Cannabinoids in Pediatric Malignancies

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Marijuana

INDIAN FAKIRS prepare bhang and ganja in this painting from the mid-1700s. The history of marijuana extends far back in history, with written records on its medical use appearing in ancient Chinese and Egyptian texts. Discovery in the 1960s of its active component, THC, eventually led to identification of the brain's own "marijuana."
The Endocannabinoid System and Receptors

Synthesizing enzymes
Transporters
Receptors
Degradation enzymes
Endocannabinoids
Novel cannabinoid receptors
Phytocannabinoids
Cannabinoids Receptors Distribution

**CB1 present:**
1. brain
2. lungs
3. vascular system
4. muscles
5. gastrointestinal tract
6. reproductive organs

**CB2 present**
1. spleen
2. bones
3. skin

**CB1+CB2 present**
1. immune system
2. liver
3. bone marrow
4. pancreas

**CB1 present in brain:**
1. cortex
2. caudate nucleus and putamen (nucleus acumbens)
3. basal ganglia
4. hypothalamus
5. cerebelum
6. hippocampus
7. amygdala
8. spinal cord

**CB2 present in brain:**
- glial cells

**CB1+CB2 present in brain:**
1. brainstem
Marijuana – Medical use

General medical use
- Sedative
- anti-inflammatory
- Antispasmodic
- Anticonvulsant

Palliative use in oncology
- Analgesic (nabiximols)
- alleviate nausea and vomit induced by chemotherapy (dronabinol, nabilone)
- appetite stimulation and attenuation of wasting (dronabinol)
- mood elevation
- relief from insomnia

Anti Cancer Treatment
- Cannabinoids are currently being tested as anticancer agents in 2 ongoing clinical studies
Is it evidence based?

Review
Medical Marijuana Use in Oncology
A Review

Gianna Wilkie, BS; Bachir Sakr, MD; Tina Rizack, MD, MPH

**IMPORTANCE** Medicinal marijuana use is currently legal in 23 states and the District of Columbia. As more states approve marijuana use for medical indications, physicians will be asked by their patients for more information regarding the risks and benefits of use. This article reviews the history, adverse effects, and proposed mechanisms of action of marijuana and summarizes the available literature regarding symptom relief and therapeutic value in patients with cancer.

**OBSERVATIONS** Marijuana in oncology may have potential for use as an antiemetic, for refractory cancer pain, and as an antitumor agent. However, much of the data are based on animal data, small trials, or are outdated.

**CONCLUSIONS AND RELEVANCE** More research is needed in all areas related to the therapeutic use of marijuana in oncology.

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- The improvements may be the result of feeling high, sedation and not real effect on pain, nausea etc...
- No comparison to current effective drugs
The use of cannabinoids is in question

- Not approved by the FDA as a treatment for cancer or any other medical condition.
- Many states have moved to legalize marijuana for medical and/or recreational use.
- Psychoactive and addictive issues.
- Physicians - mixed attitudes.
- Problem even bigger in children and adolescents.
Role of endocannabinoid system in adult tumors

