

# Targeting Inflammatory Arthralgia via Non-psychoactive Cannabinoid Mechanisms

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**DISCLOSURES:** Vinod Dasa: 2; Bioventus, Swiftpath/Pacira. 3B; Bioventus, Myoscience. 4; Myoscience, SIGHT Medical. 5; SKK, Cartiheel, KCI. 7A; Flexion Therapeutics; All other authors have no disclosures.

**INTRODUCTION:** Before total knee arthroplasty (TKA), the current non-surgical standard of care for painful, inflammatory arthropathy attributable to knee osteoarthritis (KOA) involves use of non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, and opioids. However, these treatments provide short-term pain relief and may worsen KOA severity in the long term. As TKA procedures are projected to exceed 3.4 million annually by 2040, it is crucial to develop non-addictive, long-lasting, and cost-effective methods to manage KOA-attributable inflammation and arthralgia to minimize the burden on individuals, reduce healthcare costs, and maximize equity in surgical outcomes. Further research on nociceptive receptors in the synovium, pain measures, and targeted interventions can help refine pain management, identify novel analgesic and anti-inflammatory co-therapies, and improve on individualized interventions for debilitating joint disease. The activation of the transient receptor potential vanilloid 1 (TRPV1) channel plays a role in KOA-related pain and inflammation when bound by ligands such as endocannabinoid 2-arachidonoylglycerol (2-AG), capsaicin, and 12-hydroxyeicosatetraenoic acid (HETE), resulting in calcitonin gene-related peptide (CGRP) as a byproduct. Co-activation of TRPV1 and the cannabinoid 2 receptor (CB2R) has been observed in neurodegenerative diseases and painful arthropathy. Cannabidiol (CBD), a compound that can moderately co-activate CB2R to attenuate inflammation and desensitize TRPV1, has shown potential but also binds the psychoactive CB1R due to limitations in specificity. By exploring novel CB2R analogs and TRPV1 desensitization through cross-activation, we aim to understand the effectiveness of CBD analogs to minimize painful arthropathy without a psychoactive effect. But first, we must demonstrate that assessing TRPV1 and CB2R availability in synovial material, alongside knee injury and osteoarthritis outcome scores (KOOS), may help identify individuals with severe arthralgia who will better respond to cannabinoid-related treatment. Then, testing CB2R-specific agonists, such as JWH133, for anti-inflammatory and fibrogenic effects and evaluating newly synthesized CB2R agonists with intra-articular delivery in a mouse model of KOA, will contribute valuable insights to novel approaches to pharmacotherapeutic relief of arthropathy in a patient-centric manner.

**METHODS:** This study examined TRPV1, PGP9.5, and CB2R expression in synovium samples from end-stage knee osteoarthritis (KOA) patients. Immunofluorescence analysis was performed on 59 synovium samples, randomly selected, and grouped based on the top (low pain) and bottom (high pain) quartiles of patient reported KOOS scores for pain in a database of >350 KOA patients. TRPV1 and PGP9.5 were observed by indirect immunofluorescence co-labeling of sections from all samples using specific antibodies and quantified with Slidebook™ software from confocal photomicrographs relative to cell number or tissue area determined from dapi-stained nuclei or autofluorescence, respectively. Similarly, CB2R was detected alone in serial sections from those co-stained for TRPV1 and PGP9.5 and quantified relative to cell number. Statistical analysis involved a two-tailed Student's t-test ( $\alpha=0.05$ ) to compare mean expression levels between high and low pain groups for TRPV1, PGP9.5, and CB2R.

**RESULTS:** Significant increases were observed in TRPV1 and PGP9.5 expression between low and high KOOS pain groups ( $p<0.0001$ ). Both TRPV1 and PGP9.5 were localized to nerve fibers in the subintima, while most synoviocytes in the intima and subintima expressed TRPV1. PGP9.5 was detected in some of these. Patients with high pain showed 136.90% higher TRPV1 expression and 84.11% higher PGP9.5 expression compared to patients with low pain. CB2R expression was mainly observed in synoviocytes of the intima, with higher expression in the low pain (high KOOS) group compared to the high pain (low KOOS) group ( $p<0.0001$ ). Patients with low pain exhibited 118.70% higher CB2R expression compared to patients with high pain. These findings highlight the potential role of CB2R distribution and turnover in KOA-induced synovitis and arthralgia, suggesting that targeted exposure of the synovium to CB2R analogs may cause a differential effect among patient groups based on receptor distribution and availability at the synovium level.

**DISCUSSION:** This study aimed to quantify the association between TRPV1, PGP9.5, and CB2R expression in the synovium relative to patient-reported arthralgia in KOA. Our findings demonstrate a significant increase in co-expressed TRPV1 and PGP9.5 levels among patients with high pain compared to those with low pain. These results support the hypothesis that TRPV1 and PGP9.5 levels correlate with KOOS pain scores, indicating their potential as predictors of patient responsiveness to desensitization by known ligands such as capsaicin. The upregulation of TRPV1 in patients with high pain may be attributed to elevated pro-inflammatory cytokine release, while higher PGP9.5 expression suggests increased involvement of somatosensory nerves and some synoviocytes as components of the diffuse neuroendocrine system. Additionally, we predict that the higher expression of CB2R observed in the synovium of the low pain group may be due to positive feedback on receptor turnover from higher 2-AG levels. The use of CB2R agonists, such as JWH133, may selectively target CB2R and dysregulate TRPV1, offering analgesic effects while minimizing psychotropic effects associated with CB1R activation. Intra-articular injections targeting CB2R and crosstalk with TRPV1 could provide a safer and more effective approach for managing inflammatory arthralgia.

**SIGNIFICANCE/CLINICAL RELEVANCE:** Current interventions for inflammatory arthralgia resulting from KOA involve the use of drugs shown to trigger addiction and worsen the severity of KOA. For this reason, expanding on the concentration, localization, and activation of nociceptive and cannabinoid receptors in the synovium relative to self-reported pain scores can help refine pain measures, identify responders to individualized, and combinatorial analgesic and anti-inflammatory intervention, and refine targeted intra-articular pharmacotherapy with CB-related compounds. Understanding the effect of CB2R analogs on the endocannabinoid and endovanilloid system crosstalk will help determine their benefit after targeted, intra-articular application, as an effective alternative therapy to the standard practice for KOA and other painful inflammatory arthropathies.

## FIGURES:



