Effects of Cannabinoids on Retinal Endothelial Cell Function

Max Duff, Sarah Shrader[1], Douglas, Dean[2], Ph.D., Yongqing Liu[2], Ph.D., Zhao-hui Song[1], Ph.D.
Department of Pharmacology & Toxicology[1]
Department of Ophthalmology and Visual Sciences[2]
University of Louisville School of Medicine

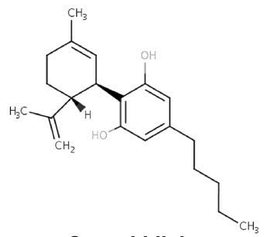
Introduction

Cannabidiol (CBD), the major non-psychoactive constituent of *Cannabis sativa*, has gained increasing attention due to its putative therapeutic uses for a wide variety of diseases and disorders.
The fatty acid amide N-Palymitoyl Dopamine (NPD) is similar to anandamide, a prototypical cannabinoid receptor agonist.
Recently, CBD was identified as inverse agonists at G protein-coupled receptors 3 (GPR3) and 6 (GPR6), two orphan receptors phylogenetically related to the cannabinoid receptors CB1 and CB2.[1,2]
Microvasculature endothelial cell function is very important for the blood-retinal barrier and control of vascular permeability. A early indicator of diabetic retinopathy and age-related macular degeneration is dysfunction of retinal vascular permeability.
Vimentin is a protein found in intermediate filaments and important in cell attachment, migration, signaling, and angiogenesis. RhoA is a molecular switch involved in controlling contractility, adhesion, and cellular polarity. ZEB1 is a transcription factor containing zinc finger and homeobox domain that is important for the regulation of both epithelial-mesenchymal transition (EMT) and endothelial-mesenchymal transition (EndoMT).
The goal of this study is to investigate the effects of CBD and NPD on retinal microvascular endothelial cell proliferation and protein expression to discover potential therapeutic applications of cannabinoids related to eye diseases and angiogenesis.

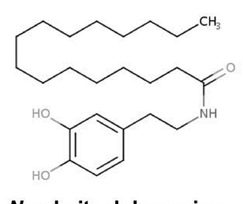
Methods

Mouse primary retinal microvascular endothelial cells (mRMVEC) were obtained from Cell Biologics Inc. and used for all the experiments.
Cell proliferation was measured by counting the cells within multiple designated areas for each treatment group on a 12-well plate.
The mRMVEC cells were treated for 4 days 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 and 4× Laemmli sample buffer added before being boiled 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) for 1 hour and then incubated overnight at 4°C with the anti-Vimentin antibody, the anti-ZEB1 antibody, or the anti-RhoA antibody. Subsequently, the membranes were washed twice 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 enhanced chemiluminescence detection kit.
Data were analyzed using GraphPad Prism (GraphPad Software, San Diego, CA) and the graphs were also generated using GraphPad Prism. Statistical analyses were performed using t test, p-values of <0.05 were considered significant.

Chemical Structures



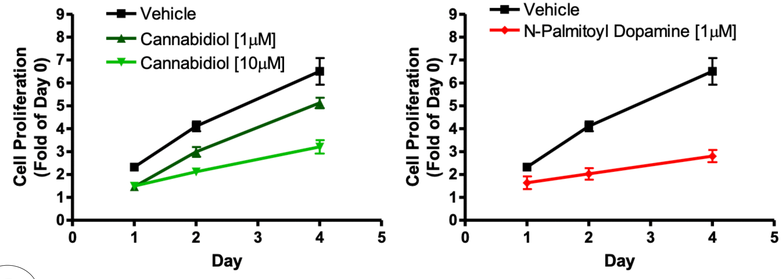
Cannabidiol



***N*-palmitoyl dopamine**

Results

1



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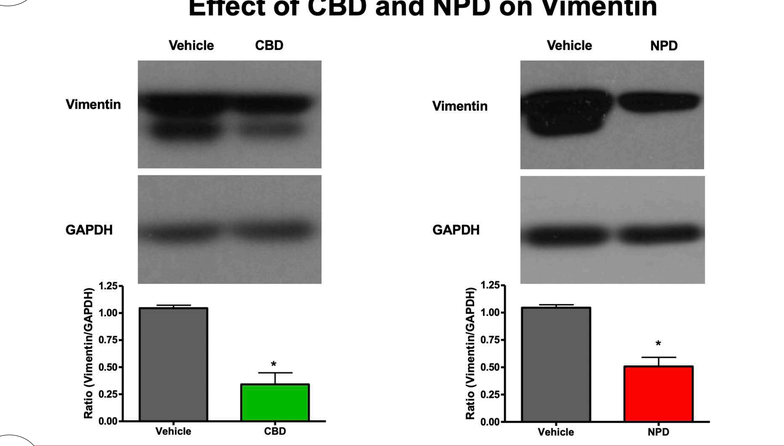

