Influence of the Synthetic Cannabinoid Agonist on Inflamed Cartilage: an in vitro Study

Jiangyinzi Shang, Hang Lin, MaCalus V. Hogan, Alan Yan
Department of Orthopaedic Surgery, University of Pittsburgh School of Medicine,
Pittsburgh, PA, United States
Email of Presenting Author: jiangyin@pitt.edu

DISCLOSURES: Nothing to report.

INTRODUCTION: Medical marijuana (versus Marijuana derivatives) has been reported to possess analgesic, immunomodulatory and anti-inflammatory properties. Recent studies in animal models of arthritis showed that cannabinoids, a group of compounds produced by marijuana, may attenuate joint damage. However, the underlying mechanism has not been completely understood. Interleukin-1β (IL-1β), a proinflammatory cytokine that can result in the degradation of cartilage, is known to be associated with the pathogenesis of osteoarthritis. While whether marijuana byproducts can suppress osteoarthritis (OA)-associated cartilage degradation has not been previously reported. In this study, we insulted human chondrocyte-derived cartilage with IL-1 β for 2 days and then applied Win into the culture to examine if it is able to suppress inflammation and cartilage degradation. The tissue phenotypes were assessed by real-time polymerase chain reaction (PCR), GAG assay, histology, and enzyme-linked immunosorbent assay (ELISA).

METHODS: With the approval from CORID, human chondrocytes were isolated from healthy articular cartilage. P2 cells were used. To generate cartilage *in vitro*, chondrocytes were pelleted and subjected to 14 days chondrogenic culture (**Fig. 1A**). To simulate cartilage degradation observed in OA, we first treated human chondrocyte-derived cartilage with IL-1β (10ng/ml) for 2 days and then applied Win, at different concentrations, into the culture. 2 days post-treatment, pellets were harvested and analyzed. The samples without IL-1 β treatment was used as the control. Statistical analysis was carried out using GraphPad Prism 9 (GraphPad, San Diego, CA). One-way or Two-way analysis of variance (ANOVA) for multi-comparison between groups.

RESULTS: In our previous study, we tested different doses of Win on human chondrocytes. Here we explored the therapeutic potential of Win when the cartilage was in an inflammatory environment, a condition that is often observed in joint injury and arthritis. When comparing the control and Win 0 groups (**Fig. 1B**), IL-1β treatment significantly suppressed the expression of anabolic genes, such as SOX9, COL2, and AGG, and conversely increased the expression of proinflammatory cytokines, such as NF-kB, IL-6, and MMP-13. The results indicated the successful generation of inflammation in the cartilage tissues. In all groups that were co-treated with Win, we did not observe the reverse of the IL-1β-induced changes of gene expression in cartilage tissues. Moreover, at a dose of 1 μM, Win induced a higher expression of catabolic genes, including COL10, NF-kB, and MMP-13.

We next assessed the GAG deposition using safranin-O (**Fig. 2A**). Surprisingly, at a low dose of 0.01 μM, Win treatment was able to preserve more GAGs than in the untreated group. This result was further confirmed by GAG (**Fig. 2B**). Finally, we measured IL-6 levels in the condition medium from different groups (**Fig. 2C**). Interestingly, at a high dose of 1 μM, Win slightly reduced the IL-6 concentration from approximately 223.82ng/ml (untreated) to 112.67ng/ml. However, its potential therapeutic value is limited since the IL-6 level in normal cartilage is approximately 3 pg/mL.

DISCUSSION: For IL-1β insulted cartilage, the IL-6 ELISA results showed evidence of downregulated inflammation with a relatively higher dose of Win; meantime, a low dose of Win showed a beneficial influence on the phenotype of IL1-β-insulted cartilage, as for upregulate results in GAG assay. When chondrocyte-derived cartilage was in an inflamed state induced by IL-1 β , Win displayed a protective effect at lower testing doses, but was not able to fully reverse the damage caused by IL-1 β . The reported anti-inflammatory effect of Win on chondrocytes may due to the global inhibition of high-dose Win on cell activities. Taken together, our results indicated the variable effects of Win on chondrocytes are dose-dependent. Whether cannabinoids can be used to treat cartilage degradation or other structure changes in OA deserves further investigation in animals.

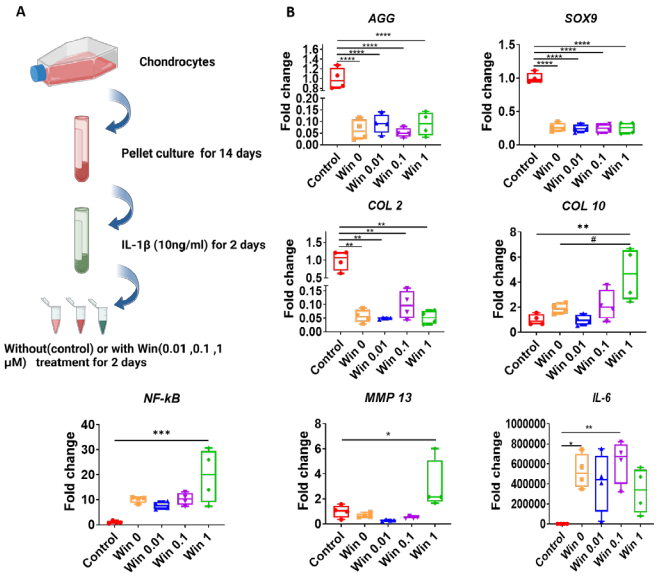

Fig. 1 Assess the influence of Win on inflamed cartilage pellets. A. Schematic to show the experimental process. The figure was created with BioRender.com. B. Relative gene expression levels in IL-1β-pretreated cartilage pellets that were co-cultured with different doses of Win (0, 0.01, 0.1, and 1μM). The tissue without IL-1β or Win treatment served as the Control group (set as 1). Win 0, 0.01, 0.1, and 1 groups were all pretreated with IL-1 β. *, p<0.05; **, p<0.01; ***, p<0.001; ****, p<0.0001; #, p<0.05. N=4.

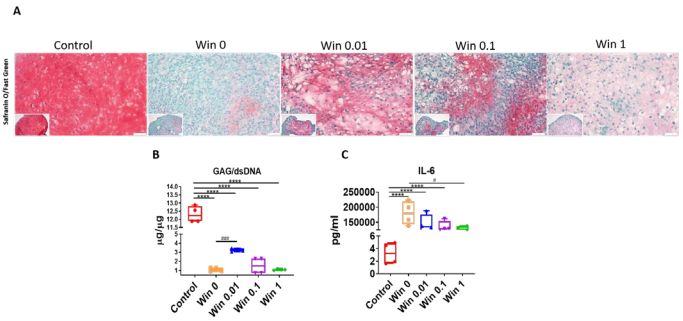

Fig. 2 A. Safranin O staining (A) and GAG assay (B) to examine cartilage pellets treated by Win at different doses (0-1 μM) under an inflamed condition. Bar=50 μm. C. ELISA to examine IL-6 levels in the condition medium from different groups. The tissue treated with IL-1β without Win served as the relative control group. *, p<0.05; ****, p<0.0001; #, p<0.05; ###, p<0.001. N = 4.

SIGNIFICANCE/CLINICAL RELEVANCE: This work investigates the influence of medical marijuana on interleukin-1β treated cartilage, and highlights the need for careful consideration when using the anti-inflammatory properties potential of medical marijuana to treat osteoarthritis.

REFERENCE: [1] 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 Mar;58(3):351-8.
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