JOURNAL OF UNDERGRADUATE RESEARCH

Effects of Cannabidiol on Intestinal Myofibroblast Fibrotic Pathways

Chloe X. Carlisle, Christopher S. Broxson, Ellen M. Zimmermann

College of Medicine, University of Florida

Dr. Ellen M. Zimmermann, Department of Medicine, Division of Gastroenterology

Abstract

Inflammatory bowel disease (IBD) includes two immune-mediated disorders, Crohn's disease (CD) and ulcerative colitis (UC). IBD is characterized by recurrent and chronic inflammation of the gastrointestinal tract. IBD is lifelong, and there is no cure. There has been an interest in new advanced medications to decrease inflammation and manage symptoms. Many of these new medications are expensive and depress the immune system, leaving many patients looking for alternatives to current medical therapies. Some IBD patients use medical marijuana to relieve the symptoms of this disease, and cannabidiol (CBD) in particular shows potential for reducing inflammation. This study investigates the potential therapeutic effects of CBD on IBD by examining its impact on fibrotic pathways in human intestinal myofibroblast (InMyoFib) cells.

Cultured InMyoFibs were pre-treated with CBD, followed by transforming growth factor-beta 1 (TGF-β1) to stimulate a pro-fibrotic phenotype and mimic human IBD in the lab. Expression of genes associated with fibrosis, inflammation, and cannabinoid signaling was measured by quantitative polymerase chain reaction (qPCR). Fibrotic protein expression in the culture media was analyzed using enzyme-linked immunosorbent assays (ELISA).

CBD pre-treatment before TGF-β1 stimulation significantly reduced the expression of pro-fibrotic genes *ACTA2*, *CCN2*, *COL1A1*, and *SERPINH1* compared to cells stimulated with TGF-β1 alone. Additionally, CBD increased the expression of inflammation-related genes *IL6* and *PTGS2* and the lipid metabolism gene *PPARG,* known to have anti-fibrotic properties. These findings suggest that CBD may modulate fibrotic and inflammatory signaling pathways, providing a potential therapeutic avenue for IBD treatment.

Keywords: inflammatory bowel disease, cannabidiol, fibrosis, inflammation

Introduction

An estimated 3.1 million adults in the United States are affected by inflammatory bowel disease (IBD) (Xu et al., 2018). Inflammatory bowel diseases are lifelong disorders characterized by chronic intestinal inflammation that frequently manifests in early adulthood and for which there are no cures (Le Berre et al., 2023). IBD encompasses Crohn's disease (CD) and ulcerative colitis (UC) and is characterized by chronic inflammation of the gastrointestinal tract. The symptoms associated with this disease include abdominal pain, partial obstruction in the intestine, and abdominal distention (Bielefeldt et al., 2009). Many IBD patients experience a multitude of these symptoms, which can significantly impact their quality of life.

Recently, there has been a growing interest in cannabis and its potential medicinal use for various medical conditions. Cannabis contains tetrahydrocannabinol (THC) as the primary psychoactive ingredient, which, along with non-intoxicating cannabidiol (CBD), has been shown to fight inflammation by lowering the production of pro-inflammatory cytokines (Henshaw et al., 2021). Henshaw et al. showed CBD alone, as well as a combination of CBD and THC, effectively reduced pro-inflammatory cytokines, an effect not observed with THC alone. In addition, some data support that CBD can suppress the activation of certain immune cells and promote regulatory cells (Nicholas & Kaplan, 2020). Due to cannabis's effect on inflammation, there has been a particular interest in its use as an alternative treatment to alleviate symptoms of IBD.

Due to restrictions on the study of CBD in the laboratory setting, much of the existing cannabis research primarily relies on survey-based questionnaires assessing subjective improvements in quality of life (Arkell et al., 2023). Despite the absence of definitive research that cannabis improves intestinal inflammation, marijuana is often considered by some individuals with IBD as a viable option for symptom relief (Storr et al., 2014). Cannabis use provides symptom relief in patients with IBD but is associated with an increased need for surgery in patients with Crohn's disease (Storr et al., 2014), suggesting possible progression of scar tissue. Further research is needed to better understand if cannabis use improves the underlying intestinal inflammation or simply masks symptoms while allowing the disease to progress.

