Osteoarthritis and Cartilage 22 (2014) 133–144

Osteoarthritis and Cartilage

Cannabinoid WIN-55,212-2 mesylate inhibits interleukin-1β induced matrix metalloproteinase and tissue inhibitor of matrix metalloproteinase expression in human chondrocytes

S.L. Dunn †, J.M. Wilkinson ‡, A. Crawford §, C.L. Le Maitre †, R.A.D. Bunning †*

† Biomedical Research Centre, Faculty of Health and Wellbeing, Sheffield Hallam University, Sheffield S1 1WB, UK
‡ Academic Unit of Bone Metabolism, Department of Human Metabolism, University of Sheffield, UK
§ Centre for Biomaterials & Tissue Engineering, University of Sheffield, School of Clinical Dentistry, UK

ARTICLE INFO

Article history:
Received 29 May 2013
Accepted 26 October 2013

Keywords:
Cannabinoid
Cartilage degradation
Chondrocytes
Interleukin 1 (IL-1)
Matrix metalloproteinases (MMPs)
Tissue inhibitors of matrix metalloproteinases (TIMP)

SUMMARY

Objective: Interleukin-1β (IL-1β) is involved in the up-regulation of matrix metalloproteinases (MMPs) leading to cartilage degradation. Cannabinoids are anti-inflammatory and reduce joint damage in animal models of arthritis. This study aimed to determine a mechanism whereby the synthetic cannabinoid WIN-55,212-2 mesylate (WIN-55) may inhibit cartilage degradation.

Methods: Effects of WIN-55 were studied on IL-1β stimulated production of MMP-3 and -13 and their inhibitors TIMP-1 and -2 in human chondrocytes. Chondrocytes were obtained from articular cartilage of patients undergoing total knee replacement. Chondrocytes were grown in monolayer and 3D alginate bead cultures. Real-time polymerase chain reaction (PCR) was used to determine the gene expression of MMP-3, -13, TIMP-1 and -2 and Enzyme Linked Immunosorbent Assay (ELISA) to measure the amount of MMP-3 and MMP-13 protein released into media. Immunocytochemistry was used to investigate the expression of cannabinoid receptors in chondrocyte cultures.

Results: Treatment with WIN-55 alone or in combination with IL-1β, decreased or abolished MMP-3, -13, TIMP-1 and -2 gene expression in human chondrocyte monolayer and alginate bead cultures in both a concentration and time dependent manner. WIN-55 treatment alone, and in combination with IL-1β, reduced MMP-3 and -13 protein production by chondrocytes cultured in alginate beads. Immunocytochemistry demonstrated the expression of cannabinoid receptors in chondrocyte cultures.

Conclusion: Cannabinoid WIN-55 can reduce both basal and IL-1β stimulated gene and protein expression of MMP-3 and -13. However WIN-55 also decreased basal levels of TIMP-1 and -2 mRNA. These actions of WIN-55 suggest a mechanism by which cannabinoids may act to prevent cartilage breakdown in arthritis.

© 2013 Osteoarthritis Research Society International. Published by Elsevier Ltd. All rights reserved.

Introduction

Osteoarthritis (OA) and rheumatoid arthritis (RA) are debilitating joint diseases, and although they have a different aetiology a key pathological feature of both is the loss of articular cartilage[1]. Cannabis-based medicine Sativex has been shown to have analgesic effects and to suppress disease activity in patients with RA[2]. Cannabinoids also have anti-inflammatory effects and reduce joint damage in animal models of arthritis[3–5]. *In vitro* studies have shown that cannabinoids reduce cytokine production from RA fibroblasts and the release of matrix metalloproteinases (MMPs) from fibroblast-like synovial cells[6–8]. Cannabinoids also have direct effects on cartilage extracellular matrix (ECM) breakdown; reducing interleukin 1 (IL-1) induced proteoglycan and collagen degradation in bovine cartilage[9]. There is thus increasing evidence to suggest that cannabinoids have chondroprotective effects and may be of value in the treatment of arthritis[10].

During OA and RA there is a shift in the equilibrium between catabolic and anabolic activities[1]. As a result the breakdown of collagen and proteoglycans may exceed the rate of synthesis of new matrix molecules resulting in cartilage degradation. Another contributing factor in cartilage breakdown in OA and RA is an

* Address correspondence and reprint requests to: R.A.D. Bunning, Biomedical Research Centre, Faculty of Health and Wellbeing, Sheffield Hallam University, Sheffield S1 1WB, UK. Tel: 44-114-225-3012; Fax: 44-114-225-3066.
E-mail addresses: sldunn@my.shu.ac.uk (S.L. Dunn), j.m.wilkinson@sheffield.ac.uk (J.M. Wilkinson), a.crawford@sheffield.ac.uk (A. Crawford), C.Lemaitre@shu.ac.uk (C.L. Le Maitre), r.a.bunning@shu.ac.uk, sciradb@exchange.shu.ac.uk (R.A.D. Bunning).

1063-4584/$ – see front matter © 2013 Osteoarthritis Research Society International. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.joca.2013.10.016

increase in inflammatory cytokines particularly IL-1 and tumour necrosis factor (TNF) produced by the articular chondrocytes or cells of the synovium[11]. This results in an increase in MMPs particularly MMP-3 and MMP-13, which are expressed in RA and OA cartilage and synovial tissue[12–20], which is not accompanied by an increase in their tissue inhibitors of matrix metalloproteinases (TIMPs)[21,22] resulting in a net increase in MMPs. MMP inhibition has been proposed as a possible mechanism to prevent breakdown of cartilage tissue in arthritis, provided the necessary functional specificity can be achieved[23].

To investigate further the potential of cannabinoids to regulate cartilage breakdown we have studied the effects of synthetic cannabinoid WIN-55,212-2 mesylate (WIN-55) on basal and IL-1β stimulated MMP-3, -13, TIMP-1 and -2 expression in human articular chondrocytes from human OA cartilage tissue in monolayer and 3D alginate bead culture. WIN-55 is an agonist at the classical cannabinoid receptors, cannabinoid receptor 1 and 2 (CB1 and 2), but also has been shown to activate other receptors including peroxisome proliferator activated receptors alpha and gamma (PPARα and γ)[24–26]. Thus the expression of these receptors in cultured OA chondrocytes was determined to identify potential targets of WIN-55.

Materials and methods

Human cartilage tissue

Human chondrocytes were obtained from articular cartilage ($n = 9$) removed from patients with symptomatic OA at the time of total knee replacement (Supplementary Table I). Cartilage was obtained under the National Research Ethics Service approval held by the Sheffield Musculoskeletal Biobank. All patients provided written, informed consent prior to participation. Cartilage blocks were taken from each anatomic compartment within the knee ($n = 6–7$) (medial and lateral tibio-femoral and patello-femoral compartments). Cartilage tissue was graded macroscopically 0–4 using the Outerbridge classification[27]. Chondrocytes were isolated from grade 0 ($n = 3$), grade 2 ($n = 6$) and grade 3 ($n = 5$) cartilage tissue as representative of non-degenerate, low degenerate and intermediate degenerate cartilage tissue. Cartilage from grade 4 severe degenerate tissue was not used in the study as the cell yield obtained was not sufficient.

Isolation of human chondrocytes

Cartilage tissue was digested in 0.25% trypsin (Sigma–Aldrich, Poole, UK) at 37°C for 30 min followed by digestion in 3 mg/ml collagenase type I (Sigma–Aldrich, Poole UK) in Dulbecco's modified Eagle's medium (DMEM)/F-12 (1:1) (Gibco, Paisley, UK) supplemented with 10% heat-inactivated foetal bovine serum (FBS) (Gibco, Paisley, UK), 2 mM glutamine (Gibco, Paisley, UK), 100 U/ml penicillin, 100 μg/ml streptomycin (Gibco, Paisley, UK), 2.5 μg/ml amphotericin B (Sigma–Aldrich, Poole, UK), and 50 μg/ml ascorbic acid (Sigma–Aldrich, Poole, UK) (complete media), at 37°C for 16 h. Cells were passed through a 70 μm cell strainer and washed twice in DMEM/F12 supplemented with 2 mM glutamine, 100 U/ml penicillin, 100 μg/ml, streptomycin, 2.5 μg/ml amphotericin B and 50 μg/ml ascorbic acid (serum free media). Cells were counted and the viability checked using trypan blue staining. Chondrocytes were cultured in monolayer in complete media in a humidified atmosphere of 5% CO_2 at 37°C and harvested at passage 2.

Monolayer culture

Chondrocytes harvested at passage 2 were seeded at a cell density of 1×10^6 cells/well in a six well plate and maintained in complete media in a humidified atmosphere of 5% CO_2 at 37°C for 24 h prior to treatment to allow cells to adhere to the cell culture plate.

Alginate bead culture

When cultured in monolayer, chondrocytes dedifferentiate into fibroblast like cells[28–30], therefore alginate beads were used to redifferentiate the chondrocytes to their native phenotype. Chondrocytes harvested at passage 2 were encapsulated in alginate beads at a cell density of 2×10^6 cells/ml, as previously described[31]. Alginate beads were cultured in complete media in a humidified atmosphere of 5% CO_2 at 37°C and the media changed every other day. Chondrocytes were redifferentiated in alginate beads for 4 weeks prior to treatment.

Cytotoxicity studies

The CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay (MTS) (Promega, Southampton, UK) was used to determine the effects of 10 μM WIN-55 on the cell viability of human chondrocytes according to the manufacturer's instructions.

