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Arthritis and cannabinoids: HU-210 and Win-55,212-2 prevent IL-1α-induced matrix degradation in bovine articular chondrocytes in-vitro

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Abstract

Cannabinoids have analgesic, immunomodulatory and anti-inflammatory properties and attenuate joint damage in animal models of arthritis. In this study the mechanisms of action of the synthetic cannabinoid agonists, HU-210 and Win-55,212-2, were studied to determine if they affected interleukin-1 alpha (IL-1α)-induced proteoglycan and collagen degradation in bovine nasal cartilage explant cultures and prostaglandin E_2 (PGE_2) production in primary cultures of bovine articular chondrocytes. The effects of the inactive enantiomer, Win-55,212-3, were compared with those of the active enantiomer, Win-55,212-2, to determine if the effects were cannabinoid (CB)-receptor mediated. The chondrocytes and explants were stimulated by IL-1α (100 U mL^{-1} ≡ 0.06 nM and 500 U mL^{-1} ≡ 0.3 nM, respectively). Proteoglycan breakdown was determined as sulfated glycosaminoglycan (sGAG) release using the dimethylmethylene blue assay. Collagen degradation was determined as hydroxyproline in the conditioned culture media and cartilage digests. PGE_2 was determined by ELISA. Expression of cannabinoid receptors, CB_1 and CB_2; cyclooxygenase-1 and -2 (COX-1 and COX-2); inducible nitric oxide synthase (iNOS); as well as activation of nuclear factor-kappa B (NF-κB) in chondrocytes were studied using immunoblotting techniques and immunofluorescence. The results showed that HU-210 and Win-55,212-2 (5–15 μM) significantly inhibited IL-1α-stimulated proteoglycan ($P < 0.001$) and collagen degradation ($P < 0.001$). Win-55,212-2 (5–10 μM) also significantly inhibited PGE_2 production ($P < 0.01$). At 5 μM, Win-55,212-2 inhibited the expression of iNOS and COX-2 and activation of NF-κB. Chondrocytes appeared to constitutively express cannabinoid receptors CB_1 and CB_2. It is concluded that biologically stable synthetic cannabinoids protect cartilage matrix from degradation induced by cytokines and this effect is possibly CB-receptor mediated and involves effects on prostaglandin and nitric oxide metabolism. Cannabinoids could also be producing these effects via inhibition of NF-κB activation.

Introduction

Cannabinoids include over 60 different natural compounds that have been identified in *Cannabis sativa* (Joy et al 1999). Cannabinoids produce their effects mainly by acting through $G_{i/o}$ protein-coupled membrane receptors (Howlett 2002; Grotenhermen 2003). Two cannabinoid receptors have been identified and cloned to date, designated as CB_1 and CB_2, and these are found mainly in the central and peripheral nervous system and immune system, respectively (Matsuda et al 1990; Munro et al 1993; Howlett et al 2002). There are indications that other subtypes of cannabinoid receptors may exist (Porter & Felder 2001).

Cannabinoids have analgesic and anti-inflammatory properties, as well as immunomodulatory effects, in animal models of arthritis (Burstein et al 1992; Dajani et al 1999; Burstein 2000; Malfait et al 2000). A central pathological feature of arthritic diseases is the resorption of cartilage. Chondrocytes maintain cartilage tissue homoeostasis, controlling the turnover rate of cartilage extracellular matrix (Steinmeyer & Daufeldt 1997). Active proteinases such as matrix metalloproteinases (MMPs) degrade major components of cartilage extracellular matrix, collagen (mainly type II collagen) and proteoglycans (mainly aggrecan) (Cawston 1998). The proteinases are inhibited by tissue inhibitors of metalloproteinases (TIMPs) (Steinmeyer & Daufeldt 1997). In rheumatic conditions, such as osteoarthritis and rheumatoid

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arthritis, there is an imbalance between the proteinases and TIMPs that accounts at least in part for the observed cartilage destruction (Steinmeyer & Daufeldt 1997; Cawston 1998). Proteoglycan degradation occurs early in cartilage resorption, followed by collagen breakdown, which may signify the point of irreparable cartilage damage (Billinghurst et al 2000).

Interleukin-1 (IL-1) is a key mediator by which the chondrocytes enhance their protease production (Cawston 1998). Chondrocytes also produce large quantities of nitric oxide (NO) from L-arginine oxidation by inducible nitric oxide synthase (iNOS or NOS-II), when stimulated by IL-1 (Amin & Abramson 1998). IL-1 also induces COX-2-dependent prostaglandin E_2 (PGE_2) production by chondrocytes (Hardy et al 2002). NO derived from iNOS and PGE_2 derived from COX-2 have been implicated in tissue injury in a variety of pathological conditions, including osteoarthritis (Amin & Abramson 1998; Hardy et al 2002; Goggs et al 2003). Inhibition of NO and PGE_2 production could, therefore, be a useful target in the search for possible treatments for arthritis. In our previous studies, cannabinoids were shown to significantly reduce NO production (Mbvundula et al 2005). Therefore, we studied the effects of the synthetic agonists, HU-210 and Win-55,212-2, as well as the inactive form of the latter, Win-55,212-3, to determine if these affect cytokine-induced cartilage extracellular matrix breakdown and cytokine-induced PGE_2 production and whether they have any protective effects in bovine chondrocyte cultures and bovine cartilage explants.