In pursuit of this goal, this study focused on cells and genes associated with human fibrosis, inflammation, and cannabinoid signaling. This study used cultured myofibroblasts,

which are contractile cells that mediate normal wound healing but are thought to be pivotal in pathologic fibrosis when not tightly regulated (Tai et al., 2021). Fibrosis is the excessive accumulation of collagen-rich fibrous tissue (scar tissue) and is common in chronic inflammatory diseases (Wynn & Ramalingam, 2012). During normal wound healing, activated myofibroblasts are characterized by neo-expression of myosin and alpha-smooth muscle actin (ACTA2) as well as other extracellular matrix (ECM) components, but when dysregulated, myofibroblasts secrete excess ECM, which contributes to fibrosis (Pakshir et al., 2020). In IBD, the thickened fibrotic tissue can cause intestinal strictures and obstructions that require surgical intervention (Yoo et al., 2020).

Transforming growth factor-beta 1 (TGF-β1) is a profibrotic cytokine that can induce fibroblast to myofibroblast differentiation, stimulate secretion of ECM and growth factors, and promote other wound repair activities, including collagen accumulation (Kim et al., 2018). In this study, recombinant human TGF-β1 was used to stimulate a fibrotic phenotype in our myofibroblast cell culture and model the environment that may be found in human IBD.

The objective of this study was to investigate the effects of CBD on fibrotic pathways in human intestinal myofibroblasts (InMyoFib), with a focus on impacts on patients with IBD. This study hypothesizes that CBD will act as an anti-inflammatory and anti-fibrotic molecule in signaling pathways. This research aimed to improve our understanding of the connections between cannabis use, symptom relief, and disease prognosis. To accomplish this, a classical reductionist approach was applied using a single cannabinoid to treat cultured human intestinal myofibroblasts in a controlled laboratory environment and analyzing gene expression followed by downstream protein expression. By examining specific cellular pathways through which CBD might influence fibrosis, insight is gained into the associated disease progression and excessive accumulation of scar tissue, which provide valuable data on the potential benefits and risks of using CBD for individuals with IBD.

Methods

Cell Culture

Normal, unaltered InMyoFibs isolated from a male of unknown race and age and frozen at passage 2 were purchased from Lonza Walkersville Inc. (Walkersville, MD) and maintained according to the manufacturer's instructions. Cells were seeded into six-well tissue culture dishes

at a density of 2,500 cells per cm^2 and fed every 2-3 days with smooth-muscle basal medium (SmBm) supplemented with 2 ng/ml human fibroblast growth factor-beta (hFGF-β), 5 µg/ml insulin, 30 mg/ml gentamicin, 15 µg/ml amphotericin (GA-1000), 3 ng/ml human epithelial growth factor (hEGF), and 5% v/v fetal bovine serum (FBS). Once InMyoFibs reached 70-80% confluence, they were washed with 1x phosphate-buffered saline (PBS) 2-3 times and maintained in a serum-free medium for 24 hours before any experimental manipulations. Cells were maintained in a humid 37°C incubator with a 5% (v/v) CO_2 environment. Cells between passage numbers 2-4 were used for all experiments.

CBD and TGF-β1 treatment

CBD in 100% methanol (Sigma Aldrich) was diluted in a serum-free SmBM without growth factors. To limit cytotoxic effects, final methanol concentrations were limited to 0.03% (v/v) for the CBD and vehicle-matched control cell culture media. InMyoFibs were serum-starved for 24 hours before exposure to 1µM CBD (or 0.03% (v/v) methanol vehicle) for 4 hours, followed by 20 hours of stimulation using recombinant human TGF-β1 (R&D systems) dissolved in a sterile vehicle of 4 mM HCl and 1 mg/ml bovine serum albumin (BSA). At the end of the experiment, culture media was collected and frozen for analysis of protein secretion, cells were harvested, and total cellular RNA was isolated for analysis of gene expression.