Cannabinoid WIN-55 and IL-1β treatments

Prior to stimulation complete media was removed, cells washed with serum free media and serum free media containing 500 μg/ml bovine serum albumin (BSA) (Sigma–Aldrich, Poole, UK) added. Chondrocytes cultured in monolayer were unstimulated or stimulated with 10 ng/ml IL-1β (Peprotech, London, UK) with and without 1 μM, 2.5 μM, 5 μM, 7.5 μM and 10 μM WIN-55 (Sigma–Aldrich, Poole, UK) for 48 h at 37°C. Chondrocytes in monolayer were treated with 10 μM WIN-55 for 3, 6, 24 and 48 h at 37°C. Chondrocytes cultured in alginate beads were unstimulated or stimulated with 10 ng/ml IL-1β with and without 10 μM WIN-55. Dimethyl sulfoxide (DMSO) (Sigma–Aldrich, Poole, UK) was used as a vehicle control at 0.1% (equivalent to that present in 10 μM WIN-55).

MMP-3 and -13 Enzyme Linked Immunosorbent Assays (ELISAs)

ELISAs (R&D Systems, Abingdon, UK) were used according to the manufacturer's instructions to measure pro and active MMP-3 protein expression (ng/ml) and pro MMP-13 protein expression (pg/ml) in alginate bead conditioned culture media following treatment with 10 μM WIN-55 with and without 10 ng/ml IL-1β stimulation for 48 h.

RNA extraction from monolayers

For each of the three patient samples obtained from Outerbridge grade 0, 2 and 3 ($n = 9$), patient samples were tested in triplicate. Following treatments, media was removed, cells washed in 1xPBS and RNA extracted in 1 ml of TRIzol reagent (Invitrogen, Paisley, UK) following the manufacturer's instructions. RNA was resuspended in 14 μl sterile deionised water.

RNA extraction from alginate beads

For each of the three patient samples obtained from Outerbridge grade 0, 2 and 3 ($n = 9$), patient samples were tested in triplicate. RNA was extracted from alginate beads using two beads per extraction as described previously[31]. The resulting RNA was then resuspended in 100 μl of sterile deionised water and the RNA was purified using RNeasy clean up columns (Qiagen, Crawley, UK) according to the manufacturer's instructions and RNA eluted in 14 μl of sterile deionised water.

Reverse transcription-real-time polymerase chain reaction (qRT-PCR)

cDNA was formed as described previously[31]. Real-time PCR was used to investigate MMP-3, -13, TIMP-1, and -2 gene expression using Applied Biosystems Taqman Gene Expression Assays (Table I). The reactions were performed for 40 cycles using Taqman Fast Universal PCR Master Mix (Applied Biosystems, Paisley, UK) on an ABI StepOnePlus real-time PCR machine (Applied Biosystems, Paisley, UK). The data were collected and the fold changes in gene expression analysed using the $2^{-\Delta\Delta Ct}$ method[32]. Gene expression was normalised to housekeeping genes GAPDH and 18S together with untreated controls.

Cannabinoid receptor expression in OA chondrocytes

Chondrocytes isolated from four patient samples of grade 2–3 cartilage at passage 2 were plated at a density of 1×10^5 cells in eight well chamber slides and allowed to adhere overnight. Chondrocytes were fixed in 4% formalin and endogenous peroxidases were quenched. Following 0.01% chymotrypsin antigen retrieval at 37°C for 20 min cells were blocked in 25% v/v goat serum/BSA. Cells were incubated with rabbit polyclonal antibodies (Abcam) overnight at 4°C against CB1 (1/100), CB2 (1/50), PPARα (1/150) and PPARγ (1/50). A rabbit isotype IgG antibody (Abcam) was used as negative control. Following washing cells were incubated with biotinylated goat anti-rabbit secondary antibody (1/300: Abcam) and binding detected using streptavidin–biotinylated horseradish peroxide complex (Vector Laboratories, Peterborough, UK) with 3,3′-diaminobenzidine tetrahydrochloride substrate (Sigma–Aldrich). Cells were counterstained using Mayer's Haematoxylin (Leica Microsystems, Milton Keynes, UK) dehydrated, cleared and mounted in Pertex (Leica Microsystems). Immunopositivity was visualised and images captured using an Olympus BX60 microscope and QCapture Pro v8.0 software (MediaCybernetics, Marlow, UK).

Statistical analysis

Data was shown to be non-parametric via a Shapiro–Wilk test hence statistical significance between DMSO vehicle control and treatment groups was determined using the non-parametric Kruskall-Wallis multiple comparisons test and Conover-Inman *post hoc* test; $P < 0.05$ was considered statistically significant for real-time PCR and ELISA analysis. All statistical analysis was performed using StatsDirect. All data analysis was performed using individual replicates for each treatment group.

Results

Cytotoxicity studies

Cytotoxicity studies showed that WIN-55 was not toxic to chondrocytes at the concentrations used (Data not shown).

Table I
Taqman gene expression assay IDs

Taqman gene expression assay	Assay ID
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	Hs9999905_m1
18S	Hs99999901_s1
MMP-3	Hs00968305_m1
MMP-13	Hs00233992_m1
TIMP-1	Hs00171558_m1
TIMP-2	Hs00234278_m1

Time-course and concentration dependent effects of WIN-55 on MMP-3, MMP-13, TIMP-1 and TIMP-2 gene expression in monolayer

Chondrocytes were treated with WIN-55 (10 μM) for 3, 6, 24 and 48 h to determine the time point at which a maximum decrease in gene expression was observed. MMP-3, -13, TIMP-1 and -2 gene expression was decreased in a time-dependent manner, the largest decrease was observed at 48 h [Supplementary Fig. 1(A–D)]; therefore this incubation time was used for all subsequent treatments. Gene expression of MMP-3, -13, TIMP-1 and -2 was decreased in a WIN-55 concentration dependent manner both with and without IL-1β (10 ng/ml) stimulation [Supplementary Fig. 2(A–D)]. A significant decrease in gene expression was observed at concentrations ≥2.5 μM WIN-55 compared to DMSO (0.1%) vehicle controls [Supplementary Fig. 2(A–D)]. WIN-55 was used at 10 μM in all following treatments, as this was the maximal inhibitory non-toxic concentration.

Effects of DMSO in monolayer and alginate bead culture

No differences in gene or protein expression were observed between untreated cells compared to cells treated with 0.1% DMSO and 10 ng/ml IL-1β treatment in combination with 0.1% DMSO compared to IL-1β treatment alone (Data not shown). Therefore 10 ng/ml IL-1β in combination with 0.1% DMSO and 0.1% DMSO controls were used for all real-time PCR and ELISA analysis.

Effect of WIN-55 on IL-1β induced MMP-3 and MMP-13 gene expression in monolayer

In order to determine the effects of WIN-55 on MMPs in the presence of the inflammatory cytokine IL-1β, real-time PCR was used to measure the gene expression of MMP-3 and -13 in chondrocytes obtained from macroscopically graded OA cartilage. For this purpose chondrocytes were stimulated with IL-1β to induce MMP expression and co-treated with WIN-55. The effects of WIN-55 alone were also investigated. IL-1β stimulation significantly induced MMP-3 gene expression in chondrocytes isolated from grade 0 ($P < 0.0001$), 2 ($P < 0.0001$) and 3 ($P < 0.0001$) cartilage and MMP-13 in chondrocytes isolated from grade 0 ($P < 0.0001$), grade 2 ($P = 0.02$) and grade 3 cartilage ($P < 0.0001$) compared to DMSO vehicle control [Fig. 1(A–F)]. Treatment with WIN-55 in combination with IL-1β significantly reduced MMP-3 and -13 gene expression in chondrocytes derived from grade 0 ($P < 0.0001$), 2 ($P < 0.0001$) and 3 ($P < 0.0001$) cartilage compared to IL-1β stimulation alone [Fig. 1(A–F)]. WIN-55 treatment in combination with IL-1β also significantly reduced MMP-3 gene expression in chondrocytes isolated from grade 0 ($P < 0.0001$), 2 ($P < 0.0001$) and 3 ($P < 0.0001$) cartilage and MMP-13 gene expression in chondrocytes isolated from grade 0 ($P < 0.0001$), grade 2 ($P = 0.01$) and grade 3 ($P < 0.0001$) compared to DMSO vehicle control [Fig. 1(A–F)]. WIN-55 treatment alone significantly reduced MMP-3 gene expression in grade 0 ($P < 0.0001$), 2 ($P < 0.0001$) and 3 ($P < 0.0001$) cartilage derived chondrocytes below basal levels compared to DMSO vehicle control [Fig. 1(A–C)]. WIN-55 treatment alone also significantly decreased MMP-13 gene expression in grade 0 ($P = 0.002$) and 3 ($P < 0.0001$) cartilage derived chondrocytes compared to DMSO control [Figs. 1(D) and 1(F)]. MMP-13 was only expressed in two samples in chondrocytes isolated from grade 2 cartilage following WIN-55 treatment therefore statistical analysis could not be performed [Fig. 1(E)].

