 30 min, rinsed with PBS and fresh media containing 0.5% FBS added prior to stimulation with BCP crystals (18 μg/cm^2) for various times between 4 and 32 h. The relative contributions of COX-1 and COX-2 to BCP crystal-stimulated 6-keto $PGF_{1\alpha}$ production were assessed using specific COX inhibitors: SC560 (COX-1) and SC236 (COX-2), and aspirin. Forty-five minutes prior to sample collection, cells were treated with 1 μM SC236, 1 μM SC560, 200 μM aspirin or left untreated. Cells were rinsed and incubated in Hanks Hepes buffer with 50 μM arachidonic acid for 15 min at 37°C. 6-Keto $PGF_{1\alpha}$ assays were carried out in duplicate at least three times.

REAL-TIME POLYMERASE CHAIN REACTION (PCR)

RNA extraction and reverse transcription were performed as previously described[14]. Real-time PCR was carried out using an ABI 7500 Sequence Detector (Applied Biosystems, UK). Using SYBR green chemistry and 18S ribosomal RNA (18S) as an internal control, relative quantitation of the amount of target copy in unknown samples is accomplished using the standard curve method. Sequences for the primers used are given in Table I. Primers were used at a final concentration of 300 nM. 18S primers and probe mix, SYBR Green master mix and universal master mix were purchased from Applied Biosystems and used as per the manufacturer's instructions.

STUDIES OF THE EFFECT OF PROSTACYCLIN IN OASF

Iloprost is a prostacyclin analogue that also has agonist activity at the EP1 receptor. For the purposes of this investigation, this EP1 receptor agonism was not considered relevant as there is negligible expression of EP1 in OASF[41]. There is no established, commercially available compound that acts as a specific IP receptor antagonist. However, a farnesyl transferase inhibitor, FPT inhibitor II [disodium-(*E*,*E*)-2-[2-oxo-2-[[(3,7,11-trimethyl-2,6,10-dodecatrienyl)oxy]amino] ethyl]phosphonic acid; Merck Biosciences], was used to antagonize the effects of iloprost, as farnesylation of the IP receptor is required for its efficient intracellular signaling[42,43]. Iloprost and the FPT inhibitor II were used at concentrations of 0.1 μg/ml and 1 μM, respectively; ethanol (0.1%) was used as vehicle control.

STATISTICAL ANALYSIS

All quantitative data were expressed as mean ± standard error of the mean (S.E.M.). Unpaired *t* tests were performed using Microsoft Excel.

Results

BCP CRYSTALS INCREASE 6-KETO $PGF_{1\alpha}$ PRODUCTION IN OASF

Figure 1 demonstrates that BCP crystals stimulated an increase in 6-keto $PGF_{1\alpha}$ levels peaking at 4 h with a second later peak at 32 h (approximately five-fold compared to untreated cells). The mean levels of 6-keto $PGF_{1\alpha}$ production

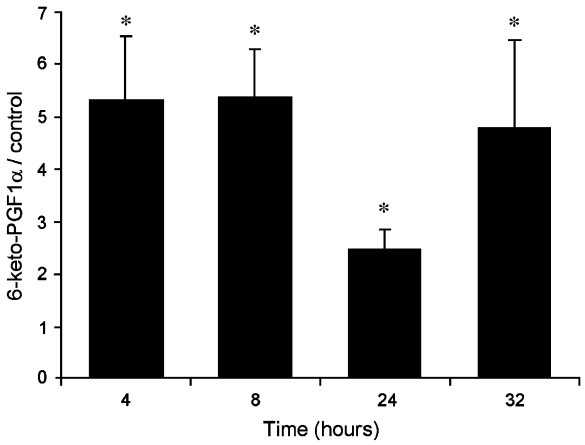

Fig. 1. BCP crystals stimulate increased prostacyclin production in OASF. OASF were pretreated with aspirin (200 μm), a non-selective COX inhibitor, to investigate newly synthesized PGs. Cells were then stimulated with BCP crystals and incubated for 4, 8, 24 or 32 h. Untreated controls were incubated for the same time intervals. Cells were then incubated with arachidonic acid (50 μM) in Hank's Hepes buffer for 15 min. 6-keto $PGF_{1\alpha}$ levels were measured (in duplicate) by enzyme immunoassay. Levels were expressed as fold change over the values obtained for the untreated control incubated for the same period. Therefore, the untreated controls at each timepoint have a value of 1, and are not shown ($n = 3$, mean ± S.E.M., $^*P < 0.05$).