Materials and Methods

Cannabinoids

Synthetic cannabinoids HU-210 (Tocris, UK), Win-55,212-2 (active enantiomer) (Sigma, UK) and Win-55,212-3 (inactive enantiomer) (Sigma, UK) were studied along with solvent control, dimethyl sulfoxide (DMSO; 0.1% final concentration) (Sigma, UK). All three cannabinoids are non-specific CB_1 and CB_2 receptor agonists. They bind both receptors with similar affinities. Win-55,212-3, however, has a low binding affinity for the cannabinoid receptors compared with Win-55,212-2. Their effects were investigated on unstimulated or IL-1α-stimulated chondrocyte PGE_2 production and cartilage proteoglycan and collagen breakdown at concentrations that were demonstrated to be non-toxic to chondrocytes. Generally cannabinoids were toxic at 100 μM. IL-1α was purchased from the National Institute for Biological Standards and Control (NIBSC; Hertfordshire, UK).

Bovine chondrocyte culture

Chondrocytes obtained from bovine metacarpophalangeal joints by sequential enzymatic digestion using 0.25% trypsin (Invitrogen Paisley, UK) and 3 mg mL^{-1} collagenase type 1 (Sigma) were plated directly into 24-well plates or 8-well chamber slides or T-flasks at 2×10^5 cells per cm^2 (Buttle et al 1997) in Dulbecco's modified Eagle's medium (DMEM) supplemented with glutamine (2 mM), penicillin (100 U mL^{-1}), streptomycin (100 μg mL^{-1}) and amphotericin B (2.5 μg mL^{-1}) in the presence of 10% heat-inactivated fetal bovine serum (FBS). The reagents were purchased from Invitrogen. The cells were incubated at 37°C in a humidified atmosphere of 5% CO_2. When the chondrocytes reached near confluence (about 7–10 days) they were stimulated with IL-1α (100 U $mL^{-1} \equiv 0.06$ nM) (Buttle et al 1997) and were incubated with either Win-55,212-2 or Win-55,212-3 (0–10 μM) for 48 h in DMEM with the supplements (except FBS).

PGE_2 determination

After 48 h incubation of the chondrocytes with the drugs, PGE_2 production was measured using an enzyme-linked immunosorbent assay (ELISA) kit (Cayman chemicals, USA) according to the manufacturer's instructions.

Cartilage proteoglycan breakdown (sGAG assay)

This was determined using cultured bovine nasal cartilage explants (Buttle et al 1993) plated out as three cartilage discs per well, in 24-well plates. The explants were incubated for 24 h in 600 μL DMEM (complete medium) before a 28-day incubation. After the 24-h incubation, the explants were unstimulated or stimulated to resorb with IL-1α (500 U $mL^{-1} \equiv 0.3$ nM) in the presence of HU-210 (0–15 μM) for 21 days and Win-55,212-2 or Win-55,212-3 (0–10 μM) for 28 days in DMEM without FBS. Culture medium was replenished accordingly every 7 days and conditioned medium was kept at –20°C until further analysis. Proteoglycan breakdown was determined as sulfated glycosaminoglycan (sGAG) in the conditioned culture media using the dimethylmethylene blue assay (Farndale et al 1986). Any residual cartilage was digested by incubation in papain solution at 65°C for 2 h (Farndale et al 1986). Proteoglycan content in the digests was determined as in the conditioned media above. Absorbance was read at 520 nm. A standard curve (0–40 μg mL^{-1}) was established using shark chondroitin sulfate (Sigma) as standard. Proteoglycan breakdown was expressed as a percentage of total sGAGs released.

Cartilage collagen breakdown (hydroxyproline assay)

Some of the conditioned culture media used in the sGAG assay above was set aside for this assay. Collagen degradation was determined as hydroxyproline in the conditioned culture media. Any residual cartilage was digested as described above before the hydroxyproline assay. The hydroxyproline assay was performed by the methods of Reddy & Enwemeka (1996). Briefly, test samples (conditioned media and digests) were hydrolysed by autoclaving at 120°C for 20 min in NaOH (2 M final concentration). The hydrolysates, as well as hydroxyproline standards, were incubated at room temperature for 25 min with chloramine-T reagent for oxidation. This was followed by addition of Ehrlich's reagent and the chromophore developed by incubating the samples at 65 °C for 20 min. Absorbance was read at 550 nm. A calibration curve (0–50 μg mL^{-1}) was established using L-hydroxyproline as standard.

