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Microsomal prostaglandin E_2 synthase 1 expression in basic calcium phosphate crystal-stimulated fibroblasts: role of prostaglandin E_2 and the EP4 receptor

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Summary

Objective: Basic calcium phosphate (BCP) crystals have been implicated in the pathogenesis of osteoarthritis (OA), in part because of their ability to upregulate cyclooxygenase and prostaglandin E_2 (PGE_2) production. The aim of this work was to investigate the expression of terminal PGE_2 synthases and PGE_2 receptors (EP) in BCP crystal-stimulated fibroblasts.

Methods: Cultured fibroblasts were stimulated with BCP crystals *in vitro*. mRNA expression was measured by real-time polymerase chain reaction, and protein production by western blotting.

Results: Basal expression of microsomal prostaglandin E_2 synthase 1 (mPGES1) in osteoarthritic synovial fibroblasts (OASF) was found to be 30-fold higher than in human foreskin fibroblasts (HFF). BCP crystals increased mPGES1 expression fourfold in HFF, but not in OASF. EP4 expression was downregulated twofold by BCP crystals in OASF, but not in HFF. Exogenous PGE_2 also downregulated EP4 expression; this effect was blocked by co-administration of L-161,982, a selective EP4 antagonist. While administration of exogenous PGE_2 significantly upregulated mPGES1 expression in OASF, mPGES1 expression was threefold higher in the OASF treated with BCP crystals and PGE_2 as compared with OASF treated with PGE_2 alone.

Conclusions: The differing effects of BCP crystals on mPGES1 expression in HFF and OASF may be explained by BCP crystal-induced EP4 downregulation in OASF, likely mediated *via* PGE_2. These data underline the complexity of the pathways regulating PGE_2 synthesis and suggest the existence of a compensatory mechanism whereby mPGES1 expression can be diminished, potentially reducing the stimulus for further PGE_2 production.

Key words: Basic calcium phosphate crystals, osteoarthritis, prostaglandin E_2, microsomal prostaglandin E_2 synthase 1, EP receptors, synovium.

Introduction

Prostaglandin E_2 (PGE_2) has been implicated in the pathogenesis of osteoarthritis (OA) and inhibition of PG synthesis *via* inhibition of cyclooxygenase (COX) by non-steroidal anti-inflammatory drugs (NSAIDs) is a cornerstone of pharmacologic therapy of OA. Prostaglandin E_2 synthases (PGES) catalyse the terminal step in synthesis of PGE_2. Microsomal PGE synthase 1 (mPGES1) was first described in 1999[1]. Although mPGES1 is constitutively expressed in certain cell types[2–4], it is considered analogous to COX-2 in that it is generally expressed at low levels at baseline and upregulated in response to pro-inflammatory stimuli. Co-ordinate upregulation of mPGES1 and COX-2 expression and resultant PGE_2 production has been reported in many cell types following treatment with pro-inflammatory stimuli[1,5–11]. mPGES2, a second form of membrane-associated PGES with a distinct structure from mPGES1[12], is expressed constitutively in a number of tissues, in particular those in which mPGES1 expression is relatively low. mPGES2 may couple with either COX-1 or COX-2[13]. Cytosolic PGES (cPGES) is expressed ubiquitously and constitutively within the cytosol, and it is considered to preferentially convert COX-1-derived PGH_2 to PGE_2[14]. However, recent data in a mouse model have cast uncertainty on the role of cPGES in PGE_2 biosynthesis *in vivo*[15]. While cPGES and mPGES2 are generally constitutively expressed, in a rat adjuvant-induced arthritis model mPGES2 was downregulated 2–3-fold and cPGES upregulated 2–6-fold[16].

A number of studies suggest a role for mPGES1 in arthritis. The features of collagen-induced arthritis (CIA), including synovitis, cartilage damage and bone erosion, are attenuated in mPGES1 knockout mice[17,18]. Stichtenoth

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et al. showed that mPGES1, concordant with COX-2, was upregulated by pro-inflammatory cytokines and downregulated by dexamethasone in primary rheumatoid synoviocytes[11]. Westman *et al.* observed strong mPGES1 immunostaining in rheumatoid arthritis (RA) synovium[19]. Kojima *et al.* showed that interleukin 1 beta (IL1β)-stimulated mPGES1 expression in RA synoviocytes was downregulated by NSAIDs[20]. This was reversed by addition of exogenous PGE_2, the administration of PGE_2 receptor (EP receptor) agonists or forskolin, a direct activator of adenylate cyclase. Upregulation of mPGES1 by pro-inflammatory cytokines such as IL1β in OA cartilage and OA chondrocytes[7,9,21] and synovial fibroblasts[5,22] has also been demonstrated.