in BCP crystal-stimulated OASF and untreated OASF were 283 ± 39 pg/μg protein and 71 ± 29 pg/μg protein, respectively (mean ± S.E.M., $n = 4$). At 4 h, the increase in 6-keto $PGF_{1\alpha}$ was inhibited by addition of aspirin or the COX-2 selective inhibitor SC236 (Fig. 2A). After 32 h, SC236 was no longer a significant inhibitor, but aspirin and the COX-1 selective inhibitor SC560 equally inhibited 6-keto $PGF_{1\alpha}$ production (Fig. 2B).

EXPRESSION OF IP RECEPTOR AND PGIS IN OASF

IP and PGIS are constitutively expressed by OASF, as measured by real-time PCR (data not shown). IP receptor messenger RNA (mRNA) expression was upregulated approximately two-fold in OASF in response to BCP crystal treatment for 24 or 32 h (Fig. 3). In contrast, expression of PGIS mRNA in OASF was unchanged by treatment with BCP crystals (data not shown).

ILOPROST MODULATES IP RECEPTOR AND mPGES1 mRNA EXPRESSION

Administration of iloprost resulted in an approximate two-fold reduction in IP receptor mRNA expression in BCP crystal stimulated OASF, an effect that was antagonized by the FPT inhibitor II (Fig. 4). Pre-treatment with the FPT inhibitor II in the absence of iloprost resulted in a slight but significant (1.2-fold) upregulation of IP receptor mRNA expression (Fig. 4). Addition of iloprost or the FPT inhibitor II did not affect basal IP receptor expression in OASF not treated with BCP crystals (data not shown).

Iloprost also resulted in a significant induction of mPGES1 mRNA expression in BCP crystal-treated OASF (Fig. 5), which was not antagonized by the FPT inhibitor II. A similar pattern of mPGES1 expression was seen in OASF treated with iloprost and the FPT inhibitor II in the absence of BCP crystals (data not shown).