Immunofluorescence

Chondrocytes were plated on 8-well chamber slides and incubated for 5 days in complete medium (for cannabinoid receptor immunofluorescence) or incubated for a further 48 h stimulated

with IL-1α (0.06 nM) with or without cannabinoids. Samples were fixed with 4% paraformaldehyde, and were permeabilized using 0.1% Triton X-100. The cells were blocked with 2% normal goat serum (NGS) (Invitrogen) for 1 h followed by overnight incubation at 4°C in primary antibody diluted in phosphate-buffered saline (PBS) with NGS. Rabbit polyclonal anti-cannabinoid receptors — CB_1 (H-150) and CB_2 (H-60) (Santa Cruz, USA); rabbit polyclonal COX-2 (1:50) (Alexis Biochemicals (now Axxora), Nottingham, UK); and rabbit polyclonal anti-NF-κB p65 (1:100) (SantaCruz) were used. Fluorescein isothiocyanate (FITC)-conjugated secondary goat anti-rabbit IgG (Santa Cruz) diluted (1:100) in PBS with NGS was used and cells were incubated in this for 1 h. NGS was used as a negative control. Cells were mounted in Slow-*Fade* anti-fade fluorescent mounting medium (Probes).

Immunoblotting

To extract whole cell lysates, cells were grown to confluence in 75-cm^2 T-flasks and incubated with or without IL-1α stimulation in the presence or absence of cannabinoid, Win-55,212-2 (1 or 5 μM) for 48 h. The chondrocytes were washed with ice-cold PBS then lysed in cold RIPA lysis buffer (Santa Cruz), including protease inhibitors. The cells were centrifuged at 10 000 *g* for 10 min at 4°C. Protein content was determined using the bicinchoninic acid assay. The whole-cell lysates were applied to SDS-polyacrylamide precast 4–12% NuPAGE Bis-Tris mini-gels (Invitrogen). SeeBlue plus2 pre-stained protein standard (Invitrogen) was used for easy and clear band identification. Then the proteins were electroblotted onto Invitolon polyvinylidene difluoride (PVDF) membranes. WesternBreeze Chromogenic Western Blot Immunodetection kit (Invitrogen) was used to detect primary antibodies immobilized on PVDF membrane, following the manufacturer's instructions. Briefly, after blocking the membranes, they were incubated in primary antibodies — rabbit polyclonal COX-1 (1:100) and COX-2 (1:100) (Alexis Biochemicals); or rabbit polyclonal anti-inducible nitric oxide (iNOS) (1:500) (Cayman chemical) for 1 h. After washing the membranes with the antibody wash, they were incubated in alkaline phosphatase-conjugated goat anti-rabbit secondary antibody for 30 min. After further washing with antibody wash and water, purple protein bands were visualized with chromogenic substrate (5-bromo-4-chloro-3-indolyl-1-phosphate (BCIP) and nitro blue tetrazolium (NBT)) for at least 1 h.

Statistical analysis

Data were presented as mean or as mean ± s.e.m. (standard error of the mean). Statistical differences from appropriate controls were determined using Kruskal–Wallis non-parametric test followed by Dunn's post test or one-way analysis of variance followed by Boniferroni's post test; $P < 0.05$ was considered statistically significant. The GraphPad Prism statistical package was used (GraphPad Software, Inc, USA).

Results

Effect of cannabinoids on IL-1α-induced proteoglycan degradation in bovine nasal cartilage explants

HU-210 (5–15 μM) reduced sGAG release at days 7–14 with approximately 20% difference compared with the vehicle controls. HU-210 at 15 μM significantly ($P < 0.05$) reduced IL-1α-induced sGAG release at day 7 (Figure 1A)

The inactive enantiomer, Win-55,212-3, did not show any effect on sGAG release when compared with the vehicle control, while Win-55,212-2 at 5 and 10 μM significantly ($P < 0.001$) reduced sGAG release compared with vehicle control and the reduction was to basal levels (Figure 1B).