RNA Isolation

To isolate total RNA, the RNeasy Plus Mini Kit (Qiagen) was used according to the manufacturer's instructions. Briefly, after collection of culture medium, cells were washed 2-3 times with ice-cold 1x PBS to remove all medium. Proprietary kit lysis buffer (RLT) was supplemented with 1% (v/v) β-mercaptoethanol to denature RNases through disulfide bond reduction. Cells were lysed directly on 6-well plates by adding 350 µl per well of the RLT-buffer premix into the dish and then verifying cell lysis under a microscope. When not processed immediately, cell lysates were frozen at -20˚C until RNA isolation. When ready to isolate, cell lysates were brought to room temperature before being transferred to gDNA eliminator spin columns to remove DNA and prevent quantitative polymerase chain reaction (qPCR) amplification of DNA gene sequences. After following the remaining manufacturer's

instructions, RNA was eluted from the spin columns using 30 µl of nuclease-free water and frozen or immediately quantified by spectrophotometry.

RNA Quantification

A Nanodrop 2000 Spectrophotometer was used to quantify RNA concentration in the samples based on the light absorbance at 260 nm. The purity of RNA was assessed by a 260 nm/280 nm absorption ratio (2.0 goal).

Generation of cDNA library

To analyze gene expression through mRNA abundance, relatively unstable RNA was first reverse-transcribed into far more stable cDNA using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems) according to the manufacturer's instructions. Briefly, the same amount of RNA (up to 2 µg maximum) was used for each sample's reverse transcription reaction, and all volumes were made equal using nuclease-free water. A single master mix was prepared for all samples and aliquoted into individual PCR reaction tubes. Reactions were pipette-mixed when adding each component. All reaction tubes were briefly centrifuged, then placed into a thermal cycler program and ran for 10 minutes at 25°C, 2 hours at 37°C, 5 minutes at 85°C, and then held at 4°C. The resulting cDNA was then frozen at -80˚C for future analysis by qPCR.

qPCR

TaqMan probes and primers (ThermoFisher) were used to assess relative gene expression via qPCR. TaqMan primers used in this study include actin alpha 2 smooth muscle (*ACTA2*), Hs00426835_g1; pro-collagen 1 alpha 1 (*COL1A1*), Hs00164004_m1; cellular communication network 2 (*CCN2*), Hs01026927_g1; prostaglandin-endoperoxide synthase 2 (*PTGS2*), Hs00153133_m1; peroxisome proliferator-activated receptor gamma (*PPARG*), Hs00234592_m1; interleukin-6 (*IL6*), Hs00985639_m1; suppressor of cytokine signaling 3 (*SOCS3*), Hs02330328_s1; serpin family h member 1 (*SERPINH1*), Hs01060397_g1; glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*), Hs99999905_m1. Individual cDNA templates from reverse transcription, as well as no cDNA-template controls (NTC), were assayed in triplicate for each gene of interest. Each 5 µl reaction consisted of 0.5µl of cDNA template,

2.5 µl TaqMan gene expression master mix, 0.25 µl TaqMan probe/primer for individual genes of interest, and 1.75 µl nuclease-free water.

Thermal cycling was performed using a QuantStudio 7 Plus instrument (Life Technologies, Carlsbad, CA) and consisted of incubations at 50°C for 2 minutes, 95°C for 10 minutes, then 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. Comparative CT analysis was based on the $2^{-\Delta\Delta Ct}$ method (Livak & Schmittgen, 2001) normalized to *GAPDH* endogenous levels. Relative gene expression for each sample was based on the mean expression level of the vehicle-exposed control group (fold-vehicle). Results are expressed as fold-vehicle ± SEM.

BCA assay

Total cell culture media protein concentration was determined using the Micro Bicinchoninic Acid (BCA) Assay (ThermoFisher) according to the manufacturer's directions. Cell culture media samples at a 1:15 dilution and a standard curve of known bovine serum albumin (BSA) concentrations were mixed with BCA working reagent and incubated at 37°C for 2 hours. The absorbance was measured at 562 nm using a microplate reader, and the total protein concentration was calculated using a 4-parameter best fit based on a standard curve.

