An Introduction to the Medical Benefits of Cannabis

Collated by Jeremy Acton **4 April 2011**

Introduction

This paper collates numerous articles on the use of Cannabis for the healing and prevention of many human diseases.

Information is also provided on the Endocannabinoid System, a most import signalling and regulatory system in the human body which is supplemented by the cannabinoids in the Cannabis plant.

In assessing the risks and benefits of Cannabis, it must be noted that the opinions in research still range widely e.g. from the view that "Cannabis causes Cancer" to the most recent research that has proved that "Cannabis cures Cancer."

Even though Cannabis is very effective in the treatment of many ailments, its side effects are not damaging (unlike prescription drugs), and that there is no known toxic or lethal effect in the use of Cannabis.

Many activists for the legalization of Cannabis are concerned that Cannabis extracts and cannabinoid synthetics will be patented for profit by pharmaceutical companies, while the use of the plant by ordinary citizens remains illegal. Legalization for the public benefit must ensure access to the plant by all citizens, whether for recreational/preventive use or as prescribed medication for the treatment of ailments.

The information provided here shows that the prohibition of Cannabis is NOT justified on the grounds of health.

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1. Cannabis as a Treatment for Cancer: Cannabis and Cannabinoids

From website of National Cancer Institute of the US National Institute of Health.

http://www.cancer.gov/cancertopics/pdq/cam/cannabis/healthprofessional/page2

History

Cannabis use for medicinal purposes dates back at least 3,000 years.[1-5] It was introduced into Western medicine in the 1840s by W.B. O'Shaughnessy, a surgeon who learned of its medicinal properties while working in India for the British East Indies Company. Its use was promoted for reported analgesic, sedative, anti-inflammatory, antispasmodic, and anticonvulsant effects.

In 1937, the U.S. Treasury Department introduced the Marihuana Tax Act. This Act imposed a levy of one dollar an ounce for medicinal use of *Cannabis* and one hundred dollars an ounce for recreational use. Physicians in the United States were the principal opponents of the Act. The American Medical Association (AMA) opposed the Act because physicians were required to pay a special tax for prescribing *Cannabis*, use special order forms to procure it, and keep special records concerning its professional use. In addition, the AMA believed that objective evidence that *Cannabis* was addictive was lacking and that passage of the Act would impede further research into its medicinal worth.[6] In 1942, *Cannabis* was removed from the U.S. Pharmacopoeia because of persistent concerns about its potential to cause harm.[2,3]

In 1951, Congress passed the Boggs Act, which for the first time, included *Cannabis* with narcotic drugs. In 1970, with the passage of the Controlled Substances Act, marijuana was classified as a Schedule I drug. Drugs in this category are distinguished as having no accepted medicinal use. Other Schedule I substances include heroin, LSD, mescaline, methaqualone, and gamma-hydroxybutyrate.

Despite its designation as having no medicinal use, *Cannabis* was distributed to patients by the U.S. government on a case-by-case basis under the Compassionate Use Investigational New Drug program established in 1978. Distribution of *Cannabis* through this program was discontinued in 1992.[1-4] In 2010, the U.S. Department of Veteran Affairs

approved marijuana use for patients in states where its medicinal use is legal.

The main psychoactive constituent of *Cannabis* was identified as delta-9-tetrahydrocannabinol (THC). In 1986, synthetic delta-9-THC in sesame oil was licensed and approved for the treatment of chemotherapy -associated nausea and vomiting under the generic name dronabinol. Clinical trials determined that dronabinol was as effective as or better than other antiemetic agents.[7] Dronabinol was also studied for its ability to stimulate weight gain in patients with AIDS in the late 1980s. Clinical trial results showed no significant weight gain, although patients reported an improvement in appetite. [8,9]

Within the past 20 years, the neurobiology of cannabinoids has been analyzed.[10-13] The first cannabinoid receptor, CB1, was pharmacologically identified in the brain in 1988. A second cannabinoid receptor, CB2, was identified in 1993. The highest concentration of CB2 receptors is located on B lymphocytes and natural killer cells, suggesting a possible role in immunity. Endogenous cannabinoids (endocannabinoids) have been identified and appear to have a role in pain modulation, control of movement, feeding behavior, and memory.[11]

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2. Marijuana Cuts Lung Cancer Tumor Growth In Half, Study Shows

http://www.sciencedaily.com/releases/2007/04/070417193338.htm

ScienceDaily (Apr. 17, 2007) — The active ingredient in marijuana cuts tumor growth in common lung cancer in half and significantly reduces the ability of the cancer to spread, say researchers at Harvard University who tested the chemical in both lab and mouse studies.

They say this is the first set of experiments to show that the compound, Delta-tetrahydrocannabinol (THC), inhibits EGF-induced growth and migration in epidermal growth factor receptor (EGFR) expressing non-small cell lung cancer cell lines. Lung cancers that over-express EGFR are usually highly aggressive and resistant to chemotherapy.

THC that targets cannabinoid receptors CB1 and CB2 is similar in function to endocannabinoids, which are cannabinoids that are naturally produced in the body and activate these receptors. The researchers suggest that THC or other designer agents that activate these receptors might be used in a targeted fashion to treat lung cancer.

"The beauty of this study is that we are showing that a substance of abuse, if used prudently, may offer a new road to therapy against lung cancer," said Anju Preet, Ph.D., a researcher in the Division of Experimental Medicine.

Acting through cannabinoid receptors CB1 and CB2, endocannabinoids (as well as THC) are thought to play a role in variety of biological functions, including pain and anxiety control, and inflammation. Although a medical derivative of THC, known as Marinol, has been approved for use as an appetite stimulant for cancer patients, and a small number of U.S. states allow use of medical marijuana to treat the same side effect, few studies have shown that THC might have anti-tumor activity, Preet says. The only clinical trial testing THC as a treatment against cancer growth was a recently completed British pilot study in human glioblastoma.

In the present study, the researchers first demonstrated that two different lung cancer cell lines as well as patient lung tumor samples express CB1 and CB2, and that non-toxic doses of THC inhibited growth and spread in the cell lines. "When the cells are pretreated with THC, they have less EGFR stimulated invasion as measured by various in-vitro assays," Preet said.

Then, for three weeks, researchers injected standard doses of THC into mice that had been implanted with human lung cancer cells, and found that tumors were reduced in size and weight by about 50 percent in treated animals compared to a control group. There was also about a 60 percent reduction in cancer lesions on the lungs in these mice as well as a significant reduction in protein markers associated with cancer progression, Preet says.

Although the researchers do not know why THC inhibits tumor growth, they say the substance could be activating molecules that arrest the cell cycle. They speculate that THC may also interfere with angiogenesis and vascularization, which promotes cancer growth.

Preet says much work is needed to clarify the pathway by which THC functions, and cautions that some animal studies have shown that THC can stimulate some cancers. "THC offers some promise, but we have a long way to go before we know what its potential is," she said.

3. Cannabinoids As Cancer Hope

by Paul Armentano
Senior Policy Analyst
NORML | NORML Foundation
www.norml.org

“Cannabinoids possess ... anticancer activity [and may] possibly represent a new class of anti-cancer drugs that retard cancer growth, inhibit angiogenesis (the formation of new blood vessels) and the metastatic spreading of cancer cells." So concludes a comprehensive review published in the October 2005 issue of the scientific journal *Mini-Reviews in Medicinal Chemistry*.

Not familiar with the emerging body of research touting cannabis' ability to stave the spread of certain types of cancers? You're not alone.

For over 30 years, US politicians and bureaucrats have systematically turned a blind eye to scientific research indicating that marijuana may play a role in cancer prevention -- a finding that was first documented in 1974. That year, a research team at the Medical College of Virginia (acting at the behest of the federal government) discovered that cannabis inhibited malignant tumor cell growth in culture and in mice. According to the study's results, reported nationally in an Aug. 18, 1974, *Washington Post* newspaper feature, administration of marijuana's primary cannabinoid THC, "slowed the growth of lung cancers, breast cancers and a virus-induced leukemia in laboratory mice, and prolonged their lives by as much as 36 percent."

Despite these favorable preclinical findings, US government officials dismissed the study (which was eventually published in the *Journal of the National Cancer Institute* in 1975), and refused to fund any follow-up research until conducting a similar -- though secret -- clinical trial in the mid-1990s. That study, conducted by the US National Toxicology Program to the tune of $2 million concluded that mice and rats administered high doses of THC over long periods experienced greater protection against malignant tumors than untreated controls.

Rather than publicize their findings, government researchers once again shelved the results, which only came to light after a draft copy of its findings were leaked in 1997 to a medical journal, which in turn forwarded the story to the national media.

Nevertheless, in the decade since the completion of the National Toxicology trial, the U.S. government has yet to encourage or fund additional, follow up studies examining the cannabinoids' potential to protect against the spread cancerous tumors.

Fortunately, scientists overseas have generously picked up where US researchers so abruptly left off. In 1998, a research team at Madrid's Complutense University discovered that THC can selectively induce apoptosis (program cell death) in brain tumor cells without negatively impacting the surrounding healthy cells. Then in 2000, they reported in the journal *Nature Medicine* that injections of synthetic THC eradicated malignant gliomas (brain tumors) in one-third of treated rats, and prolonged life in another third by six weeks.

In 2003, researchers at the University of Milan in Naples, Italy, reported that non-psychoactive compounds in marijuana inhibited the growth of glioma cells in a dose dependent manner and selectively targeted and killed malignant cancer cells.

The following year, researchers reported in the journal of the American Association for Cancer Research that marijuana's constituents inhibited the spread of brain cancer in human tumor biopsies. In a related development, a research team from the University of South Florida further noted that THC can also selectively inhibit the activation and replication of gamma herpes viruses. The viruses, which can lie dormant for years within white blood cells before becoming active and spreading to other cells, are thought to increase one's chances of developing cancers such as Karposis Sarcoma, Burkitts lymphoma, and Hodgkins disease.

More recently, investigators published pre-clinical findings demonstrating that cannabinoids may play a role in inhibiting cell growth of colectoral cancer, skin carcinoma, breast cancer, and prostate cancer, among other conditions. When investigators compared the efficacy of natural cannabinoids to that of a synthetic agonist, THC proved far more beneficial – selectively decreasing the proliferation of malignant cells and inducing apoptosis more rapidly than its synthetic alternative while simultaneously leaving healthy cells unscathed.

Nevertheless, US politicians have been little swayed by these results, and remain steadfastly opposed to the notion of sponsoring – or even acknowledging – this growing body clinical research, preferring instead to promote the unfounded notion that cannabis use causes cancer. Until this bias changes, expect the bulk of research investigating the use of cannabinoids as anticancer agents to remain overseas and, regrettably, overlooked in the public discourse.

4. The Endocannabinoid system

From Wikipedia, the free encyclopedia

The **endocannabinoid system** refers to a group of neuromodulatory lipids and their receptors that are involved in a variety of physiological processes including appetite, pain-sensation, mood, and memory. It is named for endocannabinoids, the endogenous lipids that bind cannabinoid receptors (the same receptors that mediate the psychoactive effects of cannabis). Broadly speaking, the endocannabinoid system refers to:

- The cannabinoid receptors CB_1 and CB_2, two G protein-coupled receptors primarily located in the central nervous system and periphery, respectively.
- The endogenous arachidonate-based lipids, anandamide (*N*-arachidonoylethanolamine, AEA)) and 2-arachidonoylglycerol (2-AG), collectively termed the "endocannabinoids", that are ligands for the cannabinoid receptors.
- Enzymes synthesize and degrade the endocannabinoids anandamide and 2-AG. Unlike neurotransmitters, endogenous cannabinoids are not stored in vesicles after synthesis, but are synthesized on demand (Rodriguez de Fonseca *et al.*, 2004)[*citation needed*].

The endocannabinoid system has been studied using genetic and pharmacological methods. These studies have revealed a broad role for endocannabinoid signaling in a variety of physiological processes, including neuromodulator release,[1][2][3] motor learning,[4] synaptic plasticity,[5] appetite,[6] and pain sensation.[7]

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Introduction

Endocannabinoid synthesis & release

In standard neurotransmission, the pre-synaptic neuron releases neurotransmitter into the synaptic cleft which binds to cognate receptors expressed on the post-synaptic neuron. Upon binding, the neuron depolarizes. This depolarization facilitates the influx of calcium into the neuron; this increase in calcium activates an enzyme called transacylase which catalyzes the first step of endocannabinoid biosynthesis by converting phosphatidylethanolamine, a membrane-resident phospholipid, into N-acyl-phosphatidylethanolamine (NAPE). Experiments have shown that multiple phospholipases cleave NAPE to yield anandamide [8][9]. In NAPE-phospholipase D (NAPEPLD) knockouts, the PLD-mediated cleavage of NAPE is reduced, not abolished, in low calcium concentrations, suggesting multiple, distinct pathways are involved in AEA biosynthesis (Leung et al., 2006). Once released into the extracellular space by a putative endocannabinoid transporter, messengers are vulnerable to glial inactivation. Endocannabinoids are uptaken via a putative transporter and degraded by fatty acid amide hydrolase (FAAH) which cleaves anandamide and MGLL, which cleaves 2-AG to arachidonic acid & ethanolamine and arachidonic acid & glycerol, respectively (reviewed in Pazos et al., 2005). While arachidonic acid is a substrate for leukotriene and prostaglandin synthesis, it is unclear whether this degradative byproduct has novel functions in the CNS (Yamaguchi et al., 2001; Brock, T., 2005). Emerging data in the field also points to FAAH being expressed in the postsynaptic neuron, suggesting it also contributes to the clearance and inactivation of anandamide and 2-AG by endocannabinoid reuptake.

Endocannabinoid binding & signal transduction

While there have been some papers that have linked concurrent stimulation of dopamine and CB1 receptors to an acute rise in cAMP production, it is accepted that CB1 activation causes an inhibition of cyclic adenosine monophosphate (or cAMP) when activated alone. This inhibition of cAMP is followed by phosphorylation and subsequent activation of not only a suite (p38/p42/p44) of MAP kinases but also the PI3/PKB and MEK/ERK pathway (Galve-Roperh et al., 2002; Davis et al., 2005; Jones et al., 2005; Graham et al., 2006). Results from rat hippocampal gene chip data after acute administration of tetrahydrocannabinol showed an increase in the expression of myelin basic protein, endoplasmic proteins, cytochrome oxidase, and two cell adhesion molecules: NCAM, and SC1; decreases in expression were seen in both calmodulin and ribosomal RNAs (Kittler et al., 2000). In addition, CB1 activation has been demonstrated to increase the activity of transcription factors like c-Fos and Krox-24 (Graham et al., 2006).

Endocannabinoid binding & alterations in neuronal excitability

The molecular mechanisms of CB1-mediated changes to the membrane voltage have also been studied in detail. CB1 agonists reduce calcium influx by blocking the activity of voltage-dependent N-, P/Q- and L-type calcium channels.[10][11] In addition to acting on calcium channels, Gi/o and Gs, subunits of G protein-coupled receptors, activation has also been shown to modulate potassium channel activity. Recent studies have found that CB1 activation facilitates GIRK, a potassium channel belonging to the Kir3 family.[11] Corroborating Guo and Ikeda, Binzen et al. performed a series of immunohistochemistry experiments that demonstrated CB1 co-localized with GIRK and Kv1.4 potassium channels, suggesting that these two may interact in physiological contexts[12]. In the central nervous system, CB1 receptors, for the most part, influence neuronal excitability indirectly, by reducing the impact of incoming synaptic input[13]. This mechanism ("presynaptic inhibition") is believed to occur when a neuron ("postsynaptic") releases endocannabinoids in a retrograde fashion, binding to CB1 receptors expressed on nerve terminals of an input neuron ("presynaptic"). CB1 receptors then reduce the amount of neurotransmitter released, so that subsequent input from the presynaptic neuron has less of an impact on the postsynaptic neuron. It is likely that presynaptic inhibition uses many of the same ion channel mechanisms listed above, although recent evidence has shown that CB1 receptors can also regulate neurotransmitter release by a non-ion channel mechanism, i.e. through Gi/o mediated inhibition of adenylyl cyclase and Protein Kinase A[14] Still, direct effects of CB1 receptors on membrane excitability have been reported, and strongly impact the firing of cortical neurons[15] In a series of behavioral experiments, Palazzo et al. demonstrated that NMDA, an ionotropic glutamate receptor, and the metabotropic glutamate

receptors (mGluRs) work in concert with CB1 to induce analgesia in mice, although the mechanism underlying this effect is unclear. Together, these findings suggest that CB1 influences neuronal excitability by a variety of mechanisms, and these effects are relevant to perception and behavior.

CB1 -/- phenotype

Neuroscientists often utilize transgenic CB1 knockout mice (i.e. the mice have had the gene encoding the CB1 receptor deleted or removed) to discern novel roles for the ECS. While CB1 knockout mice are healthy and live into adulthood, there are some differences among mice without CB1 and wild-type (i.e. "normal" mice with the receptor intact); When under a high-fat diet CB1 knockout mice tend to be about sixty percent leaner and slightly less hungry than wildtype[16]. Compared to wildtype, CB1 knockout mice exhibit severe deficits in motor learning, memory retrieval, and increased difficulty in completing the Morris water maze[4][17][18]. There is also evidence indicating that these knockout animals have an increased incidence and severity of stroke and seizure (Parmentier et al., 2002; Marsicano et al., 2003).

