Cannabinoid Receptor Expression in Human Osteoarthritic Cartilage

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Cannabinoids have been shown to reduce joint damage in animal models of arthritis [1-3]. In addition we have 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 primary human chondrocytes, indicating a possible mechanism via which cannabinoids may act to prevent extracellular matrix (ECM) breakdown in arthritis [4].

The actions of cannabinoids are mediated by cellular receptors, including the classical cannabinoid receptors cannabinoid receptor 1 (CB1) and cannabinoid receptor 2 (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 (PPARs) α, δ and γ. We have investigated the effects of cannabinoid WIN-55 on PPAR mRNA expression in osteoarthritic (OA) cartilage to determine whether the chondroprotective effects of WIN-55 maybe PPAR mediated. In addition the expression of cannabinoid receptors CB1, CB2, GPR55, GPR18, TRPV1, PPARα, γ and δ within OA cartilage was investigated.

Primary human chondrocytes were obtained from the articular cartilage removed from patients with symptomatic osteoarthritis at the time of total knee replacement (Ethic approvals:SMB002, SHU16060). Cartilage tissue was graded macroscopically 0-4 using the Outerbridge Classification method [5].

Chondrocytes were extracted from grade 0, 2 and 3 cartilage and cultured in monolayer (n=3 for each grade). 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, δ and γ determined using real-time PCR. Real-time PCR analysis was by $2^{-\Delta\Delta CT}$ [6]. Significance was determined using the non-parametric Kruskall Wallis multiple comparisons test and Conover Inman post hoc test; $p<0.05$ was considered statistically significant. The expression and localisation of CB1, CB2, GPR55, GPR18, TRPV1 and PPARα, δ and γ within OA cartilage was determined immunohistochemically.

Treatment of chondrocytes from grade 0 cartilage with WIN-55 had no significant effect on PPARα, δ or γ mRNA expression. Treatment of chondrocytes from grade 2 cartilage with WIN-55 had no significant effect on PPARγ mRNA expression, produced a 3 fold increase in PPARα mRNA expression ($p<0.05$), and a trend towards increase in PPARδ mRNA expression compared to 0.01% DMSO vehicle control. Treatment of chondrocytes from grade 3 cartilage with WIN-55 had no effect on PPARγ mRNA expression but induced a 6.3 fold increase in PPARα mRNA expression ($p<0.05$) and a 4.7 fold increase in PPARδ mRNA expression ($p<0.05$).

Preliminary immunohistochemical studies demonstrated that cannabinoid receptors, CB1, GPR55, GPR18, TRPV1 and PPARα, δ and γ but not CB2 are expressed in human cartilage from OA joints.

The chondroprotective effects of WIN-55 may in part be mediated by an increase in PPARα and δ expression, thus increasing the responsiveness to cannabinoids. We have shown the expression of cannabinoid receptors within OA cartilage, however it is important to investigate further the role of these receptors in cannabinoid-mediated reduction of cartilage degradation.

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