Targeted Activation of Cannabinoid 2 Receptor to Attenuate Painful Synovitis

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Introduction

Osteoarthritis (OA) causes painful joint stiffness, reducing quality of life and imposing a significant economic burden. The functional limitations associated with OA arise from cartilage degradation, inflammation, fibrosis, osteophytes, and muscle weakening around the joint. The synovial membrane produces joint fluid and is a significant source of sensory nerves that intensify pain during inflammation (synovitis). Synovitis severity is graded based on the amount of inflammatory cell foci in the synovial subintima. Underpinned by the critical need for innovative, longer-lasting anti-inflammatory therapies, non-surgical treatments for OA include opioids, steroids, and hyaluronic acid, which pose risks such as addiction and accelerated disease progression with short-term relief. Interleukin (IL)-1β promotes enzyme-mediated cartilage degradation and initiates inflammation in OA. The cannabinoid 2 receptor (CB2R), highly expressed in synoviocytes, has been associated with anti-inflammatory responses. JWH133 is a CBD analog designed for targeted activation of CB2R with 200-fold higher binding affinity than endocannabinoids and CBD. We predict that JWH133 will more effectively reduce the concentration of IL-6 secreted by IL-1β-stimulated synoviocytes compared to CBD.

Objectives

- Visualize differences in CB2R expression between high pain and low pain groups.
- Explore the relationship between CB2R expression, pain, and synovitis score.
- Assess the response of inflammatory synoviocytes to JWH133 compared to CBD.

Methods

- Synovial tissues from banked knee OA patients (n=15) were paraffin processed and sectioned for H&E and CB2R indirect immunofluorescence (IIF).
- Samples were stratified into high (n=10) and low (n=5) self-reported pain and inflammation groups based on microscopic synovitis scoring of H&E-stained sections.
- CB2R IIF signal was quantified using confocal microscopy and normalized to cell number and tissue area using Slidebook™(3i).
- Cultured human fibroblast-like synoviocyte (HFLS) replicates were serum-starved, stimulated with IL-1β (4 ng/mL), and treated with 20 μM CBD or JWH133 dissolved in Cyrene.
- IL-6 in conditioned media was measured in triplicate by sandwich ELISA (Abcam) and normalized to total protein from bicinchoninic acid assay values.
- Statistical analyses included Student's t-tests for histological comparisons, Pearson's rho (R) for associations, and one-way ANOVA to evaluate treatment effects, all with $\alpha = 0.05$.
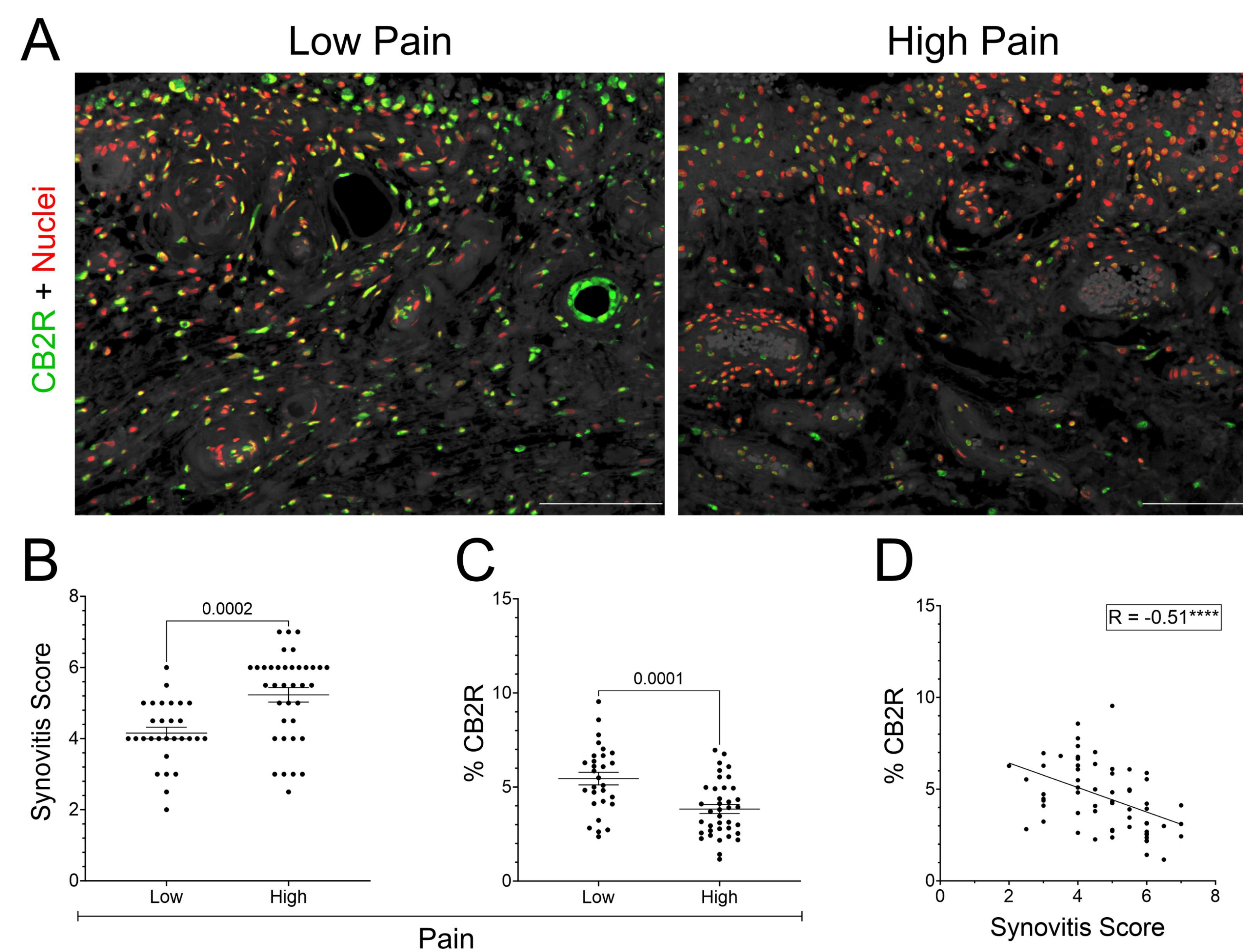
Results

