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The Effect of Cannabidiol on Cellular Inflammation

Chronic inflammation is where cells begin to attack themselves. This leads to an increase of pain markers in the body and an influx of cellular damage. Typical treatment methods for chronic inflammation include opioids and non-steroidal anti-inflammatory drugs; each of which, have benefits, as well as disadvantages, with long term use. An alternative to these methods includes applying cannabidiol, which is the non-psychoactive derivative of marijuana. Studies have suggested that cannabidiol used in vivo can alleviate cellular inflammation. To date, there have been few studies done on the use of this alternative therapy, and there is little understanding of the impact of cannabidiol on inflammation at the cellular level. The purpose of this study was to investigate the effects of cannabidiol using a cell culture model.

Given the widespread use of cannabidiol in inflammatory conditions today, we hypothesized that cannabidiol treatment of the mammalian lymphocyte HL-60 cell line would produce an anti-inflammatory effect on the cell line that is similar to non-steroidal anti-inflammatory drugs. Cytotoxicity assays were performed to confirm that drug treatment did not negatively affect the HL60 cells. Reverse Transcriptase and quantitative PCR techniques were used to identify commonly activated anti-inflammatory pathways following drug treatment. This preliminary investigation provides insight into the effectiveness of using cannabidiol as an anti-inflammatory drug.

The Effect of Cannabidiol (CBD) Showing Cellular Inflammation

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Introduction

- Chronic inflammation is where cells begin to attack themselves.
- This leads to an increase of pain markers in the body and an influx of cellular damage.
- Typical treatment methods for chronic inflammation include opioids and non-steroidal anti-inflammatory drugs; each of which have benefits, as well as disadvantages, with long term use.
- Cannabidiol (CBD), which is the non-psychoactive derivative of marijuana, has gained popularity in oil forms as a novel therapeutic to alleviate cellular inflammation.
- To date, there have been few studies done on the use of this alternative therapy, and there is little understanding of the impact of cannabidiol on inflammation at the cellular level.

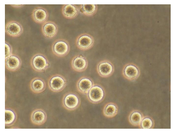
Objective

The purpose of this study was to investigate the effects of cannabidiol using a cell culture model.

We hypothesized that cannabidiol treatment of the mammalian lymphocyte HL-60 cell line would produce an anti-inflammatory effect on the cell line that is similar to non-steroidal anti-inflammatory drugs.

Methods Overview

Cell culture of Mammalian HL-60 cells
- Human leukemia cell line
- For all assays cells were grown to confluency in Modified Dulbecco's Medium, with 20% fetal bovine serum

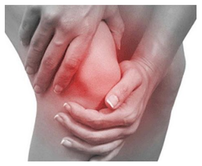


qRT-PCR to determine the expression of CBR2 in HL-60 cell line
- The initial molecular cloning of cannabinoid receptor type 2, which CBD oil is suspected to activate, was done using HL-60 cells
- TaqMan® qRT-PCR probe sets for CBR2 were used to confirm the expression of the receptor in HL-60 cell culture

CBD oil & antiinflammatory drug treatment of HL-60 cells
- A 1,000mg CBD oil solution was prepared in cell culture media
- A 100x Aspirin solution was prepared in 90% ethanol and diluted 1:10 in cell culture media (0.1µM)
- Cells were treated for 24 hours
- Samples were prepared for Trypan Blue staining for cell viability, and RNA extraction, for molecular analysis

Assessment of cytotoxicity following drug treatment
- Trypan Blue staining was performed 24 hours after drug treatment and percent viability was determined
- Quantitative MTT assays were performed to evaluate cytotoxicity: for all MTT assays cells were treated with serum free media 24 hours prior to application of drugs prepared in serum free media

Effect of CBD oil & drug treatment on inflammatory markers
- RNA was extracted from drug treated flasks
- TaqMan® qRT-PCR probe set for antiinflammatory marker A20 was used to compare the effect of CBD oil and Aspirin