ECS changes induced by cannabis consumption

Memory

Mice treated with tetrahydrocannabinol show suppression of long-term potentiation in the hippocampus - a process that is essential for the formation and storage of long-term memory[19]. These results concur with anecdotal evidence suggesting that smoked preparations of *Cannabis* attenuates short-term memory[20]. Indeed, mice without the CB1 receptor show enhanced memory and long-term potentiation indicating that the endocannabinoid system may play a pivotal role in the extinction of old memories. Recent research reported in a 2005 *Journal Of Clinical Investigation* article[21] indicate that the high-dose treatment of rats with the synthetic cannabinoid, HU-210 over a period of a few weeks resulted in stimulation of neural growth in the rats' hippocampus region, a part of the limbic system playing a part in the formation of declarative and spatial memories.

Appetite

Emerging data suggests that THC acts via CB1 receptors on hypothalamic nuclei, thus directly increasing appetite[22]. It is thought that hypothalamic neurons tonically produce endocannabinoids that work to tightly regulate hunger. The amount of endocannabinoids produced is inversely correlated with the amount of leptin in the blood[23]. For example, mice without leptin not only become massively obese but have higher-than-normal levels of hypothalamic endocannabinoids[24]. Similarly, when these mice were treated with an endocannabinoid antagonist, such as Rimonabant, food intake was

reduced[24]. When the CB1 receptor is knocked out in mice, these animals tend to be leaner and less hungry than wild-type (or "normal") mice. While there is need for more research, these results (and others) suggest that exogenous cannabinoids (as from smoking marijuana) in the hypothalamus activates a pathway responsible for food-seeking behavior[22].

ECS and multiple sclerosis

Historical records from ancient China and Greece suggest that preparations of *Cannabis Indica* were commonly prescribed to ameloriate multiple sclerosis-like symptoms such as tremors and muscle pain; unfortunately, however, treatment with marinol hasn't shown the same efficacy as inhaled Cannabis [25][26]. Due to the illegality of Cannabis and rising incidence of multiple sclerosis patients who self-medicate with the drug, there has been much interest in exploiting the endocannabinoid system in the cerebellum to provide a legal and effective relief.[20] In mouse models of multiple sclerosis, there is a profound reduction and reorganization of CB1 receptors in the cerebellum (Cabranes et al., 2006). Serial sections of cerebellar tissue subjected to immunohistochemistry revealed that this aberrant expression occurred during the relapse phase but returned to normal during the remitting phase of the disease (Cabranes et al., 2006). There is recent data indicating that CB1 agonists promote the *in vitro* survival of oligodendrocytes, specialized support glia that are involved in axonal myelination, in the absence of growth and trophic factors; in addition, these agonist have been shown to promote mRNA expression of myelin lipid protein. (Kittler et al., 2000; Mollna-Holgado et al., 2002). Taken together, these studies point to the exciting possibility that cannabinoid treatment may not only be able to attenuate the symptoms of multiple sclerosis but also improve oligodendrocyte function (reviewed in Pertwee, 2001; Mollna-Holgado et al., 2002). 2-arachidonylglycerol stimulates proliferation of a microglial cell line by a CB_2 receptor dependent mechanism, and the number of microglial cells is increased in multiple sclerosis.[27]

Role in human female reproduction

The developing embryo expresses cannabinoid receptors early in development that are responsive to anandamide which is secreted in the uterus. This signaling is important in regulating the timing of embryonic implantation and uterine receptivity. In mice, it has been shown that anandamide modulates the probability of implantation to the uterine wall. For example, in humans, the likelihood of miscarriage increases if uterine anandamide levels are too high or low[28]. These results suggest that proper intake of exogenous cannabinoids (e.g. marijuana) can decrease the likelihood for pregnancy for women with high anandamide levels, and alternatively, it can increase the likelihood for pregnancy in women whose anandamide levels were too low.[29][30].

Role in hippocampal neurogenesis

In the adult brain, the endocannabinoid system facilitates neurogenesis ("birth of new neurons") of hippocampal granule cells[21][31]. In the subgranular zone of the dentate gyrus, multipotent neural progenitors (NP) give rise to daughter cells that, over the course of several weeks, mature into granule cells whose axons project to and synapse onto dendrites on the CA3 region[32]. Very recent data suggests that the maturing granule cells are dependent on a reelin, a molecular guidance cue, for proper migration through the dentate gyrus (Gong et al., 2007). NPs in the hippocampus have been shown to possess FAAH and express CB1 and utilize 2-AG.[31] Intriguingly, CB1 activation by endogenous or exogenous promote NP proliferation and differentiation; this activation is absent in CB1 knockouts and abolished in the presence of antagonist.[21][31]

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5. Cannabinoid action induces autophagy-mediated cell death through stimulation of ER stress in human glioma cells

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Autophagy can promote cell survival or cell death, but the molecular basis underlying its dual role in cancer remains obscure. Here we demonstrate that Δ^9-tetrahydrocannabinol (THC), the main active component of marijuana, induces human glioma cell death through stimulation of autophagy. Our data indicate that THC induced ceramide accumulation and eukaryotic translation initiation factor 2α (eIF2α) phosphorylation and thereby activated an ER stress response that promoted autophagy via tribbles homolog 3–dependent (TRB3-dependent) inhibition of the Akt/mammalian target of rapamycin complex 1 (mTORC1) axis. We also showed that autophagy is upstream of apoptosis in cannabinoid-induced human and mouse cancer cell death and that activation of this pathway was necessary for the antitumor action of cannabinoids in vivo. These findings describe a mechanism by which THC can promote the autophagic death of human and mouse cancer cells and provide evidence that cannabinoid

administration may be an effective therapeutic strategy for targeting human cancers.

Introduction

Macro-autophagy, hereafter referred to as "autophagy," is a highly conserved cellular process in which cytoplasmic materials — including organelles — are sequestered into double-membrane vesicles called autophagosomes and delivered to lysosomes for degradation or recycling (1). In many cellular settings, triggering of autophagy relies on the inhibition of mammalian target of rapamycin complex 1 (mTORC1), an event that promotes the activation (de-inhibition) of several autophagy proteins (Atgs) involved in the initial phase of membrane isolation (1). Enlargement of this complex to form the autophagosome requires the participation of 2 ubiquitin-like conjugation systems. One involves the conjugation of ATG12 to ATG5 and the other of phosphatidylethanolamine to LC3/ATG8 (1). The final outcome of the activation of the autophagy program is highly dependent on the cellular context and the strength and duration of the stress-inducing signals (2–5). Thus, besides its role in cellular homeostasis, autophagy can be a form of programmed cell death, designated "type II programmed cell death," or play a cytoprotective role, for example in situations of nutrient starvation (6). Accordingly, autophagy has been proposed to play an important role in both tumor progression and promotion of cancer cell death (2–4), although the molecular mechanisms responsible for this dual action of autophagy in cancer have not been elucidated.

Δ^9-Tetrahydrocannabinol (THC), the main active component of marijuana (7), exerts a wide variety of biological effects by mimicking endogenous substances — the endocannabinoids — that bind to and activate specific cannabinoid receptors (8). One of the most exciting areas of research in the cannabinoid field is the study of the potential application of cannabinoids as antitumoral agents (9). Cannabinoid administration has been found to curb the growth of several types of tumor xenografts in rats and mice (9, 10). Based on this preclinical evidence, a pilot clinical trial has been recently run to investigate the antitumoral action of THC on recurrent gliomas (11). Recent findings have also shown that the pro-apoptotic and tumor growth–inhibiting activity of cannabinoids relies on the upregulation of the transcriptional co-activator p8 (12) and its target the pseudo-kinase tribbles homolog 3 (TRB3) (13). However, the mechanisms that promote the activation of this signaling route as well as the targets downstream of TRB3 that mediate its tumor cell–killing action remain elusive. In this study we found that ER stress–evoked upregulation of the p8/TRB3 pathway induced autophagy via inhibition of the Akt/mTORC1 axis and that activation of autophagy promoted the apoptotic death of tumor cells. The uncovering of this pathway, which we believe is novel, for promoting tumor cell death may have therapeutic implications in the treatment of cancer.

Results

Autophagy mediates THC-induced cancer cell death. As a first approach to gain insight into the morphological changes induced in cancer cells by cannabinoid administration, we performed electron microscopy analysis of U87MG human astrocytoma cells. Interestingly, double membrane vacuolar structures with the morphological features of autophagosomes were observed in THC-treated cells (Figure 1, A–C). The conversion of the soluble form of LC3 (LC3-I) to the lipidated and autophagosome-associated form (LC3-II) is considered one of the hallmarks of autophagy (1), and thus we observed the occurrence of LC3-positive dots as well as the appearance of LC3-II (Figure 1D) in cannabinoid-challenged cells. In addition, co-incubation with the lysosomal protease inhibitors E64d and pepstatin A, which blocks the last steps of autophagic degradation (14), enhanced THC-induced accumulation of LC3-II (Figure 1E), confirming that cannabinoids induce dynamic autophagy in U87MG cells. Furthermore, incubation with the cannabinoid receptor 1 (CB1) antagonist SR141716 prevented THC-induced LC3 lipidation and formation of LC3 dots (Figure 1D), indicating that induction of autophagy by cannabinoids relies on CB1 receptor activation.

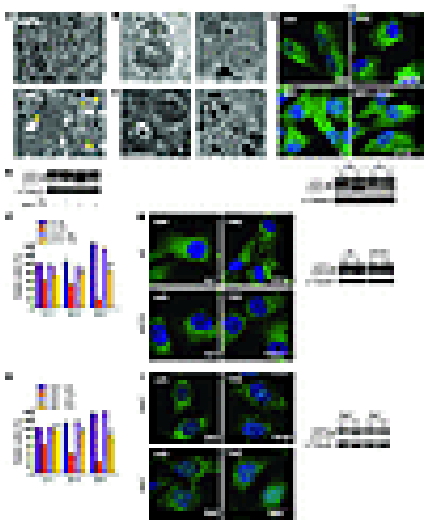
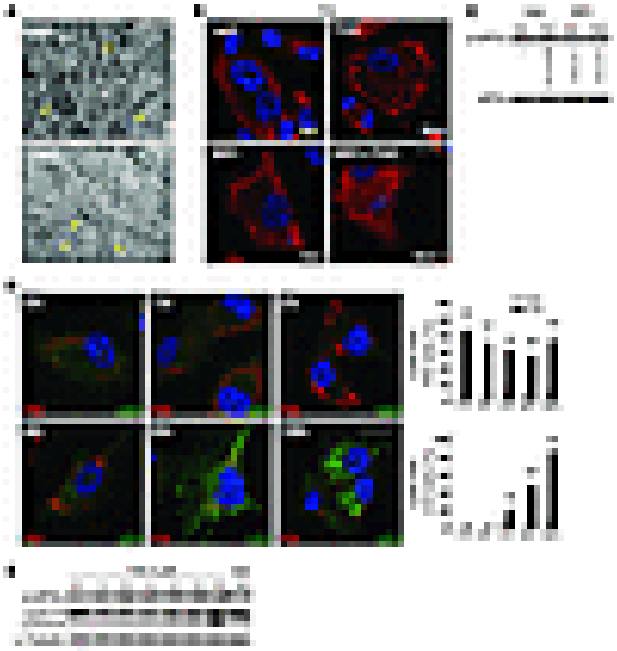


Figure 1

Inhibition of autophagy prevents THC-induced cancer cell death. (**A**–**C**) Effect of THC on U87MG cell morphology. Representative electron microscopy photomicrographs are shown (6 h). Scale bars: 500 nm. Note the presence of early (**A**, open arrows, and **B**) and late (**A**, filled arrows, and **C**) autophagosomes in THC-treated but not vehicle-treated (veh-treated) cells. (**D**) Top: Effect of SR141716 (SR1; 1 μM) and THC on LC3 immunostaining (green) in U87MG cells (18 h; *n* = 3). The percentage of cells with LC3 dots relative to the total cell number is shown in the corner of each panel (mean ± SD). Scale bar: 20 μm. Bottom: Effect of SR1 and THC on LC3 lipidation in U87MG cells (18 h; *n* = 3). (**E**) Effect of E64d (10 μM) and pepstatin A (PA; 10 μg/ml) on THC-induced LC3 lipidation in U87MG cells (18 h; *n* = 3). (**F** and **G**) Effect of THC treatment and transfection with control siRNAs (siC) or ATG1-selective siRNAs (siATG1) on cell viability (**F**; mean ± SD; *n* = 3), LC3 immunostaining (**G**, left panels; 18 h; percentage of cells with LC3 dots relative to the total number of cells cotransfected with a red fluorescent control siRNA, mean ± SD; *n* = 3; scale bar: 20 μm), and LC3 lipidation (**G**, right panel; 18 h; *n* = 3) in U87MG cells. (**H** and **I**) Effect of THC on cell viability (**H**; mean ± SD; *n* = 3), LC3 immunostaining (**I**, left panels; 18 h; percentage of cells with LC3 dots relative to the total cell number, mean ± SD; *n* = 3; scale bar: 20 μm), and LC3 lipidation (**I**, right panel; 18 h; *n* = 3) in *Atg5*$^{+/+}$ and *Atg5*$^{-/-}$ Ras^{V12}/T-large antigen MEFs. *$P < 0.05$ and **$P < 0.01$ compared with THC-treated U87MG



(**D**) and *Atg5*$^{+/+}$ (**H** and **I**) cells and compared with siC-transfected, THC-treated U87MG cells (**F** and **G**). THC concentration was 6 μM.

Since autophagy has been implicated in promotion and inhibition of cell survival, we next investigated its participation in the cancer cell death–inducing action of THC. Pharmacological inhibition of autophagy at different levels (Supplemental Figure 1, A–C; supplemental material available online with this article; doi:10.1172/JCI37948DS1) or selective knockdown of ATG1 (an essential protein in the initiation of autophagy; ref. 1) (Figure 1, F and G), ATG5 (an essential protein in the formation of the autophagosome; ref. 1) (Supplemental Figure 1, D–F), or AMBRA1 (a recently identified beclin-1–interacting protein that regulates autophagy; ref. 15) (Supplemental Figure 1, D–F) strongly reduced cannabinoid-induced autophagy and cell death. Moreover, transformed *Atg5*-deficient mouse embryonic fibroblasts (MEFs), which are defective in autophagy (16), were more resistant than their wild-type counterparts to THC-induced cell death (Figure 1H) and did not undergo autophagy upon cannabinoid treatment (Figure 1I). Taken together, these findings demonstrate that autophagy plays a prominent role in THC-induced cancer cell death.

THC induces autophagy via ER stress–dependent upregulation of p8 and TRB3. In addition to the presence of autophagosomes, electron microscopy analysis of cannabinoid-treated cells revealed the presence of numerous cells with dilated ER (Figure 2A). In line with this observation, immunostaining of the ER luminal marker protein disulphide isomerase (PDI) showed a striking dilation in the ER of THC-treated U87MG cells (Figure 2B), an event that was associated with an increased phosphorylation of the α subunit of eukaryotic translation initiation factor 2 (eIF2α), a hallmark of the ER stress response (17) (Figure 2C). In addition, THC-induced ER dilation and eIF2α phosphorylation were prevented by pharmacological blockade of the CB1 receptor (Figure 2, B and C).

Figure 2

ER stress precedes autophagy in cannabinoid action. (**A**) Effect of THC on U87MG cell morphology. Note the presence of the dilated ER in THC- but not vehicle-treated cells (6 h). Arrows point to the ER. Scale bars: 500 nm. (**B**) Effect of SR1 (1 μM) and THC on PDI immunostaining (red) in U87MG cells (8 h; $n = 3$). The percentage of cells with PDI dots relative to the total cell number is shown in the corner of each panel (mean ± SD). Scale bar: 20 μm. (**C**) Effect of SR1 (1 μM) on THC-induced eIF2α phosphorylation of U87MG cells (3 h; OD relative to vehicle-treated cells, mean ± SD; $n = 3$). (**D**) Effect of THC on PDI (red) and LC3 (green) immunostaining in U87MG cells ($n = 3$). The percentage of cells with PDI or LC3 dots relative to total cell number at each time point (mean ± SD) is shown. Scale bar: 20 μm. (**E**) Effect of THC on eIF2α

phosphorylation and LC3 lipidation in U87MG cells (n = 3). $^{**}P < 0.01$ compared with THC-treated (**B**) or vehicle-treated (**C** and **D**) cells.

Time-course analysis of PDI and LC3 immunostaining, eIF2α phosphorylation, and LC3 lipidation of cannabinoid-treated cells revealed that ER stress occurred earlier than autophagy (Figure 2, D and E). Of interest, cannabinoid administration produced similar activation of ER stress and autophagy, as well as cell death, in other human astrocytoma cell lines (Supplemental Figure 2, A–F), a primary culture of human glioma cells (Supplemental Figure 2, G–I), and several human cancer cell lines of different origin, including pancreatic cancer (Supplemental Figure 2, J–L), breast cancer, and hepatoma (data not shown). However, neither ER dilation nor eIF2α phosphorylation or autophagy was evident in normal, nontransformed primary astrocytes (Supplemental Figure 3), which are resistant to cannabinoid-induced cell death (13).