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>CB receptors or ECB degrading enzymes</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hodgkin lymphoma</td>
<td>CB1 levels increased</td>
<td>(Benz et al., 2013)</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>CB1 levels increased</td>
<td>(Gustafsson et al., 2008)</td>
</tr>
<tr>
<td>Chemically-induced cellular hepatocarcinoma</td>
<td>CB1 levels increased</td>
<td>(Mukhopadhyay et al., 2015)</td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
<td>CB1 and CB2 expression correlates with improved prognosis of patients with hepatocellular carcinoma</td>
<td>(Xu, 2006 #378)</td>
</tr>
<tr>
<td>Human epithelial ovarian tumors</td>
<td>CB1 levels increased. Correlation with disease severity</td>
<td>(Messalli et al., 2014)</td>
</tr>
<tr>
<td>Stage IV colorectal cancer</td>
<td>CB1 levels are a factor of bad prognosis following surgery</td>
<td>(Jung et al., 2013)</td>
</tr>
<tr>
<td>Colon cancer</td>
<td>CB1 levels decreased, CB1 genetic ablation increases the growth of colon carcinomas</td>
<td>(Wang et al., 2008)</td>
</tr>
<tr>
<td>Pancreatic cancer</td>
<td>CB1 and CB2 levels increased and MAGL and FAAH levels decreased associated with bad prognosis</td>
<td>(Michalski et al., 2008)</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>CB1 levels increased associated with severity of disease and poor prognosis</td>
<td>(Chung et al., 2009)</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>FAAH tumor levels (but not CB1) directly correlate with severity of the diseases</td>
<td>(Thors et al., 2010)</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>CB2 levels increased. Correlation with disease severity</td>
<td>[Caffarel, 2010 #15; Caffarel, 2006 #16; Perez-Gomez et al., 2015 #349]</td>
</tr>
<tr>
<td>Glioma</td>
<td>CB2 levels increased with degree in gliomas</td>
<td>(Sanchez et al., 2001)</td>
</tr>
<tr>
<td>Mantle cell lymphoma</td>
<td>CB1 and CB2 levels increased and FAAH levels decreased</td>
<td>(Ek et al., 2002; Islam et al., 2003; Wasik et al., 2011)</td>
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<tr>
<td>UV light induced skin carcinogenesis</td>
<td>CB1 and CB2 genetic ablation decrease UV light induced skin carcinogenesis</td>
<td>(Zheng et al., 2008)</td>
</tr>
<tr>
<td>Leukemia</td>
<td>CB2 overexpression enhances the predisposition to leukemia after leukemia virus infection.</td>
<td>(Joosten et al., 2002)</td>
</tr>
<tr>
<td>Glioma, breast cancer, skin cancer</td>
<td>GPR55 increased levels associated with higher histological tumor grade</td>
<td>(Andradas et al., 2011; Perez-Gomez et al., 2013)</td>
</tr>
</tbody>
</table>
## Cannabinoid activity in animal models of cancer

<table>
<thead>
<tr>
<th>MODEL</th>
<th>TUMOUR TYPE</th>
<th>EXPERIMENTAL SYSTEM</th>
<th>EFFECT</th>
<th>CANNABINOID</th>
<th>RECEPTOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ectopic xenografts</td>
<td>Glioma</td>
<td>SC injection of cancer cells; ID mice</td>
<td>Decreased tumour size (apoptosis, inhibition of angiogenesis, migration and invasiveness)</td>
<td>$\Delta^9$-THC, JWH-133, WIN 55212-2, CBD</td>
<td>CB1, CB2</td>
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<tr>
<td>Thyroid epithelioma</td>
<td></td>
<td></td>
<td>Decreased tumour size (inhibition of proliferation, apoptosis)</td>
<td>MET-AEA, 2-AG, CBD</td>
<td>CB1, CB2</td>
</tr>
<tr>
<td>Pancreatic carcinoma</td>
<td></td>
<td></td>
<td>Decreased tumour size</td>
<td>$\Delta^9$-THC, JWH-133</td>
<td>CB2</td>
</tr>
<tr>
<td>Breast carcinoma</td>
<td></td>
<td></td>
<td>Decreased tumour size (apoptosis, inhibition of proliferation and angiogenesis)</td>
<td>JWH-133, WIN 55212-2</td>
<td>CB1, CB2</td>
</tr>
<tr>
<td>Lung carcinoma</td>
<td></td>
<td></td>
<td>Decreased tumour size (apoptosis, inhibition of proliferation, invasiveness and angiogenesis)</td>
<td>$\Delta^9$-THC, JWH-133, WIN 55212-2, CBD</td>
<td>CB1, CB2, TRPV1</td>
</tr>
<tr>
<td>Lymphoma</td>
<td></td>
<td></td>
<td>Decreased tumour size (inhibition of proliferation, apoptosis)</td>
<td>MET-AEA</td>
<td>CB1, CB2</td>
</tr>
<tr>
<td>Cholangiocarcinoma</td>
<td></td>
<td></td>
<td>Decreased tumour size (inhibition of proliferation, apoptosis)</td>
<td>AEA</td>
<td>GPR55</td>
</tr>
<tr>
<td>Colon carcinoma</td>
<td></td>
<td></td>
<td>Decreased tumour size (apoptosis)</td>
<td>CB-13</td>
<td>CB1, CB2</td>
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<tr>
<td>Rhabdomyosarcoma</td>
<td></td>
<td></td>
<td>Decreased tumour size (apoptosis)</td>
<td>HU-210</td>
<td>CB1</td>
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<tr>
<td>Prostate carcinoma</td>
<td></td>
<td></td>
<td>Decreased tumour size (inhibition of proliferation, apoptosis)</td>
<td>JWH-015</td>
<td>CB2</td>
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<tr>
<td>Oral carcinoma</td>
<td></td>
<td></td>
<td>Decreased tumour size (inhibition of proliferation)</td>
<td>AM-1241</td>
<td>CB2</td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
<td></td>
<td></td>
<td>Decreased tumour growth (apoptosis)</td>
<td>$\Delta^9$-THC, JWH-015</td>
<td>CB2</td>
</tr>
</tbody>
</table>

