Anti-cancer efficacy of CBD Pure Isolates and Commercially Available Water-Soluble CBD in Colorectal Cancer

Gina Bishara[1], Dr. Sarah Daron-Mathis[2], & Dr. Ying Gao[2]
Middle Tennessee State University Department of Biology[1], School of Agriculture[2]

Abstract:

CBD (cannabidiol) is the non-psychotropic cannabinoid in the Cannabis sativa plant, and since the farm bill passed in December 2018 it has become the latest craze in the market. CBD is known to have a good safety profile and has been reported to have neuroprotective, antiepileptic, hypoxia-ischemia, anxiolytic, antipsychotic, analgesic, anti-inflammatory, anti-asthmatic, and antitumor properties, the public belief is that it is the "cure all", but the evidence behind these claims is quite sparse with few studies on just the CBD molecule itself. The mechanism of action for CBD in these processes is not fully understood, leading to this clear gap in information. This study addresses one particular clinical problem, colorectal cancer (CRC), which is the 3rd most common cancer in the United States. CBD as an Anti-Cancer Agent has been studied since the 1970s and there is evidence of it being antitumorigenic by slowing progression, inducing apoptosis, and having anti-proliferative effects in various cancer cells. One review article has suggested that CBD could even enhance current treatments in cancer patients. However, CBD has poor bioavailability, with bioavailability based on route of administration reported as: 5% oral, 13% sublingual, 30% inhaled. Companies are developing what is being called "Water-Soluble" CBD and claim the improvement in bioavailability. This water-soluble CBD would allow for higher concentrations of CBD to be available to a tumor, and a dosage regimen that maintained optimal levels for anti-cancer activity could then be easily identified. Given the availability of such agents, the claims being made for their efficacy, as well as the dire need for effective treatments, this study is designed to determine the efficacy, safety, and validity of water-soluble CBD compounds in colon cancer in vitro and in vivo studies.

Hypothesis: THC-free water-soluble CBD formulations that are now commercially available are more effective anti-cancer agents compared to pure CBD isolates for treating colorectal cancer due to increased bioavailability.

Objectives:

Note on reagents to be used: The CBD water-soluble formulation that will be used in this study must satisfy the following criteria: (1) It must be free of other cannabinoids or terpenes; (2) Purity must be validated by third party testing; and (3) It must be available to purchase in powder form. At this time, there are 3 possible products that fit these criteria on the market. To deidentify them to remove any bias outcome we have classified them with the state that they came from. MI, NC, and TN. The reference pure CBD isolate was a gift from a local extraction company.
This study is set up to determine the anti-tumor effects of CBD and water-soluble CBD on colon cancer cells, the human cell lines shown in Table 1 that represent an array of driver mutational pathways and to challenge with the CBD formulations and evaluated for proliferation, cell death, clonogenic survival, migration and invasion.

Table I: Mutational status of human colon cancer cell lines

Cells	Mutational Status						
	APC	**KRAS**	**BRAF**	**ERBB3**	**PIK3CA/B**	**TP53**	**SMAD4**
DLD1	mut	mut	wt	mut	mut	wt	wt
HCT116	wt	mut	wt	mut	mut/wt	wt	wt
Caco-2	mut	wt	wt	wt	wt	mut	wt
SW620	mut	mut	wt	wt	wt	mut	wt
HT29	mut	wt	mut	wt	mut/wt	mut	mut

mut= mutation; wt= wildtype; "/" is noted in cases where PIK3CA status differs from PIK3CA Refs: (Bamford et al., 2004; Kikuchi et al., 2009; Lee et al., 2011; Mouradov et al., 2014; Segditsas et al., 2009)

Methods:

Determining solubility of these compound we found that CBD isolate is soluble in EtOH and DMSO, and that the water-soluble compounds are easily mixed with PBS. DLD-1 cells have been used thus far and used to determine the parameters of these experiments. To assess the effects of CBD on proliferation, MTS assay was used on cells treated with 0-100uM[1,2] reference isolate and water-soluble CBD. Cells were seeded 8,000 cells per well in a 96 well dish, and treated the following day, then incubated 24hrs with treatments followed by adding MTS solution for 2 hrs. and run at 490nm wavelength on spectrophotometer. Wound closure assays also known as scratch assay was done to evaluate the ability of the cells to migrate after being challenged with the CBD formulations. Cells were placed in 6 well dishes and grown to confluence, then scratched with a 1 ml pipette tip, washed with PBS and then treated for 24 hours.