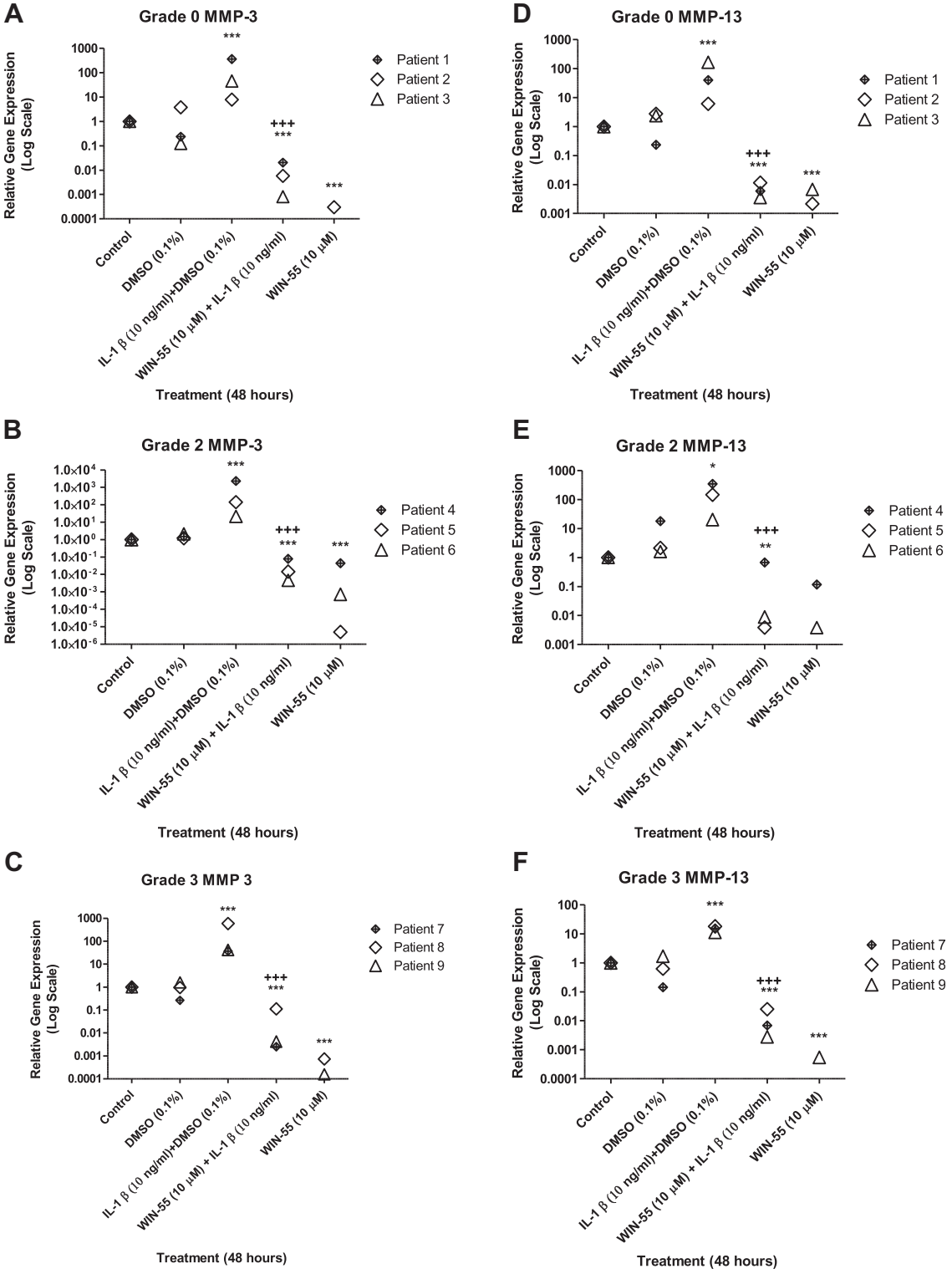

Fig. 1. **A**–**C**. The effect of WIN-55 on IL-1β induced MMP-3 gene expression in human OA chondrocytes isolated from grade 0, 2 and 3 cartilage and cultured in monolayer. **D**–**F** The effect of WIN-55 on IL-1β induced MMP-13 gene expression in human OA chondrocytes isolated from grade 0, 2 and 3 cartilage and cultured in monolayer. Each point represents the average of the repeats per patient. IL-1β (10 ng/ml) induced the gene expression of MMP-3 and -13. Following WIN-55 (10 μM) treatment in combination with IL-1β (10 ng/ml) MMP-3 and -13 gene expression was decreased. WIN-55 (10 μM) alone decreased MMP-3 and -13 gene expression. $^{***}P < 0.001$, $^{**}P < 0.01$ compared to DMSO (0.1 %) vehicle control and $^{+++}P < 0.001$ compared to IL-1β (10 ng/ml) treatment. $N = 9$ samples for each treatment group, obtained from three separate patient samples for each grade of tissue (0, 2 and 3) tested in triplicate.

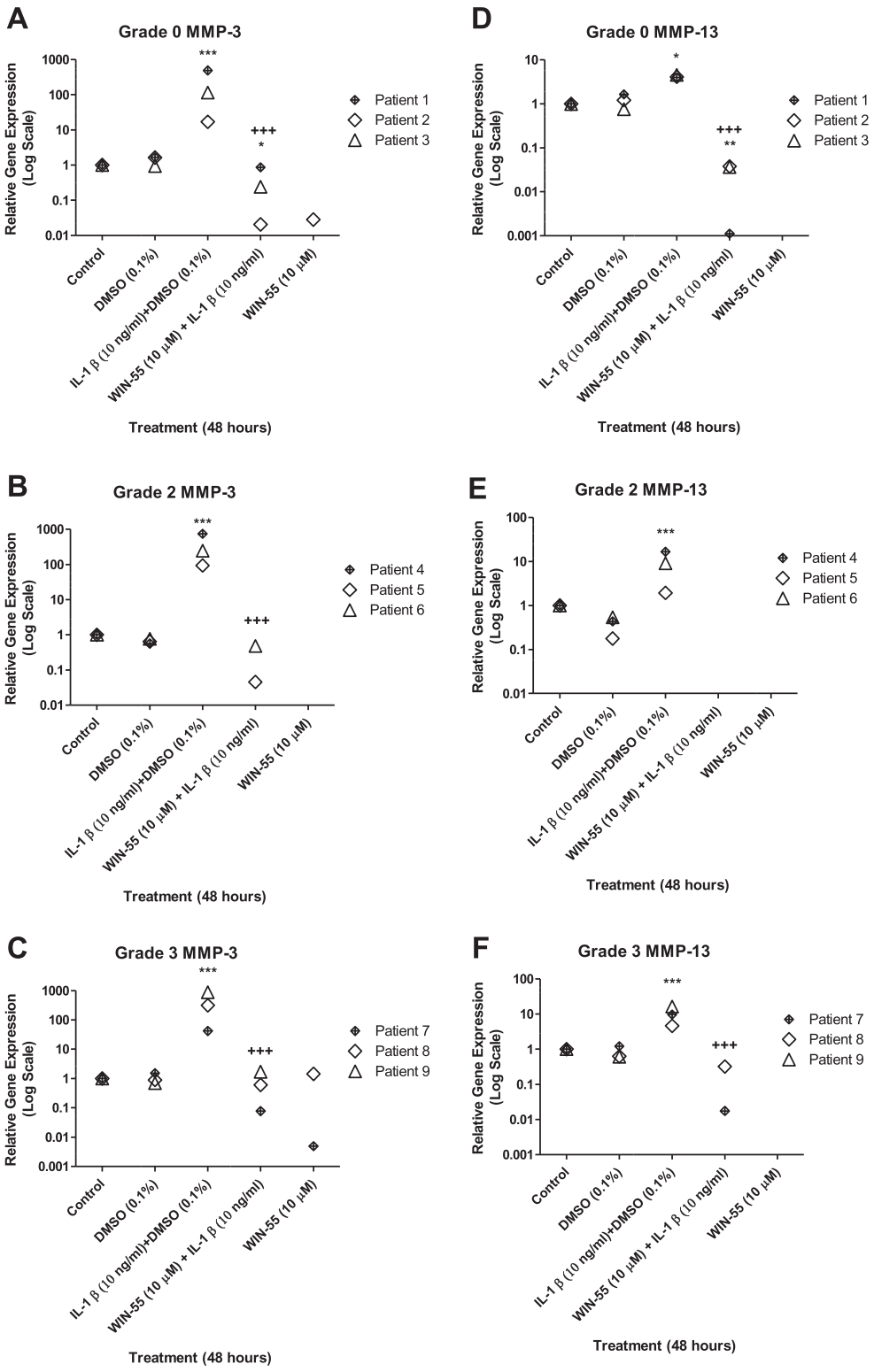

Fig. 2. **A**–**C**. The effect of WIN-55 on IL-1β induced MMP-3 gene expression in human chondrocytes isolated from grade 0, 2 and 3 cartilage and cultured in alginate beads. **D**–**F**. The effect of WIN-55 on IL-1β induced MMP-13 gene expression in human OA chondrocytes isolated from grade 0, 2 and 3 cartilage and cultured in alginate beads. Each point represents the average of the repeats per patient. IL-1β (10 ng/ml) induced the gene expression of MMP-3 and -13, following WIN-55 (10 μM) treatment in combination with IL-1β (10 ng/ml) MMP-3 and -13 gene expression was decreased or abolished. WIN-55 (10 μM) alone decreased or abolished MMP-3 and -13 gene expression. $^{*}P < 0.05$, $^{*}P < 0.01$, $^{***}P < 0.001$ compared to DMSO vehicle control and $^{+++}P < 0.001$ compared to IL-1β treatment. $N = 9$ samples for each treatment group, obtained from three separate patient samples for each grade of tissue (0, 2 and 3) tested in triplicate.