Collagen ELISA

Collagen I alpha-1 (COL1A1) protein concentrations in the culture media were analyzed using the DuoSet "sandwich" enzyme-linked immunosorbent assays (ELISA) kit (R&D systems), which utilizes antibodies against 2 different epitopes to reduce non-specific signals and more accurately identify the relative abundance of the target proteins. Capture antibody (100 µl) was added to each well of the ELISA plate and allowed to bind overnight at room temperature. The next day, any unbound antibodies were washed from the wells using the kit-provided wash buffer. To block any areas not covered in the capture antibody, 300 µl BSA protein was added to each well and allowed to bind for 1 hour at room temperature, followed by washing away any unbound protein. Culture media samples were diluted such that the same amount of total protein, 6.75 ng, was added to each well. A kit-provided control COL1A1 protein of known concentration was serially diluted to produce a standard curve. Replicate samples and standards

(100 µl per well) were incubated with the capture antibody overnight at 4℃. The next day, unbound proteins were again washed from the plate, and 100 µl per well of biotin-conjugated detection antibodies were allowed to bind at room temperature for 2 hours. After repeating the wash procedure, 100 µl of streptavidin-conjugated horseradish peroxidase (HRP) was added to each well and allowed to bind to the biotin for 20 minutes. Unbound HRP was washed from the plate as before, and 100 µl of tetramethylbenzidine (TMB) was added to each well and allowed to react for 20 minutes. The color-change reaction was stopped using 50 µl of 2 N sulfuric acid, and an optical density of 450-540 nm was measured. Sample protein concentration was determined using a 4-parameter logistic curve fit for the standard curve.