Figure 1: Microscopic images represent CB2R expression in the synovium of knee OA patients grouped by (A) low and (B) high pain. Bar = 100μm. After inter-observer calculation and (B) comparison of histological scores for synovitis that consider synovial hyperplasia, cellularity, and inflammation, the mean percentage of synovial CB2R expression of patients grouped by KOOS pain scores was (C) compared and (D) correlated to synovitis measures using Student's t-test and Spearman's rho (R).
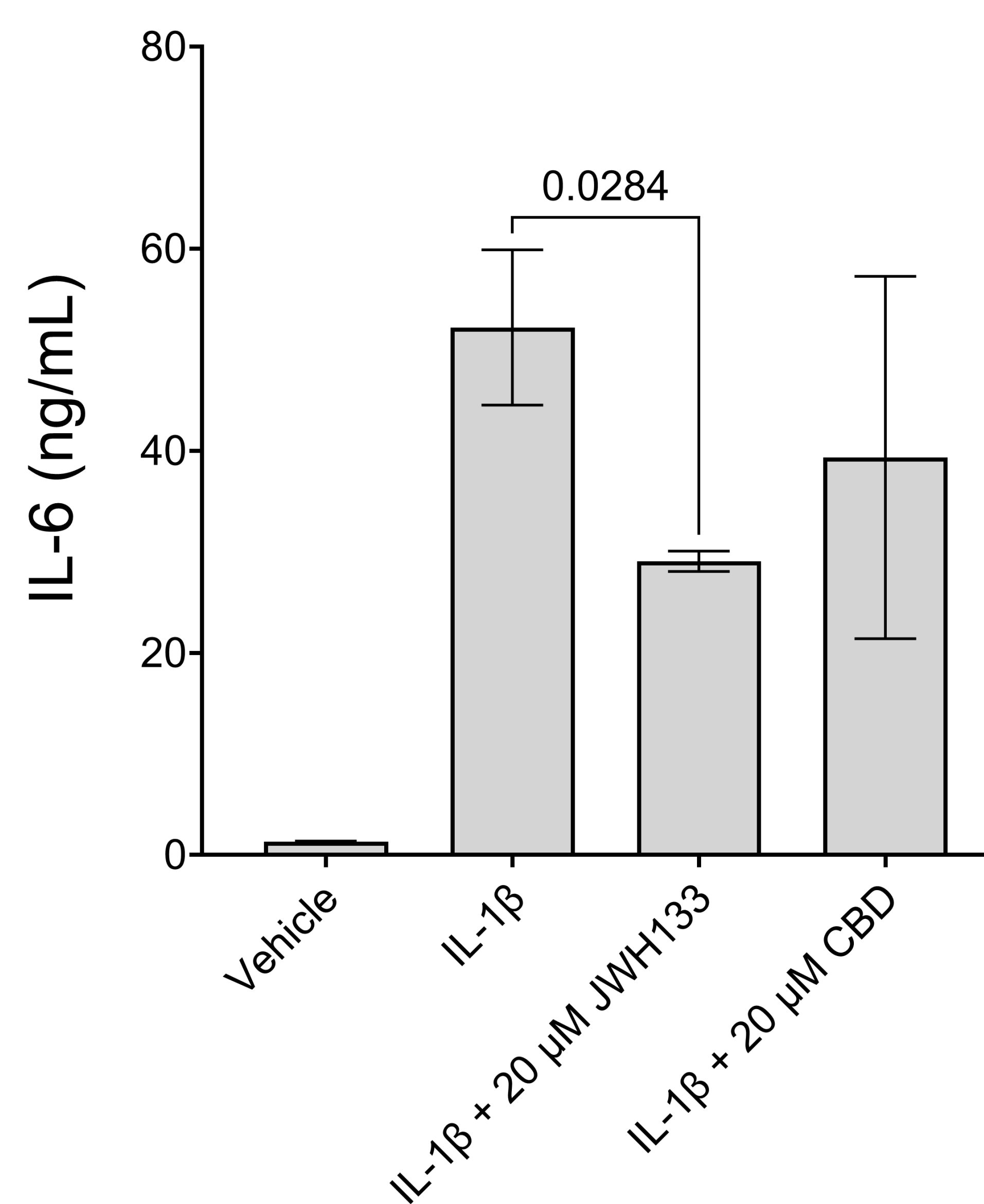

