EXTENDED REPORT

Cytokine production by infrapatellar fat pad can be stimulated by interleukin 1β and inhibited by peroxisome proliferator activated receptor α agonist

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Received 29 August 2011
Accepted 30 December 2011
Published Online First
2 February 2012

ABSTRACT

Background Infrapatellar fat pad (IPFP) might be involved in osteoarthritis (OA) by production of cytokines. It was hypothesised that production of cytokines is sensitive to environmental conditions.

Objectives To evaluate cytokine production by IPFP in response to interleukin (IL)1β and investigate the ability to modulate this response with an agonist for peroxisome proliferator activated receptor α (PPARα), which is also activated by lipid-lowering drugs such as fibrates.

Methods Cytokine secretion of IPFP was analysed in the medium of explant cultures of 29 osteoarthritic patients. IPFP (five donors) and synovium (six donors) were cultured with IL-1β and PPARα agonist Wy14643. Gene expression of IL-1β, monocyte chemoattractant protein (MCP1), (IL-6, tumour necrosis factor (TNF)α, leptin, vascular endothelial growth factor (VEGF), IL-10, prostaglandin-endoperoxide synthase (PTGS)2 and release of TNFα, MCP1 and prostaglandin E_2 were compared with unstimulated IPFP and synovium explants.

Results IPFP released large amounts of inflammatory cytokines, adipokines and growth factors. IL-1β increased gene expression of PTGS2, TNFα, IL-1β, IL-6 and VEGF and increased TNFα release in IPFP. MCP1, leptin, IL-10 gene expression and MCP1, leptin and PGE_2 release did not increase significantly. Synovium responded to IL-1β similarly to IPFP, except for VEGF gene expression. Wy14643 decreased gene expression of PTGS2, IL-1β, TNFα, MCP1, VEGF and leptin in IPFP explants and IL-1β, TNFα, IL-6, IL-10 and VEGF in synovium that responded to IL-1β.

Conclusion IPFP is an active tissue within the joint. IPFP cytokine production is increased by IL-1β and decreased by a PPARα agonist. The effects were similar to effects seen in synovium. Fibrates may represent a potential disease-modifying drug for OA by modulating inflammatory properties of IPFP and synovium.

INTRODUCTION

Osteoarthritis (OA) is the most common form of arthritis, with loss of cartilage structure as its main characteristic. In addition to subchondral bone sclerosis, synovitis with overproduction of cytokines by macrophages and fibroblasts is often seen.[1 2]

Evidence has emerged for a role of the infrapatellar fat pad (IPFP) or Hoffa's fat pad in the osteoarthritic process of the knee, since it contains adipocytes, nerve fibres, macrophages and other immune cells capable of producing cytokines.[3–5] The IPFP is located within the joint capsule of the knee close to the articular cartilage, synovium and bone, thus allowing the release of cytokines directly into the synovial fluid. IPFP has shown to produce interleukin (IL)1β, tumour necrosis factor (TNF)α, IL-6, IL-8, monocyte chemoattractant protein (MCP)1, fibroblast growth factor (FGF)2, vascular endothelial growth factor (VEGF), leptin, resistin and adiponectin.[5–8] However, the basal production of many other mediators, including anti-inflammatory cytokines, chemokines and growth factors, by the IPFP has not been investigated. It is also unclear whether the production of cytokines by the IPFP is influenced by inflammatory cytokines (eg, IL-1β) present in the osteoarthritic knee joint or if there is a cross-talk between the joint and the IPFP. In addition, modulation of production of proinflammatory mediators secreted from IPFP by potential disease-modifying drugs for OA is not known.

Recent publications[9 10] suggest that fibrates have potential as disease-modifying drugs for OA. Fibrates are peroxisome proliferator activated receptor (PPAR)α agonists. PPARα is a type I nuclear receptor, a member of the superfamily of ligand-dependent transcription factors regulating the transcription of target genes in a DNA-dependent manner by binding to PPAR response elements after heterodimerisation to retinoid X receptor. PPARα can also modulate gene transcription in a DNA binding-independent manner.[11] Agonists for PPARα are suggested as a potential therapeutic strategy for OA, since they exert anti-inflammatory effects on chondrocytes[9 10] and synovial fibroblasts,[12] which might be partially explained by their inhibitory effect on the nuclear translocation of nuclear factor κB (NF-κB).[9 13 14]

We hypothesised that next to synovium, the IPFP might contribute to the local OA disease process. We aimed to analyse whether the IPFP produces a large range of inflammatory cytokines and whether the production of cytokines is influenced by the proinflammatory environment in the OA joint as represented partially by raised levels of IL-1β, a cytokine present in the synovial fluid of osteoarthritic knee joints and known to induce cytokine production in synovium.[15 16]

In this study, we evaluated the basal production of several cytokines, growth factors and adipokines

by IPFP explants from osteoarthritic joints during the first 24 h after harvesting. Then, we evaluated whether an increased inflammatory condition, simulated by addition of IL-1β, can induce the mRNA expression and/or release of cytokines from cultured human OA IPFP; we compared this with synovium—a joint tissue that is more frequently studied. Furthermore, we examined whether activation of PPARα by a ligand could modulate the effect of IL-1β on the production and release of cytokines from IPFP.