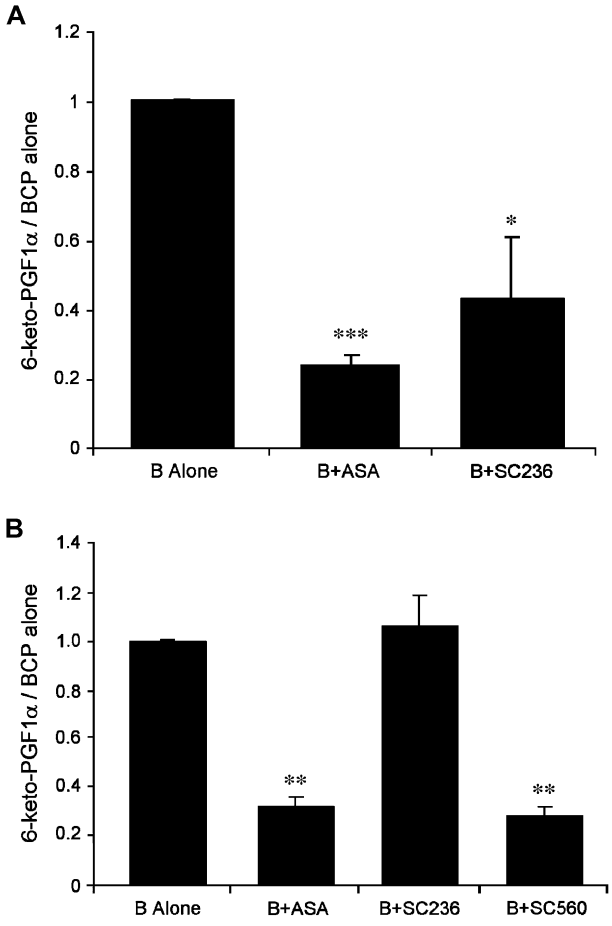

Fig. 2. Effect of COX inhibitors on BCP crystal-induced prostacyclin production (A) at 4 h and (B) at 32 h. Cells were pretreated with aspirin (200 μm) and then incubated with BCP crystals for 4 h. Cells were then treated with aspirin (B + ASA, 200 μM), SC236 (B + SC236, 1 μM), SC560 (B + SC560, 1 μM) or left untreated (B alone) for 30 min, then incubated with arachidonic acid (50 μM) in Hank's Hepes buffer for 15 min. 6-keto $PGF_{1\alpha}$ levels were measured (in duplicate) by enzyme immunoassay. Levels were expressed as fold change over the values obtained for the cells treated with BCP crystals alone, which therefore assumed a value of 1 (mean ± S.E.M., $n = 3$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).

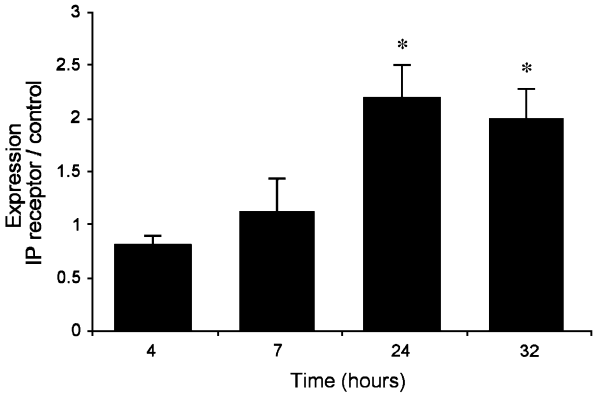

Fig. 3. Expression of the IP receptor mRNA in OASF following stimulation with BCP crystals. OASF were treated with BCP crystals and incubated for 4, 8, 24 or 32 h. Untreated controls were incubated for the same time intervals. IP receptor mRNA expression was measured by real-time PCR, normalized to 18S values and then expressed as fold change over the value obtained for the untreated control incubated for the same period. The untreated controls therefore assumed a value of 1 and are not shown (mean ± S.E.M., $n = 3$, $^{*}P < 0.05$).

ILOPROST DOWNREGULATES MMP-13 mRNA EXPRESSION IN BCP CRYSTAL-TREATED OASF

Administration of iloprost resulted in an approximate three-fold reduction in MMP-13 mRNA expression, an effect that was not altered by the FPT inhibitor II (Fig. 6). Iloprost treatment did not significantly influence MMP-1 or MMP-3 mRNA expression in BCP crystal-stimulated OASF (results not shown). Addition of iloprost or the FPT inhibitor II did not affect basal MMP-13 expression in OASF not treated with BCP crystals (data not shown).

Discussion

Our observations show for the first time that prostacyclin is produced by untreated OASF in the presence of arachidonic acid and that PGIS and the IP receptor are constitutively expressed in OASF. Furthermore, prostacyclin production was augmented in OASF in response to BCP crystal stimulation. BCP crystal-stimulated prostacyclin production was mediated primarily by COX-2 at 4 h and COX-1 at 32 h. This is similar to the observed temporal pattern of PGE_2 production in response to BCP crystal stimulation, and correlates well with the peak upregulation of COX-1 and COX-2 in response to BCP crystals[14,21]. Although PGIS and the IP receptor are not generally regulated by inflammatory stimuli, regulation of the IP receptor[27] has previously been described. BCP crystal stimulation resulted in a two-fold upregulation of IP receptor mRNA expression, but did not influence PGIS expression. While this upregulation of IP receptor mRNA expression was antagonized by pre-treatment with iloprost, the inhibitory effect of iloprost was overcome by a farnesyl transferase inhibitor. The upregulation of the IP receptor by BCP crystals is likely to augment the responses to the prostacyclin that is produced in BCP crystal-stimulated OASF, thereby potentially aggravating the effects of prostacyclin in BCP crystal-associated OA.