We next investigated whether activation of ER stress is involved in the induction of autophagy in response to cannabinoid treatment of cancer cells. We have previously shown that THC-induced accumulation of de novo–synthesized ceramide, an event that occurs in the ER (18), leads to upregulation of the stress-regulated protein p8 and its ER stress–related downstream targets, ATF4, CHOP, and TRB3, to induce cancer cell death (13). Of importance, incubation with ISP-1 (a selective inhibitor of serine palmitoyltransferase, the enzyme that catalyzes the first step of sphingolipid biosynthesis; ref. 18) prevented ceramide accumulation (Supplemental Figure 4A); THC-induced ER dilation (Supplemental Figure 4B); eIF2α phosphorylation (Figure 3A); p8, ATF4, CHOP, and TRB3 upregulation (Supplemental Figure 4C); and autophagy (Figure 3B), supporting that ceramide accumulation is involved in cannabinoid-triggered ER stress and autophagy. We also verified by means of RNA interference that CaCMKKβ — which had been previously implicated in activating autophagy in response to ER stress–associated calcium release (19) — was not involved in THC-induced autophagy and cell death (data not shown). As phosphorylation of eIF2α on Ser51 attenuates general protein synthesis while enhancing the expression of several ER stress response genes (17), we used cells derived from eIF2α S51A knockin mice to test whether eIF2α phosphorylation regulates the expression of p8 and its downstream targets. In agreement with this hypothesis, THC treatment (which promoted ceramide accumulation in both wild-type and eIF2α S51A immortalized MEFs; Supplemental Figure 5A) triggered p8, ATF4, CHOP, and TRB3 upregulation (Figure 3C) as well as autophagy (Supplemental Figure 5B) in wild-type cells but not in their eIF2α S51A counterparts.

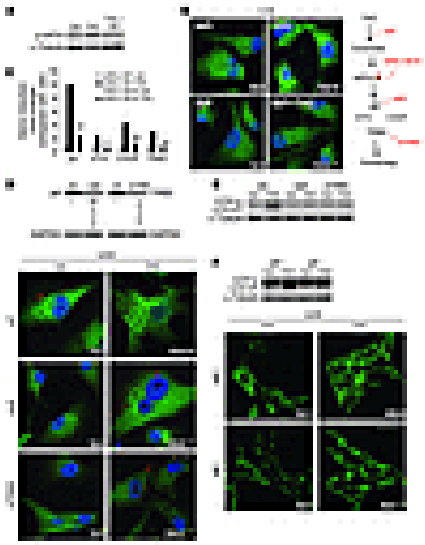
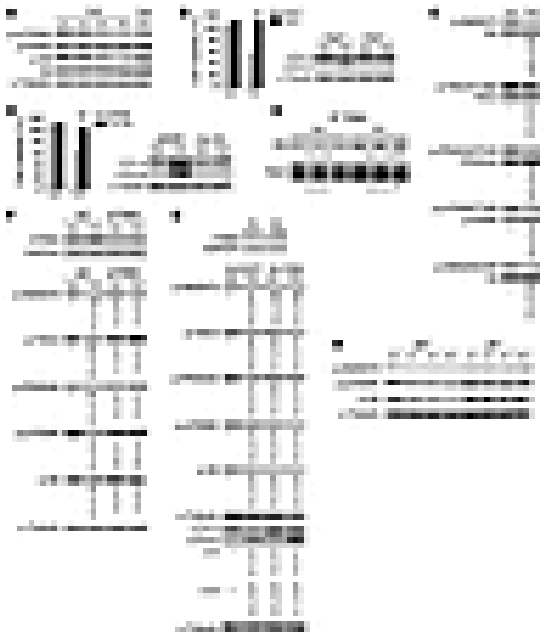


Figure 3

THC induces autophagy via ER stress–evoked p8 and TRB3 upregulation. (**A** and **B**) Effect of ISP-1 (1 μM) on THC-induced eIF2α phosphorylation (**A**; 3 h; n = 3) and LC3 immunostaining (**B**, left panels; 18 h; percentage of cells with LC3 dots

relative to the total cell number, mean ± SD; *n* = 3; scale bar: 20 μm) in U87MG cells. sip8, p8-selective siRNA; siTRB3, TRB3-selective siRNA. (**C**) Effect of THC on p8, ATF4, CHOP, and TRB3 mRNA levels of eIF2α WT and eIF2α S51A MEFs as determined by real-time quantitative PCR (8 h; *n* = 3). Numbers indicate the mean fold increase ± SD relative to vehicle-treated eIF2α WT MEFs. (**D**) Top: Analysis of p8 and TRB3 mRNA levels. Results from a representative RT-PCR experiment are shown. The numbers indicate gene expression levels as determined by real-time quantitative PCR (mean fold change ± SD relative to siC-transfected cells; *n* = 5). Bottom: Effect of THC on LC3 immunostaining (green) of U87MG cells transfected with siC, sip8, or siTRB3 (18 h; *n* = 4). The percentage of cells with LC3 dots relative to cells cotransfected with a red fluorescent control siRNA is shown in each panel (mean ± SD). Scale bar: 20 μm. (**E**) Effect of THC on LC3 lipidation in U87MG cells transfected with siC, sip8, or siTRB3 (18 h; *n* = 6). (**F**) Effect of THC on LC3 lipidation (top; 18 h; *n* = 5) and LC3 immunostaining (bottom; 18 h; percentage of cells with LC3 dots relative to the total cell number, mean ± SD; *n* = 4; scale bar: 40 μm) in $p8^{+/+}$ or $p8^{-/-}$ MEFs. **P* < 0.05 and ***P* < 0.01 compared with THC-treated U87MG (**B**), eIF2α WT (**C**), or $p8^{+/+}$ (**F**) cells and compared with siC-transfected, THC-treated U87MG cells (**D**).

We subsequently asked whether p8 and its downstream targets regulate autophagy. Knockdown of p8 or TRB3 prevented THC-induced autophagy (Figure 3, D and E) but not ER dilation (Supplemental Figure 4D) in U87MG cells. Furthermore, THC induced autophagy in $p8^{+/+}$ but not *p8*-deficient transformed MEFs (Figure 3F and Supplemental Figure 5C). Altogether, these findings reveal that THC induces autophagy of cancer cells via activation of an ER stress–triggered signaling route that involves stimulation of ceramide synthesis de novo, eIF2α phosphorylation, and p8 and TRB3 upregulation.



THC inhibits Akt and mTORC1 via TRB3. Inhibition of mTORC1 is considered a key step in the early triggering of autophagy (6). We therefore tested whether cannabinoid-induced upregulation of the p8 pathway leads to autophagy via inhibition of this complex. THC treatment of U87MG cells reduced the phosphorylation of p70S6 kinase (a well-established mTORC1 substrate) and the ribosomal protein S6 (a well-established p70S6 kinase substrate) (Figure 4, A and C), indicating that mTORC1 is inhibited in cannabinoid-challenged cells. In addition, the cannabinoid-induced decrease in p70S6 kinase and S6 phosphorylation, autophagy, and cell death were not evident in $Tsc2^{-/-}$ cells, in which mTORC1 is constitutively active (20) (Figure 4B and

Supplemental Figure 6, A and B), further supporting a major role for mTORC1 inhibition in the induction of autophagic cell death by cannabinoids.

Figure 4

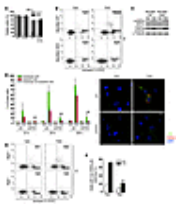
THC inhibits the Akt/mTORC1 pathway via TRB3. (**A**) Effect of THC on p70S6K and S6 phosphorylation of U87MG cells (n = 6). (**B**) Effect of THC on cell viability (left panel; 24 h; mean ± SD; n = 6) and LC3 lipidation (right panel; 18 h; n = 4) in *Tsc2*$^{+/+}$ and *Tsc2*$^{-/-}$ MEFs. (**C**) Effect of THC on Akt, TSC2, PRAS40, p70S6K, and S6 phosphorylation of U87MG cells (18 h; OD relative to vehicle-treated cells, mean ± SD; n = 7). (**D**) Effect of THC on cell viability (left panel; 24 h; mean ± SD; n = 4) and LC3 lipidation (right panel; 18 h; n = 4) of pBABE and myristoylated Akt (myr-Akt) MEFs. (**E**) Effect of THC on Akt co-immunoprecipitation with TRB3 in U87MG cell extracts (8 h; OD relative to vehicle-treated cells, mean ± SD; n = 9; input: TRB3). (**F** and **G**) Effect of THC on Akt, TSC2, PRAS40, p70S6K, and S6 phosphorylation and LC3 lipidation (**G** only) of siC- and siTRB3-transfected (**F**; 18 h; OD relative to vehicle-treated siC-transfected U87MG cells, mean ± SD; n = 7; upper panel shows an analysis of TRB3 mRNA levels) and EGFP (Ad-EGFP) or rat TRB3 (Ad-TRB3) adenoviral vector–infected (**G**; 18 h; OD relative to vehicle-treated Ad-EGFP–infected U87MG cells, mean ± SD; n = 4; upper panel shows an analysis of rTRB3 mRNA levels) U87MG cells. (**H**) Effect of THC on Akt, p70S6K, and S6 phosphorylation of *p8*$^{+/+}$ and *p8*$^{-/-}$ MEFs (n = 7). *P < 0.05 and **P < 0.01 compared with THC-treated *Tsc2*$^{+/+}$ (**B**) and pBABE (**D**) MEFs and compared with vehicle-treated (**C** and **E**), vehicle-treated siC-transfected (**F**), or Ad-EGFP–infected (**G**) U87MG cells.

The protein kinase Akt positively regulates the activity of the mTORC1 complex by phosphorylating and inhibiting TSC2 and PRAS40 (a well-established Akt substrate within the mTORC1 complex). Thus, Akt inhibition decreases mTORC1 activity and promotes autophagy (20). In line with this idea, THC decreased the phosphorylation of Akt, TSC2, and PRAS40 as well as p70S6 kinase and S6 (Figure 4C). This inhibition of the Akt/mTORC1 pathway was abrogated by incubation with a CB1 receptor antagonist (Supplemental Figure 6C) or a ceramide synthesis inhibitor (Supplemental Figure 6D). Likewise, cells overexpressing a myristoylated (constitutively active) form of Akt were resistant to THC-induced mTORC1 inhibition, autophagy, and cell death (Figure 4D and Supplemental Figure 6, E and F), further supporting that THC induces autophagy via Akt inhibition.

Since TRB3 has been shown to directly interact with and inhibit Akt (21, 22), we investigated whether upregulation of TRB3 was responsible for THC-induced Akt/mTORC1 inhibition. Several observations support that this is indeed the case: (a) THC increased the amount of Akt coimmunoprecipitated with TRB3 from U87MG extracts (Figure 4E), (b) knockdown of TRB3 prevented the effect of THC on Akt, TSC2, PRAS-40, p70S6 kinase, and S6 phosphorylation (Figure 4F), and (c) TRB3 overexpression decreased Akt, TSC2, PRAS40, p70S6 kinase, and S6 phosphorylation, enhanced the inhibitory effect of THC on the phosphorylation of these proteins, and promoted autophagy (Figure 4G). In line with these observations, THC failed to inhibit Akt, p70S6 kinase, and S6

phosphorylation of eIF2α S51A knockin or p8-deficient MEFs, in which TRB3 did not become upregulated upon cannabinoid treatment (Figure 4H and Supplemental Figure 6, G and H). Altogether, these data demonstrate that upregulation of p8 and TRB3 induce autophagy of tumor cells via inhibition of the Akt/mTORC1 pathway.

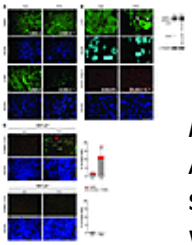
THC-induced autophagy promotes the apoptotic death of cancer cells. While analyzing the mechanism of cannabinoid cell-killing action, we observed that incubation with the pan-caspase inhibitor ZVAD-fmk prevented cell death to the same extent as genetic (Figure 5A) or pharmacological (Supplemental Figure 7) inhibition of autophagy. Furthermore, *Bax/Bak* double knockout (DKO) immortalized MEFs, which are protected against mitochondrial apoptosis (23),



were resistant to THC-induced cell death and apoptosis (Figure 5B) but underwent eIF2α phosphorylation and autophagy (Figure 5C) upon THC treatment. We therefore investigated whether cannabinoid-induced autophagy promoted the apoptotic death of cancer cells. Time-course analysis of LC3 and active caspase-3 immunostaining in U87MG cells revealed that autophagy preceded the appearance of apoptotic features in THC-treated cells (Figure 5D). In addition, selective knockdown of ATG1 (Figure 5D) as well as of AMBRA1 or ATG5 (Supplemental Figure 8) prevented THC-induced caspase-3 activation. Moreover, unlike their wild-type counterparts, *Atg5*-deficient immortalized MEFs did not undergo phosphatidylserine translocation to the outer leaflet of the plasma membrane (Figure 5E), loss of mitochondrial membrane potential (Figure 5F), or increased production of reactive oxygen species (Supplemental Figure 9) in response to cannabinoid treatment. These findings indicate that activation of the autophagy-mediated cell death pathway occurs upstream of apoptosis in cannabinoid antitumoral action.

Figure 5

Autophagy is upstream of apoptosis in cannabinoid-induced cancer cell death. (**A**) Effect of THC and the pan-caspase inhibitor ZVAD (10 μM) on the viability of *Atg5*$^{+/+}$ and *Atg5*$^{-/-}$ MEFs (36 h; percentage of viable cells relative to the corresponding *Atg5*$^{+/+}$ vehicle-treated cells, mean ± SD; *n* = 3). (**B**) Effect of THC on the apoptosis of *Bax/Bak* WT and *Bax/Bak* DKO MEFs as determined by cytofluorometric analysis of Annexin V/propidium iodide (PI) (24 h; mean ± SD; *n* = 3). The mean ± SD percentage of Annexin V–positive/PI-positive and Annexin V–positive, PI-negative cells is shown in the upper and lower corners, respectively. (**C**) Effect of THC on eIF2α phosphorylation (3 h; *n* = 3) and LC3 lipidation (18 h; *n* = 4) of *Bax/Bak* WT and DKO MEFs. (**D**) Left: Effect of THC on autophagy and apoptosis of U87MG cells transfected with siC or siATG1. Green bars, cells with LC3 dots; red bars, active caspase-3–positive cells; white bars, cells with both LC3 dots and active caspase-3 staining. Data correspond to the percentage of cells with LC3 dots (green bars), active caspase-3–positive cells (red bars), and cells with LC3 dots and active caspse-3 staining (white bars) relative to the total number of transfected cells at each time point (mean ± SD; *n* = 3). Right: Representative photomicrographs (36 h; scale bar: 20 μm). (**E** and **F**) Effect of THC on apoptosis (**E**; 24 h; *n* = 3) and loss of mitochondrial membrane potential as determined by $DiOC_6(3)$ staining (**F**; 24 h;



n = 4) of *Atg5*$^{+/+}$ and *Atg5*$^{-/-}$ MEFs. In **E**, the mean ± SD percentage of Annexin V–positive/PI-positive and Annexin V–positive, PI-negative cells is shown in the upper and lower corners, respectively. ***P* < 0.01 compared with THC-treated *Atg5*$^{+/+}$ (**A**, **E**, and **F**) and *Bax/Bak* WT (**B**) MEFs and from THC-treated, siC-transfected cells (**D**).

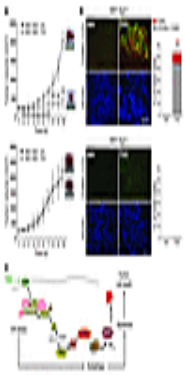
Activation of autophagy is necessary for cannabinoid antitumoral action in vivo. To determine the in vivo relevance of our findings, we first investigated whether THC promotes the activation of the above-described autophagy-mediated cell death pathway in U87MG cell–derived tumor xenografts, in which we have recently shown that cannabinoid treatment reduces tumor growth (specifically, THC administration for 14 days decreased tumor growth by 50%; ref. 13). Analysis of these tumors revealed that cannabinoid administration increases TRB3 expression and decreases S6 phosphorylation (Figure 6A). Likewise, formation of LC3 dots as well as increase in LC3-II and active caspase-3 immunostaining were observed in THC-treated, but not vehicle-treated, tumors (Figure 6B).

Figure 6

THC activates the autophagic cell death pathway in vivo. (**A**) Effect of peritumoral THC administration on TRB3 and p-S6 immunostaining in U87MG tumors. TRB3- or p-S6–stained area normalized to the total number of nuclei in each section; numbers indicate the mean fold change ± SD; 18 sections were counted for each of 3 dissected tumors for each condition. Scale bar: 50 μm. (**B**) Left: Effect of peritumoral THC administration on LC3 and active caspase-3 immunostaining in U87MG tumors. Arrows point to cells with LC3 dots. The numbers indicate the percentage of active caspase-3–positive cells relative to the total number of nuclei in each section ± SD. Ten sections were counted for each of 3 dissected tumors for each condition. Scale bars: 20 μm. Right: Effect of peritumoral THC administration on LC3 lipidation in U87MG tumors. Representative samples from 1 vehicle-treated and 1 THC-treated tumor are shown. Numbers indicate the LC3-I and LC3-II OD values relative to vehicle-treated tumors (mean ± SD). *n* = 3. (**C**) Left: Effect of THC administration on LC3 immunostaining (green) and TUNEL (red) in Ras^{V12}/E1A *p8*$^{+/+}$ and *p8*$^{-/-}$ tumor xenografts. Arrows point to cells with LC3 dots and TUNEL-positive nuclei. Right: Bar graph shows the percentage of TUNEL-positive nuclei or cells with TUNEL-positive nuclei and LC3 dots relative to the total number of nuclei in each section (mean ± SD). Eighteen sections were counted from 3 dissected tumors for each condition. Scale bars: 50 μm. Inset shows the magnification of 1 selected cell (arrows point to LC3 dots; scale bar: 10 μm). **P* < 0.05 and ***P* < 0.01 compared with vehicle-treated tumors.