There are four main PGE_2 receptors, referred to as EP receptors (EP1, EP2, EP3, and EP4). The EP2 and EP4 receptors are the predominant EP receptors expressed in human RA synoviocytes[20]; binding to either of these receptors leads to activation of adenylate cyclase and resultant cyclic adenosine monophosphate (cAMP) accumulation[23]. McCoy *et al.* characterised the disease severity of CIA in EP receptor knockout mice, and found that features of CIA were ameliorated only in the $EP4^{-/-}$ mice[24]. Other relevant effects of PGE_2- mediated *via* EP2 and/or EP4 receptors include modulation of bone turnover and of inflammatory cytokine and chemokine production[25–27]. However, the precise effects of specific prostaglandin receptors in OA synovium are unclear.

Basic calcium phosphate (BCP) crystals are predominantly composed of partially carbonate-substituted hydroxyapatite, and have been implicated in the pathogenesis of OA. *In vitro* properties of BCP crystals that may contribute to OA pathogenesis include the induction of mitogenesis, stimulation of matrix metalloproteinase secretion, increased nitric oxide production and upregulation of tumour necrosis factor alpha (TNFα) and IL1β[28]. BCP crystals also cause an increase in PGE2 production[29–31], mediated by upregulation of COX-1 and COX-2[32]. Expression of mPGES1 in response to BCP crystal stimulation has not previously been studied. However, it has been reported that BCP crystals induce early growth response 1 (Egr-1)[33], the key transcription factor in mPGES1 expression[34]. Furthermore, as COX-2 and mPGES1 are co-ordinately induced under most conditions studied to date and BCP crystals induced COX-2 expression in human foreskin fibroblasts (HFF)[32] and OA synovial fibroblasts (OASF)[35], we hypothesized that mPGES1 is also upregulated by BCP crystals. This study aimed to examine the expression of PGES and EP receptors in OASF and to ascertain if this expression is influenced by BCP crystal stimulation.

Methods

BCP CRYSTAL PREPARATION

BCP crystals were synthesised by alkaline hydrolysis of brushite, as previously described[36]. Mineral prepared by this method has a calcium/phosphate molar ratio of 1.59 and contains partially carbonate-substituted hydroxyapatite mixed with octacalcium phosphate 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 DMEM prior to use. For all experiments, cells were treated with BCP crystals at a concentration of 18 $\mu g/cm^2$, consistent with the concentrations of BCP crystals found in OA synovial fluids.

CELL CULTURE

Synovial tissue was obtained from four 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 m 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% 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 fifth or earlier passage cells. All experiments were repeated on at least three different samples.

RNA EXTRACTION, REVERSE TRANSCRIPTION AND REAL-TIME POLYMERASE CHAIN REACTION (PCR)

RNA was isolated and reverse transcribed as previously described[32]. Real-time RT-PCR was carried out using an ABI 7500 Sequence Detector (Applied Biosystems, UK). Using 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. SYBR Green chemistry was employed and primers were used at final concentration of 300 nM. Primer sequences for PGES and EP receptors are given in Table I. 18S primers and probe mix and universal master mix were purchased from Applied Biosystems and used as per the manufacturer's instructions.

PROSTANOID AGONISTS AND ANTAGONISTS

L-161982 is a selective EP4 receptor antagonist. CAY10399, is a selective EP2 receptor agonist that is 25 times more potent than butaprost. Forskolin is a direct activator of adenylate cyclase, resulting in an increase in intracellular cAMP. These compounds were used at concentrations of 1 μM, 10 μM and 30 μM respectively. PGE_2 was used at concentrations of 0.1 μM unless otherwise specified. Aspirin, SC-236 (a COX-2 selective inhibitor) and SC-560 (a COX-1 selective inhibitor) were used at concentrations of 200 μM, 1 μM and 1 μM respectively. Ethanol (0.1%) was used as vehicle control.