MATERIALS AND METHODS

Preparation of IPFP explants and study design

Human IPFPs were obtained as anonymous left-over material from 29 patients (age 76.19 years (range 54–81); body mass index (BMI) 29.54 (23–48)) with knee OA undergoing total knee replacement. The patients had the right to be asked for their consent as stated by the guidelines of the Dutch Federation of Biomedical Scientific Societies (http://www.federa.org). This study was approved by the local ethical committee (number MEC 2008-181).

To examine the basal cytokine production, the inner parts of the IPFPs were cut into pieces of approximately 50 mg, taking care to avoid obtaining synovium present at the outside of the IPFP, and immediately cultured in suspension for 24 h in a concentration of 50 mg/ml in Dulbecco's modified eagle medium (DMEM) with Glutamax (GibcoBRL, Grand Island, New York, USA) containing insulin, transferrin, selenic acid and albumin (ITS+, Becton Dickinson, Breda, The Netherlands) 100 times diluted, 50 µg/ml gentamicin and 1.5 µg/ml fungizone (both GibcoBRL). After culture, the medium was harvested, centrifuged at 200 *g* for 8 min and frozen at −80°C in aliquots.

To investigate the response of IPFP explants to IL-1β and PPARα agonist, IPFP explants of approximately 50 mg were obtained from five patients with OA. In addition, the synovia from six patients with OA were dissected and cut into pieces of 50 mg. Each IPFP and synovium sample consisted of three explants. We performed triplicate cultures for IPFP and duplicate cultures for synovium. IPFP and synovium explants were first precultured in DMEM high glucose (GibcoBRLA), supplemented with 2% fetal calf serum (Lonza, Basel, Switzerland), 50 µg/ml gentamycin (GibcoBRL) and 1.5 µg/ml fungizone (GibcoBRL) for 24 h. The explants were then washed in phosphate-buffered saline and the medium was replaced by DMEM high glucose with 1:100 ITS+. During the next 48 h, the explants were cultured with or without 10 ng/ml IL-1β and with or without 10^{-5}–10^{-3} M Wy14643 (Cayman Chemical, Ann Arbor, Michigan, USA), a potent and selective PPARα agonist. Wy14643 was dissolved in dimethyl sulphoxide (Sigma, St Louis, Missouri, USA). Detrimental effects of 10^{-5} and 10^{-4} M Wy14643 on the viability of the IPFP and synovium explants were excluded with a lactate dehydrogenase (LDH) cytotoxicity assay (online supplementary figure 1). Wy14643 (10^{-3} M) increased LDH concentration in the culture media and was therefore not used for further experiments (data not shown)). Initial studies with 10^{-5} M Wy14643 showed no apparent effect on expression of genes of interest in IL-1β-stimulated explants, therefore we only used 10^{-4} µM in further experiments. After 48 h of culture, the explants were frozen in liquid nitrogen and the harvested culture media were stored at −80 °C as described above.

RNA extraction and real-time PCR

Frozen IPFP and synovium samples were homogenised with a Mikro-Dismembrator (Braun Biotech International GmbH, Melsungen, Germany) and suspended in 1.8 ml RNA-Bee (Bioconnect) per 100 mg tissue. The RNA-bee solution was precipitated with 0.2 ml chloroform. RNA was purified using an RNeasy Micro Kit (Qiagen, Hilden, Germany). Total RNA (500 ng) was reverse-transcribed into cDNA using RevertAid First Strand cDNA Synthesis Kit (MBI Fermentas, St Leon-rot, Germany). Forward and reverse oligonucleotides are described in online supplementary table 1. TNFα, IL-1β, MCP1, IL-6 are proinflammatory cytokines involved in the OA disease process and are produced by adipose tissue.[17–19] Cyclo-oxygenase (COX)2 is an enzyme responsible for the production of inflammatory cytokines such as prostaglandin (PGE_2) and is induced by TNFα and IL-1β.[18] The gene of COX is prostaglandin-endoperoxide synthase (PTGS)2. IL-10 is described as an anti-inflammatory cytokine[20] and leptin is an important adipokine that might enhance the inflammatory processes in the joint.[19] We also analysed PPARα and PPARγ mRNA expression. TaqMan Universal PCR Master Mix (ABI, Branchburg, New Jersey, USA) or qPCR Mastermix Plus for SYBRGreen I (Eurogentec, Nederland B V, Maastricht, The Netherlands) was used to perform real-time (RT)-PCR in 20 µl reactions, according to the manufacturer's guidelines, and using the ABI PRISM 7000 Sequence Detection System with software version 1.2.3. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was compared with β-actin (ACTB), β2-microglobulin (B2M), hypoxanthine phosphoribosyltransferase 1 (HPRT1) and with the average of all tested housekeeping genes and appeared to be the most stable. With GAPDH, we observed no differences in CT values between conditions or between tissues (data not shown). GAPDH was used to calculate relative gene expression with the $2^{-\Delta CT}$ formula.