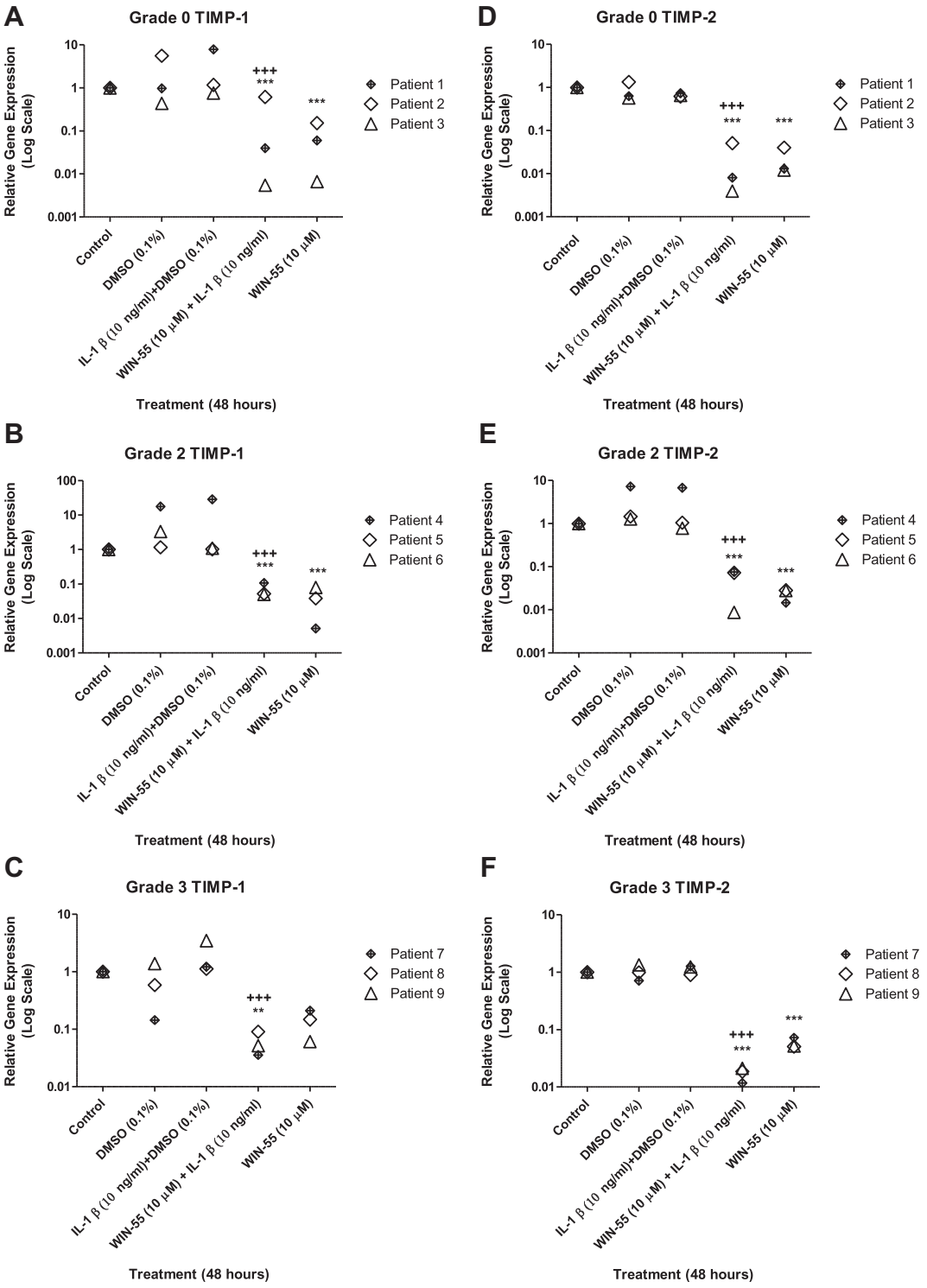

Fig. 3. **A–C** The effect of WIN-55 on TIMP-1 gene expression in human OA chondrocytes isolated from grade 0, 2 and 3 cartilage and cultured in monolayer. D–F. The effect of WIN-55 on TIMP-2 gene expression in human OA chondrocytes isolated from grade 0, 2 and 3 cartilages and cultured in monolayer. Each point represents the average of the repeats per patient. IL-1β (10 ng/ml) had no effect on TIMP-1 and -2 gene expression. WIN-55 treatment (10 μM) both alone and in combination with IL-1β (10 ng/ml) reduced TIMP-1 and -2 gene expression. $^{***}P < 0.001$ compared to DMSO vehicle control and $^{+++}P < 0.001$ compared to IL-1β treatment. $N = 9$ samples for each treatment group, obtained from 3 separate patient samples for each grade of tissue (0, 2 and 3) tested in triplicate.

Effects of WIN-55 on IL-1β induced MMP-3 and MMP-13 gene expression on chondrocytes cultured in alginate beads

The effects of WIN-55 on IL-1β induced MMP-3 and -13 gene expression were determined in chondrocytes cultured in alginate beads in order to determine gene expression in chondrocytes following redifferentiation back to their native phenotype. IL-1β stimulation significantly induced MMP-3 gene expression in chondrocytes from grade 0 ($P < 0.0001$), 2 ($P < 0.0001$) and 3 ($P < 0.0001$) cartilage and MMP-13 gene expression in grade

0 ($P = 0.018$), 2 ($P < 0.0001$) and 3 ($P < 0.0001$) cartilage derived chondrocytes compared to DMSO vehicle control [Fig. 2(A–F)]. Similarly WIN-55 treatment in combination with IL-1β significantly reduced MMP-3 gene expression in grade 0 ($P < 0.0001$), 2 ($P < 0.0001$) and 3 ($P < 0.0001$) cartilage derived chondrocytes and MMP-13 gene expression in grade 0 ($P < 0.0001$) and 3 ($P < 0.0001$) cartilage derived chondrocytes compared to IL-1β stimulation alone [Fig. 2(A–D) and (F)]. MMP-13 was not expressed in chondrocytes from grade 2 cartilage treated with IL-1β in combination with WIN-55 [Fig. 2(E)]. WIN-55 treatment in combination with IL-1β significantly reduced MMP-3 ($P = 0.03$) and -13 ($P = 0.003$) gene expression in chondrocytes from grade 0 cartilage compared to DMSO vehicle control [Fig. 2(A) and (D)]. WIN-55 treatment in combination with IL-1β reduced MMP-3 gene expression in

