Effects of Cannabidiol on Mouse Retinal Microvascular Endothelial Cells

Jenna Tinnell[1], Lucy Sloan[1], Douglas Dean[2], Ph.D., Yongqing Liu[2], Ph.D., Zhao-Hui Song[1], Ph.D.
Department of Pharmacology & Toxicology[1]
Brown Cancer Center and Department of Ophthalmology and Visual Sciences[2]
University of Louisville School of Medicine

Introduction

Angiogenesis is the processes of forming new blood vessels from existing vasculature. It occurs when there is an imbalance of inhibitory signals and/or stimulatory signals. Under pathological conditions, endothelial cells undergo a transition to become migratory and proliferative to form new vasculature (Welch-Reardon, Wu, & Hughes, 2015).

There are multiple pathways involved in angiogenesis. The mTOR signaling pathway is a central regulator of cell proliferation, growth, and survival. mTOR is activated through phosphorylation to act on downstream effectors p70SK61 and 4EBP-1 to regulate endothelial cell growth and proliferation, thus contributing to anagenesis (Kaur & Sharma, 2017). N-cadherin is a cell anchor protein that is involved in angiogenesis (Yu, Yang, Li, & Zhang, 2019). Lastly, β-catenin is a protein that is normally bound to the proteins at the cell membrane, but upon loss of cell-cell contacts it translocates to the nucleus to act as a transcription factor promoting angiogenic processes (Martowicz et al., 2019).

Cannabidiol (CBD), the major non-intoxicating constituent of *Cannabis sativa*, has gained recent attention due to its putative therapeutic uses for a wide variety of diseases. CBD was discovered in the 1940s and its structure fully characterized in the 1960s. In contrast to Δ^9-tetrahydrocannabinol (THC), the lack of intoxicating psychoactivity associated with CBD highlights the potential of this cannabinoid for clinical drug development.

CBD has numerous potential therapeutic uses. Of interest is its potential as a treatment for diseases dependent on neovascularization, such as cancer and neovascular age related macular degeneration (Aebersold, Duff, Sloan, & Song, 2021).

The present study looked at retinal microvascular endothelial cells which line the branching microvasculature that provides nutrition and protection to the retina. The goal of this study is to investigate the effects of CBD on mouse retinal microvascular endothelial cell (mRMVECs) proliferation and expression of proteins involved in the angiogenic process to identify mechanisms of potential therapeutic effects of CBD.

Methods

Cell culture: Mouse primary retinal microvascular endothelial cells (mRMVECs) were purchased from Cell Biologics Inc. and used for all the experiments.

Cell proliferation assay: Cell proliferation was measured with a Bromodeoxyuridine (BrdU) cell proliferation assay kit from Cell Signaling Technology. Cells were treated with 10 μM CBD or vehicle for 48 hours. BrdU was incubated with cells for 4 hours and then detected with primary and secondary antibody solutions with wash steps according to manufacturers protocol. Using a plate reader, spectrophometric detection at 450 nm was used to measure BrdU incorporation.

Western blot analysis: mRMVECs were treated for 48 hours before being collected in lysis buffer (20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM Na_2EDTA, 1 mM EGTA, 1% Triton, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na_3VO_4, and 1 μg/mL leupeptin). Samples were sonicated before being heated at 70 ºC and centrifuged at 12,000 rpm for 1 minute. Subsequently, proteins were resolved on a 10% SDS–polyacrylamide gel using a minigel electrophoresis system (Invitrogen) and protein bands were transferred onto a nitrocellulose membrane. The nitrocellulose membranes were blocked with 5% nonfat dried milk in TBS-T buffer (10 mM Tris-HCl, pH 8.0, 150 mM NaCl, and 0.3% Tween 20) and incubated overnight at 4°C with the antibody of choice (anti-mTOR, anti-phospho-mTOR, anti-p70SK6, anti-phospho-p70S6K1, anti-4EBP-1, anti-phospho-4EBP-1, anti-n-cadherin, anti-beta-catenin, or anti-GAPDH). The membranes were then washed three times for 5 minutes each time with TBS-T buffer and incubated with secondary antibody for 2 hours at room temperature. The membranes were then washed three times with TBS-T buffer for 5 minutes each time and the antibody-recognized protein bands were visualized by chemiluminescence substrates from Thermo Fisher Scientific and quantified using NIH image J.

Data analysis: Data were subject to analysis and graphs were also generated using GraphPad Prism (GraphPad Software, San Diego, CA. Statistical analyses were performed using t test, p-values of <0.05 were considered significant.

Results

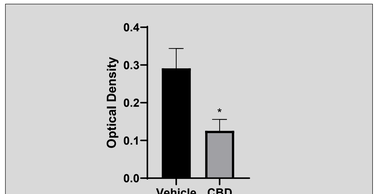


Figure 1: CBD inhibits proliferation of mRMVECs. Cell proliferation was analyzed using the BrdU assay. The cells were treated for 48 hours with 10μM CBD or vehicle. Spectrophotometric detection was performed at 450nm. The graph shows CBD significantly inhibited proliferation, as shown by a decrease in optical density (*p<0.05).

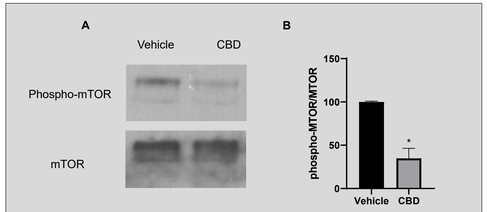


Figure 2: CBD downregulates phosphorylation of mTOR. A. Western blot analysis was used to detect phosphorylation of mTOR. 10μM CBD was used to treat mRMVECs for 48 hours. **B.** The graph shows there is a significant decrease of ratio of phospho-mTOR/total mTOR with CBD treatment comparing with vehicle treatment (*p<0.05).