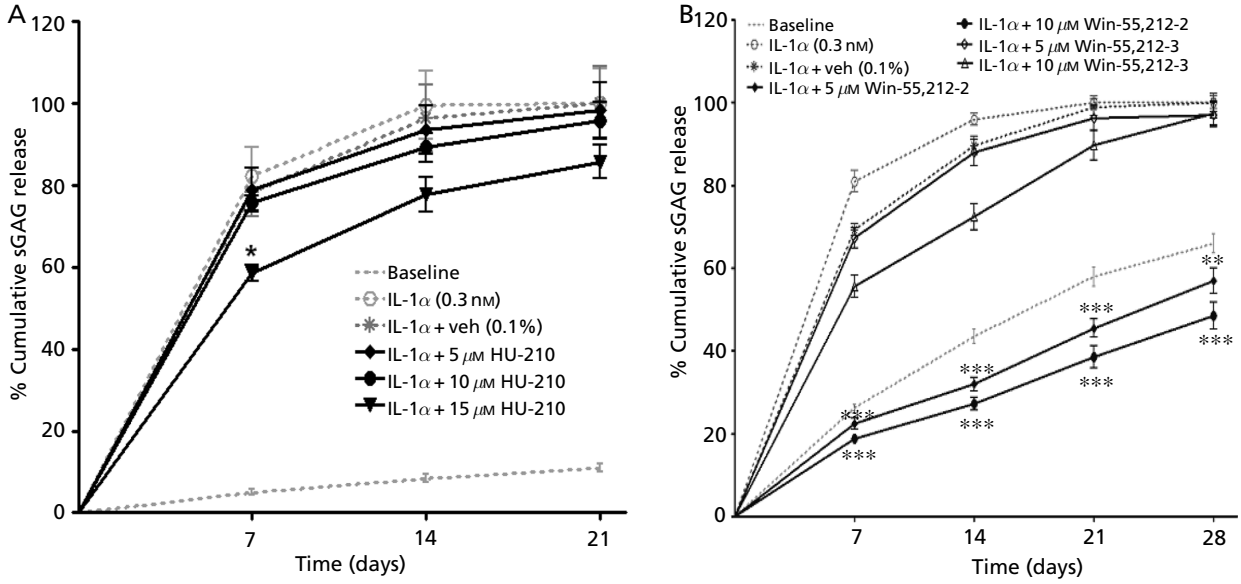

Figure 1 The effect of HU-210 (A) and Win-55,212-2 and its inactive enantiomer, Win-55,212-3 (B), on IL-1-induced proteoglycan degradation in bovine nasal cartilage explants. Results are expressed as mean ± s.e.m., n = 6 (A) or 8 (B). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with vehicle control (Kruskal–Wallis followed by Dunn's post test).

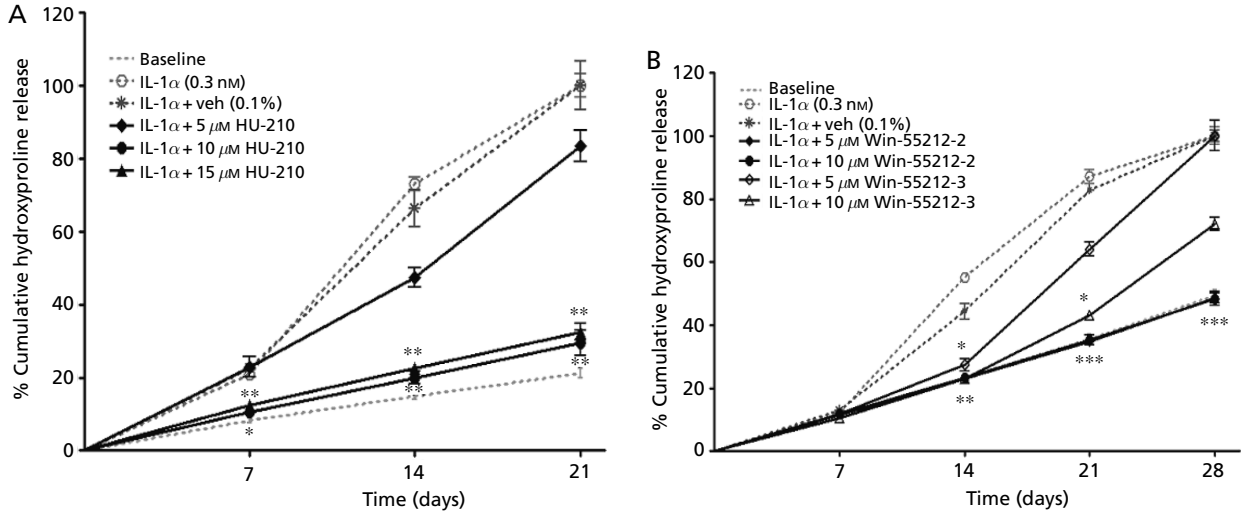

Figure 2 The effect of HU-210 (A) and Win-55,212-2 and its inactive enantiomer, Win-55,212-3 (B), on IL-1-induced collagen breakdown in bovine nasal cartilage explants. Results are expressed as mean ± s.e.m., n = 6 (A) or 8 (B). $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ compared with vehicle control (Kruskal–Wallis followed by Dunn's test).

Effect of cannabinoids on IL-1α-induced collagen degradation in bovine nasal cartilage explants

At 5 μM, HU-210 showed no effect at day 7 but later slightly reduced hydroxyproline release by up to 20% compared with the vehicle control at days 14 and 21. At 10 and 15 μM, however, HU-210 significantly reduced hydroxyproline release at day 7 ($P < 0.01$ and $P < 0.05$, respectively) and at days 14 and 21 ($P < 0.001$ and $P < 0.01$, respectively) (Figure 2A).

Win-55,212-3 did show some inhibitory effects on hydroxyproline release, although the effects were not significantly different compared with those of vehicle control by the end of the culture period (28 days). However, at 5 and 10 μM, Win-55,212-2 significantly ($P < 0.001$) reduced hydroxyproline release compared with the vehicle control throughout the culture period (Figure 2B).

Win-55,212-2 and IL-1α-induced PGE_2 production, COX-1 and COX-2 expression in bovine articular chondrocytes

Win-55,212-2 did not show any effects at 1 μM but significantly ($P < 0.01$) reduced IL-1-induced PGE_2 production (Figure 3) and COX-2 expression (Figure 4) at 5 and 10 μM, compared with the vehicle control in bovine chondrocyte cultures. COX-1 expression in the chondrocytes was not affected by IL-1 or presence of Win-55,212-2.