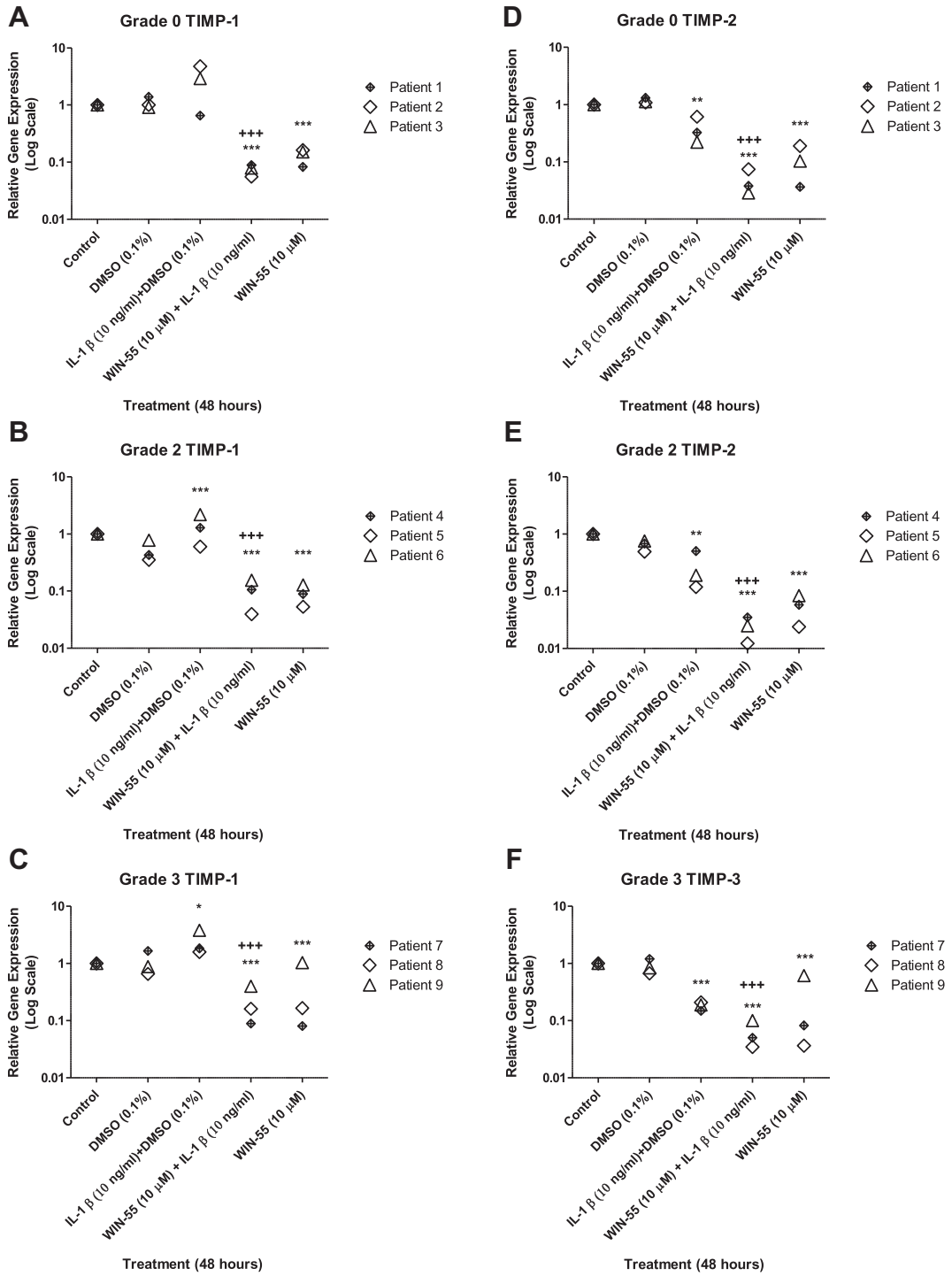

Fig. 4. **A–C**. The effect of WIN-55 on TIMP-1 gene expression in human OA chondrocytes isolated from grade 0, 2 and 3 cartilages and cultured in alginate beads. **D–F**. The effect of WIN-55 on TIMP-2 gene expression in OA human chondrocytes isolated from grade 0, 2 and 3 cartilages and cultured in alginate beads. Each point represents the average of the repeats per patient. IL-1β (10 ng/ml) stimulation increased TIMP-1 gene expression and decreased TIMP-2 gene expression below basal levels. WIN-55 treatment (10 μM) both alone and in combination with IL-1β (10 ng/ml) reduced TIMP-1 and -2 gene expression. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared to DMSO vehicle control and $^{+++}P < 0.001$ compared to IL-1β treatment. $N = 9$ samples for each treatment group, obtained from 3 separate patient samples for each grade of tissue (0, 2 and 3) tested in triplicate.

chondrocytes from grade 2 ($P = 0.11$) cartilage and both MMP-3 ($P = 0.58$) and -13 ($P = 0.11$) gene expression in chondrocytes from grade 3 cartilage compared to DMSO vehicle control; however this was not significant [Fig. 2(B), (C) and (F)]. There was no significant difference from basal levels of MMP-3 gene expression in grade 0 ($P = 0.052$) and 3 ($P = 0.16$) cartilage derived chondrocytes when treated with WIN-55 alone [Fig. 2(A and C)]. MMP-3 was not expressed in grade 2 cartilage chondrocytes treated with WIN-55 alone [Fig. 2(B)]. MMP-13 was abolished in chondrocytes from grade 2 and 3 cartilage treated with WIN-55 alone [Fig. 2(D-F)].

Effects of WIN-55 on IL-1β induced TIMP-1 and TIMP-2 gene expression in monolayer

In order to determine the effects of WIN-55 on the inhibitors of MMP-3 and -13 the gene expression of TIMP-1 and -2 was investigated following WIN-55 treatment both alone and in combination with IL-1β. IL-1β stimulation had no significant effect on TIMP-1 gene expression in chondrocytes derived from grade 0 ($P = 0.75$), 2 ($P = 0.60$) and 3 ($P = 0.12$) cartilage or TIMP-2 gene expression in chondrocytes isolated from grade 0 ($P = 0.77$), 2 ($P = 0.49$) and 3 ($P = 0.98$) cartilage [Fig. 3(A–F)]. However, WIN-55 treatment in combination with IL-1β resulted in a significant decrease in TIMP-1 gene expression compared to DMSO vehicle control and IL-1β stimulated chondrocytes derived from grade 0 ($P < 0.0001$), 2 ($P < 0.0001$) and 3 ($P = 0.001$) cartilage [Fig. 3(A–C)]. WIN-55 alone also significantly reduced TIMP-1 gene expression in chondrocytes derived from grade 0 ($P < 0.0001$) and 2 ($P < 0.0001$) cartilage below basal levels [Fig. 3(A) and (B)]. TIMP-1 gene expression was decreased in chondrocytes derived from grade 3 cartilage following WIN-55 treatment however this was not significant ($P = 0.11$) [Fig. 3(C)]. TIMP-2 gene expression was significantly reduced following WIN-55 treatment in combination with IL-1β compared to DMSO vehicle control and IL-1β stimulation in chondrocytes derived from grade 0 ($P < 0.0001$), 2 ($P < 0.0001$) and 3 ($P < 0.0001$) cartilage [Fig. 3(D–F)]. WIN-55 alone significantly reduced the gene expression of TIMP-2 below basal levels in chondrocytes isolated from grade 0 ($P < 0.0001$), 2 ($P < 0.0001$) and 3 ($P < 0.0001$) cartilage [Fig. 3(D–F)].

Effects of WIN-55 on IL-1β induced TIMP-1 and TIMP-2 gene expression on chondrocytes cultured in alginate beads

TIMP-1 gene expression was significantly increased in grade 2 ($P < 0.0009$) and 3 cartilage derived chondrocytes ($P = 0.02$) following IL-1β stimulation [Fig. 4(B) and (C)] but not in grade 0 ($P = 0.24$) cartilage chondrocytes compared to DMSO vehicle control [Fig. 4(A)]. In contrast TIMP-2 gene expression was significantly decreased following IL-1β treatment in chondrocytes extracted from grade 0 ($P = 0.009$), 2 ($P = 0.001$) and 3 ($P < 0.0001$) cartilage compared to DMSO vehicle control [Fig. 4(D–F)]. WIN-55 treatment in combination with IL-1β resulted in a significant decrease in TIMP-1 gene expression in chondrocytes derived from grades 0 ($P < 0.0001$), 2 ($P < 0.0001$), and 3 ($P < 0.0001$), cartilage and TIMP-2 gene expression in chondrocytes isolated from grades 0 ($P < 0.0001$), 2 ($P < 0.0001$), and 3 ($P = 0.0007$), cartilage compared to IL-1β stimulation [Fig. 4(A–F)]. WIN-55 treatment in combination with IL-1β also resulted in a significant decrease in TIMP-1 gene expression in chondrocytes derived from grades 0 ($P < 0.0001$), 2 ($P < 0.0001$), and 3 ($P < 0.0001$), cartilage and TIMP-2 gene expression in chondrocytes isolated from grades 0 ($P < 0.0001$), 2 ($P < 0.0001$), and 3 ($P < 0.0001$) cartilage compared to DMSO control [Fig. 4(A–F)]. WIN-55 treatment alone significantly reduced both TIMP-1 gene expression in chondrocytes derived from grade 0 ($P = 0.0005$), 2 ($P < 0.0001$) and 3 ($P = 0.0003$) cartilage below basal levels [Fig. 4(A–C)]. WIN-55 treatment alone significantly reduced both TIMP-2 gene expression in chondrocytes derived from grade 0 ($P < 0.0001$), 2 ($P < 0.0001$) and 3 ($P < 0.0001$) cartilage below basal levels [Fig. 4(D–F)].

The effects of WIN-55 on MMP-3 and -13 protein expression

In order to determine whether WIN-55 also inhibited MMP-3 and -13 expression at the protein level, pro and active MMP-3 and pro MMP-13 were measured in culture media using ELISA. Culture media obtained from chondrocytes isolated from grade 3 cartilage which had been cultured in alginate beads were used, as these demonstrated clear gene expression effects. Following stimulation of chondrocytes with IL-1β there was a significant increase in MMP-3 ($P < 0.0001$) and -13 ($P = 0.046$) protein release into the media (Table II). Treatment of chondrocytes with WIN-55 in combination with IL-1β significantly reduced both MMP-3 ($P = 0.0007$) and -13 ($P = 0.0005$) protein compared to IL-1β treatment alone (Table II). WIN-55 treatment alone significantly reduced MMP-3 protein release to below basal levels ($P = 0.04$) and MMP-13 ($P = 0.38$) protein levels remained at basal level (Table II).

Cannabinoid receptor expression in OA chondrocytes

To identify the expression of cannabinoid receptors CB1, CB2, PPARα and PPARγ in chondrocytes cultured in monolayer immunocytochemistry was used. Here we observed the expression of CB1, CB2, PPARα and γ [Fig. 5]. PPARα and γ expression was localised to the cytoplasm and nucleus [Fig. 5].

Discussion

Cartilage degradation is a pathological feature of both OA and RA[1]. The inflammatory cytokine IL-1 plays a key role in cartilage destruction and stimulates increased production of MMPs by chondrocytes, resulting in the breakdown of collagen and proteoglycan[33]. Here, we have shown that treatment of articular chondrocytes from human OA cartilage with cannabinoid WIN-55 reduces the gene and protein expression of MMP-3 and MMP-13 in the presence of IL-1β, suggesting that cannabinoids may have potential in terms of arthritis therapy. In addition we have shown that WIN-55 significantly reduces the gene expression of TIMP-1 and TIMP-2 to below basal levels.