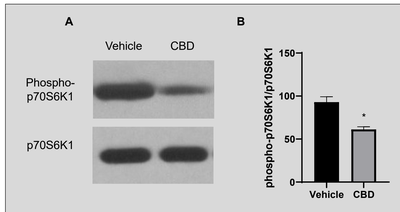


Figure 3: CBD downregulates phosphorylation of p70S6K1. A. Western blot analysis was used to detect phosphorylation of p70S6K1, a protein involved in the mTOR pathway. 10μM CBD was used to treat mRMVECs for 48 hours. **B.** The graph shows there is a significant decrease of ratio of phospho-p70S6K1/total-p70S6K1 with CBD treatment comparing with vehicle treatment (*p<0.05).

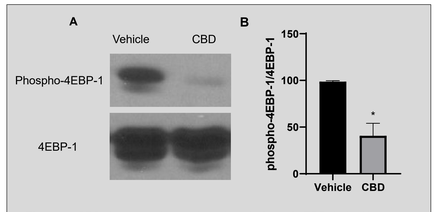


Figure 4: CBD downregulates phosphorylation of 4EBP-1. A. Western blot analysis was used to detect phosphorylation of 4EBP-1, a protein involved in the mTOR pathway. 10μM CBD was used to treat mRMVECs for 48 hours. **B.** The graph shows there is a significant decrease of ratio of phospho-4EBP-1/total-4EBP-1 with CBD treatment comparing with vehicle treatment (*p<0.05).

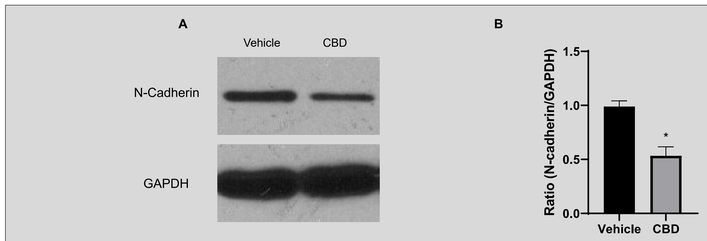


Figure 5: CBD downregulates protein expression N-cadherin. A. Western blot analysis was used to detect expression of N-cadherin. 10μM CBD was used to treat mRMVECs for 48 hours. **B.** The graph shows there is a significant decrease of ratio of N-Cadherin/GAPDH with CBD treatment comparing with vehicle treatment (*p<0.05).

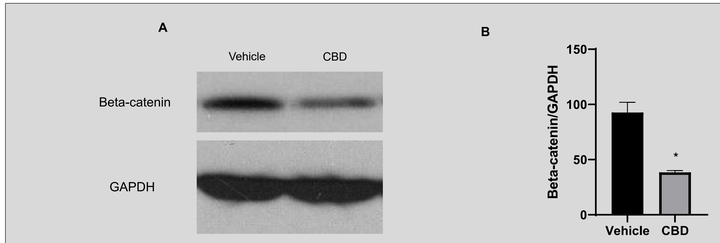
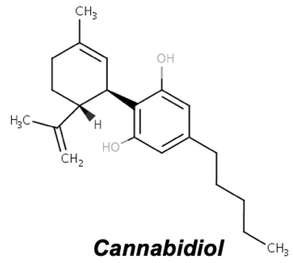


Figure 6: CBD downregulates protein expression of beta-catenin. A. Western blot analysis was used to detect expression of beta-catenin. 10μM CBD was used to treat mRMVECs for 48 hours. **B.** The graph shows there is a significant decrease of ratio of beta-catenin/GAPDH with CBD treatment comparing with vehicle treatment (*p<0.05).

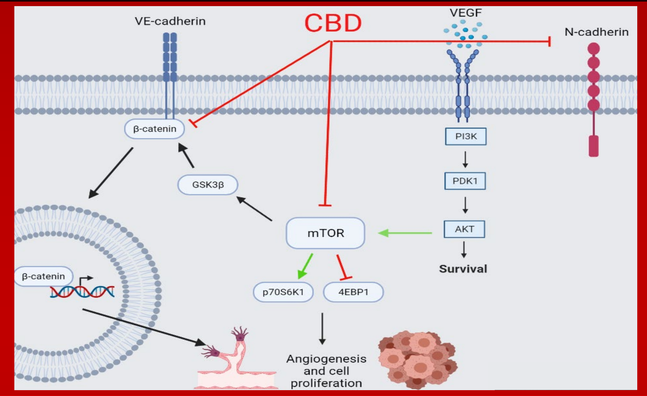
Chemical Structure



Cannabidiol

https://www.britannica.com/plant/cannabis-plant

Pathways Involved



Conclusions

- CBD inhibited mRMVEC proliferation which is important for angiogenesis.
- CBD inhibited phosphorylation of mTOR and its downstream effectors p70S6K1, and 4EBP-1, suggesting that mTOR pathway, which is involved in angiogenesis, is inhibited by CBD.
- CBD down-regulated N-cadherin which is associated with retinal angiogenesis.
- CBD down-regulated β-catenin which is involved in the angiogenic processes.
- Our data suggest that CBD might have therapeutic potentials for ocular diseases involving angiogenesis in the retina.

References

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Acknowledgements

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