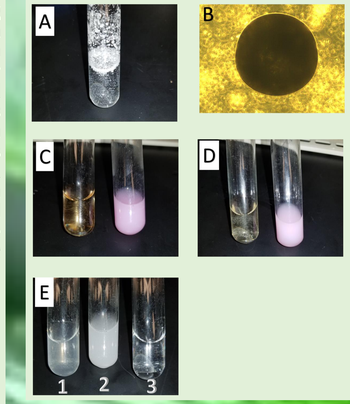

Figure 1: Solubility of different CBD formulations. A. CBD Isolate at 1M concentration in PBS. B. CBD Isolate in DMSO mixed with Cell Culture Media at 100uM under microscope at 10X. C. CBD Isolate in EtOH at 1M (left) CBD Isolate at 10mM in 4:6 ratio of EtOH and Media. D. CBD Isolate in DMSO at 1M (left) CBD Isolate at 10mM in 4:6 ratio of DMSO and Media. E. 1. CBD water-soluble solution from NC at 1mM. 2. CBD water-soluble solution from MI at 1mM. CBD water-soluble solution from TN at 1mM.

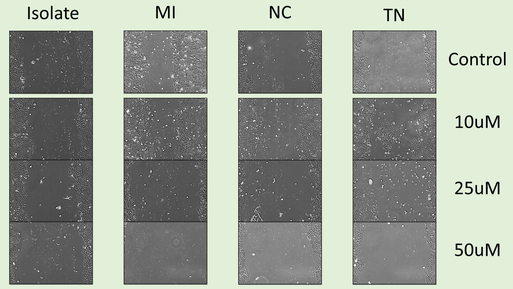

Figure 3: Wound healing assay (Scratch assay) of different CBD formulations. 6 well plates were scratched with pipette tip and then treated with different CBD formulations and amounts for 24 hrs. Plates were then captured by light microscopy 10X to determine if migration took place.

