Cannabinoid Receptor Expression in OA cartilage.

Sara L. Dunn, Aileen Crawford², J Mark Wilkinson³, Rowena A.D Bunning¹, Christine L. Le Maitre, BSc. PhD¹.

¹Biomedical Research Centre, Sheffield Hallam University, Sheffield, United Kingdom, ²School of Clinical Dentistry, University of Sheffield, Sheffield, United Kingdom, ³Department of Human Metabolism, University of Sheffield, Sheffield, United Kingdom.


Introduction: Cannabinoids have been shown to reduce joint damage in animal models of arthritis, in addition we have previously shown that synthetic cannabinoid WIN-55,212-2 mesylate (WIN-55) significantly reduces or abolishes interleukin 1 (IL-1) induced expression of matrix metalloproteinases -3 and -13 (MMP-3 and -13) in early passage human osteoarthritic (OA) chondrocytes. This suggests a possible mechanism via which cannabinoids may act to prevent extracellular matrix (ECM) breakdown. The actions of cannabinoids are mediated by cellular receptors, including the classical cannabinoid receptors cannabinoid receptor 1 and 2 (CB1 and CB2). It is now apparent that not all cannabinoid actions are mediated by these receptors. Other cannabinoid receptors have been identified including G protein-coupled receptor 55 (GPR55), G protein-coupled receptor 18 (GPR18), transient receptor potential vanilloid 1 (TRPV1) and peroxisome proliferator activated receptors alpha and gamma (PPARα and γ). Here, we investigated the effects of cannabinoid WIN-55 on PPARα and PPARγ mRNA expression in OA cartilage to determine whether the chondroprotective effects of WIN-55 may be PPAR mediated. We also examined the expression of cannabinoid receptors CB1, CB2, GPR55, GPR18, TRPV1, PPARα and PPARγ within OA cartilage.

Methods: Human chondrocytes were obtained from the articular cartilage removed from patients with symptomatic OA at the time of total knee replacement (Ethics approval:SMB002, SHU16060). Cartilage tissue was graded histologically using a modified Mankin score. Chondrocytes were isolated from cartilage and cultured in monolayer. At passage 2 chondrocytes were treated with 10 μM WIN-55 for 48 hours prior to RNA extraction. Dimethyl sulfoxide (DMSO) was used as a vehicle control at 0.1%. RNA was reverse transcribed and the mRNA expression of PPARα and PPARγ determined using real-time PCR. The expression and localisation of CB1, CB2, GPR55, GPR18, TRPV1 and PPARα and PPARγ within OA cartilage in the superficial, middle and deep zone and chondrocyte clusters together with osteocyte expression in the underlying bone was determined immunohistochemically (using commercial antibodies). Percentage immunopositivity was determined by recording the number of immunopositive and immunonegative cells within the superficial, middle and deep zones of cartilage together with osteocytes with 200 cell recorded for each zone. Kruskall Wallis test with connover ingman post hoc tests were used to compare between grades of degeneration.

Results: Treatment of OA chondrocytes with WIN-55 significantly induced the gene expression of PPARα (p<0.001) but not PPARγ (Figure 1). Immunohistochemical studies demonstrated that all the cannabinoid receptors investigated were expressed in human cartilage from OA joints (Figure 2+3). No change in the expression of CB1, CB2, GPR55 and PPARα and PPARγ was seen in chondrocytes present in the superficial zone, middle zone, deep zone or clusters compared to grade of cartilage degradation.
However, the expression of GPR18 and TRPV1 was significantly decreased in chondrocytes present in the deep zone of the cartilage which for GPR18 also correlated with increasing grade of cartilage degradation (p<0.05). Expression of all cannabinoid receptors in chondrocytes was higher than that seen in osteocytes in the underlying bone.

**Discussion:** The chondroprotective effects of WIN-55 shown previously may in part be mediated by an increase in PPARα, thus increasing the responsiveness to cannabinoids. We have shown the expression of a number of different cannabinoid receptors within OA cartilage, demonstrating that these cells have the potential to respond to endocannabinoids expressed within the joint and also to synthetic cannabinoids. These receptors could provide candidates for new drug targets which could be investigated as arthritis therapies. The range of cannabinoid receptors expressed by articular chondrocytes suggests the possibility of cannabinoid therapies which target receptors inhibiting the catabolic and pain pathways within the arthritic joint, and without mediating psychoactive effects.

**Significance:** Chondrocytes from osteoarthritic cartilage express a number of cannabinoid receptors that persist in highly degenerate cartilage, indicating that degenerative cartilage may remain responsive to cannabinoid therapy to inhibit catabolic responses. Cannabinoids could thus provide a new therapeutic target for osteoarthritis.

![](image.png)

**Figure 1:** The effect of 10 μM WIN-55 on PPARα (A) and γ (B) mRNA expression in ACs. \(n=9\). 

***p<0.001 compared to DMSO control."
Figure 2: Immunochemical staining for cannabinoid receptor expression, CB1, CB2, GPR55, GPR18 in OA articular cartilage.

Figure 3: Immunochemical staining for Cannabinoid receptor expression: PPARα, PPARγ and TRPV1 in OA articular cartilage.

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