Assays on culture media for viability and cytokines

A cytotoxicity detection kit (Roche Diagnostics, Indianopolis, USA) was used in accordance with the manufacturer's instructions to determine the presence of LDH in culture media, in order to test the effect of Wy14643 on the viability of IPFP and synovium explants. The culture media of the explants were analysed for cytokine, chemokine, adipokine and growth factor content using Milliplex kits (catalogue number: MPXHCYTO60KPMX42 and HADCYT-61K, Millipore, Massachusetts, USA). The Milliplex assays were analysed with Luminex 100 IS (Luminex Corporation, Austin, Texas, USA). PGE_2 and MCP1 content of the culture media were determined using the PGE_2 and MCP1 assay, respectively (R&D Systems, Minneapolis, USA), according to manufacturer's instructions.

Data analysis

To determine the release of cytokines directly after harvesting, the culture media of IPFPs of 29 patients with OA were measured in duplicate and averaged. Correlation with BMI was tested using a Spearman's rank correlation test and adjustment for multiple testing was done with a Bonferroni test for assessing the significance of these correlations. The effect of IL-1β with or without PPARα agonist on cytokine production was tested in the IPFPs of five patients with OA (cultured in triplicate samples) and synovium explants of six patients with OA (cultured in duplicate samples). PCR analysis was performed on each of the samples, and supernatant assays were performed on the culture media that were pooled for each condition for each patient.

Data were analysed with SPSS 18.0. A linear mixed model was used for the PCR analysis of the samples and a general linear model was used for the supernatant assays, after confirming the normal distribution of the residuals using a Wilks–Shapiro test. Both types of linear regression are robust for violation of this

assumption. Since previous studies with Wy14643 with cartilage have shown that the effect of Wy14643 is highly dependent on the response of tissue to IL-1β,[9] we also analysed the interaction between the effect of PPARα activation and the response to IL-1β in the linear mixed models by adding an interaction term. This analysis shows whether the effect of Wy14643 is dependent on the response to IL-1β.

RESULTS

IPFP explants produce a variety of cytokines and the production is increased by local cytokine stimulation

IPFP explants released leptin, adiponectin, resistin and cytokines such as IL-6, IL-8, MCP1, IL-4, fractalkine, interferon inducible protein (IP)10, hepatocyte growth factor (HGF), VEGF, growth-related protein (GRO), granulocyte-colony stimulating factor (G-CSF) and FGF2 (figure 1 and online supplementary table 2). To investigate whether the release of cytokines by the IPFP is influenced by BMI, we examined the correlation between BMI and cytokines that are secreted in high amounts by the IPFP and/or are known to be involved in the OA disease process.[15 18] Only adiponectin and MCP1 were negatively correlated with BMI, although these results were not significant after adjustment for multiple comparisons (online supplementary table 3). In addition, we dichotomised BMI (≤27.5 and >27.5), but only found a difference in release between these groups for adiponectin (p=0.05).