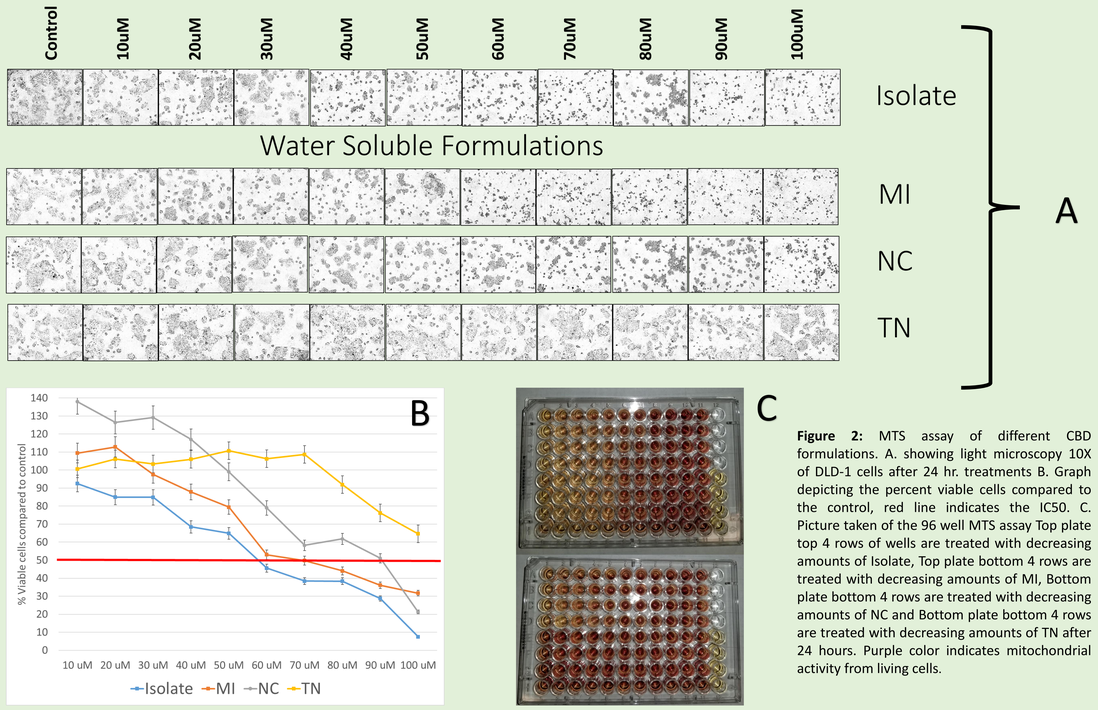

Figure 2: MTS assay of different CBD formulations. A. showing light microscopy 10X of DLD-1 cells after 24 hr. treatments B. Graph depicting the percent viable cells compared to the control, red line indicates the IC50. C. Picture taken of the 96 well MTS assay Top plate top 4 rows of wells are treated with decreasing amounts of Isolate, Top plate bottom 4 rows are treated with decreasing amounts of MI, Bottom plate bottom 4 rows are treated with decreasing amounts of NC and Bottom plate bottom 4 rows are treated with decreasing amounts of TN after 24 hours. Purple color indicates mitochondrial activity from living cells.

Results & Discussion:

While this work has only just started this past January, many discoveries have been made. First, determining the best way to get CBD Isolate into media for cell culture was a challenge. When first mixing the isolated chemical in DMSO as suggested by the Italian lab we found the compound formed spheres in the media. This also occurred when using EtOH. So, finding that if you first dilute in a mixer of 4 parts DMSO 6 parts media, while it makes a pink milky mixture, it is able to evenly disperse evenly in the media for treatments.

Secondly, we found that previous labs have reported IC50 for DLD-1 treated with Isolate was about 6 uM[3] but our results found this was to low and have finally dialed it in to be around 60uM.

We are also seeing a decrease in wound healing in these scratch assays as well and not starting until 50uM.

This discrepancy in a ten-fold amount is alarming but it is still likely in a range that can have therapeutic impact as shown is Figure 4.

Average Man

Height: 5' 9"
Weight: 198
Blood Volume: 5.47 L
Body Volume: 109.2 L

CBD intake needed

By Blood Volume
Daily dose 16mg = 9.3 uM
High suggested dose 60mg = 35 uM
Needed amount 100 mg = 58 uM
(to reach the IC50)

By Body Volume
Daily dose 16mg = 0.5 uM
High suggested dose 60mg = 1.7 uM
Needed amount 2000 mg = 58 uM
(to reach the IC50)

Note: This is with 100% bioavailability

Figure 4: Average man height, weight, blood volume and body volume. Note this is on average for men only women are completely different with blood volume and this is if there is 100% bioavailable by injection directly into the vein. This is just to give an idea of the amounts necessary and not a hard fact.

Conclusion:

This is just the starting point of this work. We now know the best way to treat cells and have found the IC50 of CBD Isolate compound. This information can now lead this research to expanding into the other human colon cancer cell lines, and other assays including Annexin V, and Cleaved Caspase 3 for apoptosis testing, CyQuant Proliferation assay and EdU incorporation assays for more sensitive proliferation assays, clonogenic survival assay, and transwell invasion assay to evaluate the ability of the cells to invade and migrate after being challenged with the CBD formulations. Some other aspects that have been brought up that will be evaluated is 1. How are normal cells at these dosages will react in culture, i.e. safety profiling, 2. Development of a cancer cell line resistant to low dosage and how that effects the IC50 in that cell line. 3. Testing the formulation of the water-soluble compounds without the CBD to see if it is the formulation that is acting on the cells or the CBD.

References:

1. Aviello, G. *et al.* Chemopreventive effect of the non-psychotropic phytocannabinoid cannabidiol on experimental colon cancer. *J. Mol. Med.* **90,** 925–934 (2012).

2. Romano, B. *et al.* Inhibition of colon carcinogenesis by a standardized Cannabis sativa extract with high content of cannabidiol. *Phytomedicine* **21,** 631–639 (2014).

3. Jeong, S. *et al.* Cannabidiol-induced apoptosis is mediated by activation of Noxa in human colorectal cancer cells. Cancer Lett. 447, 12–23 (2019).

Acknowledgements:

Department of Defense - Horizon Award and MTSU for the opportunity and funding for this research. BENMAR Extractions, Volunteer Botanicals, Nano Biotechnology Solutions for compounds and formulation gifts.

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