To further investigate whether activation of the p8 pathway mediates cannabinoid antitumoral action, we also analyzed tumors derived from *p8*$^{+/+}$ and *p8*$^{-/-}$ Ras^{V12}/E1A-transformed MEFs (in this case, THC administration for 8 days decreased by 45% the growth of *p8*$^{+/+}$ tumors but had no significant effect on *p8*$^{-/-}$ tumors; ref. 13). THC treatment increased TRB3 expression, decreased S6 phosphorylation, and increased autophagy as well as TUNEL and active caspase-3 immunostaining in p8$^{+/+}$ but not p8$^{-/-}$ tumors (Figure 6C and

Supplemental Figure 10). Moreover, THC treatment enhanced the number of cells with LC3 dots and TUNEL-positive nuclei in $p8^{+/+}$ but not in $p8^{-/-}$ tumors (Figure 6C).



In order to verify the importance of autophagy for cannabinoid antitumoral action, we next generated tumors with *Atg5*$^{+/+}$ and *Atg5*$^{-/-}$ Ras^{V12}/T-large antigen transformed MEFs. THC administration reduced by more than 80% the growth of tumors derived from wild-type cells but had no significant effect on those tumors generated by autophagy-deficient cells (Figure 7A). Furthermore, cannabinoid administration increased autophagy, TUNEL (Figure 7B), and active caspase-3 immunostaining (Supplemental Figure 11) in *Atg5*$^{+/+}$ but not *Atg5*$^{-/-}$ tumors. Likewise, cannabinoid administration increased the number of cells with LC3 dots and TUNEL-positive nuclei in *Atg5*$^{+/+}$ but not *Atg5*$^{-/-}$ tumors (Figure 7B). Taken together, these findings demonstrate that activation of the autophagy-mediated cell death pathway is indispensable for cannabinoid antitumoral action.

Figure 7

Autophagy is essential for cannabinoid antitumoral action. (**A**) Effect of peritumoral THC administration on the growth of *Atg5*$^{+/+}$ (upper panel) and *Atg5*$^{-/-}$ (lower panel) Ras^{V12}/T-large antigen MEF tumor xenografts generated in nude mice (mean ± SD; $n = 7$ for each condition). Photographs show representative images of vehicle- and THC-treated tumors. (**B**) Left: Effect of THC administration on LC3 immunostaining (green) and apoptosis as determined by TUNEL (red) in *Atg5*$^{+/+}$ and *Atg5*$^{-/-}$ MEF tumor xenografts. Representative images from 1 vehicle-treated and 1 THC-treated *Atg5*$^{+/+}$ and *Atg5*$^{-/-}$ tumors are shown. Right: Bar graphs show the percentage of TUNEL-positive nuclei and cells with TUNEL-positive nuclei and LC3 dots relative to the total number of nuclei in each section (mean ± SD). Eighteen sections were counted from 3 dissected tumors for each condition (vehicle-treated and THC-treated). Scale bar: 50 μm. (**C**) Schematic of the proposed mechanism of THC-induced cell death (see text for details). $^{**}P < 0.01$ compared with vehicle-treated tumors.

Finally, we analyzed the tumors of 2 patients enrolled in a clinical trial aimed at investigating the effect of THC on recurrent glioblastoma multiforme. The patients were subjected to intracranial THC administration, and biopsies were taken before and after the treatment (11). In the 2 patients, cannabinoid inoculation increased TRB3 immunostaining and decreased S6 phosphorylation (Figure 8A). Interestingly, the number of cells with autophagic phenotype (Figure 8B) as well as with active caspase-3 immunostaining (Figure 8C) was increased in the tumor samples obtained after THC treatment. Although these studies were only conducted in specimens from 2 patients, they are in line with the preclinical evidence shown above and suggest that cannabinoid administration might also trigger autophagy-mediated cell death in human tumors.

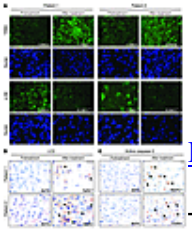


Figure 8

THC administration promotes autophagy in glioblastomas of 2 patients. Analysis of different parameters in 2 patients with glioblastoma multiforme before and after intracranial THC treatment (it was estimated that doses of 6–10 μM were reached at the site of administration). (**A**) TRB3 and p-S6 immunostaining. Representative photomicrographs are shown. Numbers indicate the TRB3- or p-S6–stained area normalized to the total number of nuclei in each section (mean fold change ± SD) relative to the corresponding pre-treatment sample. Fifteen sections were counted for each tumor and each condition (before and after treatment). Scale bar: 50 μm. (**B**) Representative photomicrographs of LC3 diaminobenzidine immunostaining. The mean percentage of cells with LC3 dots ± SD relative to the total number of nuclei in each section is noted in the corner of each panel. Ten sections were counted from each biopsy for each condition. Arrows point to cells with LC3 dots. Scale bar: 20 μm. (**C**) Representative photomicrographs of active caspase-3 diaminobenzidine immunostaining. Numbers indicate the percentage of cells with active caspase-3 staining ± SD relative to the total number of nuclei in each section. Ten sections were counted from each biopsy for each condition. Arrows point to cells with active caspase-3 staining. Scale bar: 20 μm. **P* < 0.05 and ***P* < 0.01 compared with before treatment.

Discussion

In this study we show that cannabinoids, a new family of potential antitumoral agents, induce autophagy of cancer cells and that this process mediates the cell death–promoting activity of these compounds. Several observations strongly support this idea: (a) THC induced autophagy and cell death in different types of cancer cells but not in nontransformed astrocytes, which are resistant to cannabinoid killing action, (b) pharmacological or genetic inhibition of autophagy prevented THC-induced cell death, (c) autophagy-deficient tumors were resistant to THC growth-inhibiting action, and (d) THC administration activated the autophagic cell death pathway in 3 different models of tumor xenografts as well as in 2 human tumor samples.

Depending on the cellular context and the strength and duration of the triggering stimulus, autophagy is involved in the promotion or inhibition of cancer cell survival (4, 5, 24, 25). However, the molecular bases of this dual role of autophagy in cancer remain unknown. Data presented here demonstrate that induction of autophagy by cannabinoids leads to cancer cell death and identify the signaling route responsible for the activation of this cellular process. Thus, our findings suggest that THC — via activation of the CB1 receptor and stimulation of ceramide synthesis de novo — activates an early ER stress response that leads to increased phosphorylation of eIF2α on Ser51. Experiments performed with eIF2α S51A mutant cells have shown that phosphorylation of this residue, which is known to attenuate general protein translation while enhancing the expression of several genes related with the ER stress response (17), is required for the upregulation of the stress protein p8 and its ER stress–related downstream targets ATF4, CHOP, and TRB3 as well as for the induction of autophagy by cannabinoids. Furthermore, we demonstrate

that the upregulation of p8 and TRB3, which has been previously implicated in cannabinoid-evoked cell death (13), is a crucial event in the triggering of autophagy. Ceramide accumulation has been proposed to induce ER stress (26, 27) and autophagy (28), and eIF2α phosphorylation has been implicated in the induction of autophagy in response to different situations (29–31). However, the molecular mechanisms responsible for these actions have not been clarified. Findings presented here now suggest that upregulation of the p8-TRB3 pathway constitutes a mechanism by which de novo–synthesized ceramide and eIF2α phosphorylation promote autophagy, thus identifying what we believe is a novel connection between ER stress and autophagy.

Our data also demonstrate that the autophagy-promoting activity of the p8-regulated pathway is based on its ability to inhibit the Akt/mTORC1 axis. Regulation of mTORC1 largely relies on the activity of the prosurvival kinase Akt, whose inhibition leads to mTORC1 inactivation and, in turn, to autophagy (20). Our findings reveal that THC upregulates TRB3, promoting its interaction with Akt and leading to decreased phosphorylation of this kinase as well as of its direct substrates TSC2 and PRAS40, which triggers mTORC1 inhibition and induction of autophagy. TRB3 has been previously shown to inhibit Akt (21, 22), although the precise contribution of this pseudo-kinase to the regulation of Akt activity in different cellular contexts is unclear (32). Here we demonstrate that TRB3 inhibition of the Akt/mTORC1 axis is essential for cannabinoid-induced autophagy of cancer cells. Moreover, we show that this pathway is essential for cannabinoid antitumoral action. Thus, THC administration leads to TRB3 upregulation, mTORC1 inhibition, induction of autophagy, and reduction of tumor growth in different models of tumor xenografts, but not in p8-deficient tumors that are defective in the upregulation of the p8/TRB3 pathway. Furthermore, activation of this pathway was also evident in 2 glioma patients that had been treated with THC. These results thus uncover a role for TRB3 that may be of great importance in the regulation of cancer cell death.

Autophagy has been proposed to protect from apoptosis, act as an apoptosis-alternative pathway to induce cell death, or act together with apoptosis as a combined mechanism for cell death (6, 33). However, very little is known about the role of the interplay between these 2 cellular processes in the control of tumor growth in response to anticancer agents. Our results now clearly demonstrate that induction of autophagy is involved in the mechanism by which cannabinoids promote the activation of the mitochondrial pro-apoptotic pathway. Thus, neither tumors in which the p8-regulated pathway has been ablated (and in which, therefore, THC treatment does not induce autophagy) nor tumors intrinsically deficient in autophagy undergo apoptosis in response to THC, and so they are resistant to THC antitumoral action. These findings reveal that autophagy is required for the activation of apoptosis in response to cannabinoid treatment in vivo.

It is worth noting that the concentrations of THC used in this study are in the same range as those administered intracranially to the patients in which we observed activation of the autophagy-mediated cell death pathway (11) and could be thus considered clinically relevant. Of interest, intraperitoneal administration of THC to U87MG tumor xenografts produces a similar decrease

in tumor growth (that occurs in concert with increased autophagy and apoptosis) to that observed when the cannabinoid is administered peritumorally (our unpublished observations). Considering that no signs of toxicity were observed in the clinical trial patients (11) or in tumor-bearing animals treated intracranially, peritumorally, or intraperitoneally with THC (refs. 34 and 35 and data not shown), and that no overt toxic effects have been reported in other clinical trials of cannabinoid use in cancer patients for various applications (e.g., inhibition of nausea, vomiting, and pain) and using different routes of administration (e.g., oral, oro-mucosal) (9, 36), our findings support that safe, therapeutically efficacious doses of THC may be reached in cancer patients.

In summary, in this study we identify what we believe is a new route that links the ER stress response to the activation of autophagy and promotes the apoptotic death of tumor cells (Figure 7C). The identification of this pathway will help to understand the molecular events that lead to activation of autophagy-mediated cell death by anticancer drugs and may contribute to the design of new therapeutic strategies for inhibiting tumor growth.

Methods

Cell culture and viability. Cortical astrocytes were prepared from 24-hour-old mice as previously described (13). Primary cultures of brain tumor cells were prepared and cultured as described in the Supplemental Methods. U87MG, T98G, U373MG, and MiaPaCa2 cells, $p8^{+/+}$ and $p8^{-/-}$ Ras^{V12}/E1A MEFs, $Atg5^{+/+}$ and $Atg5^{-/-}$ T-large antigen MEFs (provided by Noboru Mizushima, Tokyo Medical and Dental University, Tokyo, Japan), *Bax/Bak* wild-type and *Bax/Bak* DKO T-large antigen MEFs (provided by Luca Scorrano, Dulbecco Telethon Institute, Milan, Italy, and Patrizia Agostinis, Catholic University of Leuven, Leuven, Belgium), eIF2α S51S WT and eIF2α S51A T-large antigen MEFs (provided by Richard Kaufman, University of Michigan, Ann Arbor, Michigan, USA, and Cesar de Haro and Juan J. Berlanga, Centro de Biología Molecular Severo Ochoa, Autonoma University, Madrid, Spain), $Tsc2^{+/+}$ and $Tsc2^{-/-}$ $p53^{-/-}$ MEFs, empty vector (pBABE) and pBABE-myr-Akt MEFs, and $Atg5^{+/+}$ and $Atg5^{-/-}$ Ras^{V12}/T-large antigen MEFs were cultured in DMEM containing 10% FBS and transferred to medium containing 0.5% FBS (except Ras^{V12}/E1A-transformed MEFs, which were transferred to medium containing 2% FBS) 18 h before performing the different treatments. $p8^{+/+}$ and $p8^{-/-}$ Ras^{V12}/E1A MEFs as well as $Atg5^{+/+}$ and $Atg5^{-/-}$ Ras^{V12}/T-large antigen MEFs correspond to a polyclonal mix of at least 20 different selected clones. Unless otherwise indicated, THC was used at a final concentration of 5 μM. Cell viability was determined by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] test (Sigma-Aldrich).

Flow cytometry. Briefly, cells (approximately 5×10^5 cells per assay) were trypsinized, divided in 2 tubes, washed, and collected by centrifugation at 1,500 *g* for 5 min. One aliquot was incubated for 10 min at 37°C with Annexin V–FITC (BD Biosciences). Propidium iodide (1 μg/ml) was added just before cytofluorometric analysis. The other aliquot was simultaneously labeled with 3,3ı-dihexyloxacarbocyanine iodide ($DiOC_6$[3], 40 nM; Invitrogen) and hydroethidium (5 μM; Invitrogen) for 10 minutes at 37°C, followed by

cytofluorometric analysis. Cells (10,000) were recorded in each analysis. Fluorescence intensity was analyzed in an EPICS XL flow cytometer (Beckman Coulter).

Western blot. Western blot analysis was performed following standard procedures. A list of the antibodies used can be found in Supplemental Methods. Densitometric analysis was performed with Quantity One software (Bio-Rad).

Transfections. U87MG cells (75% confluent) were transfected with siRNA duplexes using the DharmaFECT 1 Transfection reagent (Dharmacon). Cells were trypsinized and seeded 24 h after transfection, at a density of 5,000 cells/cm^2. Transfection efficiency was greater than 70% as monitored with a control fluorescent (red) siRNA (siGLO RISC-Free siRNA; Dharmacon). In immunofluorescence experiments, control and selective siRNAs were used in a 1:5 ratio, and cells with red spots were scored as transfected.

Infections with adenoviral vectors. U87MG cells (75% confluent) were transduced for 1 h with supernatants obtained from HEK293 cells infected with adenoviral vectors carrying EGFP (provided by Javier G. Castro, Hospital Infantil Universitario Niño Jesús, Madrid, Spain), rat HA-tagged TRB3 (donated by Patrick Iynedjian, University of Geneva, Geneva, Switzerland) (32), or human EGFP-LC3 (provided by Aviva Tolkovsky and Christoph Goemans, University of Cambridge, Cambridge, United Kingdom). Infection efficiency was greater than 80% as determined by EGFP fluorescence.

RNA interference. Double-stranded RNA duplexes were purchased from Dharmacon. A list of sequences can be found in the Supplemental Methods.

RT-PCR analysis. RNA was isolated using Trizol Reagent (Invitrogen). cDNA was obtained with Transcriptor Reverse transcriptase (Roche Applied Science). Primers and amplification conditions can be found in the Supplemental Methods.

Real-time quantitative PCR. cDNA was obtained using Transcriptor (Roche Applied Science). Real-time quantitative PCR assays were performed using the FastStart Universal Probe Master mix with Rox (Roche Applied Science), and probes were obtained from the Universal ProbeLibrary Set (Roche Applied Science). Primer sequences can be found in the Supplemental Methods. Amplifications were run in a 7900 HT-Fast Real-Time PCR System (Applied Biosystems). Each value was adjusted by using 18S RNA levels as a reference.

Immunoprecipitation. U87MG cells were lysed in HEPES lysis buffer (see Supplemental Methods for buffer composition). Lysate (1–4 mg) was precleared by incubating with 5–20 µl of protein G–Sepharose conjugated to pre-immune IgG. The lysate extracts were then incubated with 5–20 µl of protein G–Sepharose conjugated to 5–20 µg of the anti-TRB3 antibody or pre-immune IgG. TRB3 antibody (aminoterminal end, ab50516; Abcam) was covalently conjugated to protein G–Sepharose using dimethyl pimelimidate. Immunoprecipitations were carried out for 1 h at 4°C on a rotatory wheel. The immunoprecipitates were washed 4 times with HEPES lysis buffer, followed by 2 washes with HEPES kinase buffer. The immunoprecipitates were resuspended in

30 µl of sample buffer (not containing 2-mercaptoethanol) and filtered through a 0.22-µm Spin-X filter, and 2-mercaptoethanol was added to a concentration of 1% (vol/vol). Samples were subjected to electrophoresis and immunoblot analysis.

Ceramide levels. Ceramide levels were determined as previously described (37).

Confocal laser scanning microscopy. Standard protocols for immuno-fluorescence microscopy were used (see Supplemental Methods for the antibodies used). To quantify the percentage of cells with LC3 or PDI dots, at least 200 cells per condition were counted in randomly selected fields. In all cases, only those cells with 4 or more prominent dots of either LC3 or PDI were scored positively.

In vivo treatments. Tumors derived from U87MG cells and $p8^{+/+}$ and $p8^{-/-}$ MEFs were induced and treated as previously described (13). Tumors derived from $Atg5^{+/+}$ or $Atg5^{-/-}$ Ras^{V12}/T-large antigen MEFs (see Supplemental Methods for the procedure used to generate these cells) were induced in nude mice by subcutaneous injection of 10^7 cells in PBS supplemented with 0.1% glucose. Tumors were allowed to grow until an average volume of 200–250 mm^3, and animals were assigned randomly to the different groups. At this point, vehicle or THC (15 mg/kg/d) in 100 µl of PBS supplemented with 5 mg/ml BSA was administered daily in a single peritumoral injection. Tumors were measured with an external caliper, and volume was calculated as $(4\pi/3) \times (\text{width}/2)^2 \times (\text{length}/2)$. All procedures involving animals were performed with the approval of the Complutense University Animal Experimentation Committee according to Spanish official regulations.