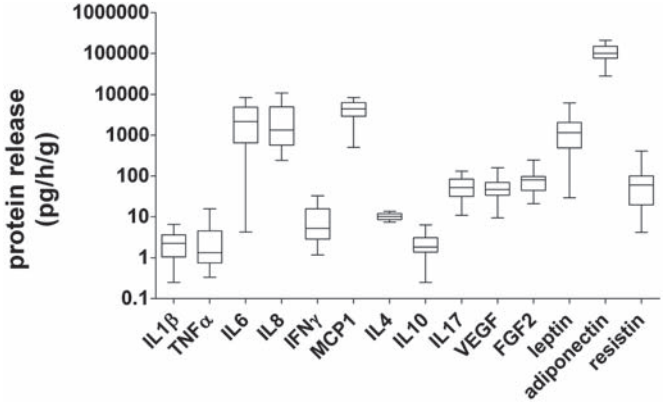
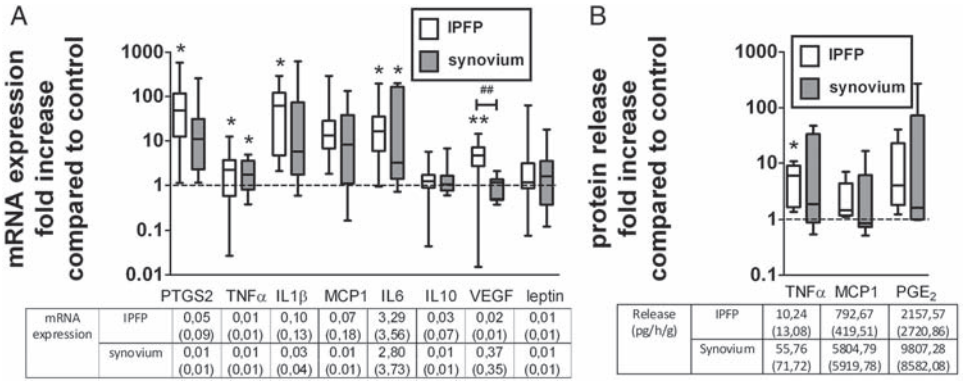


Figure 1 Cytokine release by osteoarthritic infrapatellar fat pad explants. Multiplex ELISA analysis of culture media of infrapatellar fat pad explants from 29 donors that were cultured for 24 h (50 mg adipose tissue/ml). Whisker box plots with minimum and maximum values. FGF, fibroblast growth factor; IFN, interferon; IL, interleukin; MCP, monocyte chemoattractant protein; TNF, tumour necrosis factor; VEGF, vascular endothelial growth factor. (See also online supplementary table 1.)

To investigate whether the cytokine production by IPFP explants changes owing to local inflammatory stimuli, we added 10 ng/ml IL-1β and analysed mRNA expression and release of cytokines produced by IPFP. Similar experiments were performed on synovium explants (figure 2). In the IPFP explants, the addition of IL-1β increased the mRNA expression 132.4 times for PTGS2 (p=0.02), 2.8 times for TNFα (p=0.04), 74.0 times for IL-1β (p=0.01), 43.1 times for IL-6 (p=0.02) and 4.6 times for VEGF (p=0.01). Leptin mRNA expression showed a trend towards an increase of 10.1 times (p=0.09), respectively, while IL-10 (p=0.12) and MCP1 (p=0.16) were not altered after treatment with IL-1β. To confirm these results, we analysed protein release of TNFα, a cytokine involved in OA disease, and MCP1 and PGE_2 since these cytokines are known to be released in high amounts by adipose tissue and synovium. Adding IL-1β to the IPFP explants increased the release of TNFα 5.5 times (p=0.04), while there were no significant differences for MCP1 (p=0.20) and PGE_2 (p=0.23).

The effect of IL-1β on IPFP was comparable with the stimulatory effect of IL-1β in the synovium samples, where mRNA expression was increased 2.1 times for TNFα (p=0.02), 69.8 times for IL-6 (p=0.01) and a trend towards an increase of 35.5 times for PTGS2 (p=0.08) and 102.1 times for IL-1β (p=0.08). MCP1 gene expression was not altered (p=0.17). The relative gene expression of leptin in synovium explants was very low and did not increase (p=0.16) by adding IL-1β as shown in figure 2. There was no increase in IL-10 mRNA expression (p=0.23) and VEGF mRNA expression (p=0.87). Only VEGF mRNA expression was significantly differently affected by IL-1β in synovium compared with IPFP samples (p<0.0001). The addition of IL-1β to synovium samples did not increase the release of TNFα (p=0.15), MCP1 (p=0.22) or PGE_2 (p=0.23). We found no differences in mRNA expression of PPARα and PPARγ between IPFP and synovium or between controls and explants cultured with IL-1β (data not shown).