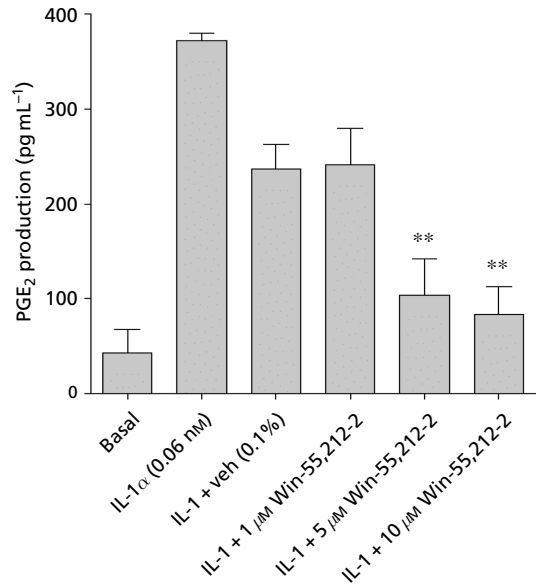

Figure 3 Effect of Win-55,212-2 on IL-1α-induced PGE_2 production in primary bovine articular chondrocyte culture. The results are presented as mean ± s.e.m., n = 6. $**P < 0.01$ compared with vehicle control (analysis of variance followed by Boniferroni's post-hoc test).

Cannabinoid receptor expression in bovine articular chondrocytes

The bovine articular chondrocytes showed punctate expression of cannabinoid receptors CB1 and CB2 through immunofluorescence (Figure 5). The expression of these receptors appeared to be constitutive. The expression of the receptors in these cells was also observed in immunoblot experiments (results not shown).

Win-55,212-2 and iNOS expression in bovine articular chondrocytes

The expression of iNOS was enhanced by the presence of IL-1 in the chondrocytes. Win-55,212-2 did not show any effects at 1 μM but blocked iNOS expression at 5 μM (Figure 6).