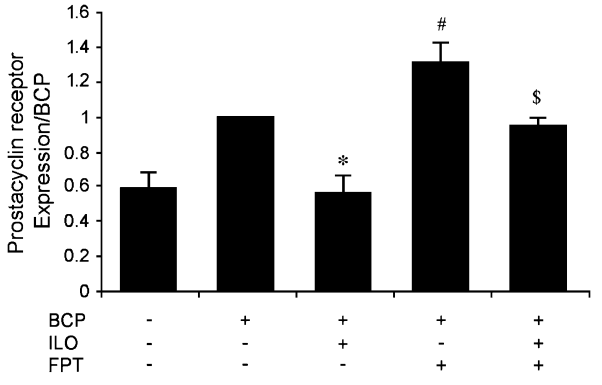

Fig. 4. Effect of iloprost on IP receptor mRNA expression. OASF were treated for 32 h with BCP crystal with or without pre-treatment with iloprost (ILO), FPT inhibitor II (FPT) or both for 30 min prior to the addition of BCP crystals. mRNA expression was measured by real-time PCR. Results were normalized to 18S values then expressed as fold change over values obtained for OASF treated with BCP crystals alone, which therefore assumed a value of 1 (mean ± S.E.M., $n = 3$, $^{*}P < 0.05$ vs BCP alone, $\#P = 0.05$ vs BCP alone, $\$P < 0.05$ vs BCP + ILO).

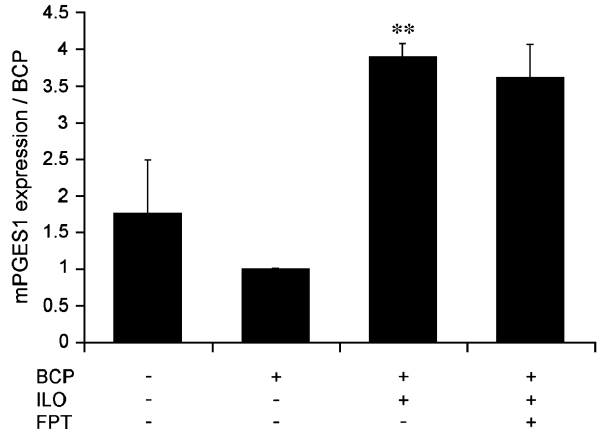

Fig. 5. Effect of iloprost on mPGES1 mRNA expression. OASF were treated for 32 h with BCP crystal with or without pre-treatment with iloprost (ILO), FPT inhibitor II (FPT) or both for 30 min prior to the addition of BCP crystals. mRNA expression was measured by real-time PCR. Results were normalized to 18S values then expressed as fold change over values obtained for OASF treated with BCP crystals alone, which therefore assume a value of 1 (mean ± S.E.M., $n = 3$, $^{**}P < 0.01$).

These results also suggest that BCP crystal-stimulated prostacyclin production may modulate OASF responses to BCP crystals. The downregulatory effect of iloprost on IP receptor expression may indicate the presence of a negative feedback loop on prostacyclin function. However, at least in BCP crystal-stimulated OASF, this negative feedback loop does not appear to have the capacity to overcome the upregulatory effect of BCP crystals on IP receptor expression. The mPGES1 upregulation in response to iloprost suggests the presence of a hitherto unsuspected crosstalk between the terminal pathways leading to PGE_2 and prostacyclin production and emphasizes the complex regulation of the pathways leading to prostaglandin synthesis. Iloprost inhibited BCP crystal-stimulated MMP-13 expression, but did not affect BCP crystal-induced MMP-1 or MMP-3 expression. Therefore, the prostacyclin production observed in OASF in response to BCP crystals may limit some of the deleterious effects of BCP crystals on articular cartilage.