Figure 2: IL-6 levels were compared between treatment groups using Student's t-test and one-way ANOVA ($\alpha = 0.05$). Compared to the cells pre-stimulated with IL-1β and treated with Cyrene vehicle, IL-6 levels were 44% lower in the HFLS pre-stimulated with IL-1β and treated with 20 μM of JWH133. CBD decreased IL-6 by 24.65% compared to stimulated Cyrene vehicle controls but without calculated significance.

Discussion and Limitations

- The inverse relationship between CB2R expression and synovitis highlights a therapeutic need in patients with heightened painful inflammation.
- JWH133 has shown superior efficacy in suppressing IL-6 production compared to CBD, positioning it as a potential anti-inflammatory agent for synovitis.
- Ongoing evaluation of knee OA patient-derived synoviocytes is necessary to explore the therapeutic potential of JWH133.
- The anti-inflammatory effect of CB2R helps modulate cartilage-degrading proteases and fibrous collagen deposition; therefore, JWH133 must be evaluated in animal models of OA.
- Future studies should investigate its broader effects on inflammatory pathways, mechanisms of action, and interaction with pain receptors in the synovium.
- A larger sample size is needed to validate these findings.
- CBD's known low binding affinity to CB2R and its off-target effects may account for the large standard error of the means (SEM) seen in Figure 2.

Conclusion and Significance

Intra-articular injections containing JWH133 could effectively address the need for alternative pain treatment for patients experiencing knee OA, reforming current pain treatment across the orthopaedic field.

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