The Effect of Cannabis Compound Tetrahydrocannabinol on Fibrotic Pathways; Implications Beyond Fibrosis

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Conclusions

1. In intestinal myofibroblasts, THC suppresses expression of many mediators of fibrosis consistent with an anti-fibrotic role
2. THC increases TGFB1 expression consistent with a pro-fibrotic effect
3. THC suppresses TGFB1 signaling through the AKT pathway. This pathway impacts many disease processes from atherosclerosis to cancer
4. Translational studies are needed to determine the efficacy and safety of cannabinoids in patients with Crohn's disease

Background

- Crohn's disease (CD) is a chronic inflammatory disease of the intestine that typically begins in teens or young adults. Over years, pro-fibrotic factors like transforming growth factor beta-1 (TGFB1) become activated leading to strictures that require surgery.
- CD is treated with potent immunosuppressive medications. Patients have sought alternative treatments like cannabinoids that improve symptoms.
- In other organ systems, cannabinoids are anti-inflammatory and anti-fibrotic. Whether cannabinoids decrease inflammation or effect fibrosis in CD is not known.
- Our previous data show that in human intestinal myofibroblasts, the cell responsible for fibrosis in CD, tetrahydrocannabinol (THC) decreases mediators of fibrosis like *ACTA2* & *TIMP1* mRNAs (Figure 1 A,B).

Aims

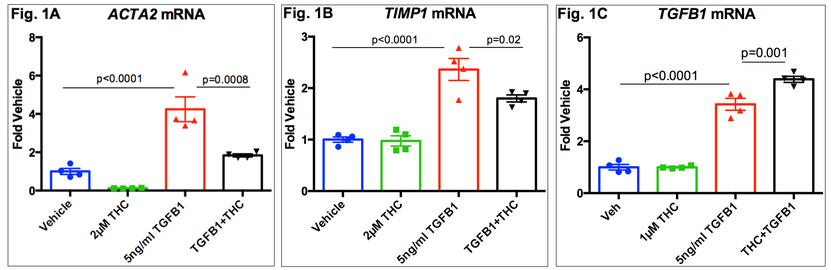
- Understand the mechanism by which THC affects the pro-fibrotic TGFB1 pathway in intestinal myofibroblasts.

Methods

- Here we used human intestinal myofibroblasts, exposed to TGFB1 to mimic fibrotic Crohn's disease and study the effects of THC.
- Interrogate gene expression by quantitative polymerase chain reaction (qPCR).
- Analyze protein expression and phosphorylation by Western blot.
- Results are expressed as fold-vehicle ± S.E.M. using 1 way ANOVA to obtain p-values.

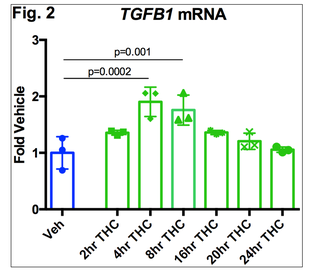
Results

Figure 1. THC inhibited TGFB1-induced pro-fibrotic factors except *TGFB1* mRNA.



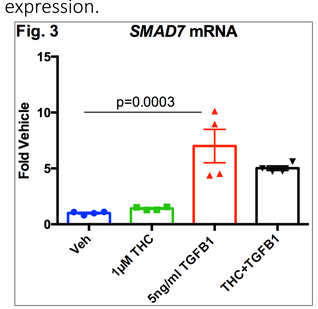
24 hours of THC inhibited fibrotic markers *ACTA2*, and *TIMP1*, but increased the pro-fibrotic *TGFB1* mRNA.

Figure 2. Time-dependent effect of THC on *TGFB1* mRNA



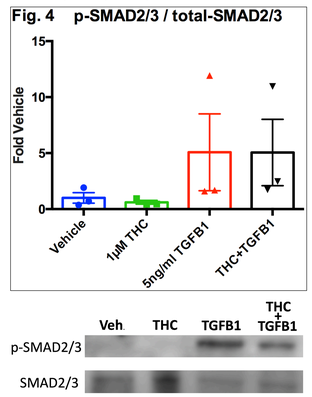
Time-course confirmed 1μM THC increases *TGFB1* mRNA. Largest effect at 4 hours THC.

Figure 3. THC does not alter *SMAD7* gene expression.



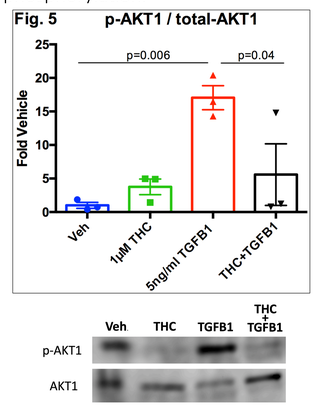
Mothers against decapentaplegic homolog 7 (SMAD7) is a negative regulator of TGFB1 signaling. 24 hours THC pre-treatment did not change *SMAD7* mRNA levels suggesting that THC was not acting through the canonical SMAD pathway.

Figure 4. THC does not alter SMAD2/3 protein phosphorylation.



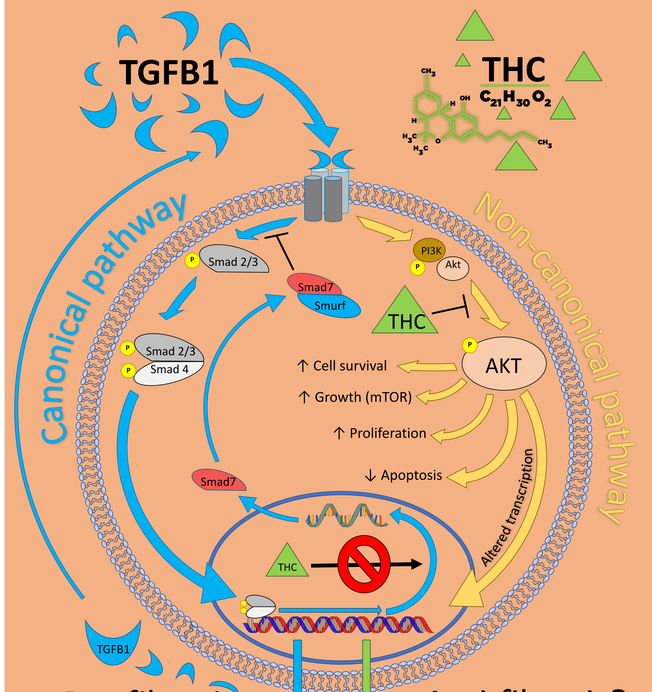
TGFB1 activates SMAD2/3 protein by phosphorylation (p-SMAD2/3). THC did not alter p-SMAD2/3 protein, further implicating a SMAD-independent pathway (3 independent experiments; representative Western blot).

Figure 5. THC reduces AKT protein phosphorylation.



RAC-alpha serine/threonine-protein kinase (AKT1) is a SMAD-independent pathway also phosphorylated (activated) by TGFB1.
24 hours THC pretreatment reduced P-AKT compared to TGFB1 stimulated cells (3 independent experiments; representative Western blot).

Working model of THC mechanism



Summary

- TGFB1 stimulates many pro-fibrotic factors and increases expression of its own mRNA
- THC inhibits TGFB1-stimulated effects on pro-fibrotic factors
- THC increases TGFB1 gene expression in a time-dependent manner
- THC exerts its effects through the non-canonical pathway

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