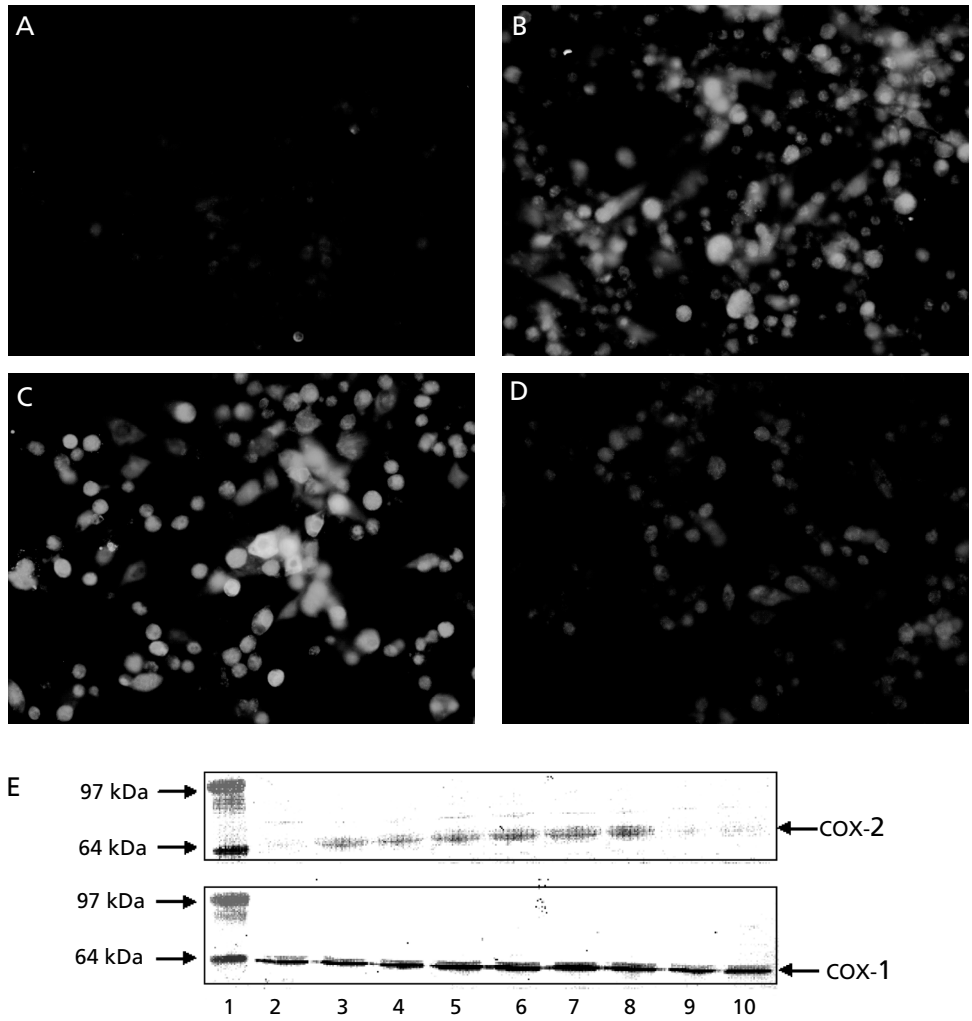

Figure 4 A–D. Immunofluorescence analysis of COX-2 expression in bovine articular chondrocytes cultured for 7 days. The cells were unstimulated (B) or stimulated with IL-1α (0.06 nM) in the presence of 0.1% vehicle – DMSO (C) or 5 μM Win-55,212-2 (D). Negative control (NGS used instead of primary antibody) (A). Primary antibody against COX-2 was diluted 1:50 and FITC-conjugated secondary antibody was used to visualize COX-2 expression. E. Immunoblot analysis of COX-1 and COX-2 expression in bovine articular chondrocytes. The cells were unstimulated or stimulated with IL-1α (0.06 nM) for 48 h in the presence or absence of Win-55,212-2. Lane 1, marker; lane 2, basal; lanes 3 & 4, IL-1α; lanes 5 & 6, vehicle control (IL-1 + 0.1% DMSO); lanes 7 & 8, IL-1 + Win-55,212-2 (1 μM); lanes 9 & 10, IL-1 + Win-55,212-2 (5 μM).

Win-55,212-2 and NF-κB activation in bovine articular chondrocytes

Win-55,212 (5 μM) attenuated the IL-1-induced activation of NF-κB in bovine articular chondrocytes (Figure 7).

Discussion

These results show that synthetic cannabinoids have the potential to protect against IL-1α-induced cartilage breakdown as demonstrated by inhibition of cartilage matrix degradation in bovine nasal cartilage explants. The synthetic cannabinoids significantly inhibited proteoglycan degradation early in the process of cartilage resorption and potently inhibited collagen breakdown later in the process (Figures 1A, and B, 2A and B). In preliminary studies, the cannabinoids were shown to have little or no effect on basal sGAG release, although HU-210 and Win-55,212-2 significantly inhibited IL-1-induced proteoglycan and collagen breakdown in bovine cartilage explants. Win-55,212-2 was able to reduce matrix degradation to about basal levels at the concentrations tested, thus showing its ability to abrogate completely the degradative effects of IL-1 in cartilage.

In this study, Win-55,212-2 appears to have anti-inflammatory effects. In bovine articular chondrocytes, it was shown to inhibit PGE_2 production (Figure 3) and appears to attenuate the production of PGE_2 by blocking COX-2 expression (Figure 4). Win-55,212-2 in this study has been shown to be a specific COX-2 inhibitor since it did not affect expression of COX-1. This implies that the physiological roles of COX-1 may not be affected by use of the drug, and thus may have less side-effects, if any, compared with other COX-2 inhibitors that also affect COX-1 expression. In earlier studies, Win-55,212-2 was shown to significantly reduce NO production as well (Mbvundula et al 2005). The

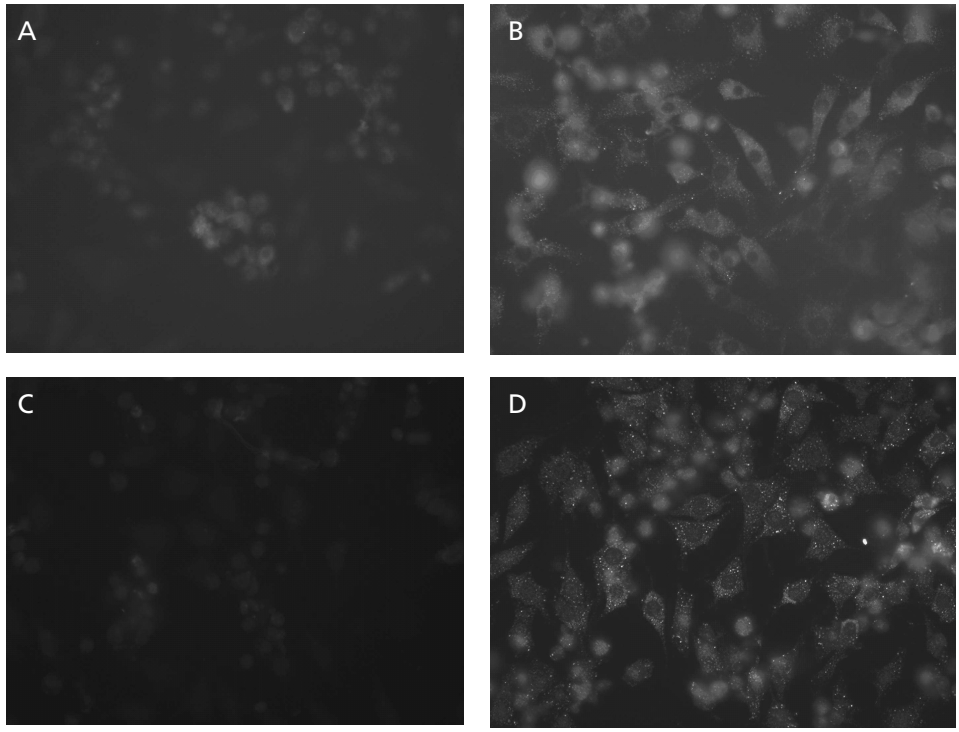

Figure 5 Immunofluorescence analysis of CB1 and CB2 cannabinoid receptors in bovine articular chondrocytes cultured for 7 days. A & C. Negative controls (NGS used instead of primary antibody). B. CB1 receptor expression. D. CB2 receptor expression. Primary antibodies against the receptors were diluted 1:100. FITC-conjugated secondary antibody was used to visualize the receptor's expression.