In this study we have used both monolayer and a 3D culture system for culture of chondrocytes. Chondrocytes that have been isolated from articular cartilage dedifferentiate in monolayer culture changing their matrix synthesis, with a decrease in type II collagen and aggrecan, developing a fibroblast like phenotype and an increase in collagen type I[28–30]. Dedifferentiation can be

Table II
The effect of WIN-55 on pro and active MMP-3 and pro-MMP-13 protein release into culture media from grade 3 OA chondrocytes cultured in alginate beads

Treatment (48 h)	MMP-3 (ng/alginate bead)	MMP-13 (pg/alginate bead)
DMSO (0.1%)	1.4 (0.0659)	327.5 (1.6407)
IL-1β (10 ng/ml) + DMSO (0.1%)	38.4*** (0.5092)	1,092.0* (16.8345)
WIN-55 (10 μM) + IL-1β (10 ng/ml)	1.4+++ (0.0178)	219.4+++ (1.2038)
WIN-55 (10 μM)	0.2* (0.0051)	270.7 (3.8866)

Results are expressed as mean and 95% confidence interval; *$P < 0.05$, ***$P < 0.001$,compared to DMSO vehicle control and +++$P < 0.001$ compared to IL-1β treatment alone.

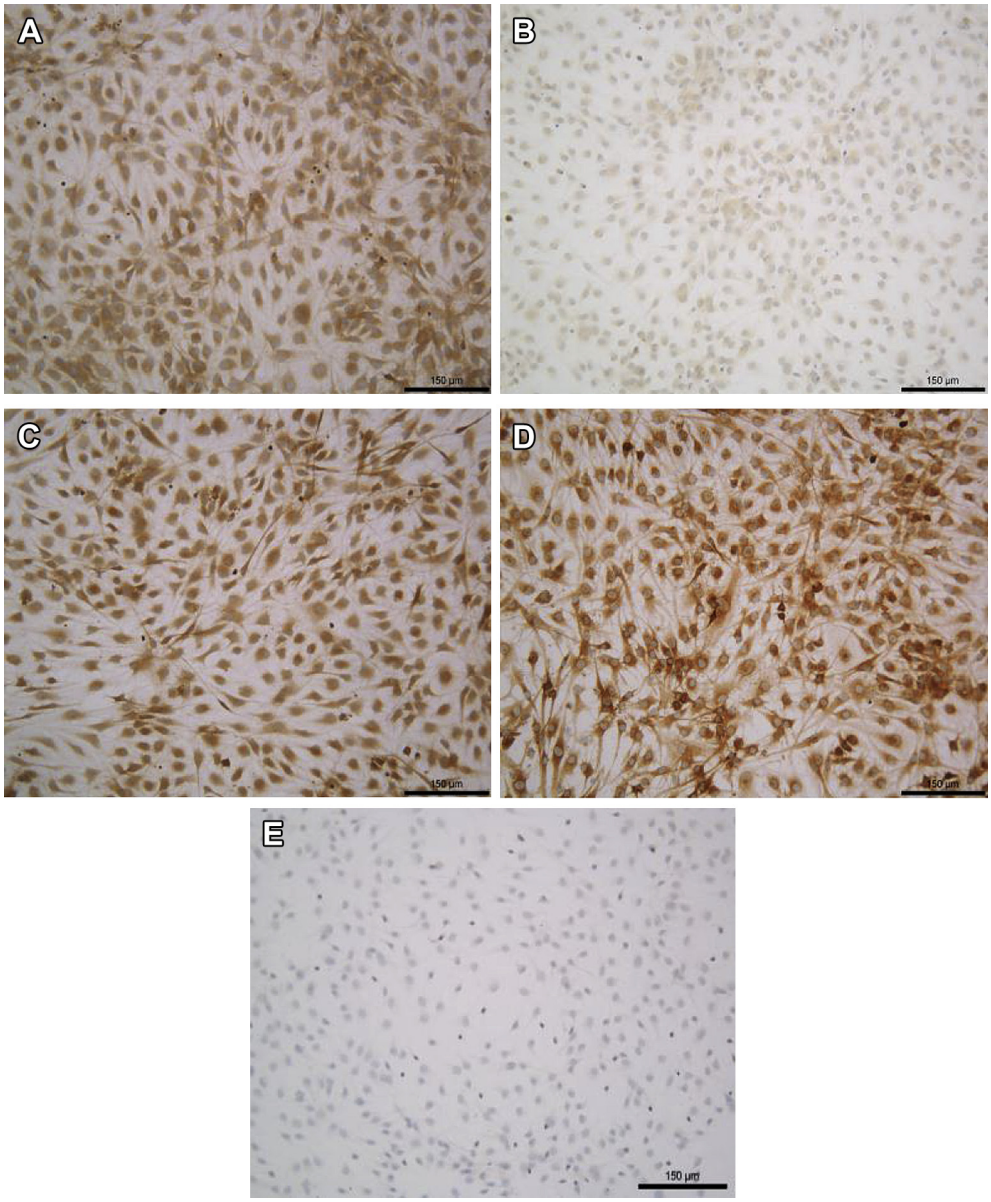

Fig. 5. Cannabinoid receptor expression in OA chondrocytes cultured in monolayer. Representative photomicrographs of immunocytochemistry of (A) CB1, (B) CB2, (C) PPARα, (D) PPARγ, (E) IgG control (10 µg) ($n = 4$ cultures).

reversed with the key phenotypic features of chondrocytes being preserved when cultured in a 3D system such as alginate beads[34]. In this study, chondrocytes were treated with IL-1β to mimic inflammatory processes in an *in vitro* model of OA[35].

Synthetic cannabinoids WIN-55 and HU-210 reduce IL-1α induced proteoglycan and collagen degradation in bovine nasal cartilage tissue suggesting a chondroprotective effect of these compounds[9]. Here, we have shown a possible mechanism by which WIN-55 may prevent IL-1β induced ECM breakdown in OA cartilage tissue via preventing the expression of MMPs. We have demonstrated that chondrocytes from different grades of OA cartilage modulate MMP-3 and MMP-13 expression in response to WIN-55 with and without IL-1β stimulation. These findings together with others, suggest that cannabinoids may be of importance in the treatment of arthritis[3–5,7,8,36]. Selvi *et al.* 2008 demonstrated that WIN-55 and CP55,940 inhibit IL-1β induced secretion of IL-6 and IL-8 in RA fibroblast like synovial cells, suggesting an anti-inflammatory activity of cannabinoids[7]. In another study non-psychoactive cannabinoid ajulemic acid (AJA) reduced MMP-1, MMP-3 and MMP-9 release from fibroblast like synovial cells stimulated with IL-1α and TNFα[8]. *In vivo*, AJA has also been shown to reduce the severity of adjuvant-induced arthritis[4] and other non-psychoactive cannabinoids, cannabidiol (CBD) and HU-320 reduced inflammation and joint damage in murine collagen-induced arthritis[3,5].

The effects of WIN-55 on articular chondrocytes did not appear to be influenced by the grade of the cartilage they were isolated from when cultured in monolayer. Chondrocytes cultured in monolayer express MMP-3 and MMP-13 at very low levels following WIN-55 treatment. However when comparing the effects of WIN-55 in monolayer to alginate bead culture, a greater inhibitory effect on MMP-3 and MMP-13 gene expression was seen in alginate bead cultured chondrocytes. Interestingly a biphasic expression pattern of MMP-3 and MMP-13 in response to WIN-55 was observed. MMP-3 was expressed in grade 0 and grade 3 cartilage derived chondrocytes and abolished in grade 2 cartilage

chondrocytes and MMP-13 was abolished in chondrocytes from all grades of cartilage when cultured in alginate beads. These varying responses to WIN-55 treatment in alginate bead culture may indicate that the expression of MMPs may be differentially regulated depending on the grade and extent of cartilage degradation and the culture method utilised. Studies have shown that cartilage tissue derived from different OA grades or normal aged cartilage may influence the response of the chondrocytes to different treatments[37–42]. Interestingly biphasic effects have also been seen with other cannabinoids namely AJA[43].

During OA there is thought to be an imbalance between MMP and TIMP expression which in part contributes to cartilage breakdown[21]. We have shown that WIN-55 inhibits expression of both destructive MMPs and protective TIMPs involved in the pathogenesis of OA, indicating that inhibition may occur via a signalling pathway, which regulates both at the transcription level. Human MMPs and TIMPs share a common activating protein 1 (AP-1) site in their promoters which regulates their transcription[44]. WIN-55 may have a differential effect on AP-1 activation via peroxisome proliferator activated receptors (PPARs). WIN-55 has been shown to activate AP-1 via PPARα. In addition AP-1 may be involved in the activation of interferon β (IFNβ)[45]. Production of IFNβ may result in reduced levels of MMPs and TIMPs. IFNβ reduced MMP-1, -3 and TIMP-1 in fibroblast-like synovial cells both with and without IL-1β stimulation and synovial tissue from patients with RA, treated with IFNβ, showed reduced levels of MMP-1 and TIMP-1[46]. Furthermore IFNβ has been shown to have anti-inflammatory properties in the treatment of arthritis[47–49]. Conversely PPARγ agonists have been shown to reduce IL-1β induced MMP-1 expression in human synovial fibroblasts via inhibiting DNA binding of AP-1[50]. WIN-55 also binds to PPARγ so could also act in this way[51]. The biological activities of cannabinoids and the signal transduction pathways they induce or inhibit need to be further investigated in OA. Since TIMP-1 and -2 are decreased by WIN-55 in human OA chondrocytes it is unclear whether there is a change in MMP and TIMP balance following cannabinoid treatment. However the inhibitory effect of WIN-55 on MMP-3 and -13 expression would indicate a possible role of cannabinoids in suppressing IL-1β induced ECM degradation by MMPs.

