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Effect of Phytocannabinoids and Endocannabinoids on Ovarian Cancer Cell Proliferation

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Abstract

The phytocannabinoids tetrahydrocannabinol (THC) and cannabidiol (CBD) and the endocannabinoids 2-arachidonylglycerol (2-AG) and anandamide (AEA) exhibit antiproliferative effect on ovarian cancer cells derived from different organs, including thyroid, brain, prostate and breast. THC and the endocannabinoids are known agonists at the G-protein coupled receptors (CB1 and CB2), which appear to mediate the antiproliferative effect on some of the cancer cells. CBD’s antiproliferative effect, on the other hand, is mediated via CB receptor-independent mechanism. In this study, I hypothesized that ovarian cancer cell proliferation will be also inhibited by these cannabinoids. For my study, I used the SKOV3 adenocarcinoma ovarian cell line, which exhibits both rapid proliferative and highly invasive properties. Both normal and cancer cells respond and react to both phytocannabinoids (e.g., THC, CBD) and endocannabinoids (e.g., 2-AG, AEA/anandamide). The response to these cannabinoids involve both phytocannabinoids (e.g., THC, CBD) and endocannabinoids (e.g., 2-AG, AEA/anandamide). The response to these cannabinoids involve both phytocannabinoid receptor (CB) dependent and independent mechanisms (Pertwee, Brit J Pharmacol 153:158, 2008). The effect of cannabinoids on many cancer cells (e.g., from breast, glioma, lung, lymphoma, pancreas, prostate and skin) is primarily antiproliferative (Nikan et al., Life Sci 146:124, 2016). A combination of high expression of cannabinoid receptors (Hanneman and Marnett, Cancer Metastasis Rev 30:599, 2011) and the reliance of tumors on aerobic glycolysis (Kroemer and Pouyssegur, Cancer Cell 13:472, 2008) may account for the preferential cytotoxic effect of cannabinoids on cancer cells. In this study I investigated the effect of endocannabinoids and the phytocannabinoids THC and CBD on SKOV3 ovarian cancer cells.

Background

Both normal and cancer cells respond and react to both phytocannabinoids (e.g., THC, CBD) and endocannabinoids (e.g., 2-AG, AEA/anandamide). The response to these cannabinoids involve both phytocannabinoid receptor (CB) dependent and independent mechanisms (Pertwee, Brit J Pharmacol 153:158, 2008). The effect of cannabinoids on many cancer cells (e.g., from breast, glioma, lung, lymphoma, pancreas, prostate and skin) is primarily antiproliferative (Nikan et al., Life Sci 146:124, 2016). A combination of high expression of cannabinoid receptors (Hanneman and Marnett, Cancer Metastasis Rev 30:599, 2011) and the reliance of tumors on aerobic glycolysis (Kroemer and Pouyssegur, Cancer Cell 13:472, 2008) may account for the preferential cytotoxic effect of cannabinoids on cancer cells. In this study I investigated the effect of endocannabinoids and the phytocannabinoids THC and CBD on SKOV3 ovarian cancer cells.

Methods

SKOV3 cancer cell culture: The cells were plated in 96-well plates in 180 µl at 2 x 10^3 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 wells and incubated for 2 hours. The reduced WST-1 reagent formazan by cellular dehydrogenase was quantified by measuring its absorbance at 450 nm.

Confluent Fluorescence microscopy: Subconfluent cells (25,000/mL) growing in 6-well plates were incubated with Nuclear Green and a dye that only enters dead cells, Mitotracker Green FM, for mitochondrial membrane potential (Δψm) and for mitochondrial identification or tetramethylrhodamine methyl (TMRF) that stains for membrane potential (Δψm). Zymography of conditioned medium: Equal amounts of protein from conditioned medium from confluent cells were separated and the proteins in the gels were renatured, activated overnight in 2% SDS and visualized with Coommassie Brilliant blue dye.

Effect of M-AEA on Cell Viability

In response to M-AEA cells retract but remain attached to the culture dish (24 hours). In spite of the fact that they remain attached, the cells take up the Nuclear Green Dead dye, a fluorescent dye, which only enters the plasma membrane compromised cells and intercalates with DNA (arrow).

Effect of 2-AG on SKOV3 Viability

Effect of M-AEA on SKOV3 Viability

40.00
60.00
80.00
100.00
120.00
Percent Cell Viability

Control

ABA 50 µM

At low concentrations, both phytocannabinoids cause non-significant increase in viability. However, at 8 µM they significantly inhibit the viability of the cells (*p = <0.05).

Confluent cells were incubated with the cannabinoids for 24 hours. Under confluent cell culture conditions, the cannabinoids at above concentrations are not toxic to the cells. Both CBD and THC appear to increase the amount of MMP-2 secreted by the cells.

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 morphology.

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.