Results

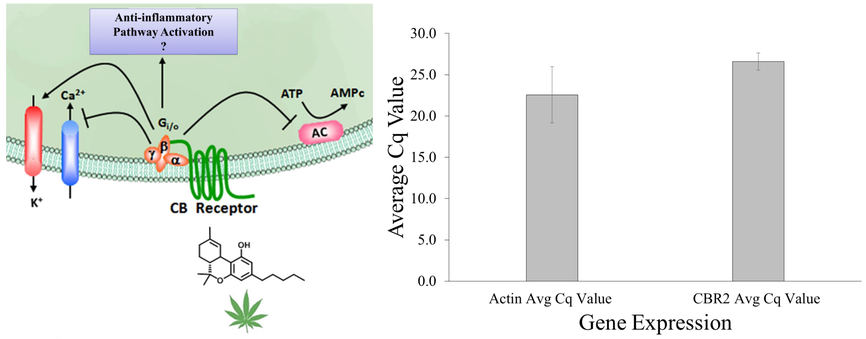


Figure 1. HL60 cells express the CBR2 receptor. Previously literature has shown that HL-60 cells express cannabinoid receptor type 2 (CBR2) [1]. To confirm the HL-60 cell line expresses the receptor, and is thus able to respond to treatment with the drug, RNA was extracted from HL-60 cells grown in culture. Resulting RNA was converted to cDNA and used in a qRT-PCR assay to detect the expression of the CBR2 receptor. In both PCRs a total of 1µg of the same RNA sample was used and a TaqMan® primer/probe set for either Actin and CBR2 was used. As expected, the average Cq value for actin was 22.6 cycles, whereas the average Cq value for CBR2 was 26.6 cycles. This data confirms that HL-60 cells express the CBR2 receptor and are able to respond to the drug. Each sample was run in ≥5 replicates. Standard deviation amongst samples was ±3.4 for actin and ±1.0 for CBR2 as shown by error bars on the graph.

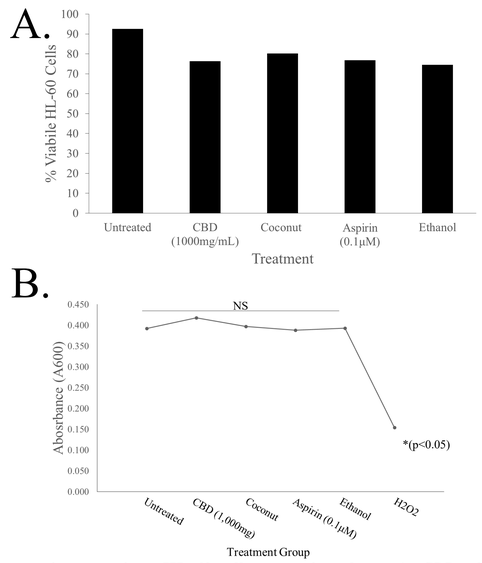
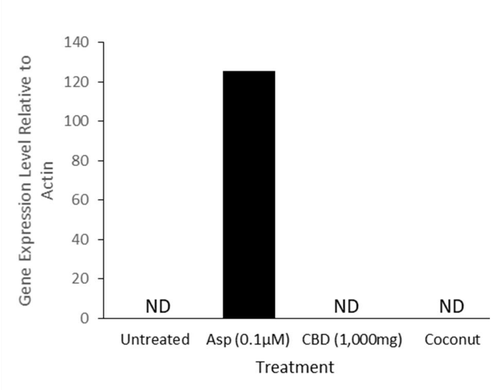