Results

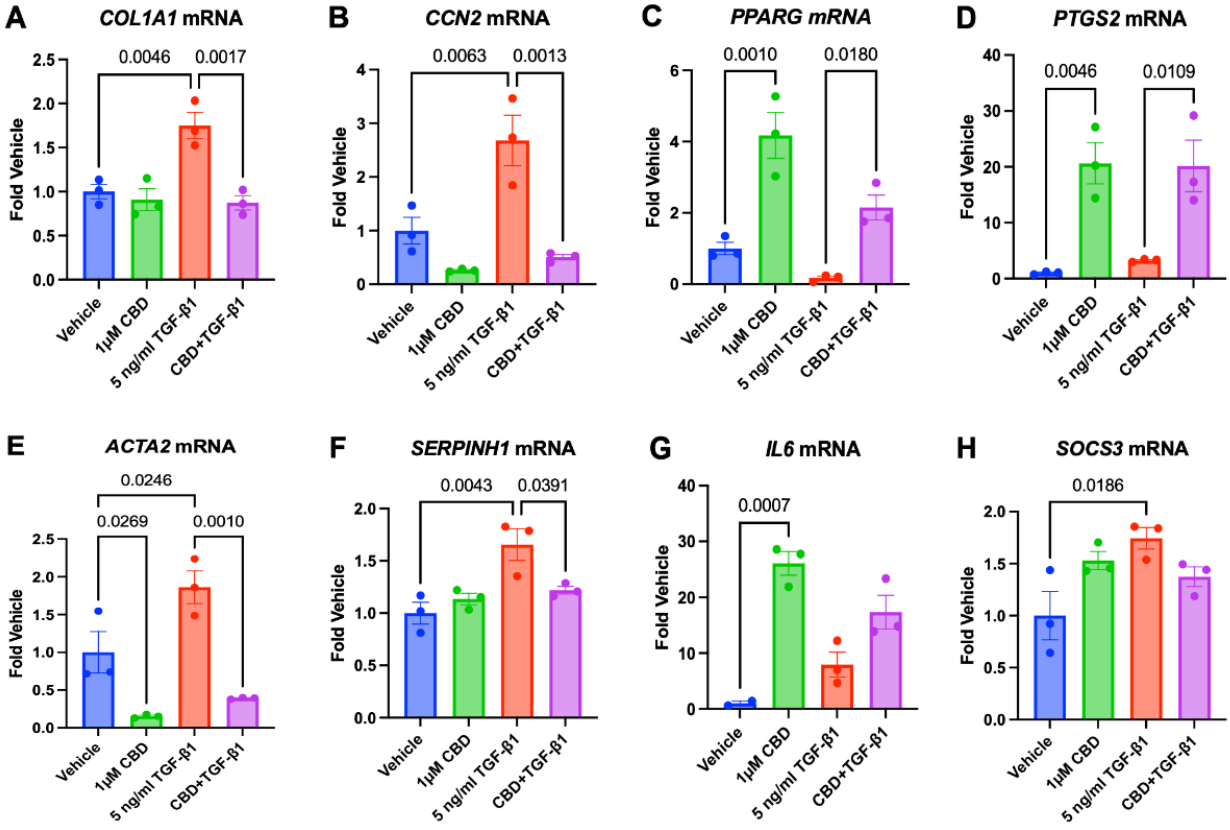

Figure 1. Relative gene expression as measured by qPCR. Experiment contained n=3 samples per treatment group. Analysis by 1 way ANOVA with Sidak's multiple comparison correction using GraphPad Prism statistical software; $p<0.05$ considered significant; results expressed as fold-vehicle +/- SEM.

As expected, stimulation with TGF-β1 increased expression of every pro-fibrotic gene measured and *SOCS3* when compared to cells only exposed to the vehicle (Fig. 1A, 1B, 1E, 1F, 1H). Compared to InMyoFibs solely stimulated by TGF-β1, there was a significant reduction in every pro-fibrotic gene measured when CBD was first introduced to cells 4 hours before stimulation by TGF-β1 (Fig. 1A, 1B, 1E, 1F).

Intriguingly, *PPARG* and *PTGS2* gene expression were higher in TGF-β1-stimulated cells when CBD was applied before TGFβ1, indicating a noteworthy influence of CBD on the expression of these genes as shown in Figure 1C and 1D.

Examining the gene *ACTA2*, the most frequently used marker of myofibroblast differentiation, showed that the addition of CBD 4 hours before stimulation by TGF-β1 resulted in reduced expression compared to cells only stimulated with TGF-β1 as shown in Figure 1E. Even in the absence of TGF-β1, CBD treatment alone decreased *ACTA2* expression compared to the vehicle-treated cells, emphasizing its potential to mitigate fibrosis. Additionally, a reduction in gene expression of *SERPINH1* was observed with TGF-β1 stimulation and CBD treatment as compared to TGF-β1-stimulation only, as shown in Figure 1 F. This shows a repression in the collagen production pathway, indicating a potential decrease in fibrogenesis.

Figures 1A-C, 1E, and 1F show CBD treatment to have anti-fibrotic effects on gene expression. Interestingly, when compared to vehicle-treated cells, InMyoFibs exposed to CBD alone also demonstrated an increase in gene expression of *PPARG*, *PTGS2*, and *IL6*, even in the absence of external stimulation by TGF-β1 (Fig. 1C, 1D, 1G). The InMyoFib cells stimulated solely by TGF-β1 showed an increase in *SOCS3* gene expression, suggesting an anti-inflammatory effect.

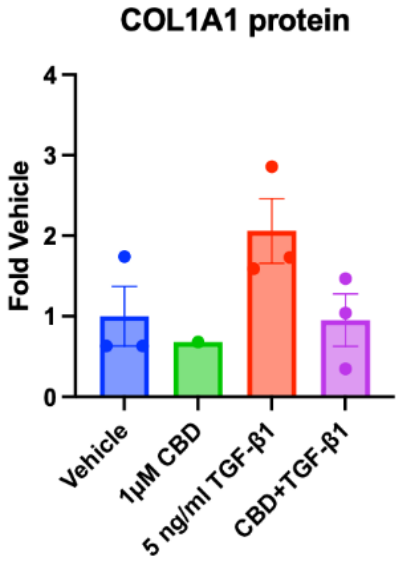

Figure 2. ELISA results for COL1A1 protein media.