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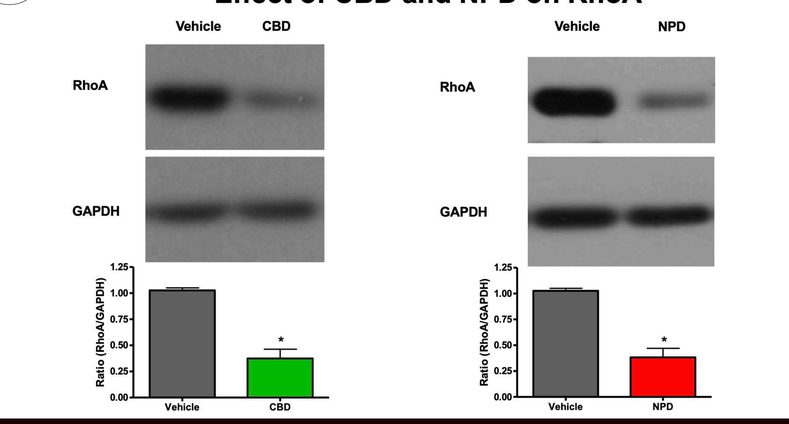

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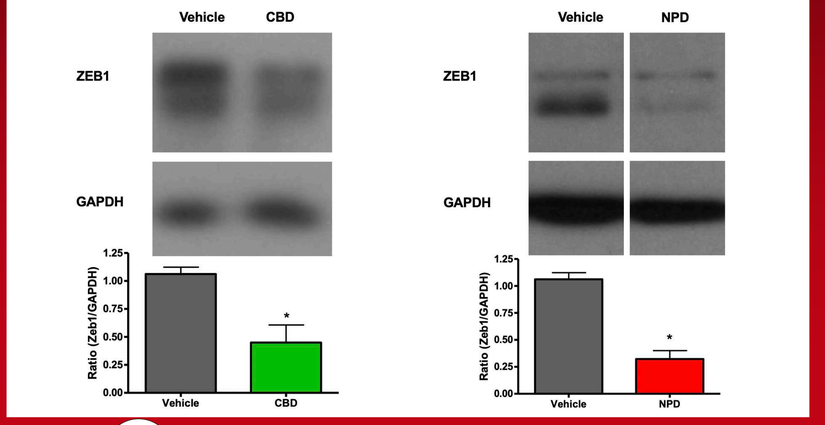

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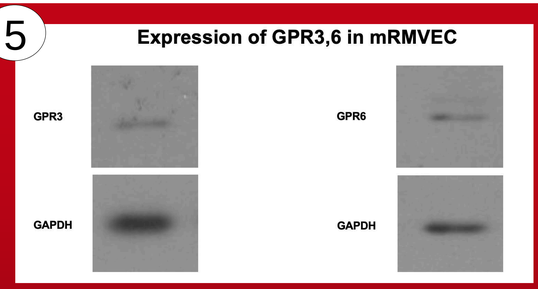

Conclusions

Figure 1 demonstrates that both CBD and NPD decreased the proliferation of the mRMVEC cells. Figures 2, 3, and 4 show that CBD and NPD downregulated protein expression of Vimentin, RhoA, and Zeb1 with statistical significance. Figure 5 shows that the G-protein-coupled receptors 3 and 6 are expressed in the mRMVEC cells.

This data indicates that these two cannabinoids can modulate the function of mRMVEC cells and also suggest cannabinoids could be further explored as potential therapeutic agents. Further study is needed to determine possible therapeutic effects that relate to eye diseases such as diabetic retinopathy and AMD, as well as angiogenesis and cancer metastasis.

References

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2. Laun AS, Shrader SH, Brown KJ and Song ZH (2019) GPR3, GPR6, and GPR12 as novel molecular targets: their biological functions and interaction with cannabidiol. *Acta pharmacologica Sinica* **40**:300-308.

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"Team work makes the Dream work"

"Good, better, best. Never let it rest until your good is better and your better is best."