		PTGS2	TNFα	IL1β	MCP1	IL6	IL10	VEGF	leptin
mRNA expression	IPFP	0,05 (0,09)	0,01 (0,01)	0,10 (0,13)	0,07 (0,18)	3,29 (3,56)	0,03 (0,07)	0,02 (0,01)	0,01 (0,01)
	synovium	0,01 (0,01)	0,01 (0,01)	0,03 (0,04)	0,01 (0,01)	2,80 (3,73)	0,01 (0,01)	0,37 (0,35)	0,01 (0,01)

		TNFα	MCP1	PGE_2
Release (pg/h/g)	IPFP	10,24 (13,08)	792,67 (419,51)	2157,57 (2720,86)
	Synovium	55,76 (71,72)	5804,79 (5919,78)	9807,28 (8582,08)

Figure 2 Interleukin 1β increases the production of cytokines by infrapatellar fat pad and synovium explants. IPFP samples (n=15 samples, obtained from five patients with OA) and synovium samples (n=12 samples, obtained from six patients with OA) were cultured for 48 h with or without 10 ng/ml interleukin 1β. (A) mRNA expression, normalised to glyceraldehyde-3-phosphate dehydrogenase, fold increase relative to control. (B) Protein release into culture media, fold increase relative to control. Table indicates absolute protein release with SD between brackets. Bars show the fold increase compared with control without IL-1β that is set at 1 (dotted line). Light bars indicate IPFP samples, dark bars indicate synovium samples. * and ** indicate a significant difference from control without IL-1β (p<0.05 and p<0.0001, respectively). Significant differences (p<0.0001) between synovium and IPFP are indicated by ##. IL, interleukin; IPFP, infrapatellar fat pad; MCP, monocyte chemoattractant protein; OA, osteoarthritis; PTGS, prostaglandin-endoperoxide synthase; TNF, tumour necrosis factor; VEGF, vascular endothelial growth factor.

In summary, treatment with IL-1β leads to a significant increase in expression of mainly proinflammatory cytokines in IPFP and synovium explants. The increase in cytokine production by IPFP explants was comparable to the synovium explants, except for VEGF, which was increased by IL-1β in IPFP but not in synovium.

PPARα activation inhibits IL-1β-induced cytokine production by IPFP

Wy14643, a PPARα agonist,[21] had no effect on production of cytokines by IPFP and synovium in culture conditions without IL-1β (data not shown). To investigate whether PPARα activation could inhibit IL-1β induced cytokine production, we treated the IL-1β stimulated IPFP explants with 10^{-4} M Wy14643. The addition of 10^{-4} M Wy14643 decreased the IL-1β induced mRNA expression of VEGF 2.6 fold (p=0.01) in IPFP samples (figure 3a). In addition, a trend towards decrease for IL-1β mRNA expression with 1.5-fold (p=0.10), IL-10 with 1.4-fold (p=0.10) and leptin with 3.0-fold (p=0.08) was seen, whereas no significant differences for PTGS2 (p=0.11), TNFα (p=0.40), MCP1 (p=0.44) and IL-6 (p=0.97) were seen. There was a significant interaction between IL-1β response and the effect of PPARα for PTGS2, IL-1β, TNFα, MCP1, VEGF and leptin, which might indicate that the effect of PPARα activation was dependent on the effect of IL-1β. No interaction for IL-6 and IL-10 was seen.

In addition, we analysed the secretion of TNFα, MCP1 and PGE_2 to the culture media to confirm the mRNA expression data. We also analysed leptin since this adipokine is mainly secreted by adipose tissue.[17] We observed no significant differences for TNFα (p=0.88), MCP1 (p=0.52), PGE_2 (p=0.45) and leptin (p=0.64) release when adding Wy14643 to the IL-1β-stimulated explants of IPFP (figure 3b). There was an interaction between IL-1β response and PPARα activation for leptin, indicating that the effect of Wy14643 depends on the IL-1β response, but not for TNFα, MCP1 and PGE_2.

In synovium, there was no effect of Wy14643 on the IL-1β-induced changes in mRNA expression of PTGS2 (p=0.74), IL-1β (p=0.85), TNFα (p=0.76), MCP1 (p=0.62), IL-6 (p=0.74), IL-10 (p=0.19), VEGF (p=0.93) and leptin (p=0.69) (figure 4a). Conceivably, PPARα might exhibit its activity in a highly proinflammatory environment in the synovium tissue samples that are highly responsive to IL-1β. To investigate this hypothesis we evaluated the effects of PPARα ligand in synovium tissue from donors with a high response to IL-1β, and investigated the interaction between both. A statistical interaction between IL-1β response and PPARα effect for IL-1β, TNFα, IL-6, IL-10 and VEGF was found, but not for PTGS and leptin.

