**Effect of Phytocannabinoids and Endocannabinoids on Ovarian Cancer Cell Proliferation**

Bert Crawford  
Mentor: P. Bagavandoss  
Department of Biological Sciences, Kent State University @ Stark, North Canton, OH

---

**Abstract**

The phytocannabinoids β-tetrahydrocannabinol (â-THC) and cannabidiol (CBD) and the endocannabinoids 2-arachidonoyl glyceryl (2-AG) exhibit antiproliferative effect in various cancer cells derived from different organs, including thyroid, brain, prostate and breast. The response to these cannabinoids involve both cannabinoid receptor-dependent or independent signaling pathways and preserved mitochondrial membrane potential. In this study I investigated the effect of endocannabinoids and the reliance of tumors on aerobic glycolysis and the pathways that the cancer cells use to generate energy. A combination of higher expression of both THC and CBD in ovarian cancer cell lines, exhibits both rapid proliferative and highly invasive properties. The cells were plated at subconfluent density in DMEM-F12 medium containing 10% fetal bovine serum (FBS). After overnight cell attachment to the culture dish, the cells were incubated for 24 hours in serum-free medium. Subsequently, the cells were exposed to various concentrations of the cannabinoids in serum-free medium for 48 hours. Within 24 hours of incubation with phytocannabinoids at approximately 10 µM, both THC and CBD inhibited apoptosis and swelling of the cells. Incubation of these cells with a DNA-binding fluorochrome revealed intact nuclei. R1 methanandamide, a metabolically stable analog of the endocannabinoid arachidonoylethanolamide, also induced cell death at a higher concentration (1-30 µM). On the other hand, the endocannabinoid 2-AG increased the proliferation of the cells. The observed differential effects of cannabinoids on SKOV3 cell proliferation support my hypothesis in part.

**Methods**

**SKOV3 cancer cell culture:** The cells were plated in 96-well plates in 100 µl at 2 x 10⁵ cells/well (subconfluent) in DMEM-F12 medium containing antibiotics, 10% cell serum, and 2 mM glutamine. The experiments, however, were performed in serum-free medium. After 48-hour incubation with test compounds, 10 µl of WST-1 reagent was added to the cells and incubated for 2 hours. The reduction of WST-1 reagent to formazan by cellular dehydrogenase was quantified by measuring its absorbance at 490 nm.

**Confocal Fluorescence microscopy:** Subconfluent cells (25,000/ml) growing in 6-well plates were incubated with NucGreen Dead dye, a dye that only enters dead cells, Mitotracker Green FM, for mitochondria identification or tetramethylrhodamine methyl (TMRM) stain for mitochondrial membrane potential (Δψm). Zymography of conditioned medium: Equal amounts of protein from conditioned media from confluent cell cultures were separated and the gels in the gels were renatured, activated overnight at 37°C and visualized with Coomassie Brilliant blue dye.

**Statistical analysis:** Data were analyzed by a-way ANOVA followed by Bonferroni post hoc analysis. Significance level was established at less than 0.05 ( p < 0.05).

**Background**

Both normal and cancer cells respond and react to both phytocannabinoids (e.g., THC, CBD) and endocannabinoids (e.g., AEA, anandamide). The response to these cannabinoids involve both cannabinoid receptor (CB) dependent and independent mechanisms (Hermanson and Marnett, Cancer Metastasis Rev 30:599, 2011) and the reliance of tumors on aerobic glycolysis (anandamide) and 2-arachidonoylglycerol (2-AG) exhibit antiproliferative effects on various cancer cells. The observed differential effects of cannabinoids on SKOV3 cell proliferation support my hypothesis in part.

**Summary and Future Directions**

- The results of my investigation suggest that both phytocannabinoids and endocannabinoids, with the exception of 2-AG, inhibit the proliferation of SKOV3 ovarian cancer cells. At lower concentrations of cannabinoids, an increase in viable cells was observed. This may represent the typical hormetic effect observed with many compounds on biological systems.
- Both phytocannabinoids decreased the cell viability at lower concentrations than any of the endocannabinoids. However, the ability of the endocannabinoids to decrease the viability of cells may depend partly on their metabolism by the presence of endocannabinoid degrading enzymes in the cells.
- In AEA and M-AEA treated cultures, even the attached cells are dead as evident from the binding of the Nuclear Green Dead dye to DNA.
- At concentrations similar to AEA, the endocannabinoid 2-AG appears non-toxic to the cells. The cells maintain functional mitochondria as evident from the preservation of mitochondrial membrane potential. Upon treatment with 2-AG, the cells also develop perinuclear granular structures.
- Future experiments will test: a) if the effects of cannabinoids are mediated via cannabinoid receptor-dependent or independent signaling pathways and b) if the endocannabinoid metabolizing enzymes fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase ( MAGL) modulate the antiproliferative effects of endocannabinoids.