Choice of concentrations and timing of administration of inhibitors was informed by prior experiments, cytotoxicity assays, the available literature and the manufacturers' instructions.

WESTERN BLOTTING

Samples containing equal protein concentrations were electrophoresed on 15% sodium dodecyl sulfate (SDS) polyacrylamide gels and proteins were transferred to nitrocellulose membranes over 2 h. Membranes were blocked in 3% bovine serum albumin. The membranes were then incubated for 3 h with 1:500 dilution of mouse monoclonal anti-human mPGES1 primary antibody (SPI-Bio, France). Secondary peroxidase-conjugated anti-mouse immunoglobulin was used at a dilution of 1/10,000. Immunoreactive bands were detected using enhanced chemiluminescence reagents enhanced chemiluminescence (ECL)-plus (Amersham, UK).

STATISTICAL ANALYSIS

To simplify the graphical presentation of the data, all quantitative data are expressed as mean fold change ± the standard error of the mean (S.E.M.)

Table I
Real-time PCR primer sequences for PGE_2 synthases and receptors

Gene of interest	Primer sequence (5′ → 3′)
mPGES1	Forward CCT AAC CCT TTT GTC GCC TG
	Reverse CAG GTA GGC CAC GGT GTG T
mPGES2	Forward GGA GAA AGC TCG CAA CAA CTA AAT
	Reverse AGC CTT CAT GGC TGG GTA GTA G
cPGES	Forward AGC ATA AAA GAA CGG ACA GAT CAA
	Reverse CGA CAC TAA GCC AAT TAA GCT TTG
EP1	Forward ATG GTG GGC CAG CTT GTC
	Reverse GCC ACC AAC ACC AGC ATT G
EP2	Forward TGC CTT TCA CGA TTT TTG CA
	Reverse TTA ATT GAT AAA AAC CTA AGA GCT TGG A
EP3	Forward TCT CCG CTC CTG ATA ATG ATG TT
	Reverse TCT GCT TCT CCG TGT GTG TCT T
EP4	Forward CGA CCT TCT ACA CGC TGG TAT G
	Reverse CCG GGC TCA CCA ACA AAG T

relative to the corresponding untreated control value. Where applicable, the *t*-test was employed for statistical analysis. *P* values less than 0.05 were considered significant.

Results

BCP CRYSTALS UPREGULATE mPGES1 IN HFF BUT NOT IN OASF

mPGES1 mRNA expression in OASF was measured by real-time PCR. Incubation of OASF with BCP crystals for various times (1−32 h) did not significantly influence mPGES1 mRNA expression in OASF [Fig. 1(A)]. In addition, stimulation with phorbol myristate acetate (PMA), previously reported to upregulate mPGES1 mRNA expression[37,38], did not augment mPGES1 mRNA expression in OASF [Fig. 1(A)]. In contrast, in HFF, a significant threefold and fourfold induction of mPGES1 mRNA expression was evident at 4 and 7 h following BCP crystal stimulation, respectively [Fig. 1(B)].

mPGES1 IS OVER-EXPRESSED IN OASF AS COMPARED TO HFF

mPGES1 mRNA expression was compared in HFF and OASF by real-time PCR [Fig. 2(A)]. Basal mPGES1 expression in untreated OASF was 30-fold higher than in untreated HFF. Even at 7 h post-stimulation with BCP crystals, the timepoint of maximum induction of mPGES1 in HFF by BCP crystals, mPGES1 expression is approximately 10-fold lower than in untreated OASF.

