Influence of the Synthetic Cannabinoid Agonist on Inflamed Cartilage: an in vitro Study
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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 isolated human chondrocyte-derived cartilage with IL-1β for 2 days and then applied Win into the culture to examine if it is able to suppress inflammation and cartilage degradation. This work investigates the influence of medical marijuana on interleukin-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-κB, and MMP-13.

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 (10µg/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-1β 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 IL-1β-untreated cartilage, as for upregulate results in GAG assay. When chondrocyte-derived cartilage was in an inflamed state induced by IL-1β, Win displayed a protective effect at lower testing doses, but was not able to fully reverse the damage caused by IL-1β. 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.

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.


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