We have observed the expression of both classical cannabinoid receptors CB1 and CB2 in human chondrocytes from OA cartilage at passage 2. CB1 and CB2 have previously been shown to be expressed at similar levels both at the protein and RNA level in synovia of patients with OA and RA and their expression is thought to play a role in the pathology of joint disease[52]. PPARα and γ were also expressed in OA chondrocytes at passage 2 and their expression appears to be both cytoplasmic and nuclear. PPARs are nuclear receptors however studies have also shown that the localisation of PPARα receptors in chondrocytes is also cytoplasmic[53]. WIN-55 activates CB1 and CB2 with Kis of 1.89–123 nM and 0.28–16.2 nM respectively[24]. In addition, WIN-55 has also been shown to activate PPARα and γ[25,26]. Targeting PPARs using both specific and cannabinoid agonists for the treatment of OA and RA has been reported previously[8,26,36,50,54–56]. The observed effects of WIN-55 on MMP and TIMP expression may be mediated by one or more of these receptors or by receptors as yet unknown.

In conclusion, in human OA chondrocytes, the synthetic cannabinoid WIN-55 inhibits the expression of matrix degrading enzymes MMP-3 and -13 and their inhibitors TIMP-1 and -2 in the presence or absence of IL-1β. This suggests a possible mechanism by which cannabinoids may act to prevent ECM breakdown in arthritis. Cannabinoids could provide a dual role in the treatment of arthritis as disease modifying agents in addition to having anti-inflammatory properties[10]. Although there is increasing evidence to suggest that cannabinoids may be of therapeutic value in the treatment of arthritis, further studies into the receptors and signalling pathways involved in the actions of cannabinoids need to be investigated in order to elucidate their effects on catabolic and anabolic mediators in both normal and arthritic cartilage.

Authors contributions

SD participated in its design, performed all the laboratory work and analysis and co-wrote the manuscript. JMW helped to conceive the study, secure funding, contributed to its design and co-ordination, participated in interpretation of data and co-wrote the manuscript. AC helped to conceive the study, secure funding, contributed to its design and co-ordination and co-wrote the manuscript. CLM helped to conceive the study, secure funding, contributed to its design and co-ordination, participated in interpretation of data and co-wrote the manuscript. RADB helped to conceive the study, secure funding, contributed to its design and co-ordination, participated in interpretation of data and co-wrote the manuscript. All authors read and approved the final manuscript.

Competing interest

None declared.

Acknowledgements

This work was funded by a Ph.D. Studentship from the Biomedical Research Centre, Faculty of Health and Well Being, Sheffield Hallam University, Sheffield, UK. Tissue samples were supplied by the Sheffield Biorepository.

Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.joca.2013.10.016.

References

1. Goldring MB, Marcu KB. Cartilage homeostasis in health and rheumatic diseases. Arthritis Res Ther 2009;11:224, http://dx.doi.org/10.1186/ar2592.
2. Blake DR, Robson P, Ho M, Jubb RW, McCabe CS. Preliminary assessment of the efficacy, tolerability and safety of a cannabis-based medicine (Sativex) in the treatment of pain caused by rheumatoid arthritis. Rheumatology (Oxford) 2006;45:50–2, http://dx.doi.org/10.1093/rheumatology/kei183.
3. Sumariwalla PF, Gallily R, Tchilibon S, Fride E, Mechoulam R, Feldmann M. A novel synthetic, nonpsychoactive cannabinoid acid (HU-320) with antiinflammatory properties in murine collagen-induced arthritis. Arthritis Rheum 2004;50:985–98, http://dx.doi.org/10.1002/art.20050.
4. Zurier RB, Rossetti RG, Lane JH, Goldberg JM, Hunter SA, Burstein SH. Dimethylheptyl-THC-11 oic acid: a nonpsychoactive antiinflammatory agent with a cannabinoid template structure. Arthritis Rheum 1998;41:163–70, http://dx.doi.org/10.1002/1529-0131(199801)41:1<163::AID-ART20>3.0.CO;2-9.
5. Malfait AM, Gallily R, Sumariwalla PF, Malik AS, Andreakos E, Mechoulam R, *et al*. The nonpsychoactive cannabis constituent cannabidiol is an oral anti-arthritic therapeutic in murine collagen-induced arthritis. Proc Natl Acad Sci U S A 2000;97:9561–6, http://dx.doi.org/10.1073/pnas.160105897.
6. Zurier RB, Rossetti RG, Burstein SH, Bidinger B. Suppression of human monocyte interleukin-1beta production by ajulemic acid, a nonpsychoactive cannabinoid. Biochem Pharmacol 2003;65:649–55.

7. Selvi E, Lorenzini S, Garcia-Gonzalez E, Maggio R, Lazzerini PE, Capecchi PL, *et al*. Inhibitory effect of synthetic cannabinoids on cytokine production in rheumatoid fibroblast-like synoviocytes. Clin Exp Rheumatol 2008;26:574–81.
8. Johnson DR, Stebulis JA, Rossetti RG, Burstein SH, Zurier RB. Suppression of fibroblast metalloproteinases by ajulemic acid, a nonpsychoactive cannabinoid acid. J Cell Biochem 2007;100:184–90, http://dx.doi.org/10.1002/jcb.21046.
9. Mbvundula EC, Bunning RA, Rainsford KD. Arthritis and cannabinoids: HU-210 and Win-55,212-2 prevent IL-1alpha-induced matrix degradation in bovine articular chondrocytes in-vitro. J Pharm Pharmacol 2006;58:351–8, http://dx.doi.org/10.1211/jpp.58.3.0009.
10. Dunn SL, Wilkinson JM, Crawford A, Le Maitre CL, Bunning RA. Cannabinoids: novel therapies for arthritis? Future Med Chem 2012;4:713–25, http://dx.doi.org/10.4155/fmc.12.20.
11. Goldring MB, Otero M. Inflammation in osteoarthritis. Curr Opin Rheumatol 2011;23:471–8, http://dx.doi.org/10.1097/BOR.0b013e328349c2b1.
12. Davidson RK, Waters JG, Kevorkian L, Darrah C, Cooper A, Donell ST, *et al*. Expression profiling of metalloproteinases and their inhibitors in synovium and cartilage. Arthritis Res Ther 2006;8:R124, http://dx.doi.org/10.1186/ar2013.
13. Bau B, Gebhard PM, Haag J, Knorr T, Bartnik E, Aigner T. Relative messenger RNA expression profiling of collagenases and aggrecanases in human articular chondrocytes in vivo and in vitro. Arthritis Rheum 2002;46:2648–57, http://dx.doi.org/10.1002/art.10531.
14. Hembry RM, Bagga MR, Reynolds JJ, Hamblen DL. Immunolocalisation studies on six matrix metalloproteinases and their inhibitors, TIMP-1 and TIMP-2, in synovia from patients with osteo- and rheumatoid arthritis. Ann Rheum Dis 1995;54:25–32.
15. Okada Y, Shinmei M, Tanaka O, Naka K, Kimura A, Nakanishi I, *et al*. Localization of matrix metalloproteinase 3 (stromelysin) in osteoarthritic cartilage and synovium. Lab Invest 1992;66:680–90.
16. Wolfe GC, MacNaul KL, Buechel FF, McDonnell J, Hoerrner LA, Lark MW, *et al*. Differential in vivo expression of collagenase messenger RNA in synovium and cartilage. Quantitative comparison with stromelysin messenger RNA levels in human rheumatoid arthritis and osteoarthritis patients and in two animal models of acute inflammatory arthritis. Arthritis Rheum 1993;36:1540–7.
17. Chubinskaya S, Kuettner KE, Cole AA. Expression of matrix metalloproteinases in normal and damaged articular cartilage from human knee and ankle joints. Lab Invest 1999;79:1669–77.
18. Yoshihara Y, Nakamura H, Obata K, Yamada H, Hayakawa T, Fujikawa K, *et al*. Matrix metalloproteinases and tissue inhibitors of metalloproteinases in synovial fluids from patients with rheumatoid arthritis or osteoarthritis. Ann Rheum Dis 2000;59:455–61.
19. Koshy PJ, Lundy CJ, Rowan AD, Porter S, Edwards DR, Hogan A, *et al*. The modulation of matrix metalloproteinase and ADAM gene expression in human chondrocytes by interleukin-1 and oncostatin M: a time-course study using real-time quantitative reverse transcription-polymerase chain reaction. Arthritis Rheum 2002;46:961–7.
20. Tetlow LC, Adlam DJ, Woolley DE. Matrix metalloproteinase and proinflammatory cytokine production by chondrocytes of human osteoarthritic cartilage: associations with degenerative changes. Arthritis Rheum 2001;44:585–94, http://dx.doi.org/10.1002/1529-0131(200103)44:3<585::AID-ANR107>3.0.CO;2-C.
21. Dean DD, Martel-Pelletier J, Pelletier JP, Howell DS, Woessner Jr JF. Evidence for metalloproteinase and metalloproteinase inhibitor imbalance in human osteoarthritic cartilage. J Clin Invest 1989;84:678–85, http://dx.doi.org/10.1172/JCI114215.
22. Martel-Pelletier J, McCollum R, Fujimoto N, Obata K, Cloutier JM, Pelletier JP. Excess of metalloproteases over tissue inhibitor of metalloprotease may contribute to cartilage degradation in osteoarthritis and rheumatoid arthritis. Lab Invest 1994;70:807–15.
23. Murphy G, Nagase H. Reappraising metalloproteinases in rheumatoid arthritis and osteoarthritis: destruction or repair? Nat Clin Pract Rheumatol 2008;4:128–35, http://dx.doi.org/10.1038/ncprheum0727.
24. Pertwee RG, Howlett AC, Abood ME, Alexander SP, Di Marzo V, Elphick MR, *et al*. International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid receptors and their ligands: beyond CB and CB. Pharmacol Rev 2010;62:588–631, http://dx.doi.org/10.1124/pr.110.003004.
25. Sun Y, Alexander SP, Kendall DA, Bennett AJ. Cannabinoids and PPARalpha signalling. Biochem Soc Trans 2006;34:1095–7, http://dx.doi.org/10.1042/BST0341095.
26. O'Sullivan SE, Kendall DA. Cannabinoid activation of peroxisome proliferator-activated receptors: potential for modulation of inflammatory disease. Immunobiology 2010;215:611–6, http://dx.doi.org/10.1016/j.imbio.2009.09.007.
27. Cameron ML, Briggs KK, Steadman JR. Reproducibility and reliability of the outerbridge classification for grading chondral lesions of the knee arthroscopically. Am.J.Sports Med 2003;31:83–6.
28. Mayne R, Vail MS, Mayne PM, Miller EJ. Changes in type of collagen synthesized as clones of chick chondrocytes grow and eventually lose division capacity. Proc Natl Acad Sci U S A 1976;73:1674–8.
29. von der Mark K, Gauss V, von der Mark H, Muller P. Relationship between cell shape and type of collagen synthesised as chondrocytes lose their cartilage phenotype in culture. Nature 1977;267:531–2.
30. Benya PD, Padilla SR, Nimni ME. Independent regulation of collagen types by chondrocytes during the loss of differentiated function in culture. Cell 1978;15:1313–21.
31. Le Maitre CL, Freemont AJ, Hoyland JA. The role of interleukin-1 in the pathogenesis of human intervertebral disc degeneration. Arthritis Res Ther 2005;7:R732–45, http://dx.doi.org/10.1186/ar1732.
32. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 2001;25:402–8, http://dx.doi.org/10.1006/meth.2001.1262.
33. Burrage PS, Mix KS, Brinckerhoff CE. Matrix metalloproteinases: role in arthritis. Front Biosci 2006;11:529–43.
34. Caron MM, Emans PJ, Coolsen MM, Voss L, Surtel DA, Cremers A, *et al*. Redifferentiation of dedifferentiated human articular chondrocytes: comparison of 2D and 3D cultures. Osteoarthritis Cartilage 2012;20:1170–8, http://dx.doi.org/10.1016/j.joca.2012.06.016.
35. Goldring MB. The role of the chondrocyte in osteoarthritis. Arthritis Rheum 2000;43:1916–26, http://dx.doi.org/10.1002/1529-0131(200009)43:9<1916::AID-ANR2>3.0.CO;2-I.
36. Clockaerts S, Bastiaansen-Jenniskens YM, Feijt C, Verhaar JA, Somville J, De Clerck LS, *et al*. Peroxisome proliferator activated receptor alpha activation decreases inflammatory and destructive responses in osteoarthritic cartilage. Osteoarthritis Cartilage 2011;19:895–902, http://dx.doi.org/10.1016/j.joca.2011.03.010.