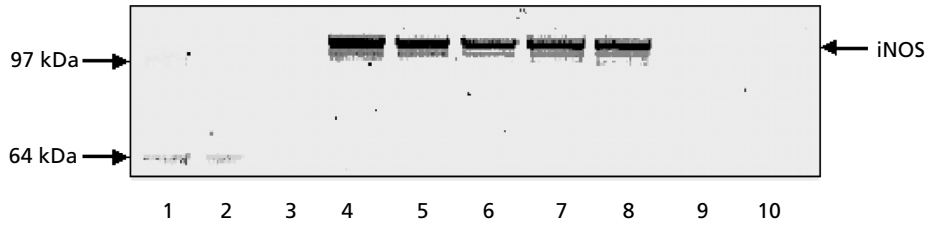

Figure 6 Immunoblot analysis of iNOS expression in bovine articular chondrocytes. The cells were unstimulated or stimulated with IL-1α (0.06 nM) for 48 h in the presence or absence of Win-55,212-2. Lanes 1 & 2, marker; lane 3, basal; lane 4, IL-1α; lanes 5 & 6, vehicle control (IL-1 + 0.1% DMSO); lanes 7 & 8, IL-1 + Win-55,212-2 (1 μM); lanes 9 & 10, IL-1 + Win-55,212-2 (5 μM).

follow-up study on this has revealed that Win-55,212-2 blocks the IL-1-induced expression of iNOS (Figure 6). Thus its reduction of NO production in chondrocytes is, at least in part, through its ability to block iNOS expression. This ability to inhibit both PGE_2 and NO production in bovine articular chondrocytes by blocking COX-2 and iNOS presents an attractive therapeutic potential for this compound. These pro-inflammatory mediators have been implicated in the pathogenesis of osteoarthritis (Studer et al 1999; Martel-Pelletier et al 2003). They have different effects in articular cells that may lead to cartilage resorption. These effects include inhibition of matrix synthesis, upregulation of MMP production and induction of chondrocyte apoptosis (Goggs et al 2003; Henrotin et al 2003; Martel-Pelletier et al 2003; Studer et al 1999, 2003). Apoptosis induced by PGE_2 in chondrocytes is linked with the cAMP-dependent pathway (Miwa et al 2000) and since cannabinoids inhibit cAMP production by inhibiting adenylate cyclase (Howlett et al 2002), cannabinoids could inhibit PGE_2-dependent chondrocyte apoptosis by directly inhibiting PGE_2 production or by inhibiting the cAMP-dependent pathway. Since apoptotic death of articular chondrocytes has been implicated in cartilage degradation (Goggs et al 2003), its inhibition would protect the cartilage and that could be one of the ways in which cannabinoids are able to protect cartilage from cytokine-induced degradation.

PGE_2 has been shown to up-regulate the expression and synthesis of insulin-like growth factor binding proteins (IGFBPs) and is involved in the activation of the IGF-1/IGFBP axis (Di Battista et al 1996, 1997), suggesting that it has a vital role in chondrocytes metabolism. These IGFBPs sequester IGF-1, thus reducing the anabolic stimulation of this tissue and contributing to the net loss of cartilage in inflammatory and degenerative arthritides (Olney et al 1996; Neidel et al 1997). Cannabinoids,