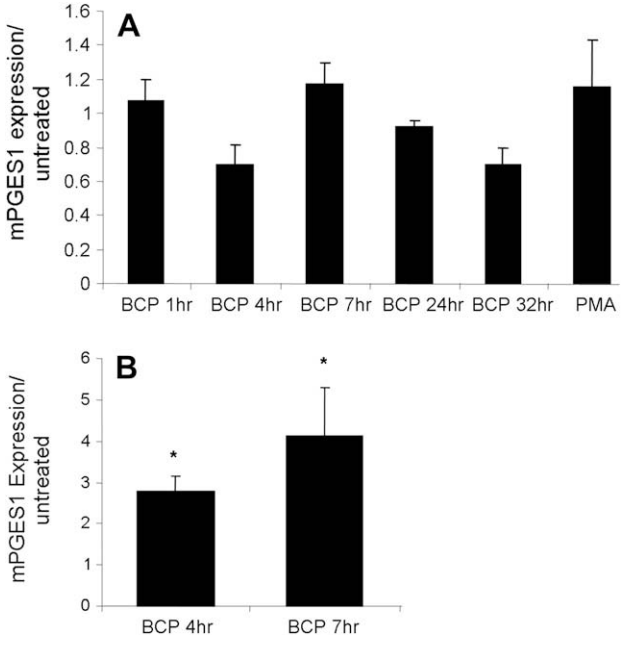

Fig. 1. mPGES1 mRNA expression in HFF but not OASF is augmented by treatment with BCP crystals. (A) OASF were treated with BCP crystals (BCP 1 h-BCP 32 h) or left untreated for 1−32 h, or stimulated with PMA. (B) HFF were treated with BCP crystals or left untreated for 4 (BCP 4 h) or 7 h (BCP 7 h). mPGES1 mRNA expression was measured by real-time PCR, normalised to 18S values and expressed as fold change in the treated cells over untreated control incubated for the same period of time. Therefore, the values of the untreated controls are 1. (A: mean ± S.E.M., $n = 4$; B: mean ± S.E.M., $n = 3$, $^*P < 0.05$).

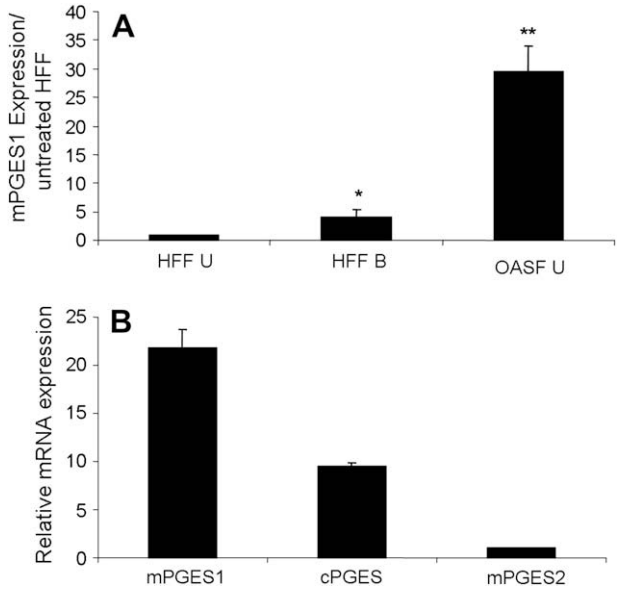

Fig. 2. (A) Comparison of mPGES1 mRNA expression between HFF and OASF. Untreated HFF (HFF U), BCP crystal-treated HFF (HFF B) and untreated OASF (OASF U) were incubated for 7 h. mPGES1 mRNA expression was measured by real-time PCR. Results are expressed as mPGES1 mRNA expression normalised to 18S levels, then expressed as fold change over the values for the untreated HFF. Therefore, the untreated HFF assumes a value of 1 (mean ± S.E.M., $n = 3$, $^*P < 0.05$, $^{**}P < 0.01$ vs HFF U). (B) Relative expression of PGE_2 synthases in OASF. Real-time PCR was used to quantify the mRNA expression levels of mPGES1, cPGES and mPGES2 in untreated OASF. Levels were normalised to 18S and then mRNA expression for each synthase was calculated relative to the expression of mPGES2 mRNA (mean ± S.E.M., $n = 4$).

RELATIVE EXPRESSION OF PROSTAGLANDIN SYNTHASES IN OASF

The relative expression of mPGES1, mPGES2 and cPGES in untreated OASF was measured by real-time PCR [Fig. 2(B)]. The mean relative mRNA expression was mPGES1: cPGES: mPGES2 = 22: 9: 1. Expression of cPGES and mPGES2 mRNA in OASF was unchanged by treatment with BCP crystals (data not shown).