Human tumor samples. Tumor biopsies were obtained from 2 recurrent glioblastoma multiforme patients who had been treated with THC. The characteristics of the patients and the clinical study have been described in detail elsewhere (11). Briefly, THC dissolved in 30 ml of physiological saline solution plus 0.5% (wt/vol) human serum albumin was administered intratumorally to the patients. Patient 1 received a total of 1.46 mg of THC for 30 days, while patient 2 received a total of 1.29 mg of THC for 26 days (it was estimated that doses of 6–10 µM THC were reached at the site of administration; ref. 11). Samples were fixed in formalin, embedded in paraffin, and used for immunomicroscopy.

Immunomicroscopy of tumor samples. Samples from tumor xenografts were dissected, Tissue-Tek (Sakura) embedded, frozen, and, before the staining procedures were performed, fixed in acetone for 10 min at room temperature. Samples from human tumors were subjected to deparaffinization, rehydration, and antigen retrieval before the staining procedures were performed. Standard protocols for immunofluorescence or immunohistochemistry microscopy were used (see Supplemental Methods). Nuclei were counterstained with TOTO-3 iodide (U87MG and human tumor samples; Invitrogen) or Hoechst 33342 (MEF tumors; Invitrogen). Fluorescence images were acquired using Metamorph-Offline 6.2 software (Universal Imaging) and Zeiss Axioplan 2 Microscope.

TUNEL. Tumor samples were fixed, blocked, and permeabilized, and TUNEL was performed as previously described (13).

Electron microscopy. Ultrastructural analysis of vehicle- and THC-treated cells was assessed by conventional embedding in the epoxy-resin EML-812 (Taab Laboratories). Ultrathin (20- to 30-nm-thick) sections of the samples were obtained using a Leica-Reichert-Jung ultramicrotome and then stained with saturated uranyl acetate–lead citrate by standard procedures. Ultrathin sections were analyzed in a JEOL 1200-EX II transmission electron microscope operating at 100 kV.

Statistics. Statistical analysis was performed by ANOVA with a post-hoc analysis using the Student-Neuman-Keuls test. Differences were considered significant when the *P* value was less than 0.05.

Supplemental data

View Supplemental data

Acknowledgments

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Footnotes

Conflict of interest: The authors have declared that no conflict of interest exists.

Nonstandard abbreviations used: Atg, autophagy protein; eIF2α, eukaryotic translation initiation factor 2α; MEF, mouse embryonic fibroblast; THC, Δ^9-tetrahydrocannabinol; mTORC1, mammalian target of rapamycin complex 1; PDI, protein disulphide isomerase; TRB3, tribbles homolog 3.

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6. New US Study Affirms Smoked Marijuana Protects Against Cancer.

In 1974, University of Virginia researchers discovered something very unlikely. Cannabis, banned in the United States in 1937, and further demonized by the Nixon administration in 1968, had an unexpected property: it inhibited the growth of lung cancer cells. But, even more surprising was the response from the government: an apparent complete absence, even discouragement of any follow-up studies. The results were briefly mentioned in news reports at the time, but with the end of the Carter administration, cannabis became a step-child as far as scientific research was concerned.

Like any unloved step-child cannabis was treated with different rules, and made a scape-goat for social ills.

There was still research being done on cannabis, but funding was only available if the intent was to prove harm. In fact, it wasn't until the pioneering work done by Dr. Raphael Mechoulam, in Israel, and Dr. Manuel Guzman in Spain, that this startling anti-cancer property of cannabis sativa became public again.

What is even more troubling is that the United States Government actually did a secret follow up-study on the Virginia findings, in the mid '90's. When it only served to confirm the results of the 1974 research, and showed that THC (one of the main active ingredient in cannabis – and the one the government loves to hate), when administered to mice, protected them against malignancy, true to form, our government attempted to bury the results. Fortunately, a draft copy of the study was leaked to the journal, AIDS Treatment News, and the media covered the story. An excellent article by Paul Armentano, Deputy Director of NORML, covers this part of our shameful history.

By 2003, the cat was pretty much out of the bag, and a quick search on PubMed brings up at least 262 results when you put in "cannabis and cancer" in the search string. But, as late as this year, the US Government was still funding research meant to prove that cannabis causes cancer. The extremely flawed survey which attempted to link cannabis smoking with testicular cancer falls into this category. In fact, in 2008, two years after Dr. Donald Tashkin research which showed that not only does cannabis not cause lung cancer, but appears to protect against it, three respected doctors from the cannabis research group felt compelled to write a letter to the European Respiratory

Journal debunking a New Zealand study which claimed that smoking cannabis led to an increased risk of lung cancer.

Now, this month in Cancer Prevention Research Journal one can find a study demonstrating that chronic, long term of cannabis actually reduces the incidence of head and neck cancer. Specifically:

"10 to 20 years of marijuana use was associated with a significantly reduced risk of HNSCC" [head and neck squamous cell carcinoma].

Knowing this, are you angry? You should be. It's a safe bet to say you know someone who has cancer. Or died of it.

It's also a safe bet that you didn't hear any coverage of this story in the mainstream media.

For my money, it's way past time for the politics of prohibtion to be thrown aside, and hard science applied to what promises to be an extraordinary new era in the treatment and cure of cancer.

And... we need all the voices we can get saying: That time is now!

Requests for reprints of the study cited above can be made here: Karl T. Kelsey, Department of Community Health, Department of Pathology and Laboratory Medicine, Division of Biology and Medicine, Brown University, Providence, RI. Phone: 401-863-6420 _skype_ 401-863-6420 ; Fax: 401-863-9008; E-mail:Karl_Kelsey@brown.edu.

7. Pot Shrinks Tumors - Government Knew in '74

By Raymond Cushing
Source: San Antonio Current

Wednesday, March 28, The United States Supreme Court rules on whether marijuana use for medicinal purposes can be a valid defense on charges of marijuana possession. The following article was listed as one of the top 25 censored stories of the year 2000. We reprint it here and pose the question, why would the government want to keep us from knowing this?

The term medical marijuana took on dramatic new meaning in February 2000, when researchers in Madrid announced they had destroyed incurable brain tumors in rats by injecting them with THC, the active ingredient in cannabis.

The Madrid study marks only the second time that THC has been administered to tumor-bearing animals. In 1974, researchers at the Medical College of Virginia, who had been funded by the National Institutes of Health to find evidence that marijuana damages the immune system, found instead that THC slowed the growth of three kinds of cancer in mice -- lung and breast cancer, and a virus-induced leukemia.

The DEA quickly shut down the Virginia study and all further cannabis/tumor research, according to Jack Herer, who reports on the events in his book, The Emperor Wears No Clothes. In 1976, President Gerald Ford put an end to all public cannabis research and granted exclusive research rights to major pharmaceutical companies, who set out -- unsuccessfully -- to develop synthetic forms of THC that would deliver all the medical benefits without the "high."

The Madrid researchers reported in the March issue of Nature Medicine that they injected the brains of 45 rats with cancer cells, producing tumors whose presence they confirmed through magnetic resonance imaging (MRI). On the 12th day they injected 15 of the rats with THC and 15 with Win-55,212-2, a synthetic compound similar to THC. "All the rats left untreated uniformly died 12-18 days after glioma (brain cancer) cell inoculation ... Cannabinoid (THC)-treated rats survived significantly longer than control rats. THC administration was ineffective in three rats, which died by days 16-18. Nine of the THC-treated rats surpassed the time of death of untreated rats, and survived up to 19-35 days. Moreover, the tumor was completely eradicated in three of the treated rats." The rats treated with Win-55,212-2 showed similar results.

The Spanish researchers, led by Dr. Manuel Guzman of Complutense University, also irrigated healthy rats' brains with large doses of THC for seven days, to test for harmful biochemical or neurological effects. They found none.

"Careful MRI analysis of all those tumor-free rats showed no sign of damage related to necrosis, edema, infection or trauma ... We also examined other potential side effects of cannabinoid administration. In both tumor-free and tumor-bearing rats, cannabinoid administration induced no substantial change in behavioral parameters such as motor coordination or physical activity. Food and water intake, as well as body weight gain, were unaffected during and after cannabinoid delivery. Likewise, the general hematological profiles of cannabinoid-treated rats were normal. Thus, neither biochemical parameters nor markers of tissue damage changed substantially during the seven-day delivery period or for at least two months after cannabinoid treatment ended."

Guzman's investigation is the only time since the 1974 Virginia study that THC has been administered to live, tumor-bearing animals. (The Spanish researchers cite a 1998 study in which cannabinoids inhibited breast cancer cell proliferation, but that was a "petri dish" experiment that didn't involve live subjects.)

In an e-mail interview for this story, the Madrid researcher said he had heard of the Virginia study, but had never been able to locate literature on it. Hence, the Nature Medicine article characterizes the new study as the first on tumor-laden animals and doesn't cite the 1974 Virginia investigation.

"I am aware of the existence of that research. In fact I have attempted many times to obtain the journal article on the original investigation by these people, but it has proven impossible," Guzman said.

In 1983, the Reagan/Bush Administration tried to persuade American universities and researchers to destroy all 1966-76 cannabis research work, including compendiums in libraries, reports Jack Herer, who states, "We know that large amounts of information have since disappeared."

Guzman provided the title of the work -- "Antineoplastic activity of cannabinoids," an article in a 1975 Journal of the National Cancer Institute -- and this writer obtained a copy at the University of California medical school library in Davis and faxed it to Madrid.

The summary of the Virginia study begins, "Lewis lung adenocarcinoma growth was retarded by the oral administration of tetrahydrocannabinol (THC) and cannabinol (CBN)" -- two types of cannabinoids, a family of active components in marijuana. "Mice treated for 20 consecutive days with THC and CBN had reduced primary tumor size."

The 1975 journal article doesn't mention breast cancer tumors, which are featured in the only newspaper story ever to appear about the 1974 study -- in the "Local" section of The Washington Post on Aug. 18, 1974. Under the headline, "Cancer Curb Is Studied," it read in part:

"The active chemical agent in marijuana curbs the growth of three kinds of cancer in mice and may also suppress the immunity reaction that causes rejection of organ transplants, a Medical College of Virginia team has discovered." The researchers "found that THC slowed the growth of lung cancers, breast cancers, and a virus-induced leukemia in laboratory mice, and prolonged their lives by as much as 36 percent."

Guzman, writing from Madrid, was eloquent in his response after this writer faxed him the clipping from The Washington Post of a quarter century ago. In translation, he wrote:

"It is extremely interesting to me, the hope that the project seemed to awaken at that moment, and the sad evolution of events during the years following the discovery, until now we once again draw back the veil, over the anti-tumoral power of THC, 25 years later. Unfortunately, the world bumps along between such moments of hope and long periods of intellectual castration."

News coverage of the Madrid discovery has been virtually nonexistent in this country. The news broke quietly on Feb. 29, 2000 with a story that ran once on the UPI wire about the Nature Medicine article. This writer stumbled on it through a link that appeared briefly on the Drudge Report Web page. The New York Times, The Washington Post, and Los Angeles Times all ignored the story, even though its newsworthiness is indisputable: a benign substance occurring in nature destroys deadly brain tumors.

Raymond Cushing is a regular contributor to the Sacramento News & Review and the Anderson Valley (CA) Advertiser.

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8. Emerging Clinical Applications For Cannabis & Cannabinoids
A Review of the Recent Scientific Literature, 2000 — 2009

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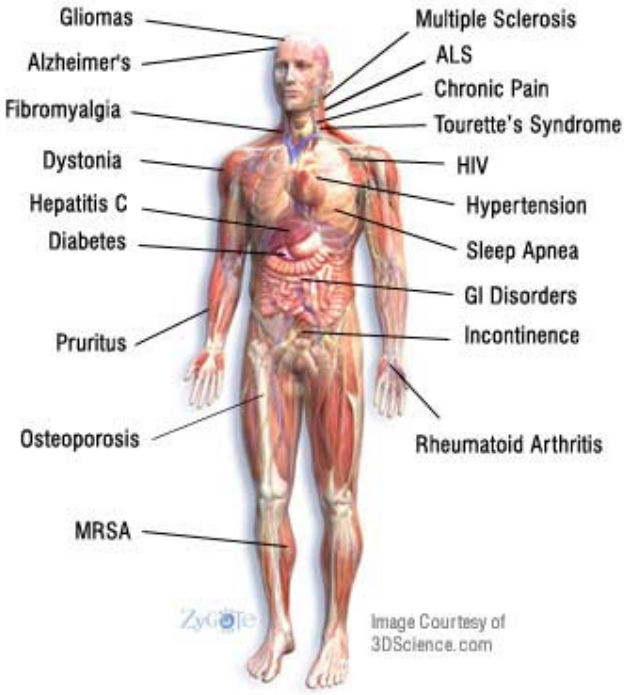
Washington, DC
January 15, 2009



Potential Therapeutic Uses of Medical Marijuana

Despite the ongoing political debate regarding the legality of medicinal marijuana, clinical investigations of the therapeutic use of cannabinoids are now more prevalent than at any time in history. A search of the National Library of Medicine's PubMed website quantifies this fact. A keyword search using the terms "cannabis, 1996" (the year California voters became the first of 13 states to allow for the drug's medical use under state law) reveals just 258 scientific journal articles published on the subject during that year. Perform this same search for the year 2008, and one will find over 2,100 published scientific studies.

While much of the renewed interest in cannabinoid therapeutics is a result of the discovery of the endocannabinoid regulatory system, some of this increased attention is also due to the growing body of testimonials from medicinal cannabis patients and their physicians. Nevertheless, despite this influx of anecdotal reports, much of the modern investigation of medicinal cannabis remains limited to preclinical (animal) studies of individual cannabinoids (e.g. THC or cannabidiol) and/or synthetic cannabinoid agonists (e.g., dronabinol or WIN 55,212-2) rather than clinical trial investigations involving whole plant material. Predictably, because of the US government's strong public policy stance against any use of cannabis, the bulk of this modern cannabinoid research is taking place outside the United States.

As clinical research into the therapeutic value of cannabinoids has proliferated – there are now more than 17,000 published papers in the scientific literature analyzing marijuana and its constituents — so too has investigators' understanding of cannabis' remarkable capability to combat disease. Whereas researchers in the 1970s, 80s, and 90s primarily assessed cannabis' ability to temporarily alleviate various disease symptoms — such as the nausea

associated with cancer chemotherapy — scientists today are exploring the potential role of cannabinoids to modify disease.

Of particular interest, scientists are investigating cannabinoids' capacity to moderate autoimmune disorders such as multiple sclerosis, rheumatoid arthritis, and inflammatory bowel disease, as well as their role in the treatment of neurological disorders such as Alzheimer's disease and amyotrophic lateral sclerosis (a.k.a. Lou Gehrig's disease.)

Investigators are also studying the anti-cancer activities of cannabis, as a growing body of preclinical and clinical data concludes that cannabinoids can reduce the spread of specific cancer cells via apoptosis (programmed cell death) and by the inhibition of angiogenesis (the formation of new blood vessels). Arguably, these latter trends represent far broader and more significant applications for cannabinoid therapeutics than researchers could have imagined some thirty or even twenty years ago.

THE SAFETY PROFILE OF MEDICAL CANNABIS

Cannabinoids have a remarkable safety record, particularly when compared to other therapeutically active substances. Most significantly, the consumption of marijuana – regardless of quantity or potency -- cannot induce a fatal overdose. According to a 1995 review prepared for the World Health Organization, "There are no recorded cases of overdose fatalities attributed to cannabis, and the estimated lethal dose for humans extrapolated from animal studies is so high that it cannot be achieved by … users."

In 2008, investigators at McGill University Health Centre and McGill University in Montreal and the University of British Columbia in Vancouver reviewed 23 clinical investigations of medicinal cannabinoid drugs (typically oral THC or liquid cannabis extracts) and eight observational studies conducted between 1966 and 2007. Investigators "did not find a higher incidence rate of serious adverse events associated with medical cannabinoid use" compared to non-using controls over these three decades.

That said, cannabis should not necessarily be viewed as a 'harmless' substance. Its active constituents may produce a variety of physiological and euphoric effects. As a result, there may be some populations that are susceptible to increased risks from the use of cannabis, such as adolescents, pregnant or nursing mothers, and patients who have a family history of mental illness. Patients with Hepatitis C, decreased lung function (such as chronic obstructive pulmonary disease), or who have a history of heart disease or stroke may also be at a greater risk of experiencing adverse side effects from marijuana. As with any medication, patients should consult thoroughly with their physician before deciding whether the medicinal use of cannabis is safe and appropriate.