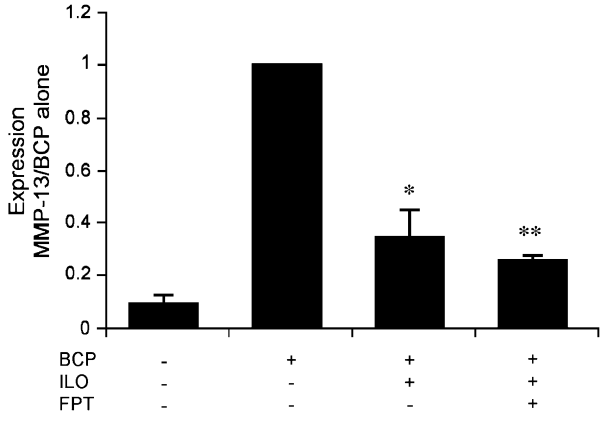

Fig. 6. Effect of iloprost on BCP crystal-induced MMP-13 upregulation. OASF were incubated with BCP crystals for 32 h, with or without iloprost (ILO) with or without the farnesyl transferase inhibitor II (FPT). MMP-13 mRNA expression was measured by real-time PCR, normalized to 18S values and then expressed as fold change over the value obtained for the sample treated with BCP crystals alone. Therefore the samples treated with BCP crystals alone assumed a value of 1 (mean ± S.E.M., $n = 3$, $^{*}P < 0.05$, $^{**}P < 0.01$ vs samples treated with BCP crystals alone).

The possibility that the observed effects of iloprost on mPGES1, IP and MMP-13 expression are mediated through binding the EP1 receptor is unlikely given the very low level of expression of this receptor in OASF[41]. The inconsistent antagonistic effect of the FPT inhibitor may indicate that different activities of the IP receptor may differ in their dependence on IP farnesylation. As the physiology of prostacyclin–IP receptor signaling is still not well understood, definitive characterization of the effects of prostacyclin in OASF may require the commercial availability of specific IP agonists and antagonists, or the use of other techniques such as siRNA. The potential role of prostacyclin in OA certainly warrants further study, given its known involvement in nociception and inflammatory responses and potential to promote angiogenesis, modulate MMP expression and contribute to cell proliferation[25–29]. In this regard, it is interesting to note the putative role of angiogenesis in OA pathogenesis[31].

Therefore, prostacyclin may have beneficial as well as deleterious effects in BCP crystal-associated OA. While PGE_2, the other major prostaglandin produced in OA joints, has been shown to mediate IL-1β-associated cartilage degradation *in vitro*[44], it has also been shown to lead to reduced cytokine production[45–47] and diminished chemokine production[48–50]. Inhibition of PG synthesis with non-steroidal anti-inflammatory drugs (NSAIDs) is the most widely used pharmacologic strategy for the treatment of OA. Many OA patients will have associated intra-articular BCP crystal deposition. While NSAIDs provide symptomatic relief, their overall impact on OA disease progression is unclear. Although the intricacies of the effects of PGs in OA remain to be fully elucidated, these studies suggest that such a broad, indiscriminate inhibition of PG production as that provided by NSAIDs is unwise. Identification of strategies preferentially targeting the deleterious effects of PGs over their beneficial actions may result in more effective, potentially disease-modifying, treatments for OA.

Acknowledgements

This work was funded by the Health Research Board, Wellcome Trust and the PRTLI Cycle III.

References

1. Fam AG. Basic calcium phosphate (calcium apatite) crystal deposition diseases. In: Smyth CJ, Holers VM, Eds. Gout, Hyperuricemia and Other Crystal-associated Arthropathies. New York: Marcel Dekker 1999:333–358.
2. Molloy ES, McCarthy GM. Calcium crystal deposition diseases: update on pathogenesis and manifestations. Rheum Dis Clin North Am 2006;32:383–400. vii.
3. Dieppe PA, Crocker PR, Corke CF, Doyle DV, Huskisson EC, Willoughby DA. Synovial fluid crystals. Q J Med 1979;48:533–53.
4. Gibilisco PA, Schumacher HR Jr, Hollander JL, Soper KA. Synovial fluid crystals in osteoarthritis. Arthritis Rheum 1985;28:511–5.
5. Swan A, Chapman B, Heap P, Seward H, Dieppe P. Submicroscopic crystals in osteoarthritic synovial fluids. Ann Rheum Dis 1994;53:467–70.