EP4 RECEPTOR EXPRESSION IS DOWNREGULATED BY BCP CRYSTALS IN OASF BUT NOT HFF

Real-time PCR was used to quantify the mRNA expression levels of the EP1, EP2, EP3, and EP4 receptors in untreated OASF. EP4 was the most highly expressed EP receptor with appreciable expression of EP2 also found; there was negligible expression of EP1 and EP3 (data not shown). This pattern of EP receptor expression is consistent with that previously reported for RA synoviocytes[20]. EP4 receptor mRNA expression was downregulated up to 2.5-fold in OASF in response to BCP crystal treatment [Fig. 3(A)]. In contrast, EP4 expression was increased approximately twofold in HFF by BCP crystal stimulation [Fig. 3(B)]. Expression of EP1, EP2 and EP3 receptor mRNA was not significantly altered when OASF were treated with BCP crystals (data not shown).

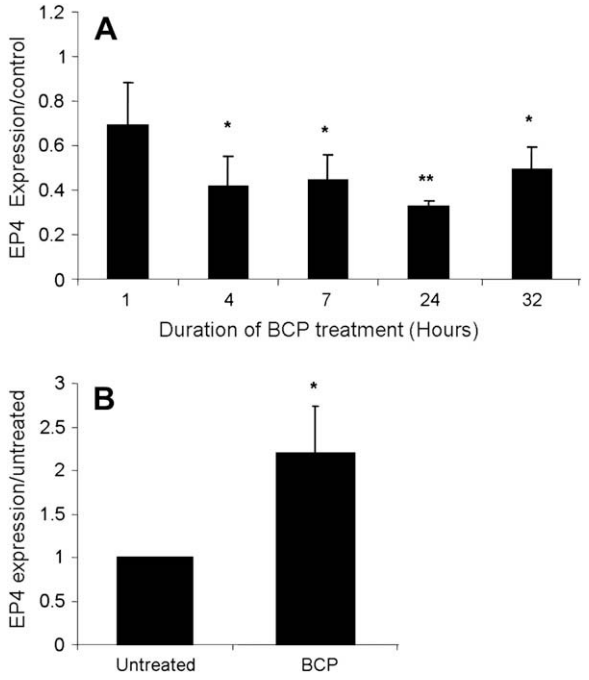

Fig. 3. Expression of the EP4 receptor mRNA in [A] OASF and [B] HFF following stimulation with BCP crystals. OASF were treated with BCP crystals for 1–32 h. HFF were treated with BCP crystals for 4 h or left untreated. EP4 receptor mRNA expression was measured by real-time PCR, normalised to 18S values and then expressed as fold change over the value obtained for the untreated control incubated for the same period of time (mean ± S.E.M., $n = 3$, $^{*}P < 0.05$, $^{**}P < 0.01$).

PGE_2 DOWNREGULATES EP4 EXPRESSION IN BCP CRYSTAL-TREATED OASF

In BCP crystal-treated OASF, EP4 receptor mRNA expression was downregulated approximately twofold in OASF in response to exogenous PGE_2 treatment and upregulated by aspirin and SC-236 (Fig. 4). The addition of exogenous PGE_2 overcame the effect of aspirin and SC-236. The downregulatory effect of PGE_2 on EP4 mRNA expression was diminished by treatment with L-161982, a selective EP4 receptor antagonist.

PGE_2 UPREGULATES mPGES1 EXPRESSION *VIA* THE cAMP-DEPENDENT EP2/EP4 RECEPTORS

Treatment with PGE_2 significantly increased mPGES1 expression in both BCP crystal-treated and untreated OASF [Fig. 5(A)]. Increased mPGES1 protein production following addition of PGE_2 to BCP crystal-stimulated OASF was demonstrated by western blotting [Fig. 5(B)]. Pre-treatment with L-161982 diminished the upregulation of mPGES1 mRNA expression seen in OASF in response to PGE_2 stimulation in both untreated and BCP crystal-treated OASF [Fig. 5(A)]. Following the addition of PGE_2, there was an approximately threefold higher level of mPGES1 expression in BCP crystal-treated OASF vs OASF not treated with BCP crystals, which was comparable to the mPGES1 upregulation seen with BCP crystal stimulation in HFF. In contrast to its effect on mPGES1, PGE_2 stimulation did not affect mRNA expression of mPGES2 or cPGES either in untreated or BCP crystal-treated OASF (data not shown).