HOW TO USE THIS REPORT

As states continue to approve legislation enabling the physician-supervised use of medicinal marijuana, more patients with varying disease types are exploring the use of therapeutic cannabis. Many of these patients and their physicians are now discussing this issue for the first time, and are seeking guidance on whether the therapeutic use of cannabis may or may not be advisable. This report seeks to provide this guidance by summarizing the most recently published scientific research (2000-2009) on the therapeutic use of cannabis and cannabinoids for 19 clinical indications:

* Alzheimer's disease
* Amyotrophic lateral sclerosis
* Chronic Pain
* Diabetes mellitus
* Dystonia
* Fibromyalgia
* Gastrointestinal disorders
* Gliomas
* Hepatitis C
* Human Immunodeficiency Virus
* Hypertension
* Incontinence
* Methicillin-resistant Staphyloccus aureus (MRSA)
* Multiple sclerosis
* Osteoporosis
* Pruritus
* Rheumatoid arthritis
* Sleep apnea
* Tourette's syndrome

In some of these cases, modern science is now affirming longtime anecdotal reports of medicinal cannabis users (e.g., the use of cannabis to alleviate GI disorders). In other cases, this research is highlighting entirely new potential clinical utilities for cannabinoids (e.g., the use of cannabinoids to modify the progression of diabetes.)

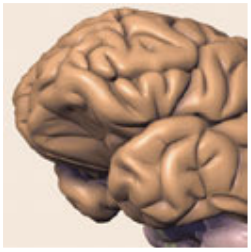
The conditions profiled in this report were chosen because patients frequently inquire about the therapeutic use of cannabis to treat these disorders. In addition, many of the indications included in this report may be moderated by cannabis therapy. In several cases, preclinical data and clinical indicates that cannabinoids may halt the progression of these diseases in a more efficacious manner than available pharmaceuticals. In virtually all cases, this report is the most thorough and comprehensive review of the recent scientific literature regarding the therapeutic use of cannabis and cannabinoids.

For patients and their physicians, let this report serve as a primer for those who are considering using or recommending medicinal cannabis. For others, let this report serve as an introduction to the broad range of emerging clinical applications for cannabis and its various compounds.

Paul Armentano
Deputy Director
NORML | NORML Foundation
Washington, DC
January 15, 2009

Alzheimer's Disease

Alzheimer's disease (AD) is a neurological disorder of unknown origin that is characterized by a progressive loss of memory and learned behavior. Patients with Alzheimer's are also likely to experience depression, agitation and appetite loss, among other symptoms. Over 4.5 million Americans are estimated to be afflicted with the disease. No approved treatments or medications are available to stop the progression of AD, and few pharmaceuticals have been FDA-approved to treat symptoms of the disease.


Courtesy of 3DScience.com

A review of the recent scientific literature indicates that cannabinoid therapy may provide symptomatic relief to patients afflicted with AD while also moderating the progression of the disease.

Writing in the February 2005 issue of the *Journal of Neuroscience*, investigators at Madrid's Complutense University and the Cajal Institute in Spain reported that the intracerebroventricular administration of the synthetic cannabinoid WIN 55,212-2 prevented cognitive impairment and decreased neurotoxicity in rats injected with amyloid-beta peptide (a protein believed to induce Alzheimer's). Additional synthetic cannabinoids were also found to reduce the inflammation associated with Alzheimer's disease in human brain tissue in culture. "Our results indicate that ... cannabinoids succeed in preventing the neurodegenerative process occurring in the disease," investigators concluded.[1] Follow up studies by investigators demonstrated that the administration of the nonpsychotropic plant cannabinoid cannabidiol (CBD) also mitigated memory loss in a mouse model of the disease.[2]

Investigators at The Scripps Research Institute in California in 2006 reported that THC inhibits the enzyme responsible for the aggregation of amyloid plaque — the primary marker for Alzheimer's disease — in a manner "considerably superior" to approved Alzheimer's drugs such as donepezil and tacrine. "Our results provide a mechanism whereby the THC molecule can directly impact Alzheimer's disease pathology," researchers concluded. "THC and its analogues may provide an improved therapeutic [option] for Alzheimer's disease [by]... simultaneously treating both the symptoms and the progression of [the] disease."[3]

More recently, investigators at Ohio State University, Department of Psychology and Neuroscience, reported that older rats administered daily doses of WIN 55,212-2 for a period of three weeks performed significantly better than non-treated controls on a water-maze memory test. Writing in the journal

Neuroscience in 2007, researchers reported that rats treated with the compound experienced a 50 percent improvement in memory and a 40 to 50 percent reduction in inflammation compared to controls.[4]

Previous preclinical studies have demonstrated that cannabinoids can prevent cell death by anti-oxidation.[5] Some experts believe that cannabinoids' neuroprotective properties could also play a role in moderating AD.[6] Writing in the September 2007 issue of the *British Journal of Pharmacology*, investigators at Ireland's Trinity College Institute of Neuroscience concluded, "[C]annabinoids offer a multi-faceted approach for the treatment of Alzheimer's disease by providing neuroprotection and reducing neuroinflammation, whilst simultaneously supporting the brain's intrinsic repair mechanisms by augmenting neurotrophin expression and enhancing neurogenesis. ... Manipulation of the cannabinoid pathway offers a pharmacological approach for the treatment of AD that may be efficacious than current treatment regimens."[7]

In addition to potentially modifying the progression of AD, clinical trials also indicate that cannabinoid therapy can reduce agitation and stimulate weight gain in patients with the disease. Most recently, investigators at Berlin Germany's Charite Universitatmedizin, Department of Psychiatry and Psychotherapy, reported that the daily administration of 2.5 mg of synthetic THC over a two-week period reduced nocturnal motor activity and agitation in AD patients in an open-label pilot study.[8]

Clinical data presented at the 2003 annual meeting of the International Psychogeriatric Association previously reported that the oral administration of up to 10 mg of synthetic THC reduced agitation and stimulated weight gain in late-stage Alzheimer's patients in an open-label clinical trial.[9] Improved weight gain and a decrease in negative feelings among AD patients administered cannabinoids were previously reported by investigators in the *International Journal of Geriatric Psychiatry* in 1997.[10]

Additional study assessing the use of cannabinoids for Alzheimer's would appear to be warranted.

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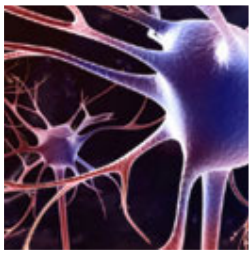
Amyotrophic Lateral Sclerosis (ALS)

Amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig's disease, is a fatal neurodegenerative disorder that is characterized by the selective loss of motor neurons in the spinal cord, brain stem, and motor cortex. An estimated 30,000 Americans are living with ALS, which often arises spontaneously and afflicts otherwise healthy adults. More than half of ALS patients die within 2.5 years following the onset of symptoms.



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A review of the scientific literature reveals an absence of clinical trials investigating the use of cannabinoids for ALS treatment. However, recent preclinical findings indicate that cannabinoids can delay ALS progression, lending support to anecdotal reports by patients that cannabinoids may be efficacious in moderating the disease's development and in alleviating certain ALS-related symptoms such as pain, appetite loss, depression and drooling.[1]

Writing in the March 2004 issue of the journal *Amyotrophic Lateral Sclerosis & Other Motor Neuron Disorders*, investigators at the California Pacific Medical Center in San Francisco reported that the administration of THC both before and after the onset of ALS symptoms staved disease progression and prolonged survival in animals compared to untreated controls.[2]

Additional trials in animal models of ALS have shown that the administration of other naturally occurring and synthetic cannabinoids can also moderate ALS progression but not necessarily impact survival.[3-4] One recent study demonstrated that blocking the CB1 cannabinoid receptor did extend life span in an ALS mouse model, suggesting that cannabinoids' beneficial effects on ALS may be mediated by non-CB1 receptor mechanisms.[5]

As a result, experts are calling for clinical trials to assess cannabinoids for the treatment of ALS. Writing in the *American Journal of Hospice & Palliative Medicine* in 2010, a team of investigators reported, "Based on the currently available scientific data, it is reasonable to think that cannabis might significantly slow the progression of ALS, potentially extending life expectancy and substantially reducing the overall burden of the disease." They concluded, "There is an overwhelming amount of preclinical and clinical evidence to

warrant initiating a multicenter randomized, double-blind, placebo-controlled trial of cannabis as a disease-modifying compound in ALS."[6]

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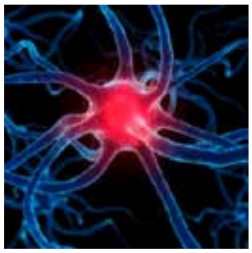
Chronic Pain

PDF



As many as one in five Americans lives with chronic pain.[1] Many of these people suffer from neuropathic pain (nerve-related pain) -- a condition that is associated with numerous diseases, including diabetes, cancer, multiple sclerosis, and HIV. In most cases, the use of standard analgesic medications such as opiates and NSAIDS (non-steroidal anti-inflammatory drugs) is ineffective at relieving neuropathic pain.

Survey data indicates that the use of cannabis is common in chronic pain populations[2] and several recent FDA-designed clinical trials indicate that inhaled marijuana can significantly alleviate neuropathic pain. These include a pair of randomized, placebo-controlled clinical trials demonstrating that smoking cannabis reduces neuropathy in patients with HIV by more than 30 percent compared to placebo.[3-4] (Additional details on these studies appear in the HIV section of this book.) In addition, a 2007 University of California at San Diego double-blind, placebo-controlled trial reported that inhaled cannabis significantly reduced capsaicin-induced pain in healthy volunteers[5] A 2008 University of California at Davis double-blind, randomized clinical trial reported both high and low doses of inhaled cannabis reduced neuropathic pain of diverse causes in subjects unresponsive to standard pain therapies.[6] Finally, a 2010 McGill University study finding that smoked cannabis significantly improved measures of pain, sleep quality and anxiety in participants with refractory pain for which conventional therapies had failed.[7]

Preclinical data indicates that cannabinoids, when administered in concert with one another, are more effective at ameliorating neuropathic pain than the use

of a single agent. Investigators at the University of Milan reported in 2008 that the administration of single cannabinoids such as THC or CBD produce limited relief compared to the administration of plant extracts containing multiple cannabinoids, terpenes (oils), and flavonoids (pigments).

Researchers concluded: "[T]he use of a standardized extract of Cannabis sativa ... evoked a total relief of thermal hyperalgesia, in an experimental model of neuropathic pain, ... ameliorating the effect of single cannabinoids," investigators concluded. ... "Collectively, these findings strongly support the idea that the combination of cannabinoid and non-cannabinoid compounds, as present in [plant-derived] extracts, provide significant advantages in the relief of neuropathic pain compared with pure cannabinoids alone."[8]

In 2009, an international team of investigators from the United Kingdom, Belgium and Romania affirmed these preclinical findings in a clinical study of intractable cancer pain patients. They concluded: "[I]n this study, the THC/CBD extract showed a more promising efficacy profile than the THC extract alone. This finding is supported by evidence of additional synergy between THC and CBD. CBD may enhance the analgesic potential of THC by means of potent inverse agonism at CB2 receptors, which may produce anti-inflammatory effects, along with its ability to inhibit immune cell migration. ... These results are very encouraging and merit further study."[9]

Additional clinical trials assessing inhaled cannabis and chronic pain remain ongoing.[10]

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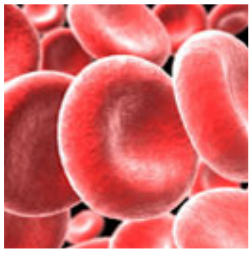
Diabetes Mellitus

Diabetes mellitus is a group of autoimmune diseases characterized by defects in insulin secretion resulting in hyperglycemia (an abnormally high concentration of glucose in the blood). There are two primary types of diabetes. Individuals diagnosed with type 1 diabetes (also known as juvenile diabetes) are incapable of producing pancreatic insulin and must rely on insulin medication for survival. Individuals diagnosed with type 2 diabetes (also known as adult onset diabetes) produce inadequate amounts of insulin. Type 2 diabetes is a less serious condition that typically is controlled by diet. Over time, diabetes can lead to blindness, kidney failure, nerve damage, hardening of the arteries and death. The disease is the third leading cause of death in the United States after heart disease and cancer.



Courtesy of 3DScience.com

A search of the scientific literature reveals no clinical investigations of cannabis for the treatment of diabetes, but does identify a small number of preclinical studies indicating that cannabinoids may modify the disease's progression and provide symptomatic relief to those suffering from it.[1-2] A 2006 study published in the journal *Autoimmunity* reported that injections of 5 mg per day of the non-psychoactive cannabinoid CBD significantly reduced the incidence of diabetes in mice. Investigators reported that 86% of untreated control mice in the study developed diabetes. By contrast, only 30% of CBD-treated mice developed the disease.[3] In a separate experiment, investigators reported that control mice all developed diabetes at a median of 17 weeks (range 15-20 weeks), while a majority (60 percent) of CBD-treated mice remained diabetes-free at 26 weeks.[4]

Other preclinical trials have demonstrated cannabinoids to possess additional beneficial effects in animal models of diabetes. Writing in the March 2006 issue of the *American Journal of Pathology*, researchers at the Medical College of Virginia reported that rats treated with CBD for periods of one to four weeks experienced significant protection from diabetic retinopathy.[5] This condition, which is characterized by retinal oxygen deprivation and a breakdown of the blood-retinal barrier, is the leading cause of blindness in working-age adults.

Cannabinoids have also been shown to alleviate neuropathic pain associated with the disease. A pair of studies published in the journal *Neuroscience Letters* in 2004 reported that mice administered a cannabis receptor agonist experienced a reduction in diabetic-related tactile allodynia (pain resulting from non-injurious stimulus to the skin) compared to non-treated controls.[6-7] The

findings suggest that "cannabinoids have a potential beneficial effect on experimental diabetic neuropathic pain."

A 2001 trial demonstrated that delta-9-THC could moderate an animal model of the disease by reducing artificially-elevated glucose levels and insulitis in mice compared to non-treated controls.[8] Most recently, an international team of researchers from the United States, Switzerland and Israel reported in the Journal of the American College of Cardiology that the administration of CBD reduces various symptoms of diabetic cardiomyopathy (weakening of the heart muscle) in a mouse model of type 1 diabetes. Authors concluded, "[T]hese results coupled with the excellent safety and tolerability profile of CBD in humans, strongly suggest that it may have great therapeutic potential in the treatment of diabetic complications."[9]

With the incidence of diabetes steadily increasing in both the adult and juvenile population, it would appear that further cannabinoid research is warranted in the treatment of this disease.

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Dystonia

Dystonia is a neurological movement disorder characterized by abnormal muscle tension and involuntary, painful muscle contractions. It is the third most common movement disorder after Parkinson's disease and tremor, affecting more than 300,000 people in North America.


Courtesy of 3DScience.com

A small number of case reports and preclinical studies investigating the use of cannabis and cannabinoids for symptoms of dystonia are referenced in the recent scientific literature. A 2002 case study published in the July issue of the *The Journal of Pain and Symptom Management* reported improved symptoms of dystonia after smoking cannabis in a 42-year-old chronic pain patient. Investigators reported that subject's subjective pain score fell from 9 to zero (on a zero-to-10 visual analog scale) following cannabis inhalation, and that the subject did not require any additional analgesic medication for the following 48 hours. "No other treatment intervention to date had resulted in such dramatic overall improvement in [the patient's] condition," investigators concluded.[1]

A second case study reporting "significant clinical improvement" following cannabis inhalation in a single 25-year-old patient with generalized dystonia due to Wilson's disease was documented by an Argentinian research team in the August 2004 issue of the journal *Movement Disorders*.[2]

Also in 2004, a German research team at the Hannover Medical School reported successful treatment of musician's dystonia in a 38-year-old professional pianist following administration of 5 mg of THC in a placebo-controlled single-dose trial.[3] Investigators reported "clear improvement of motor control" in the subject's affected hand, and noted, "[Two] hours after THC intake, the patient was able to play technically demanding literature, which had not been possible before treatment." Prior to cannabinoid treatment, the subject had been unresponsive to standard medications and was no longer performing publicly. "The results provide evidence that … THC intake … significantly improves [symptoms of] … focal dystonia," investigators concluded.

By contrast, a 2002 randomized, placebo-controlled study investigating the use of the synthetic oral cannabinoid naboline (Cesamet) in 15 patients afflicted with generalized and segmental primary dystonia did not show a significant reduction in dystonic symptoms.[4] Investigators speculated that this result may have been dose-related, and that administration of a higher dosage may have yielded a different outcome.

At least one recent preclinical trial indicates that both synthetic cannabinoids as well as high doses of the natural non-psychoactive cannabinoid cannabidiol (CBD) could moderate the disease progression of dystonia in animals.[5] Limited references regarding the use of cannabinoids for dystonia in humans[6] and animals[7] in the 1980s and the 1990s also appear in the scientific literature. It

would appear that additional, larger clinical trials are warranted to investigate the use of cannabis and cannabinoids for this indication.

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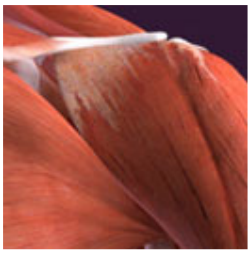
Fibromyalgia

Fibromyalgia is a chronic pain syndrome of unknown etiology. The disease is characterized by widespread musculoskeletal pain, fatigue and multiple tender points in the neck, spine, shoulders and hips. An estimated 3 to 6 million Americans are afflicted by fibromyalgia, which is often poorly controlled by standard pain medications.



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Fibromyalgia patients frequently self-report using cannabis therapeutically to treat symptoms of the disease,[1-2] and physicians – where legal to do so – often recommend the use of cannabis to treat musculoskeletal disorders.[3-4] To date however, there are few clinical trials assessing the use of cannabinoids to treat the disease.