6. Nalbant S, Martinez JA, Kitumnuaypong T, Clayburne G, Sieck M, Schumacher HR Jr. Synovial fluid features and their relations to osteoarthritis severity: new findings from sequential studies. Osteoarthritis Cartilage 2003;11:50–4.
7. Halverson PB, McCarty DJ. Patterns of radiographic abnormalities associated with basic calcium phosphate and calcium pyrophosphate dihydrate crystal deposition in the knee. Ann Rheum Dis 1986;45:603–5.
8. Carroll GJ, Stuart RA, Armstrong JA, Breidahl PD, Laing BA. Hydroxyapatite crystals are a frequent finding in osteoarthritic synovial fluid, but are not related to increased concentrations of keratan sulfate or interleukin 1 beta. J Rheumatol 1991;18:861–6.
9. Cheung HS, Story MT, McCarty DJ. Mitogenic effects of hydroxyapatite and calcium pyrophosphate dihydrate crystals on cultured mammalian cells. Arthritis Rheum 1984;27:668–74.
10. McCarthy GM, Westfall PR, Masuda I, Christopherson PA, Cheung HS, Mitchell PG. Basic calcium phosphate crystals activate human osteoarthritic synovial fibroblasts and induce matrix metalloproteinase-13 (collagenase-3) in adult porcine articular chondrocytes. Ann Rheum Dis 2001;60:399–406.
11. Ea H-K, Uzan B, Rey C, Lioté F. Octacalcium phosphate crystals directly stimulate expression of inducible nitric oxide synthase through p38 and JNK mitogen-activated protein kinases in articular chondrocytes. Arthritis Res Ther 2005;7:R915–26.
12. Whelan L, O'Byrne JM, Sallis JD, McCarthy GM. Basic calcium phosphate crystals stimulate nitric oxide synthase and modulate bcl-2/bax expression in human synovial fibroblasts. Arthritis Rheum 2002;46:S140.
13. Nadra I, Mason JC, Philippidis P, Florey O, Smythe CD, McCarthy GM, *et al*. Proinflammatory activation of macrophages by basic calcium phosphate crystals via protein kinase C and MAP kinase pathways: a vicious cycle of inflammation and arterial calcification? Circ Res 2005;96:1248–56.
14. Morgan MP, Whelan LC, Sallis JD, McCarthy CJ, Fitzgerald DJ, McCarthy GM. Basic calcium phosphate crystal-induced prostaglandin E2 production in human fibroblasts: role of cyclooxygenase 1, cyclooxygenase 2, and interleukin-1beta. Arthritis Rheum 2004;50:1642–9.
15. McCarthy GM, Mitchell PG, Struve JA, Cheung HS. Basic calcium phosphate crystals cause coordinate induction and secretion of collagenase and stromelysin. J Cell Physiol 1992;153:140–6.
16. Cheung HS, Halverson PB, McCarty DJ. Release of collagenase, neutral protease, and prostaglandins from cultured mammalian synovial cells by hydroxyapatite and calcium pyrophosphate dihydrate crystals. Arthritis Rheum 1981;24:1338–44.
17. Reuben PM, Wenger L, Cruz M, Cheung HS. Induction of matrix metalloproteinase-8 in human fibroblasts by basic calcium phosphate and calcium pyrophosphate dihydrate crystals: effect of phosphocitrate. Connect Tissue Res 2001;42:1–12.
18. Bai G, Howell DS, Howard GA, Roos BA, Cheung HS. Basic calcium phosphate crystals up-regulate metalloproteinases but down-regulate tissue inhibitor of metalloproteinase-1 and -2 in human fibroblasts. Osteoarthritis Cartilage 2001;9:416–22.