Figure 5(C) demonstrates that treatment with CAY10399, an EP2 selective agonist, and forskolin, a direct adenylate cyclase agonist, both replicated the upregulation of mPGES1 mRNA expression seen in OASF in response to PGE_2 stimulation. Administration of aspirin, SC-236 or SC-560 did not significantly influence the level of mPGES1 mRNA expression in either untreated or BCP crystal-treated OASF (data not shown).

Discussion

Enhanced PGE_2 synthesis in the setting of inflammation is thought to be predominantly mediated by the sequential action of COX-2 and mPGES1. Both of these enzymes are generally expressed at low levels at baseline, are co-ordinately upregulated by pro-inflammatory stimuli[6,7,9–11,39] and downregulated by dexamethasone[11]. The functional coupling between COX-2 and mPGES1 is underlined by the preferential utilisation of COX-2 derived PGH_2 by mPGES1[10] and their co-localisation to the perinuclear membrane[10,40].

Given the demonstrated ability of BCP crystals to upregulate COX-2 in HFF[32] and OASF[35], it was anticipated that upregulation of mPGES1 would also occur in response to BCP crystals. Indeed, there was up to fourfold upregulation of mPGES1 noted in BCP crystal-treated HFF. Consistent

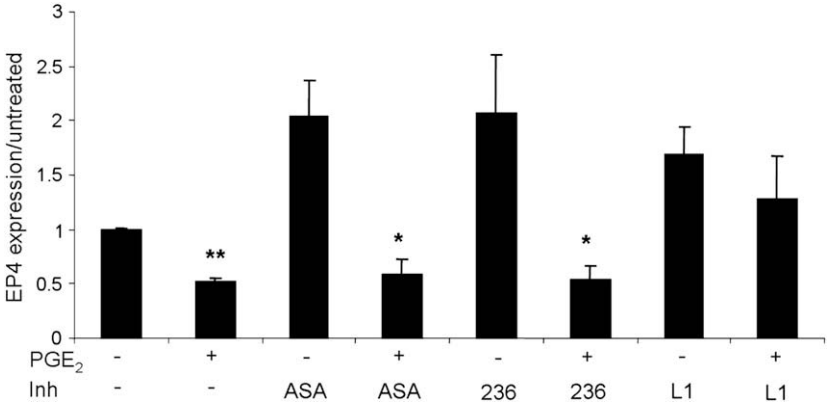

Fig. 4. Effect of PGE_2 on EP4 mRNA expression in BCP crystal-stimulated OASF. OASF were treated with 1 μM PGE_2 and/or 200 μM aspirin (ASA), 1 μM SC-236 (236) and 10 μM L-161982 (L1), prior to treatment with BCP crystals. EP4 receptor mRNA expression was measured by real-time PCR, normalised to 18S values and then expressed as fold change over the value obtained for the untreated control incubated for the same period of time. Therefore the untreated control (far left column) assumed a value of 1 (mean ± S.E.M., $n = 3$, $^{*}P < 0.05$, $^{**}P < 0.01$).