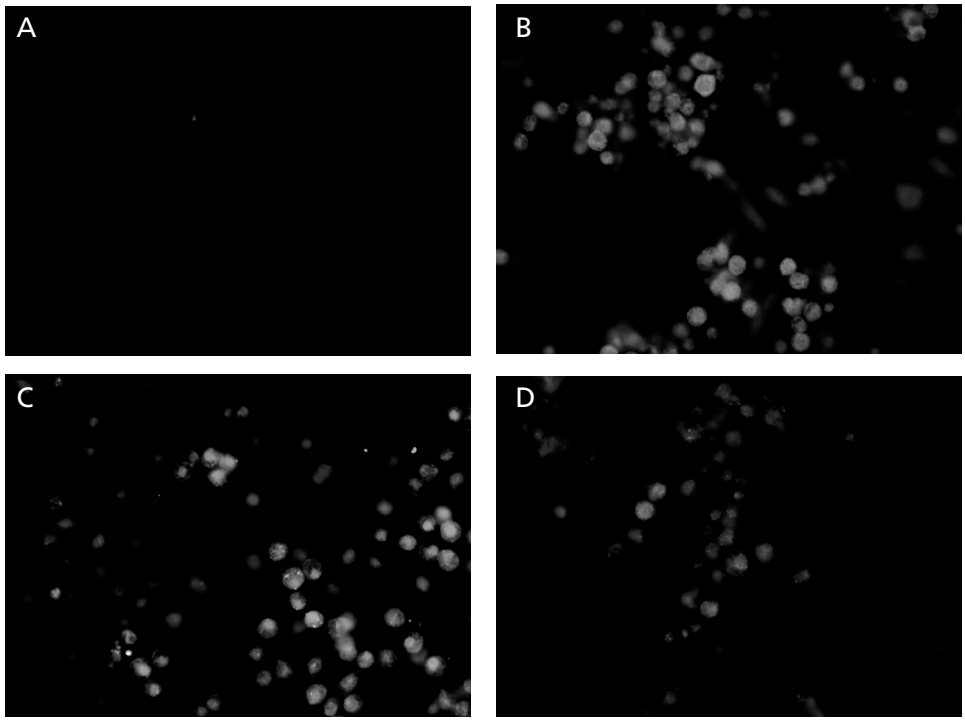

Figure 7 Immunofluorescence analysis of NF-κB activation in bovine articular chondrocytes cultured for 7 days. The cells were unstimulated (B) or stimulated with IL-1α (0.06 nM) in the presence of: 0.1% vehicle – DMSO (C) or 5 μM Win-55,212-2 (D). In the negative control, NGS was used instead of primary antibody (A). Primary antibody against NF-κB p65 was diluted 1:100 and FITC-conjugated secondary antibody was used to visualize NF-κB activation.

by inhibiting PGE_2, may be interfering with this enhancement of expression of IGFBPs and enhancing IGF-1 bioactivity or bioavailability, thus protecting cartilage from resorption.

In chondrocytes, NO is a key regulator of the signalling pathways downstream of IL-I, involving NF-κB activation and leading to the expression of genes that are involved in the pathophysiology of arthritic diseases (Mendes et al 2002). NO is not per se involved in the immediate cytokine-stimulated activation of NF-κB in chondrocytes, but rather sustains nuclear translocation, and this persistent activation of NF-κB may be responsible for NO-sustained catabolic processes that lead to cartilage resorption in arthritic diseases (Clancy et al 2004). The ability of Win-55,212 to block NO production implies that this drug may also be capable of modulating the signalling pathways downstream of IL-1, including the activation of NF-κB. In this study, Win-55,212-2 appeared to reduce NF-κB nuclear translocation stimulated by IL-1 (Figure 7). This could be partly through its ability to abrogate NO production but also could be partly through direct effects on the NF-κB activation mechanisms. It is possible that the effects produced by the cannabinoids are partly through their capability to attenuate NF-κB activation.

HU-210 and Win-55,212-2 are agonists to both the CB_1 and the CB_2 cannabinoid receptors. It is possible these cannabinoids are producing the cartilage protective effects via cannabinoid receptor(s). The cannabinoid receptor inactive enantiomer, Win-55,212-3, did not inhibit NO production (Mbvundula et al 2005) and did not protect cartilage from the degradative effects of IL-1, although it significantly inhibited collagen degradation at 10 μM at days 14 and 21. It is not unusual for the inactive enantiomer to produce some effects, since it has less affinity for the receptor(s) than the active enantiomer. It is also possible that at high concentrations, the cannabinoids were exhibiting non-specific effects. However, the stereoselectivity observed suggests that Win-55,212-2 could be acting through the cannabinoid receptor(s). The bovine articular chondrocytes used were shown, for the first time, to constitutively express both CB_1 and CB_2 cannabinoid receptors (Figure 5). Since Win-55,212-2 is a non-specific cannabinoid receptor agonist, it is therefore not possible to tell exactly at which receptor it is acting without carrying out further studies. The use of AM 281 and AM 630 (CB_1 and CB_2 cannabinoid receptor antagonists, respectively) did not help sort this dilemma because these antagonists did not antagonize, but instead enhanced, Win-55,212-2's effects and did not act as inverse agonists in the system tested, as would be expected (Mbvundula et al 2005). It is also possible that the cannabinoids were acting through a non-CB1/CB2 receptor yet to be identified.

Conclusions

Cannabinoids appear to have protective effects toward cytokine-induced extracellular matrix degradation in cartilage. They reduced the breakdown of both cartilage collagen and proteoglycan and this could be partly through their ability to inhibit the production of inflammatory mediators, such as PGE_2 and NO, by blocking expression of inducible forms of the enzymes that

produce these mediators, COX-2 and iNOS. Cannabinoids may also be protecting cartilage from resorption by modulating NF-κB activation, thus modulating activation of genes of catabolic factors involved in cartilage breakdown. These cannabinoids appear to be acting through some receptor(s) since their effects were concentration dependent and stereoselective. Now, since the chondrocytes have been shown to express the cannabinoid receptors, there is a strong possibility that the cannabinoids acted through these receptors.

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