19. Rothenberg RJ. Modulation of prostaglandin E2 synthesis in rabbit synoviocytes. Arthritis Rheum 1987;30: 266–74.
20. McCarty DJ, Cheung HS. Prostaglandin (PG) E2 generation by cultured canine synovial fibroblasts exposed to microcrystals containing calcium. Ann Rheum Dis 1985;44:316–20.
21. Molloy ES, Morgan M, Doherty G, Whelan L, Misstear K, O'Byrne J, *et al*. Basic calcium phosphate crystals activate prostaglandin synthetic pathways in human osteoarthritic synovial fibroblasts. Arthritis Rheum 2004;50(9):S246.
22. Murakami M, Kudo I. Recent advances in molecular biology and physiology of the prostaglandin E2-biosynthetic pathway. Prog Lipid Res 2004;43:3–35.
23. Molloy ES, Morgan MP, Whelan LC, Fitzgerald DJ, Crofford LJ, McCarthy GM. Basic calcium phosphate (BCP) crystals upregulate microsomal prostaglandin E2 synthase 1 (mPGES1) and peroxisome proliferator-activated receptor (PPARgamma) mRNA in human fibroblasts. Ir J Med Sci 2003;172:11.
24. Molloy ES, Doherty G, Morgan M, O'Byrne J, Fitzgerald D, Crofford LC, *et al*. The EP4 receptor mediates a positive feedback mechanism on basic calcium phosphate crystal-induced PGE_2 production in human osteoarthritic synovial fibroblasts. Arthritis Rheum 2004;50(9):S332.
25. Clohisy JC, Connolly TJ, Bergman KD, Quinn CO, Partridge NC. Prostanoid-induced expression of matrix metalloproteinase-1 messenger ribonucleic acid in rat osteosarcoma cells. Endocrinology 1994; 135:1447–54.
26. Kitahara M, Ichikawa M, Kinoshita T, Shiozawa S, Shigematsu H, Komiyama A. Prostacyclin inhibits the production of MMP-9 induced by phorbol ester through protein kinase A activation, but does not affect the production of MMP-2 in human cultured mesangial cells. Kidney Blood Press Res 2001;24:18–26.
27. Nasrallah R, Hebert RL. Reduced IP receptors in STZ-induced diabetic rat kidneys and high-glucose-treated mesangial cells. Am J Physiol Renal Physiol 2004; 287:F673–81.
28. Gately S, Li WW. Multiple roles of COX-2 in tumor angiogenesis: a target for antiangiogenic therapy. Semin Oncol 2004;31:2–11.
29. Simmons DL, Botting RM, Hla T. Cyclooxygenase isozymes: the biology of prostaglandin synthesis and inhibition. Pharmacol Rev 2004;56:387–437.
30. Haywood L, McWilliams DF, Pearson CI, Gill SE, Ganesan A, Wilson D, *et al*. Inflammation and angiogenesis in osteoarthritis. Arthritis Rheum 2003;48: 2173–7.
31. Bonnet CS, Walsh DA. Osteoarthritis, angiogenesis and inflammation. Rheumatology (Oxford) 2005;44: 7–16.
32. Neuman P, Hulth A, Linden B, Johnell O, Dahlberg L. The role of osteophytic growth in hip osteoarthritis. Int Orthop 2003;27:262–6.
33. Stichtenoth DO, Selve N, Tsikas D, Gutzki FM, Frolich JC. Increased total body synthesis of prostacyclin in rats with adjuvant arthritis. Prostaglandins 1995; 50:331–40.
34. Claveau D, Sirinyan M, Guay J, Gordon R, Chan CC, Bureau Y, *et al*. Microsomal prostaglandin E synthase-1 is a major terminal synthase that is selectively up-regulated during cyclooxygenase-2-dependent prostaglandin E2 production in the rat adjuvant-induced arthritis model. J Immunol 2003;170:4738–44.
35. McQueen DS, Iggo A, Birrell GJ, Grubb BD. Effects of paracetamol and aspirin on neural activity of joint