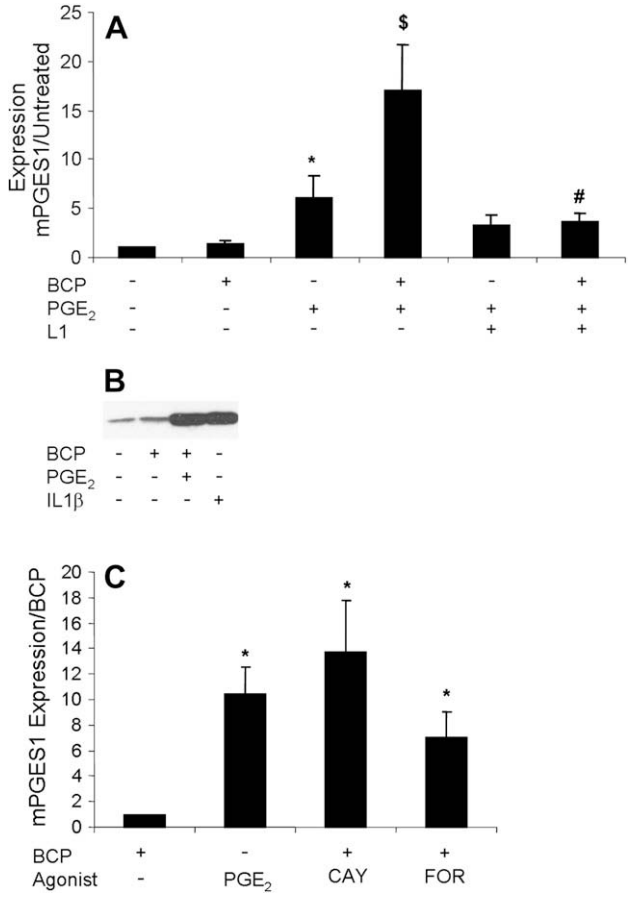

Fig. 5. Effect of PGE_2 on expression of mPGES1. (A) OASF were treated with 1 μM PGE_2 and/or 10 μM L-161982 (L1), prior to treatment with BCP crystals. mPGES1 mRNA expression was measured by real-time PCR, normalised to 18S values and expressed as fold change over the values obtained for the untreated control cells. Therefore the value for the untreated samples (most leftward column) was 1 (mean ± S.E.M., $n = 3$, $^*P < 0.05$ vs untreated OASF, $\$P < 0.05$ vs OASF treated only with BCP crystals, $\#P < 0.05$ vs OASF treated only with BCP crystals and PGE_2). (B) Western blotting for mPGES1. OASF were treated as indicated and incubated for 24 h. PGES1 protein was measured by western blotting of cell lysates, using a monoclonal antibody to mPGES1. Equal protein loading, as determined by Bradford assay were loaded onto 15% SDS_PAGE gels. (C) Effect of EP2 and cAMP agonists on mPGES1 mRNA expression in OASF. OASF were incubated with BCP crystals with or without PGE_2, CAY10399 (CAY) or forskolin (FOR). mPGES1 mRNA expression was measured by real-time PCR, normalised to 18S values and expressed as fold change of the values for the agonist treated cells over the values seen with BCP alone. Therefore the value for OASF treated with BCP crystals alone is 1 (mean ± S.E.M., $n = 3$, $^*P < 0.05$).

with this is the observation that BCP crystals upregulate Egr-1, a key transcription factor for mPGES1 expression, in human fibroblasts[33]. However, mPGES1 mRNA expression is not enhanced in OASF in response to BCP crystal stimulation. This differential response to BCP crystal stimulation may be explained by the fact that baseline expression of mPGES1 in OASF is over 30-fold greater than in HFF. Indeed, mPGES1 is more abundantly expressed in unstimulated OASF than the 'constitutive' PGE synthases, cPGES and mPGES2. Increased expression of mPGES2 in tissues with low levels of mPGES1 expression and vice versa has been previously reported[12]. Strong mPGES1 expression has been demonstrated on immunohistochemical analysis of RA synovium[19]. Cheng *et al.* found that mPGES1 expression could be induced by IL1β in OASF, but mPGES1 was detectable in untreated cells[5]. OASF have not previously been compared with other cell types as regards mPGES1 expression. mPGES1 is constitutively expressed in certain tissues[2,3], but the level of mPGES1 expression in non-diseased synovium has not been established. It is conceivable that chronic upregulation of mPGES1 occurs in OASF *in vivo*, as a result of pro-inflammatory stimuli, such as cytokines (e.g., IL1) and BCP crystals.

Similar to RA synoviocytes[20], EP4 and EP2 are the only EP receptors appreciably expressed in OASF. Regulation of the EP receptors[41–43] has previously been described; differential regulation of the EP2 and EP4 receptors has previously been demonstrated in response to lipopolysaccharide[43]. Expression of EP4 (but not EP2) receptor mRNA is downregulated by BCP crystals in OASF, but not in HFF. This downregulatory effect of BCP crystals on EP4 receptor expression was replicated by treatment of OASF with PGE_2. EP4 receptor mRNA expression was increased by treatment with aspirin and SC-236, an effect that was overcome by treatment with PGE_2. However, the downregulatory effect of PGE_2 on EP4 expression was blocked by L-161982, a specific EP4 receptor antagonist. This suggests that the likely mechanism of downregulation of EP4 receptor mRNA expression seen in response to BCP crystals in OASF is through a direct effect of PGE_2 on the EP4 receptor.