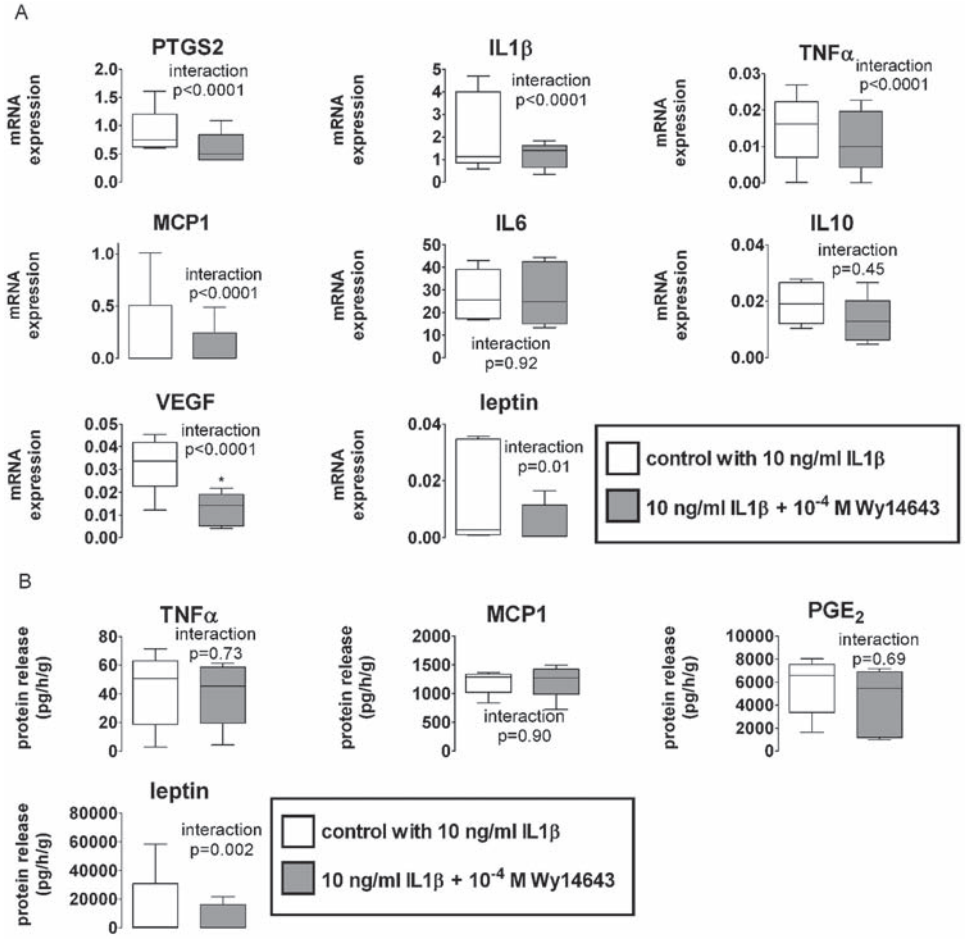


Figure 3 Peroxisome proliferator activated receptor α (PPARα) agonist Wy14643 on cytokine mRNA expression and protein release of infrapatellar fat pad. Explants of infrapatellar fat pad were cultured for 48 h in the presence of interleukin (IL)1β with or without PPARα agonist Wy14643. (A) mRNA expression relative to housekeeping gene of prostaglandin-endoperoxide synthase (PTGS)2, IL-1β, tumour necrosis factor (TNF)α, monocyte chemoattractant protein (MCP)1, IL-6, vascular endothelial growth factor (VEGF), leptin and IL-10. N=15 samples, obtained from five patients with osteoarthritis. (B) Release of TNFα, MCP1, prostaglandin (PG)E2 and leptin in the culture media.