mechanonociceptors in adjuvant arthritis. Br J Pharmacol 1991;104:178–82.
36. Murata T, Ushikubi F, Matsuoka T, Hirata M, Yamasaki A, Sugimoto Y, *et al*. Altered pain perception and inflammatory response in mice lacking prostacyclin receptor. Nature 1997;388:678–82.
37. Yuhki K, Ueno A, Naraba H, Kojima F, Ushikubi F, Narumiya S, *et al*. Prostaglandin receptors EP2, EP3, and IP mediate exudate formation in carrageenin-induced mouse pleurisy. J Pharmacol Exp Ther 2004;311:1218–24.
38. Pietila P, Moilanen E, Seppala E, Nissila M, Lepisto P, Laitinen O, *et al*. Differences in the production of arachidonic acid metabolites between healthy and rheumatic synovial fibroblasts *in vitro*. A preliminary study. Scand J Rheumatol 1984;13:243–6.
39. Hamilton JA, Clarris BJ, Fraser JR, Niall MC. Peripheral blood mononuclear cells stimulate prostacyclin levels of human synovial fibroblast-like cells. Rheumatol Int 1985;5:121–5.
40. Evans RW, Cheung HS, McCarty DJ. Cultured human monocytes and fibroblasts solubilize calcium phosphate crystals. Calcif Tissue Int 1984;36:645–50.
41. Molloy ES. Basic calcium phosphate crystals and osteoarthritis: role of prostaglandins, Molecular and Cellular Therapeutics. Dublin: National University of Ireland 2006. 260 pp.
42. O'Meara SJ, Kinsella BT. Investigation of the effect of the farnesyl protein transferase inhibitor R115777 on isoprenylation and intracellular signalling by the prostacyclin receptor. Br J Pharmacol 2004;143:318–30.
43. O'Meara SJ, Kinsella BT. The effect of the farnesyl protein transferase inhibitor SCH66336 on isoprenylation and signalling by the prostacyclin receptor. Biochem J 2005;386:177–89.
44. Hardy MM, Seibert K, Manning PT, Currie MG, Woerner BM, Edwards D, *et al*. Cyclooxygenase 2-dependent prostaglandin E2 modulates cartilage proteoglycan degradation in human osteoarthritis explants. Arthritis Rheum 2002;46:1789–803.
45. Vassiliou E, Jing H, Ganea D. Prostaglandin E2 inhibits TNF production in murine bone marrow-derived dendritic cells. Cell Immunol 2003;223:120–32.
46. Iwasaki K, Noguchi K, Endo H, Kondo H, Ishikawa I. Prostaglandin E2 downregulates interleukin-12 production through EP4 receptors in human monocytes stimulated with lipopolysaccharide from Actinobacillus actinomycetemcomitans and interferon-gamma. Oral Microbiol Immunol 2003;18:150–5.
47. Di Battista JA, Martel-Pelletier J, Pelletier J. Suppression of tumor necrosis factor (TNF-alpha) gene expression by prostaglandin E(2). Role Of early growth response protein-1 (Egr-1). Osteoarthritis Cartilage 1999;7:395–8.
48. Takayama K, Garcia-Cardena G, Sukhova GK, Comander J, Gimbrone MA Jr, Libby P. Prostaglandin E2 suppresses chemokine production in human macrophages through the EP4 receptor. J Biol Chem 2002;277:44147–54.
49. Largo R, Diez-Ortego I, Sanchez-Pernaute O, Lopez-Armada MJ, Alvarez-Soria MA, Egido J, *et al*. EP2/EP4 signalling inhibits monocyte chemoattractant protein-1 production induced by interleukin 1beta in synovial fibroblasts. Ann Rheum Dis 2004;63:1197–204.
50. Jing H, Vassiliou E, Ganea D. Prostaglandin E2 inhibits production of the inflammatory chemokines CCL3 and CCL4 in dendritic cells. J Leukoc Biol 2003;74:868–79.