37. Freemont AJ, Hampson V, Tilman R, Goupille P, Taiwo Y, Hoyland JA. Gene expression of matrix metalloproteinases 1, 3, and 9 by chondrocytes in osteoarthritic human knee articular cartilage is zone and grade specific. Ann Rheum Dis 1997;56:542–9.
38. Vincenti MP. The matrix metalloproteinase (MMP) and tissue inhibitor of metalloproteinase (TIMP) genes. Transcriptional and posttranscriptional regulation, signal transduction and cell-type-specific expression. Methods Mol Biol 2001;151:121–48.
39. Page CE, Smale S, Carty SM, Amos N, Lauder SN, Goodfellow RM, *et al*. Interferon-gamma inhibits interleukin-1beta-induced matrix metalloproteinase production by synovial fibroblasts and protects articular cartilage in early arthritis. Arthritis Res Ther 2010;12:R49, http://dx.doi.org/10.1186/ar2960.
40. Dozin B, Malpeli M, Camardella L, Cancedda R, Pietrangelo A. Response of young, aged and osteoarthritic human articular chondrocytes to inflammatory cytokines: molecular and cellular aspects. Matrix Biol 2002;21:449–59.
41. Hickery MS, Bayliss MT, Dudhia J, Lewthwaite JC, Edwards JC, Pitsillides AA. Age-related changes in the response of human articular cartilage to IL-1alpha and transforming growth factor-beta (TGF-beta): chondrocytes exhibit a diminished sensitivity to TGF-beta. J Biol Chem 2003;278:53063–71, http://dx.doi.org/10.1074/jbc.M209632200.
42. Fan Z, Bau B, Yang H, Soeder S, Aigner T. Freshly isolated osteoarthritic chondrocytes are catabolically more active than normal chondrocytes, but less responsive to catabolic stimulation with interleukin-1beta. Arthritis Rheum 2005;52:136–43, http://dx.doi.org/10.1002/art.20725.
43. Burstein S. PPAR-gamma: a nuclear receptor with affinity for cannabinoids. Life Sci 2005;77:1674–84, http://dx.doi.org/10.1016/j.lfs.2005.05.039.
44. Borden P, Heller RA. Transcriptional control of matrix metalloproteinases and the tissue inhibitors of matrix metalloproteinases. Crit Rev Eukaryot Gene Expr 1997;7:159–78.
45. Downer EJ, Clifford E, Amu S, Fallon PG, Moynagh PN. The synthetic cannabinoid R(+)WIN55,212-2 augments interferon-beta expression via peroxisome proliferator-activated receptor-alpha. J Biol Chem 2012;287:25440–53, http://dx.doi.org/10.1074/jbc.M112.371757.
46. Smeets TJ, Dayer JM, Kraan MC, Versendaal J, Chicheportiche R, Breedveld FC, *et al*. The effects of interferon-beta treatment of synovial inflammation and expression of metalloproteinases in patients with rheumatoid arthritis. Arthritis Rheum 2000;43:270–4, http://dx.doi.org/10.1002/1529-0131(200002)43:2<270::AID-ANR5>3.0.CO;2-H.
47. Tak PP, Hart BA, Kraan MC, Jonker M, Smeets TJ, Breedveld FC. The effects of interferon beta treatment on arthritis. Rheumatology (Oxford) 1999;38:362–9.
48. van Holten J, Plater-Zyberk C, Tak PP. Interferon-beta for treatment of rheumatoid arthritis? Arthritis Res 2002;4:346–52.
49. van Holten J, Reedquist K, Sattonet-Roche P, Smeets TJ, Plater-Zyberk C, Vervoordeldonk MJ, *et al*. Treatment with recombinant interferon-beta reduces inflammation and slows cartilage destruction in the collagen-induced arthritis model of rheumatoid arthritis. Arthritis Res Ther 2004;6:R239–49, http://dx.doi.org/10.1186/ar1165.
50. Fahmi H, Pelletier JP, Di Battista JA, Cheung HS, Fernandes JC, Martel-Pelletier J. Peroxisome proliferator-activated receptor gamma activators inhibit MMP-1 production in human synovial fibroblasts likely by reducing the binding of the activator protein 1. Osteoarthritis Cartilage 2002;10:100–8, http://dx.doi.org/10.1053/joca.2001.0485.
51. O'Sullivan SE. Cannabinoids go nuclear: evidence for activation of peroxisome proliferator-activated receptors. Br J Pharmacol 2007;152:576–82, http://dx.doi.org/10.1038/sj.bjp.0707423.
52. Richardson D, Pearson RG, Kurian N, Latif ML, Garle MJ, Barrett DA, *et al*. Characterisation of the cannabinoid receptor system in synovial tissue and fluid in patients with osteoarthritis and rheumatoid arthritis. Arthritis Res Ther 2008;10:R43, http://dx.doi.org/10.1186/ar2401.
53. Bordji K, Grillasca JP, Gouze JN, Magdalou J, Schohn H, Keller JM, *et al*. Evidence for the presence of peroxisome proliferator-activated receptor (PPAR) alpha and gamma and retinoid Z receptor in cartilage. PPARgamma activation modulates the effects of interleukin-1beta on rat chondrocytes. J Biol Chem 2000;275:12243–50.
54. Fahmi H, Martel-Pelletier J, Pelletier JP, Kapoor M. Peroxisome proliferator-activated receptor gamma in osteoarthritis. Mod Rheumatol 2011;21:1–9, http://dx.doi.org/10.1007/s10165-010-0347-x.
55. Giaginis C, Giagini A, Theocharis S. Peroxisome proliferator-activated receptor-gamma (PPAR-gamma) ligands as potential therapeutic agents to treat arthritis. Pharmacol Res 2009;60:160–9, http://dx.doi.org/10.1016/j.phrs.2009.02.005.
56. Fahmi H, Di Battista JA, Pelletier JP, Mineau F, Ranger P, Martel-Pelletier J. Peroxisome proliferator–activated receptor gamma activators inhibit interleukin-1beta-induced nitric oxide and matrix metalloproteinase 13 production in human chondrocytes. Arthritis Rheum 2001;44:595–607, http://dx.doi.org/10.1002/1529-0131(200103)44:3<595::AID-ANR108>3.0.CO;2-8.