Mechanisms of cannabinoid antitumor action

Cannabinoid-induced apoptosis relies on the stimulation of ER stress and autophagy
Clinical experience

• No clinical trials of Cannabis as a treatment for cancer in humans were identified in a PubMed search

• a single, small study of intratumoral injection of delta-9-THC in patients with recurrent glioblastoma multiforme reported potential antitumoral activity (Guzmán et al)

• Clinical data in pediatric use is limited to a few case reports
Summary of current published studies

• Endocannabinoid system plays a tumor suppressor role in different cancer types

• Endocannabinoid system may play a pro-tumorigenic role

• The focus is mainly on THC which is psychotropic

• Role of CBD

• Role in pediatric tumors
Δ⁹-Tetrahydrocannabinol inhibits cell cycle progression by downregulation of E2F1 in human glioblastoma multiforme cells

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Abstract

Background: The active components of Cannabis sativa L., Cannabinoids, traditionally used in the field of cancer for alleviation of pain, nausea, wasting and improvement of well-being have received renewed interest in recent years due to their diverse pharmacologic activities such as cell growth inhibition, anti-inflammatory activity and induction of tumor regression. Here we used several experimental approaches, which identified delta-9-tetrahydrocannabinol (Δ⁹-THC) as an essential mediator of cannabinoid antitumoral action. Methods and results: Administration of Δ⁹-THC to glioblastoma multiforme (GBM) cell lines results in a significant decrease in cell viability. Cell cycle analysis showed G₀/G₁ arrest and did not reveal occurrence of apoptosis in the absence of any sub-G₁ populations. Western blot analyses revealed a THC altered cellular content of proteins that regulate cell progression through the cell cycle. The cell content of E2F1 and Cyclin A, two proteins that promote cell cycle progression, were suppressed in both U251-MG and U87-MG human glioblastoma cell lines, whereas the level of p16INK4A, a cell cycle inhibitor was upregulated. Transcription of thymidylate synthase (TS) mRNA, which is promoted by E2F1, also declined as evident by QRT-PCR. The decrease in E2F1 levels resulted from proteasome mediated degradation and was prevented by proteasome inhibitors. Conclusions: Δ⁹-THC is shown to significantly affect viability of GBM cells via a mechanism that appears to elicit G₁ arrest due to downregulation of E2F1 and Cyclin A. Hence, it is suggested that Δ⁹-THC and other cannabinoids be implemented in future clinical evaluation as a therapeutic modality for brain tumors.
Glioblastoma Multiforme (GBM)

- GBM is the most aggressive form of brain tumor
- Standard therapy: operation, Temozolomide, irradiation - relatively ineffective
- Survival - 1 year (or less...)
- Novel therapeutics are needed
- Renewed interest in the role of cannabinoids in cancer therapy
Δ⁹-THC inhibits proliferation of human glioblastoma multiforme cell lines

**U-251MG**

- **Δ⁹THC 48h**
- **Δ⁹THC 72h**

**U-87MG**

- **Δ⁹THC 48h**
- **Δ⁹THC 72h**
$\Delta^9$-THC inhibits cell cycle progression through G0-G1 transition (G1 arrest)
## Cell Cycle Regulators

<table>
<thead>
<tr>
<th>Protein</th>
<th>U-87 Control</th>
<th>U-87 48h</th>
<th>U-251 Control</th>
<th>U-251 48h</th>
</tr>
</thead>
<tbody>
<tr>
<td>E2F1</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Cyclin A</td>
<td></td>
<td></td>
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<tr>
<td>p16</td>
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<td>p53</td>
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<td>p27</td>
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<td>p21</td>
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</tr>
<tr>
<td>β actin</td>
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</tbody>
</table>
Δ⁹-THC decreases E2F1 protein and RNA expression levels in both human GBM cell lines
E2F1 protein levels might be mediated via the proteasome degradation
Δ⁹-THC effect on protein levels and on genes that are involved in cell cycle progression