Writing in the July 2006 issue of the journal *Current Medical Research and Opinion*, investigators at Germany's University of Heidelberg evaluated the analgesic effects of oral THC in nine patients with fibromyalgia over a 3-month period. Subjects in the trial were administered daily doses of 2.5 to 15 mg of THC and received no other pain medication during the trial. Among those participants who completed the trial, all reported a significant reduction in daily recorded pain and electronically induced pain.[5]

A 2008 study published in the *The Journal of Pain* reported that the administration of the synthetic cannabinoid nabilone significantly decreased

pain in 40 subjects with fibromylagia in a randomized, double-blind, placebo-controlled trial. "As nabilone improved symptoms and was well-tolerated, it may be a useful adjunct for pain management in fibromyalgia," investigators concluded.[6] A separate 2010 trial performed at McGill University in Montreal reported that low doses of nabilone significantly improved sleep quality in patients diagnosed with the disease.[7]

Previous clinical and preclinical trials have shown that both naturally occurring and endogenous cannabinoids hold analgesic qualities,[8-11] particularly in the treatment of pain resistant to conventional pain therapies. (Please see the 'Chronic Pain' section of this book for further details.) As a result, some experts have suggested that cannabinoids are applicable for the treatment of chronic pain conditions such as fibromyalgia, and have theorized that the disease may be associated with an underlying clinical deficiency of the endocannabinoid system.[12]

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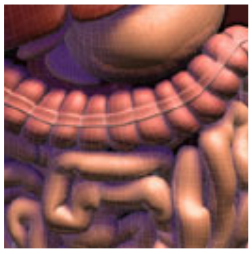
Gastrointestinal Disorders

Gastrointestinal (GI) disorders, including functional bowel diseases such as irritable bowel syndrome (IBS) and inflammatory bowel diseases such as Crohn's disease and colitis, afflict more than one in five Americans, particularly women. While some GI disorders may be controlled by diet and pharmaceutical medications, others are poorly moderated by conventional treatments. Symptoms of GI disorders often include cramping, abdominal pain, inflammation of the lining of the large and/or small intestine, chronic diarrhea, rectal bleeding and weight loss.



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Although several anecdotal reports[1-2] and a handful of case reports[3-4] exist in the scientific literature supporting the use of cannabinoids to treat symptoms of GI disorders, virtually no clinical trial work has been performed in this area, aside from a 2007 clinical study assessing the impact of oral THC on colonic motility.[5]

However, numerous preclinical studies demonstrate that activation of the CB1 and CB2 cannabinoid receptors exert biological functions on the gastrointestinal tract.[6] Effects of their activation in animals include suppression of gastrointestinal motility,[7] inhibition of intestinal secretion,[8] reduced acid reflux,[9] and protection from inflammation,[10] as well as the promotion of epithelial wound healing in human tissue.[11] As a result, many experts now believe that cannabinoids and/or modulation of the endogenous cannabinoid system represents a novel therapeutic approach for the treatment of numerous GI disorders — including inflammatory bowel diseases, functional bowel diseases, gastro-oesophagael reflux conditions, secretory diarrhea, gastric ulcers and colon cancer.[12-14]

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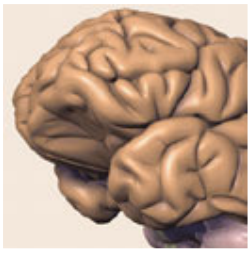
Gliomas/Cancer

Gliomas (tumors in the brain) are especially aggressive malignant forms of cancer, often resulting in the death of affected patients within one to two years following diagnosis. There is no cure for gliomas and most available treatments provide only minor symptomatic relief.



Courtesy of 3DScience.com

A review of the modern scientific literature reveals numerous preclinical studies and one pilot clinical study demonstrating cannabinoids' ability to act as antineoplastic agents, particularly on glioma cell lines.

Writing in the September 1998 issue of the journal *FEBS Letters*, investigators at Madrid's Complutense University, School of Biology, first reported that delta-9-THC induced apoptosis (programmed cell death) in glioma cells in culture.[1] Investigators followed up their initial findings in 2000, reporting that the administration of both THC and the synthetic cannabinoid agonist WIN 55,212-2 "induced a considerable regression of malignant gliomas" in animals.[2] Researchers again confirmed cannabinoids' ability to inhibit tumor growth in animals in 2003.[3]

That same year, Italian investigators at the University of Milan, Department of Pharmacology, Chemotherapy and Toxicology, reported that the non-psychoactive cannabinoid, cannabidiol (CBD), inhibited the growth of various human glioma cell lines *in vivo* and *in vitro* in a dose dependent manner. Writing in the November 2003 issue of the *Journal of Pharmacology and Experimental Therapeutics Fast Forward*, researchers concluded, "Non-psychoactive CBD ... produce[s] a significant anti-tumor activity both *in vitro* and *in vivo*, thus suggesting a possible application of CBD as an antineoplastic agent."[4]

In 2004, Guzman and colleagues reported that cannabinoids inhibited glioma tumor growth in animals and in human glioblastoma multiforme (GBM) tumor samples by altering blood vessel morphology (e.g., VEGF pathways). Writing in the August 2004 issue of *Cancer Research*, investigators concluded, "The present laboratory and clinical findings provide a novel pharmacological target for cannabinoid-based therapies."[5]

More recently, investigators at the California Pacific Medical Center Research Institute reported that the administration of THC on human glioblastoma multiforme cell lines decreased the proliferation of malignant cells and induced cell death more rapidly than did the administration of WIN 55,212-2. Researchers also noted that THC selectively targeted malignant cells while ignoring healthy ones in a more profound manner than the synthetic alternative.[6]

Most recently, Guzman and colleagues reported that THC administration decreases recurrent glioblastoma multiforme tumor growth in patients diagnosed with recurrent GBM. In the first ever pilot clinical trial assessing the use of cannabinoids and GBM, investigators found that the intratumoral administration of THC was associated with reduced tumor cell proliferation in two of nine subjects. "The fair safety profile of THC, together with its possible anti-proliferative action on tumor cells reported here and in other studies, may set the basis for future trials aimed at evaluating the potential antitumoral activity of cannabinoids," investigators concluded.[7] Several additional investigators have also recently called for further exploration of cannabis-based therapies for the treatment of glioma.[8-10]

In addition to cannabinoids' ability to moderate glioma cells, separate studies demonstrate that cannabinoids and endocannabinoids can also inhibit the proliferation of other various cancer cell lines, including breast carcinoma,[11-15] prostate carcinoma,[16-18] colorectal carcinoma,[19] gastric adenocarcinoma,[20] skin carcinoma,[21] leukemia cells,[22-23]neuroblastoma,[24] lung carcinoma,[25-26] uterus carcinoma,[27] thyroid epithelioma,[28] pancreatic adenocarcinoma,[29-30], cervical carcinoma,[31] oral cancer,[32] biliary tract cancer (cholangiocarcinoma)[33] and lymphoma.[34-35]

Studies also indicate that the administration of cannabinoids, in conjunction with conventional anti-cancer therapies, can enhance the effectiveness of standard cancer treatments.[36] Most recently, investigators at the University of California, Pacific Medical Center reported that cannabinoids possess synergistic anti-cancer properties -- finding that the administration of a combination of the plant's constituents is superior to the administration of isolated compounds alone.[37]

Consequently, many experts now believe that cannabinoids "may represent a new class of anticancer drugs that retard cancer growth, inhibit angiogenesis and the metastatic spreading of cancer cells."[38-39]

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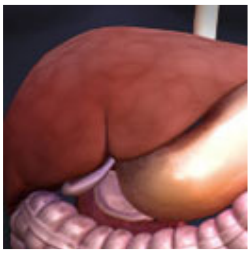
Hepatitis C

Hepatitis C is a viral disease of the liver that afflicts an estimated four million Americans. Chronic hepatitis C is typically associated with fatigue, depression, joint pain and liver impairment, including cirrhosis and liver cancer.



Patients diagnosed with hepatitis C frequently report using cannabis to treat both symptoms of the disease as well as the nausea associated with antiviral therapy.[1-2] An observational study by investigators at the University of California at San Francisco (UCSF) found that hepatitis C patients who used cannabis were significantly more likely to adhere to their treatment regimen than patients who didn't use it.[3] Nevertheless, no clinical trials assessing the use of cannabinoids for this indication are available in the scientific literature.

Preclinical data indicates that the endocannabinoid system may moderate aspects of chronic liver disease[4-5] and that cannabinoids may reduce inflammation in experimental models of hepatitis.[6] However, other clinical reviews have reported a positive association between daily cannabis use and the progression of liver fibrosis (excessive tissue build up) and steatosis (excessive fat build up) in select hepatitis C patients.[7-9]

As a result, experts hold divergent opinions regarding the therapeutic use of cannabinoids for hepatitis C treatment. Writing in the October 2006 issue of the *European Journal of Gastroenterology*, investigators from Canada and Germany concluded that cannabis' "potential benefits of a higher likelihood of treatment success [for hepatitis c patients] appear to outweigh [its] risks."[10] By contrast, other experts discourage the use of cannabis in patients with chronic hepatitis until further studies are performed.[11-15]

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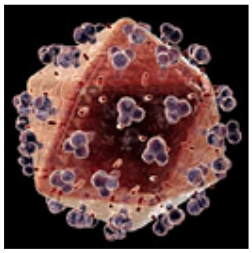
Human Immunodeficiency Virus (HIV)

The human immunodeficiency virus is a retrovirus that invades cells in the human immune system, making it highly susceptible to infectious diseases. According to the World Health Organization, over 500,000 Americans have died from HIV/AIDS and over one million US citizens are living with the disease.



Courtesy of 3DScience.com

Survey data indicates that cannabis is used by as many one in three North American patients with HIV/AIDS to treat symptoms of the disease as well as the side-effects of various antiretroviral medications.[1-4] One recent study reported that more than 60 percent of HIV/AIDS patients self-identify as "medical cannabis users."[5] Patients living with HIV/AIDS most frequently report using cannabis to counter symptoms of anxiety, appetite loss and nausea, and at least one study has reported that patients who use cannabis therapeutically are 3.3 times more

likely to adhere to their antiretroviral therapy regimens than non-cannabis users.[6]

Clinical trial data indicates that cannabis use does not adversely impact CD4 and CD8 T cell counts[7-8] and may even improve immune function.[9-10]

In 2007, investigators at Columbia University published clinical trial data in 2007 reporting that HIV/AIDS patients who inhaled cannabis four times daily experienced "substantial ... increases in food intake ... with little evidence of discomfort and no impairment of cognitive performance." They concluded, "Smoked marijuana ... has a clear medical benefit in HIV-positive [subjects]."[11]

That same year, investigators at San Francisco General Hospital and the University of California's Pain Clinical Research Center reported in the journal *Neurology* that inhaling cannabis significantly reduced HIV-associated neuropathy compared to placebo. Researchers reported that inhaling cannabis three times daily reduced patients' pain by 34 percent. They concluded, "Smoked cannabis was well tolerated and effectively relieved chronic neuropathic pain from HIV-associated neuropathy [in a manner] similar to oral drugs used for chronic neuropathic pain."[12]

In 2008, researchers at the University of California at San Diego reported similar findings. Writing in the journal *Neuropsychopharmacology*, they concluded: "Smoked cannabis … significantly reduced neuropathic pain intensity in HIV-associated … polyneuropathy compared to placebo, when added to stable concomitant analgesics. … Mood disturbance, physical disability and quality of life all improved significantly during study treatment. … Our findings suggest that cannabinoid therapy may be an effective option for pain relief in patients with medically intractable pain due to HIV."[13]

As a result, many experts now believe that "marijuana represents another treatment option in [the] health management" of patients with HIV/AIDS.[14]

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Hypertension

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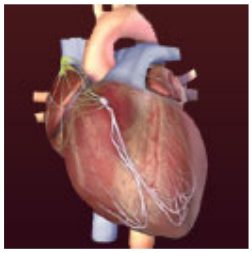

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High blood pressure, or hypertension, afflicts an estimated one in four American adults. This condition puts a strain on the heart and blood vessels and greatly increases the risk of stroke and heart disease.

Emerging research indicates that the endogenous cannabinoid system plays a role in regulating blood pressure, though its mechanism of action is not well understood.[1] Animal studies demonstrate that anandamide and other endocannabinoids profoundly suppress cardiac contractility in hypertension and can normalize blood pressure,[2-3] leading some experts to speculate that the manipulation of the endocannabinoid system "may offer novel therapeutic approaches in a variety of cardiovascular disorders."[4]

The administration of natural cannabinoids has yielded conflicting cardiovascular effects on humans and laboratory animals.[5-9] The vascular response in humans administered cannabis in experimental conditions is typically characterized by a mild increase in heart rate and blood pressure. However, complete tolerance to these effects develops quickly and potential health risks appear minimal.[10-11]

In animals, cannabinoid administration in animals is typically associated with vasodilation, transient bradycardia and hypotension,[12] as well as an inhibition of atherosclerosis (hardening of the arteries) progression.[13-15] The administration of synthetic cannabinoids have also been shown to lower blood pressure in animals and have not been associated with cardiotoxicity in humans.[16]

At this time, research assessing the clinical use of cannabinoids for hypertension is in its infancy though further investigation appears warranted.[17]

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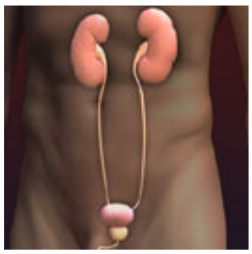
Incontinence

Urinary incontinence is defined as a loss of bladder control. Incontinence can result from several biological factors, including weak bladder muscles and inflammation, as well as from nerve damage associated with diseases such as multiple sclerosis (MS) and Parkinson's disease. More than one in ten Americans over age 65 is estimated to suffer from incontinence, particularly women.


Courtesy of 3DScience.com

Several recent clinical trials indicate that cannabinoid therapy may reduce incidents of incontinence. Writing in the February 2003 issue of the journal *Clinical Rehabilitation*, investigators at Oxford's Centre for Enablement in Britain reported that self-administered doses of whole-plant cannabinoid extracts improved bladder control compared to placebo in patients suffering from MS and spinal cord injury.[1]

Investigators at London's Institute for Neurology followed up these initial findings in an open-label pilot study of cannabis-based extracts for bladder dysfunction in 15 patients with advanced multiple sclerosis. Following cannabinoid therapy, "urinary urgency, the number of and volume of incontinence episodes, frequency and nocturia all decreased significantly," investigators determined. "Cannabis-based medicinal extracts are a safe and effective treatment for urinary and other problems in patients with advanced MS."[2]

These findings were confirmed in 2006 in a multi-center, randomized placebo-controlled trial involving 630 patients administered oral doses of cannabis extracts or THC. Researchers reported that subjects administered cannabis extracts experienced a 38 percent reduction in incontinence episodes from baseline to the end of treatment, while patients administered THC experienced a 33 percent reduction, suggesting a "clinical effect of cannabis on incontinence episodes."[3]

Most recently, preclinical data presented at the 2006 annual meeting of the American Urological Association indicated that cannabis analogs can reduce bladder inflammation and bladder over-activity in animals.[4]

In light of these findings, experts have recommended the use of cannabinoids as potential 'second-line' agents for treating incontinence.[5]

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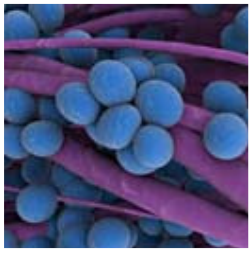
Methicillin-resistant *Staphyloccus aureus* (MRSA)



Many bacterial infections possess multi-drug resistance. Arguably the most significant of these bacteria is methicillin-resistant *Staphyloccus aureus*, more commonly known as MRSA or 'the superbug.' This bacterium is resistant to standard antibiotics, including penicillin. According to the *Journal of the American Medical Association*, MRSA is responsible for nearly 20,000 hospital-stay related deaths annually in the United States.[1]

Published data demonstrates that cannabinoids possess strong antibacterial properties. In 2008, investigators at Italy's Universita del Piemonte Orientale and Britain's University of London, School of Pharmacy assessed the germ-fighting properties of five separate cannabinoids against various strains of multidrug-resistant bacteria, including MRSA. They reported that all of the compounds tested showed "potent antibacterial activity" and that cannabinoids were "exceptional" at halting the spread of MRSA.[2]

A second study published that same year reported that non-cannabinoid constituents in the plant also possess antibacterial properties against MRSA and malaria.[3]

Clinical trials regarding the use of cannabinoids for MRSA have been recommended, with some experts stating, "Cannabis sativa ... represents an interesting source of antibacterial agents to address the problem of multidrug resistance in MRSA and other pathogenic bacteria."[4]

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Multiple Sclerosis

Multiple sclerosis (MS) is a chronic degenerative disease of the central nervous system that causes inflammation, muscular weakness and a loss of motor coordination. Over time, MS patients typically become permanently disabled and, in some cases, the disease can be fatal. According to the US National Multiple Sclerosis Society, about 200 people are diagnosed every week with the disease — often striking those 20 to 40 years of age.