To confirm the gene expression data, levels of secreted COL1A1 protein were measured in the culture media. TGF-β1 stimulation increased COL1A1 protein levels in the culture media compared to the vehicle-treated group ($p>0.05$), and CBD treatment of TGF-β1-stimulated cells reduced these protein levels compared to the cells only stimulated with TGF-β1 ($p>0.05$) as shown in Figure 2. Note that the relative levels of COL1A1 protein between treatment groups match the trends seen in the gene expression data; however, the short TGF-B1 stimulation time of this experiment (20 hours) is likely not long enough for the production and secretion of significantly different volumes of COL1A1 protein.

Discussion

IBD patients often endure progressive and irreversible bowel damage for most of their adult lives. Repeated rounds of inflammation and healing can lead to progressive fibrosis of the intestine and potential obstruction requiring surgical intervention to remove the damaged or blocked intestine (Le Berre et al., 2023). While a host of new, potent medications decrease intestinal inflammation, anti-inflammatory medications are not effective at preventing fibrosis, and there are currently no medications to treat or reverse the pathological fibrosis associated with IBD (Yoo et al., 2020; Liu et al., 2024). There is a great unmet need for anti-fibrotic therapies.

Our data show that CBD suppresses the key pro-fibrotic genes *ACTA2*, *CCN2*, *COL1A1*, and *SERPINH1*, making it an appealing candidate as an anti-fibrotic drug. These same trends were also evident in the COL1A1 secreted protein analysis, although the relatively short time of only 20 hours of TGF-β1 stimulation was likely too short to see significant differences in secreted COL1A1 protein levels, which typically require 48 hours to achieve maximal stimulation (Petrov et al., 2002). Directly related is the collagen-specific molecular chaperone *SERPINH1,* which is shown here to mimic the COL1A1 mRNA and protein trends, providing further supporting evidence of CBD's influence on this signaling pathway. As the most frequently used marker of myofibroblast differentiation and activation, *ACTA2* expression might be the best proxy for evaluating CBD's ability to repress myofibroblast fibrogenesis (Rockey et al., 2013). Here, CBD significantly reduced *ACTA2* expression in both TGF-β1 stimulated and unstimulated cells, further supporting CBD's potential as an anti-fibrotic agent.

Perhaps most importantly, CBD inhibited *CCN2* expression, which implies a reduction in mature connective tissue growth factor (CTGF) protein, although direct measurement of the protein is required to verify this. This is important because CTGF is known to induce fibrosis and is associated with virtually all fibrotic diseases (Fu et al., 2022). Additionally, CTGF has been shown to enhance TGF-β1 signaling, thereby increasing the intensity and duration of pro-fibrotic signaling (Abreu et al., 2002). The ability of CBD to repress *CCN2* expression further supports its potential as an anti-fibrotic agent. Taken together, these data implicate CBD as a potential inhibitor of fibrosis development.

This data also revealed that CBD increased the expression of *PPARG* and *PTGS2* in both non-stimulated and TGF-β1 stimulated cells. Agonists of the PPARG nuclear receptor have been shown to have anti-fibrogenic properties (Koo et al., 2017), suggesting this same mechanism may be inhibiting fibrotic signaling in this study. While it is known that TGF-β1 downregulates PPARG (Wei et al., 2010), our results show that CBD upregulates the expression of *PPARG* in unstimulated cells and can partially offset TGFβ's suppressive effect.

The increased expression of *PTGS2* in CBD-exposed cells was an unexpected result, as the COX2-PGE_2 signaling cascade has long been considered pro-inflammatory (Simon 1999). Additionally, others have shown PPARG activation to significantly inhibit COX2 gene expression (Hou et al., 2012). However, more recent studies have reported overwhelmingly

opposite effects implying *PTGS2*/COX2-PGE_2 involvement in the resolution of inflammation (Martín-Vázquez et al., 2023). More research into this intriguing finding is certainly warranted.

Analogous to the *PTGS2* results, exposure to CBD in the absence of TGF-β1 also increased *IL6* expression with levels rising by 25-fold over the vehicle-treated control group. While this may seem counterintuitive given IL6's pro-inflammatory role through trans-signaling, IL6 is pleiotropic with diverse biological effects, which include well-established anti-inflammatory activities mediated through classical signaling (Sheller et al., 2011). The pro-inflammatory trans-signaling is dependent upon a soluble form of the IL6 receptor, while classical signaling relies on a membrane-bound IL6 receptor, so future studies to distinguish which signaling is occurring might employ identification of the presence and relative abundance of both receptor types.