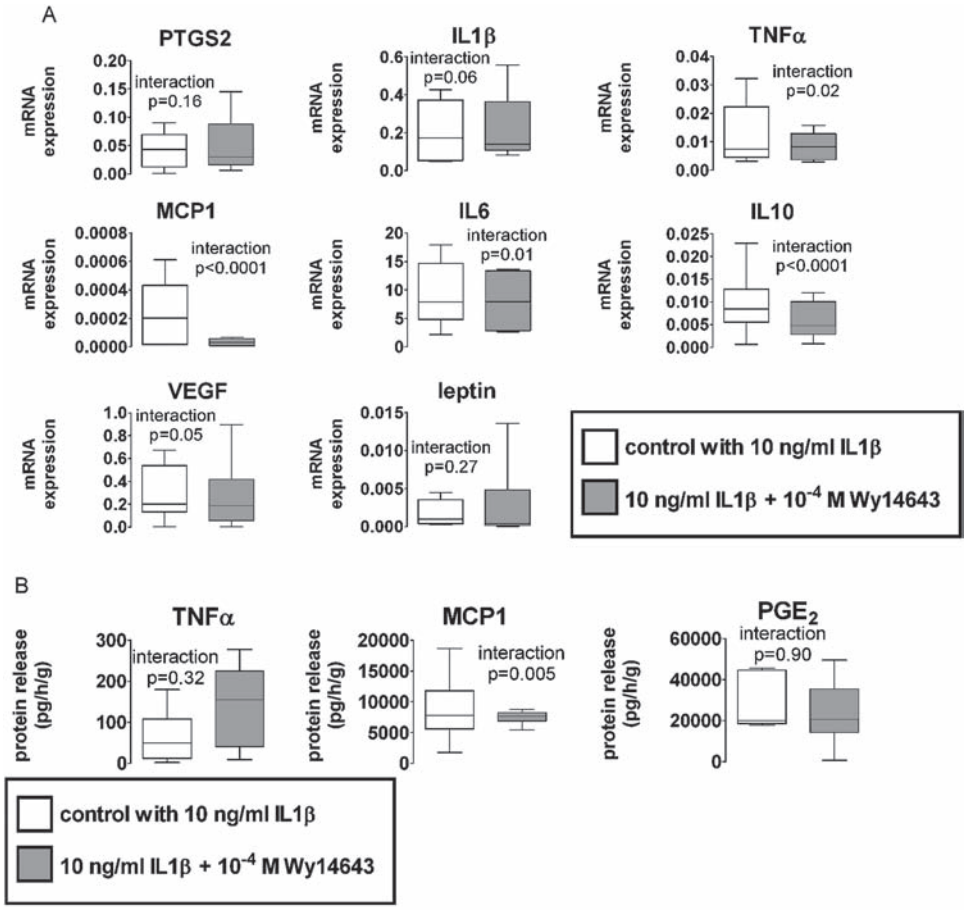


Figure 4 Peroxisome proliferator activated receptor α (PPARα) agonist Wy14643 on cytokine mRNA expression and protein release of synovium. Explants of synovium were cultured during 48 h in the presence of interleukin (IL)1β with or without PPARα agonist Wy14643. (A) mRNA expression relative to housekeeping gene of prostaglandin-endoperoxide synthase (PTGS)2, IL-1β, tumour necrosis factor (TNF)α, monocyte chemoattractant protein (MCP)1, IL-6, vascular endothelial growth factor (VEGF), leptin and IL-10. N=12 samples, obtained from six patients with osteoarthritis. (B) Release of TNFα, MCP1 and prostaglandin (PG)E2 in the culture media. *Indicates a significant difference ($p<0.05$). The interaction term indicates that the effect of PPARα agonist Wy14643 depends on the response of the patient to IL-1β.

Wy14643 did not decrease TNFα (p=0.14), MCP1 (p=0.30) or PGE_2 release (p=0.63) by synovium explants to the culture medium. There seemed to be an interaction with IL-1β response for MCP1 release, but not for PGE_2 or TNFα (figure 4b).

DISCUSSION

There is increasing evidence that the IPFP contributes to the OA disease process in the knee joint by the production of cytokines that may induce destructive and inflammatory responses in cartilage.[4 6–8 17] The secretion of IL-1β, TNFα, IL-6, IL-8, MCP1, FGF2, VEGF, leptin, resistin and adiponectin by IPFP has been described previously.[5–8] Our study confirms the production of these cytokines and shows that additional cytokines, such as IL-4, IL-10, are produced by osteoarthritic IPFP explants. We found large variation in cytokine production between donors, possibly related to the presence of immune cells.[3 5] Although no studies have investigated the diffusion of synovial fluid cytokines to the IPFP, the anatomical location and the presence of immune cells (eg, macrophages, T cells) in osteoarthritic IPFP[4 5 22] make it likely that the IPFP itself is influenced by cytokines present in the synovial fluid. We therefore examined the effect of IL-1β, a proinflammatory cytokine present in osteoarthritic synovial fluid,[16] on production of COX2, IL-1β, TNFα, MCP1, IL-6, VEGF, leptin and PGE_2 and found an increased production of PTGS2 and all cytokines, except IL-10 and leptin. Since IL-10 is a cytokine with anti-inflammatory and chondroprotective properties,[20] the lack of increase in IL-10 gene expression confirms the proinflammatory phenotype that seems to develop in the IPFP by adding IL-1β. Leptin is produced by adipocytes while all other cytokines are mainly produced by the non-adipocyte fraction—mostly infiltrated immune cells.[17] Therefore, the absence of a significant effect on leptin production in the IPFP samples may indicate that the IL-1β effect on cytokine production mainly occurs through an effect on immune cells and not on adipocytes. The effects of IL-1β on cytokine production in IPFP samples were comparable with the responses in the synovium explants, except for VEGF and PGE_2. Although the explants were not obtained from the same donor, this demonstrates that in addition to synovium, the IPFP is also an important joint tissue that can be stimulated by local inflammatory responses in the joint and might contribute to the OA disease process.