Treatment of OASF with PGE_2 results in a significant upregulation of mPGES1 transcript, in particular in cells also treated with BCP crystals, implying the presence of a positive feedback loop in the generation of PGE_2 in OASF. This response was not observed for cPGES or mPGES2. This effect of PGE_2 is significantly diminished by pre-treatment with L-161982, implicating the EP4 receptor in this response. In this regard it is interesting to note that PGE_2-EP4 signalling has been shown to induce functional expression of Egr-1[44], a key transcription factor for mPGES1 expression[34]. Thus, the downregulation of the EP4 receptor seen in BCP crystal-treated OASF may suppress the positive feedback mechanism described above and could potentially explain the discordant responses to BCP crystals between OASF and HFF as regards mPGES1 upregulation, as well as the lack of an observed inhibitory effect of COX inhibition on mPGES1 expression in BCP crystal-stimulated OASF. The effects of the EP4 receptor downregulation is only overcome by the addition of an excess of PGE_2, at which time a stimulatory effect of BCP crystals on mPGES1 expression in OASF becomes apparent. One potential hypothesis is that the mechanism of EP4 receptor downregulation exists as a restraint on this positive feedback loop and is facilitated in OASF by the increased basal expression of mPGES1 and consequent PGE_2 production. The upregulation of mPGES1 by PGE_2 is replicated by CAY10399, an EP2 receptor agonist, suggesting that EP2 may also contribute to the upregulation of mPGES1 by PGE_2. Treatment with forskolin, a direct adenylate cyclase activator also leads to a significant induction of mPGES1 in OASF, reflecting the fact that the EP2 and EP4 receptors mediate their cellular effects principally *via* increased cAMP production. Similar results were obtained in RA synoviocytes[20].

As mPGES1 appears to be the major PGE synthase contributing to PGE_2 production in the setting of inflammation, it has been proposed as a therapeutic target in inflammatory diseases[45]. Selective inhibition of mPGES1 could prevent

PGE_2 production in response to inflammatory stimuli, while permitting ongoing production of PGE_2 (by the constitutive PGES) and of other prostanoids, that may be required for homeostatic purposes. This could theoretically result in significantly less toxicity than is seen with NSAIDs. The relative overexpression of mPGES1 in OASF identifies it as a potential therapeutic target in OA. In this regard it is interesting to note the findings in the mPGES1 knockout mouse[17,18]. There were no apparent adverse consequences of the lack of mPGES1, but there was significant amelioration of the clinical and histological features of an inflammatory arthritis model. However, the precise contribution of PGE_2 to conditions such as OA is not known, and PGE_2 can mediate an attenuation of inflammation in certain situations[26,27]. Furthermore, as mPGES1 is constitutively expressed in the kidney, urogenital tract and reproductive organs[2,3] and the potential for a compensatory increase in the activity of other PGES (as occurs with COX) has not been examined, caution must be maintained at present in promoting mPGES1 inhibition as a therapeutic anti-inflammatory strategy.

This data suggests that EP4 may also be a therapeutic target in OA. However, enthusiasm for this approach is tempered by the fact that the effects of PGE_2 are often mediated through more than one EP receptor, and that PGE_2-EP4 signalling may mediate anti-inflammatory effects in some settings[26,27] and is involved in gastric mucosal protection[46,47].

In summary, we have demonstrated discordance between COX-2 and mPGES1 responses to BCP crystal stimulation in OASF; this may be explained by PGE_2-mediated downregulation of EP4 receptor expression in BCP crystal-stimulated OASF. These observations emphasize the complex nature of the regulation of PG production, and underline the challenges faced in selectively targeting pathways that could more specifically inhibit the deleterious effects of PGs in OA.

Conflict of interest

None of the authors has a conflict of interest to report regarding this paper.

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