Our research suggests that CBD has potential anti-fibrotic effects by downregulating pro-fibrotic genes and upregulating anti-fibrotic genes in myofibroblasts. Further exploration of the long-term effects of cannabis and its components, such as CBD, on the progression and management of IBD is necessary.

Future studies should focus on assessing the protein expression levels of the genes identified in this study using techniques such as Western blotting or immunohistochemistry to better understand their roles in the observed phenotypes. Additionally, it is crucial to investigate the specific types of IL-6 receptors involved, distinguishing between pro-inflammatory and anti-inflammatory receptors. Furthermore, examining the type of collagen cross-linking will help determine whether the collagen tissue is more permanent and pathologic fibrotic or not, providing insights into the nature of the fibrotic response.

Conclusion

Based on the findings of this study, the hypothesis that CBD acts as an anti-inflammatory and anti-fibrotic molecule is supported. These results suggest that CBD has the potential to modulate fibrotic pathways, thereby offering a promising therapeutic approach for preventing the progression of IBD.

References

Abreu, J. G., Ketpura, N. I., Reversade, B., & De Robertis, E. M. (2002). Connective-tissue growth factor (CTGF) modulates cell signalling by BMP and TGF-beta. *Nature cell biology*, *4*(8), 599–604. https://doi.org/10.1038/ncb826

Arkell, T. R., Downey, L. A., Hayley, A. C., & Roth, S. (2023). Assessment of Medical Cannabis and Health-Related Quality of Life. *JAMA network open, 6*(5), e2312522. https://doi.org/10.1001/jamanetworkopen.2023.12522

Bielefeldt, K., Davis, B., & Binion, D. G. (2009). Pain and inflammatory bowel disease. *Inflammatory Bowel Diseases, 15*(5), 778–788. https://doi.org/10.1002/ibd.20848

Fu, M., Peng, D., Lan, T., Wei, Y., & Wei, X. (2022). Multifunctional regulatory protein connective tissue growth factor (CTGF): A potential therapeutic target for diverse diseases. *Acta pharmaceutica Sinica. B*, *12*(4), 1740–1760. https://doi.org/10.1016/j.apsb.2022.01.007

Henshaw, F. R., Dewsbury, L. S., Lim, C. K., & Steiner, G. Z. (2021). The effects of cannabinoids on pro- and anti-inflammatory cytokines: A systematic review of in vivo studies. *Cannabis and Cannabinoid Research, 6*(3), 177–195. https://doi.org/10.1089/can.2020.0105

Hou, Y., Moreau, F., & Chadee, K. (2012). PPARγ is an E3 ligase that induces the degradation of NFκB/p65. *Nature communications*, *3*, 1300. https://doi.org/10.1038/ncomms2270

Kim, K. K., Sheppard, D., & Chapman, H. A. (2018). TGF-β1 signaling and tissue fibrosis. *Cold Spring Harbor Perspectives in Biology, 10*(4). https://doi.org/10.1101/cshperspect.a022293

Koo, J. B., Nam, M. O., Jung, Y., Yoo, J., Kim, D. H., Kim, G., Shin, S. J., Lee, K. M., Hahm, K. B., Kim, J. W., Hong, S. P., Lee, K. J., & Yoo, J. H. (2017). Anti-fibrogenic effect of PPAR-γ agonists in human intestinal myofibroblasts. *BMC gastroenterology*, *17*(1), 73. https://doi.org/10.1186/s12876-017-0627-4

Le Berre, C., Danese, S., & Peyrin-Biroulet, L. (2023). Can we change the natural course of inflammatory bowel disease?. *Therapeutic advances in gastroenterology*, *16*, 17562848231163118. https://doi.org/10.1177/17562848231163118

Liu, Y., Zhang, T., Pan, K., & Wei, H. (2024). Mechanisms and therapeutic research progress in intestinal fibrosis. *Frontiers in medicine*, *11*, 1368977. https://doi.org/10.3389/fmed.2024.1368977