We investigated whether the basal production of cytokines by IPFP correlated with BMI, since this might provide an explanation for the association between obesity and the incidence/progression of knee OA.[23] However, no correlation between BMI and IPFP cytokine production was demonstrated and any correlation tended to be negative for many cytokines. We concluded that intra-articular influences such as IL-1β may be more important in regulating cytokine production than systemic influences

such as BMI. Additional analysis in more patients and with a lower mean BMI should confirm the lack of correlation between BMI and cytokine production by the IPFP. Since we did not use IPFP of healthy joints, it remains unclear whether BMI is correlated with IPFP production in the healthy joint.

We examined whether PPARα activation could inhibit the IL-1β stimulatory effect on IPFP explants. This nuclear receptor is expressed in white adipose tissue, but also cartilage synovium and bone, and is a target for agonists such as fibrates, which are potential disease-modifying drugs for OA.[9 12 24] In our study, addition of Wy14643 led to a decrease in IL-1β-induced gene expression of VEGF. Additional statistical analyses demonstrated an interaction between response to IL-1β and effect of PPARα activation, indicating that the PPARα agonist Wy14643 had an effect only in those donors who responded to IL-1β, and not in the non-responsive donors. This was supported by the absence of an effect of PPARα agonist Wy14643 in cultures without IL-1β. Furthermore, we have observed similar results in a study on cartilage explants,[9] where we demonstrated that PPARα activation inhibits the nuclear translocation of NF-κB. Immune cells such as macrophages[5 17 22] may be responsible for the increase in cytokine production when IL-1β is added. The inhibition of NF-κB in this cell fraction by PPARα activation has been described and may be an important mechanism for the effects seen in our study.[13] Other intracellular signalling pathways such as mitogen-activated protein kinase phosphorylation or cleavage of inflammasome/caspase-1 may also be involved.[13 25] Future experiments could elucidate the underlying mechanisms and the potential influence of other pro- and anti-inflammatory cytokines on IPFP.

When Wy14643 was added to IL-1β-stimulated synovium explants, we observed a trend towards a decrease for MCP1 gene expression and interaction between IL-1β and Wy14643 in five genes of interest. Previous in vitro experiments have shown that PPARα agonists decrease the production of IL-6 and IL-8 in IL-1β-induced synoviocytes and also decrease the release of cytokines by macrophages, which are also present in osteoarthritic synovium.[13 14 26] The less pronounced effect of analyses of supernatants did not confirm all gene expression results of cultures with Wy14643. This might be owing to the high secretion of cytokines by the explants, making the effects of a relatively short period of culture with Wy14643 insufficient to result in significant differences between conditions. The protein data also had a large variation between samples. This might be due to differences in the number of cells between explants, although we did standardise the weight of the explants per volume medium. Differences in cell numbers between the explants were easily corrected with housekeeper gene expression in the mRNA expression analyses but we could not correct the secreted protein data for these variations.

PPARα agonists such as fibrates are used as lipid-lowering drugs. In addition to their lipid-lowering effect on plasma triglyceride levels, they exert anti-inflammatory systemic effects. PPARα agonists have also been shown to be effective in reducing inflammatory responses in osteoarthritic cartilage.[9] This study provides a proof of principle that PPARα activation also leads to a decrease in the production of cytokines by the IPFP. Since OA pathogenesis probably involves systemic and metabolic factors such as dyslipidaemia and atherosclerosis,[27–29] the combination of local antidestructive and anti-inflammatory effect in the joint, with systemic effects on serum lipid levels and vascular pathology, makes fibrates of interest as a therapeutic strategy for OA.[30 31]

CONCLUSION

The IPFP is a source of cytokines. The production of cytokines can be stimulated by IL-1β, an important cytokine present in the synovial fluid of osteoarthritic joints. PPARα activation significantly decreases the production of inflammatory cytokines in the IPFP explants and to a lesser extent in the synovium explants. This study reinforces the potential role of adipose tissue in the aetiopathogenesis of OA and shows that PPARα agonists such as fibrates are a potential therapeutic strategy for OA.

Acknowledgements The authors thank the orthopaedic surgeons and the nursing team for their assistance in obtaining infrapatellar fat pad and synovium of patients undergoing total knee replacement. This study/work was performed within the framework of the Dutch Top Institute Pharma project #T1-213. SC received a scholarship of the University of Antwerp and the Anna Foundation.

Competing interests None.

Provenance and peer review Not commissioned; externally peer reviewed.

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