Figure 2. CBD oil treatment is not toxic to HL-60 cells grown in culture. A. *CBD oil treatment does not negatively affect HL-60 cell viability.* Maximum strength commercially CBD oil was purchased from C4HealthLabs LLC, at a concentration of 2,000mg/mL. A 1:2 dilution of the oil into cell culture media provided a 1,000mg/mL solution that was used for all cell treatments. A coconut vehicle control (v/v) was also included. To compare the effects of CBD with the leading anti-inflammatory treatment Asprin (0.1µM in 90% ethanol) was also included, along with an ethanol vehicle control (v/v). HL-60 cells were treated with the drug containing and control solutions for 24hrs, after which a sample was collected for analysis by Trypan Blue staining according to manufactures instructions. Untreated cells had a percent viability of 92%, CBD oil treated cells had 76.4%, coconut oil had 80.1%, Aspirin (0.1µM) had 76.8% and ethanol alone had 74.5%. Thus indicating that CBD oil does not negatively affect cell viability. B. CBD is not cytotoxic to HL-60 cells. In a complementary experiment using a commercially available quantitative MTT assay, all treatment groups had similar A600 values as the untreated cells, all of which were greater than the absorbance of cells treated with the cytotoxic agent, hydrogen peroxide. This data confirmed that CBD oil was not cytotoxic with treated cells having an A600 of 0.4 compared with the untreated A600 of 0.4 and the hydrogen peroxide treated cells A600 of 0.1. Assays were completed using an n=5 replicates. NS difference was detected between untreated and anti-inflammatory treatment conditions (p>0.05, Student T-test, two-tailed). Treatment with hydrogen peroxide resulted in significant cell death, Student T-test, two-tailed, p<0.05.



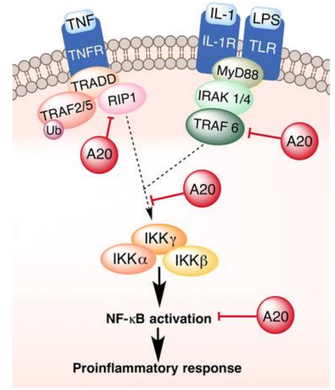


Figure 3. HL-60 cells do not upregulate the anti-inflammatory marker A20 following CBD treatment. A20 protein is an inhibitor of the pro-inflammatory TNFα cytokine and is commonly upregulated following anti-inflammatory treatment.. The HL-60 cells were grown in cell culture with different treatment variants; untreated negative control, Aspirin (0.1µM in 90% ethanol) 1000mg/1ml CBD, and coconut oil vehicle control (v/v). From the cultures RNA was isolated and converted into cDNA for use in qt-PCR to test the expression of the anti-inflammatory marker, A20 relative to Actin. All resulting treatments except aspirin had non-detectable expression. This data only confirms A20 as an anti-inflammatory marker is produced only following aspirin treatment. Confirming that CBD does not utilize the same anti-inflammatory pathway as aspirin.

Conclusion

- Through the experiments, it is shown that CBD is not toxic to cells. However, in a cell culture model, the anti-inflammatory properties that are typically ascribed to CBD were not as readily detectable as the effects of aspirin.
- For the A20 anti-inflammatory pathway, treatment with CBD failed to show any detectable activation. Whereas, Aspirin showed activation of this anti-inflammatory pathway.
- HL-60 cells are immature leukemia cells, so there are very limited cytokines that are expressed during pro/anti-inflammatory conditions. This made it difficult to determine the effects of CBD on several inflammatory and anti-inflammatory mediators.
- In an HL-60 cell culture model, Aspirin was shown to be more effective in activation of anti-inflammatory pathways comparison to CBD.

Future Directions

- Using a cell line that expresses more cytokines to observe changes in the activation of the pro-inflammatory/anti-inflammatory pathways would be useful for future research as the HL-60 cell line is limited in its expression capability.
- Cells could then be challenged with an inflammatory stimulus like LPS and/or serum starvation to produce an inflammatory response. We would be able to see if CBD could actively treat or reduce inflammation.
- Utilizing human subjects who use CBD could further investigate if CBD on a systemic/organismal level could reduce inflammation.
- A study of people who currently use CBD would be effective, to see where it has the potential to accumulate in the body in fat cells to amplify the response.
- Treatment should be monitored over a longer time point. This would help show if CBD is effective over a long period of time. If shown noneffective over a course of 7 days, then it would provide insights into the effectiveness in the long term.

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