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods, 25*(4), 402–408. https://doi.org/10.1006/meth.2001.1262

Martín-Vázquez, E., Cobo-Vuilleumier, N., López-Noriega, L., Lorenzo, P. I., & Gauthier, B. R. (2023). The PTGS2/COX2-PGE_2 signaling cascade in inflammation: Pro or anti? A case study with type 1 diabetes mellitus. *International journal of biological sciences*, *19*(13), 4157–4165. https://doi.org/10.7150/ijbs.86492

Nichols, J. M., & Kaplan, B. L. F. (2020). Immune responses regulated by Cannabidiol. *Cannabis and Cannabinoid Research, 5*(1), 12–31. https://doi.org/10.1089/can.2018.0073

Pakshir, P., Noskovicova, N., Lodyga, M., Son, D. O., Schuster, R., Goodwin, A., Karvonen, H., & Hinz, B. (2020). The myofibroblast at a glance. *Journal of Cell Science, 133*(13) 2. https://doi.org/10.1242/jcs.227900

Petrov, V. V., Fagard, R. H., & Lijnen, P. J. (2002). Stimulation of collagen production by transforming growth factor-beta1 during differentiation of cardiac fibroblasts to myofibroblasts. *Hypertension*, *39*(2), 258–263. https://doi.org/10.1161/hy0202.103268

Rockey, D. C., Weymouth, N., & Shi, Z. (2013). Smooth muscle α actin (Acta2) and myofibroblast function during hepatic wound healing. *PloS one, 8*(10), e77166. https://doi.org/10.1371/journal.pone.0077166

Scheller, J., Chalaris, A., Schmidt-Arras, D., & Rose-John, S. (2011). The pro- and anti-inflammatory properties of the cytokine interleukin-6. *Biochimica et Biophysica Acta, 1813*(5), 878–888. https://doi.org/10.1016/j.bbamcr.2011.01.034

Simon L. S. (1999). Role and regulation of cyclooxygenase-2 during inflammation. *The American Journal of Medicine*, *106*(5B), 37S–42S. https://doi.org/10.1016/s0002-9343(99)00115-1

Storr, M., Devlin, S., Kaplan, G. G., Panaccione, R., & Andrews, C. N. (2014). Cannabis use provides symptom relief in patients with inflammatory bowel disease but is associated with worse disease prognosis in patients with Crohn's disease. *Inflammatory Bowel Diseases, 20*(3), 472–480. https://doi.org/10.1097/01.mib.0000440982.79036.d6

Tai, Y., Woods, E. L., Dally, J., Kong, D., Steadman, R., Moseley, R., & Midgley, A. C. (2021). Myofibroblasts: Function, Formation, and Scope of Molecular Therapies for Skin Fibrosis. *Biomolecules, 11*(8), 1095. https://doi.org/10.3390/biom11081095

Wei, J., Ghosh, A. K., Sargent, J. L., Komura, K., Wu, M., Huang, Q. Q., Jain, M., Whitfield, M. L., Feghali-Bostwick, C., & Varga, J. (2010). PPARγ downregulation by TGFß in fibroblast and impaired expression and function in systemic sclerosis: a novel mechanism for progressive fibrogenesis. *PloS one*, *5*(11), e13778. https://doi.org/10.1371/journal.pone.0013778

Wynn, T. A., & Ramalingam, T. R. (2012). Mechanisms of fibrosis: therapeutic translation for fibrotic disease. *Nature medicine, 18*(7), 1028–1040. https://doi.org/10.1038/nm.2807

Xu, F., Dahlhamer, J. M., Zammitti, E. P., Wheaton, A. G., & Croft, J. B. (2018). Health-risk behaviors and chronic conditions among adults with inflammatory bowel disease — United States, 2015 and 2016. *Morbidity and Mortality Weekly Report, 67*(6), 190–195. https://doi.org/10.15585/mmwr.mm6706a4

Yoo, J. H., Holubar, S., & Rieder, F. (2020). Fibrostenotic strictures in Crohn's disease. *Intestinal research, 18*(4), 379–401. https://doi.org/10.5217/ir.2019.09148