U251-MG and U87-MG

Cyclin A
Actin

C 24h 48h
100 28 20
100 32 31

p16
Actin

C 24h 48h
100 214 306
100 165 57

Cyclin A mRNA levels (% of control)

24h
Δ⁹-THC
48h

U-251

Cytoplasm

Cyclin A
β actin

C 6h 19h 30h

Nuclear

Cyclin A
β actin

C 6h 19h 30h

Thymidylate synthase mRNA levels (% of control)

24h
Δ⁹-THC
48h
Cannabinoids and Pediatric Tumors
Cannabinoid receptor 1 (CB1) is a potential drug target for treatment of translocation-positive rhabdomyosarcoma.

Cannabinoids reduce viability of RMS cells

HU210 reduces tumor growth in vivo

Susanne Oesch et al. Mol Cancer Ther 2009;8:1838-1845
In vitro and in vivo efficacy of non-psychoactive cannabidiol in neuroblastoma

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N. Peshes-Yalom PhD,# A. Castiel PhD,‡ D. Waldman MD,## R. Gallily PhD,** R. Mechoulam PhD,‖
and A. Toren MD PhD****

ABSTRACT

Background Neuroblastoma (NBL) is one of the most common solid cancers in children. Prognosis in advanced
NBL is still poor despite aggressive multimodality therapy. Furthermore, survivors experience severe long-term
multi-organ sequelae. Hence, the identification of new therapeutic strategies is of utmost importance. Cannabinoids
and their derivatives have been used for years in folk medicine and later in the field of palliative care. Recently, they
were found to show pharmacologic activity in cancer, including cytostatic, apoptotic, and antiangiogenic effects.

Methods We investigated, in vitro and in vivo, the anti-NBL effect of the most active compounds in Cannabis,
Δ⁹-tetrahydrocannabinol (THC) and cannabidiol (CBD). We set out to experimentally determine the effects of those
compounds on viability, invasiveness, cell cycle distribution, and programmed cell death in human NBL SK-N-SH cells.

Results Both compounds have antitumourigenic activity in vitro and impeded the growth of tumour xenografts
in vivo. Of the two cannabinoids tested, CBD was the more active. Treatment with CBD reduced the viability and
invasiveness of treated tumour cells in vitro and induced apoptosis (as demonstrated by morphology changes, sub-G1
cell accumulation, and annexin V assay). Moreover, CBD elicited an increase in activated caspase 3 in treated cells
and tumour xenografts.

Conclusions Our results demonstrate the antitumourigenic action of CBD on NBL cells. Because CBD is a non-
psychoactive cannabinoid that appears to be devoid of side effects, our results support its exploitation as an effective
anticancer drug in the management of NBL.

Key Words Neuroblastoma, cannabidiol, Δ⁹-tetrahydrocannabinol, apoptosis, tumour xenograft models, non-
psychoactive cannabinoids

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NEUROBLASTOMA

- Neuroblastoma is the most common extracranial solid tumor of infancy.
- It is an embryonal malignancy of the sympathetic nervous system arising from neuroblasts.
- 40% are high risk at presentation.
- HR patients continue to have very poor outcomes despite intensive therapy.
Δ⁹-Tetrahydrocannabinol (THC) and cannabidiol (CBD) reduce viability of neuroblastoma (NBL) cell lines in vitro with CBD having a better effect.
Alteration of SK-N-SH cell cycle progression induced by cannabidiol (CBD)
Apoptotic effects of CBD on SK-N-SH cells
Anti-invasiveness effect of cannabidiol (CBD) on SK-N-SH cells
CBD suppresses tumor growth in a mouse xenograft model and increases cleaved caspase-3 staining in treated xenografts.
Conclusions and future directions

• There exist solid scientific evidences supporting that cannabinoids exhibit a remarkable anticancer activity in preclinical models of cancer

• These agents also show an acceptable safety profile

• Clinical studies aimed at testing cannabinoids as single agents or in combinational therapies are needed.
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Raphael Mechoulam
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Ruth Gallily