Clinical and anecdotal reports of cannabinoids' ability to reduce MS-related symptoms such as pain, spasticity, depression, fatigue, and incontinence are plentiful in the scientific literature.[1-12] Most recently, investigators at the University of California at San Diego reported in 2008 that inhaled cannabis significantly reduced objective measures of pain intensity and spasticity in patients with MS in a placebo-controlled, randomized clinical trial. Investigators concluded that "smoked cannabis was superior to placebo in reducing spasticity and pain in patients with multiple sclerosis and provided some benefit beyond currently prescribed treatment."[13] Not surprisingly, patients with multiple sclerosis typically report engaging in cannabis therapy,[14] with one survey indicating that nearly one in two MS patients use the drug therapeutically.[15]

Other recent clinical and preclinical studies suggest that cannabinoids may also inhibit MS progression in addition to providing symptom management. Writing in the July 2003 issue of the journal *Brain*, investigators at the University College of London's Institute of Neurology reported that administration of the synthetic cannabinoid agonist WIN 55,212-2 provided "significant neuroprotection" in an animal model of multiple sclerosis. "The results of this study are important because they suggest that in addition to symptom management, ... cannabis may also slow the neurodegenerative processes that ultimately lead to chronic disability in multiple sclerosis and probably other disease," researchers concluded.[16]

Investigators at the Netherland's Vrije University Medical Center, Department of Neurology, also reported for the first time in 2003 that the administration of oral THC can boost immune function in patients with MS. "These results suggest pro-inflammatory disease-modifying potential of cannabinoids [for] MS," they concluded.[17]

Clinical data reported in 2006 from an extended open-label study of 167 multiple sclerosis patients found that use of whole plant cannabinoid extracts relieved symptoms of pain, spasticity and bladder incontinence for an extended period of treatment (mean duration of study participants was 434 days) without requiring subjects to increase their dose.[18] Results from a separate two-year open label extension trial in 2007 also reported that the administration of cannabis extracts was associated with long-term reductions in neuropathic pain in select MS patients. On average, patients in the study required fewer daily doses of the drug and reported lower median pain scores the longer they took it.[19] These results would be unlikely in patients suffering from a progressive

disease like MS unless the cannabinoid therapy was halting its progression, investigators have suggested.

In recent years, health regulators in Canada,[20] the United Kingdom,[21] Spain[22] and New Zealand[23] have approved the prescription use of plant cannabis extracts to treat symptoms of multiple sclerosis. Regulatory approval in the European Union and in the United States remains pending.

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Osteoporosis

Osteoporosis is a degenerative skeletal disease characterized by a deterioration of bone tissue. Patients with osteoporosis are at risk for suffering multiple fractures and other serious disabilities. Approximately 10 million Americans over age 50 suffer from osteoporosis, according to the US Surgeon General's office, and another 34 million are at risk for developing the disease.

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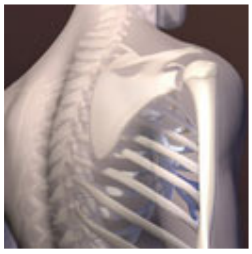

Courtesy of 3DScience.com

Initial references regarding the potential use of cannabinoids to protect against the onset of osteoporosis are available in the scientific literature beginning in the early 1990s.[1] To date, however, no clinical work has taken place investigating the use of cannabis for this indication.

Writing in the January 2006 issue of the *Proceedings of the National Academy of Sciences*, investigators at the Bone Laboratory of the Hebrew University in Jerusalem reported that the administration of the synthetic cannabinoid agonist HU-308 slowed the development of osteoporosis, stimulated bone building and reduced bone loss in animals.[2] Follow up research published in the *Annals of the New York Academy of Sciences* in 2007 reported that the activation of the CB2 cannabinoid receptor reduced experimentally-induced bone loss and stimulated bone formation.[3] Investigators have previously reported that mice deficient in the CB2 cannabinoid receptor experienced age-accelerated bone loss reminiscent of human osteoporosis.[4]

Scientists now speculate that the main physiologic involvement of specific endocannabinoid receptors (CB2 receptors) is to maintain "bone remodeling at balance, thus protecting the skeleton against age-related bone loss,"[5] leading some experts to believe that cannabinoids may be "a promising target novel target for anti-osteoporotic drug development."[6]

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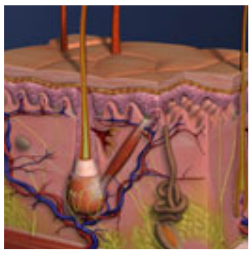
Pruritus

PDF



Courtesy of 3DScience.com

Itching (pruritus) is a common symptom associated with numerous skin diseases, as well as a secondary symptom of numerous serious conditions such as renal failure and liver disease. Itching, unlike other skin sensations, is generally a result of CNS activities and typically goes untreated by standard medical therapies.

A review of the scientific literature reveals three clinical trials investigating the use of cannabinoids in the treatment of pruritus. Writing in the August 2002 issue of the *American Journal of Gastroentrology*, investigators from the University of Miami Department of Medicine reported successful treatment of pruritus with 5 mg of THC in three patients with cholestatic liver disease.[1] Prior to cannabinoid therapy, subjects had failed to respond to standard medications and had lost their ability to work. Following evening cannabinoid administration, all three patients reported a decrease in pruritus, as well as "marked improvement" in sleep and were eventually able to return to work. Resolution of depression was also reported in two out of three subjects. "Delta-9-tetrahydrocannabinol may be an effective alternative in patients with intractable cholestatic pruritus," investigators concluded.

The following year, British researchers reported in the June 2003 issue of the journal *Inflammation Research* that the peripheral administration of the synthetic cannabinoid agonist HU-211 significantly reduced experimentally-induced itch in 12 subjects.[2] Investigators had previously reported that topical

application of HU-210 on human skin reduced experimentally-induced pain and acute burning sensations.[3]

Most recently, researchers at Wroclaw, Poland's University of Medicine, Department of Dermatology, reported that application of an endocannabinoid-based topical cream reduced uremic pruritus and xerosis (abnormal dryness of the skin) in hemodialysis patients.[4] Three weeks of twice-daily application of the cream "completely eliminated" pruritus in 38 percent of trial subjects and "significantly reduced" itching in others. Eighty-one percent of patients reported a "complete reduction" in xerosis following cannabinoid therapy.

In light of these encouraging preliminary results, some dermatology experts now believe that cannabinoids and the cannabinoid system may represent "promising new avenues for managing itch more effectively."[5]

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Rheumatoid Arthritis

Rheumatoid arthritis (RA) is an inflammatory disease of the joints characterized by pain, stiffness, and swelling, as well as an eventual loss of limb function. Rheumatoid arthritis is estimated to affect about one percent of the population, primarily women.

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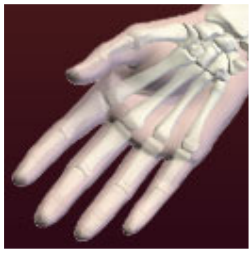

Courtesy of 3DScience.com

Use of cannabis to treat symptoms of RA is commonly self-reported by patients with the disease. In a 2005 anonymous questionnaire survey of medicinal cannabis patients in Australia, 25 percent reported using cannabinoids to treat RA.[1] A survey of British medical cannabis patients found that more than 20 percent of respondents reported using cannabis for symptoms of arthritis.[2] Nevertheless, few clinical trials investigating the use of cannabis for RA appear in the scientific literature.

In January 2006, investigators at the British Royal National Hospital for Rheumatic Disease reported successful treatment of arthritis with cannabinoids in the first-ever controlled trial assessing the efficacy of natural cannabis extracts on RA.[3] Investigators reported that administration of cannabis extracts over a five week period produced statistically significant improvements in pain on movement, pain at rest, quality of sleep, inflammation and intensity of pain compared to placebo. No serious adverse effects were observed. Similar results had been reported in smaller Phase II trials investigating the use of orally administered cannabis extracts on symptoms of RA.[4]

Preclinical data also indicates that cannabinoids can moderate the progression of RA. Writing in the August 2000 issue of the *Journal of the Proceedings of the National Academy of Sciences*, investigators at London's Kennedy Institute for Rheumatology reported that cannabidiol (CBD) administration suppressed progression of arthritis *in vitro* and in animals.[5] Administration of CBD after the onset of clinical symptoms protected joints against severe damage and "effectively blocked [the] progression of arthritis," investigators concluded. Daily administration of the synthetic cannabinoid agonist HU-320 has also been reported to protect joints from damage and to ameliorate arthritis in animals.[6]

Summarizing the available literature in the September 2005 issue of the *Journal of Neuroimmunology*, researchers at Tokyo's National Institute for Neuroscience concluded, "Cannabinoid therapy of RA could provide symptomatic relief of joint pain and swelling as well as suppressing joint destruction and disease progression."[7]

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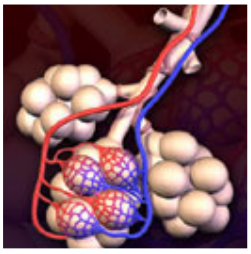
Sleep Apnea

Sleep apnea is a medical disorder characterized by frequent interruptions in breathing of up to ten seconds or more during sleep. The condition is associated with numerous physiological disorders, including fatigue, headaches, high blood pressure, irregular heartbeat, heart attack and stroke. Though sleep apnea often goes undiagnosed, it is estimated that approximately four percent of men and two percent of women ages 30 to 60 years old suffer from the disease.



Courtesy of 3DScience.com

One preclinical study is cited in the scientific literature investigating the role of cannabinoids on sleep-related apnea. Writing in the June 2002 issue of the journal of the American Academy of Sleep Medicine, researchers at the University of Illinois (at Chicago) Department of Medicine reported "potent suppression" of sleep-related apnea in rats administered either exogenous or endogenous cannabinoids.[1] Investigators reported that doses of delta-9-THC and the endocannabinoid oleamide each stabilized respiration during sleep and blocked serotonin-induced exacerbation of sleep apnea in a statistically significant manner. No follow up investigations have taken place assessing the use of cannabinoids to treat this indication. However, several recent preclinical and clinical trials have reported on the use of THC, natural cannabis extracts and endocannabinoids to induce sleep[2-3] and/or improve sleep quality.[4]

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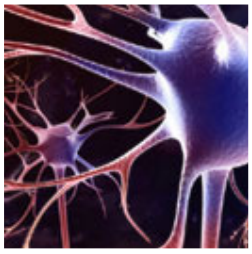
Tourette's Syndrome

Tourette's syndrome (TS) is a complex neuropsychiatric disorder of unknown etiology that is characterized by involuntary vocal tics. Severity of this condition varies widely among patients. Though there is no cure for Tourette's syndrome, the condition often improves with age. Experts estimate that 100,000 Americans are afflicted with TS.


Courtesy of 3DScience.com

A review of the scientific literature reveals several clinical trials investigating the use of cannabinoids for the treatment of TS. Writing in the March 1999 issue of the *American Journal of Psychiatry*, investigators at Germany's Medical School of Hanover, Department of Clinical Psychiatry and Psychotherapy, reported successful treatment of Tourette's syndrome with a single dose of 10 mg of delta-9-THC in a 25-year-old male patient in an uncontrolled open clinical trial.[1] Investigators reported that the subject's total tic severity score fell from 41 to 7 within two hours following cannabinoid therapy, and that improvement was observed for a total of seven hours. "For the first time, patients' subjective experiences when smoking marijuana were confirmed by using a valid and reliable rating scale," authors concluded.

Investigators again confirmed these preliminary results in a randomized, double-blind, placebo-controlled, crossover, single dose trial of THC in 12 adult TS patients. Researchers reported a "significant improvement of tics and obsessive-compulsive behavior (OCB) after treatment with delta-9-THC compared to placebo."[2] Investigators reported no cognitive impairment in subjects following THC administration[3] and concluded, "THC is effective and safe in treating tics and OCB in TS."[4]

Investigators confirmed these results in a second randomized, double-blind, placebo-controlled trial involving 24 patients administered daily doses of up to 10 mg of THC over a six-week period. Researchers reported that subjects experienced a significant reduction in tics following long-term cannabinoid treatment,[5] and suffered no detrimental effects on learning, recall or verbal memory.[6] A trend toward significant improvement of verbal memory span during and after therapy was also observed.

Summarizing their findings in the October 2003 issue of the journal *Expert Opinions in Pharmacotherapy*, investigators concluded that in adult TS patients, "Therapy with delta-9-THC should be tried ... if well established drugs either fail to improve tics or cause significant adverse effects."[7]

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9. Worth Repeating: Body's Own Cannabinoids Are The Bliss Within

By Ron Marczyk, R.N.

Health Education Teacher (Retired)

Did you see the medicinal cannabis science report in *The New York Times* on February 16?

In summary, the report says the great sense of euphoria and calm that many people report experiencing after prolonged exercise ("the runner's high") is not so much governed by the endorphins as "now an emerging field of neuroscience indicates that an altogether different neurochemical system within the body and brain, the endocannabinoid system, may be responsible for that feeling" of "pure happiness, elation, a feeling of unity with one's self and/or nature, endless peacefulness," and "inner harmony."

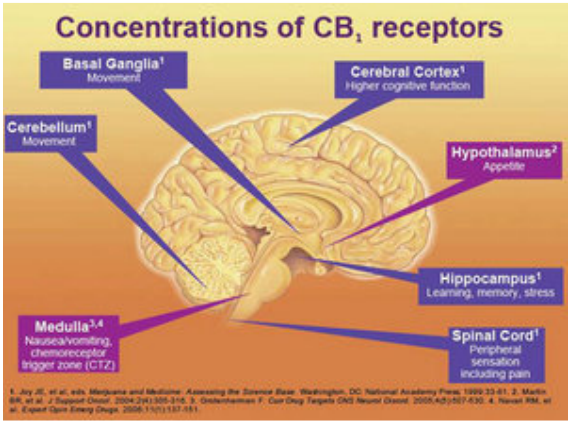
I have always been fascinated by how exercise and positive mood states go together. Having a master's degree in exercise physiology and cardiac rehabilitation, being a runner for 45 years, and as a rock climber with a background in Zen, I feel qualified to discuss how the endocannabinoid system can be activated by exercise and/or THC ingestion.

If you aren't familiar with the endocannabinoid system, the body's own internal source of cannabis-like chemicals, I suggest you read "Introduction to the Endocannabinoid System" from NORML.

To rehabilitate out-of-shape, sedentary individuals and motivate them to get their heart rate into the target zone and sweat, as a health practitioner, I would give them a new cognitive approach to understanding exercise -- not for competition, but as a medicine that gets you "high" on exercise, that gives you that red-cheeked, smiling, glowing face. This is the very expression of the bliss of wellness.

Ongoing discoveries, which are starting to dominate research on the endocannabinoid system, are validating the ancient stories of healing mind and body with this non-toxic plant.

The four parts of the endocannabinoid system that have been discovered piecemeal in the past 19 years, but have only recently been gaining the attention of the pharmaceutical companies, who are now positioning themselves to profit by manufacturing drugs that activate the system but still keep cannabis illegal for the masses.



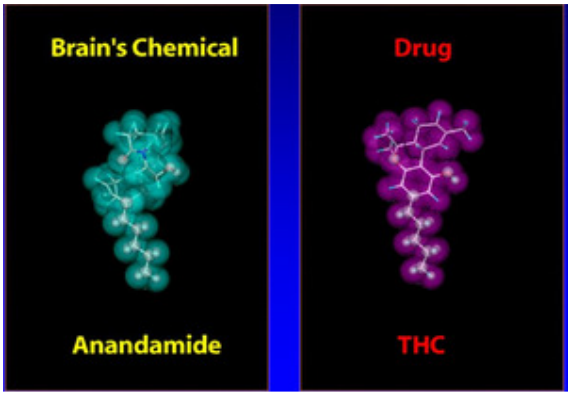
Graphic: Marijuana: Should It Be Legal?

Only as recently as 1992 did medical researchers discover this previously unknown, body-wide neurocellular receptor system that controls or regulates

almost every function in the body, by apparently bringing the mind and body back to a state of homeostasis after being stressed by the environment. This system **is** the "wisdom of the body" that we all experience as the body "just knowing how to fix itself" after illness. This system is the very definition of wellness!

Researchers at that time hadn't found an internally made neurotransmitter that fit these receptors, labeled CB1 and CB2 (CB stands for *cannabinoid*). The only molecule that fit and activated them perfectly was the THC molecule from cannabis, so they labeled these body-wide receptors, collectively, as the endocannabinoid system (*endo-* meaning "made in the body").

The search was on to find a naturally made neurotransmitter that also fit these receptors, and in 1992, anandamide, an endogenous cannabinoid neurotransmitter, was discovered. The name is taken from the Sanskrit word ananda, which means bliss, delight, or "the bliss within."



Graphic: Addiction Inbox

The whole system is controlled by four players -- two bliss receptors and two bliss molecules. CB1 bliss receptors are found primarily in the brain, controlling the euphoric effects of aerobic exercise and of cannabis ingestion. CB2 bliss receptors are found everywhere else in the body, but in highest concentration in the immune system. In addition, CB2 receptor activation also seems to play a large part in controlling inflammation and pain modulation.

Why I am using the term "bliss receptor"? Because when these receptors are activated, the individual experiences an internal state of bliss! When the toggle on a light switch is thrown, the switch doesn't care what flipped it; the same light is always produced. The CB1 receptor is the same; exercise or THC ingestion yields the same result.

The first bliss molecule, THC, was isolated in 1964, but only with the discovery of the second bliss molecule, anandamide, in 1992 did the significance of THC's discovery make sense.

Apparently there existed in nature a non-toxic phytochemical plant family which, when ingested, brings the body and mind back into equilibrium and homeostasis, as important as any other phytochemical, vitamin, or mineral we take in from our environment.

Many individuals may have a sub-optimal anandamide production capability, perhaps due to PTSD, child abuse, poverty, poor nutrition, or genetics. Think of THC as a non-toxic natural phytochemical like Resveratrol. THC ingestion by individuals may be a form of self-selection in which they are boosting the function of the endocannabinoid system through titration to optimally balance their internal state.

Why is anandamide called the "bliss molecule"? Because it describes exactly what happens when the system is activated. When anandamide or THC locks into the CB1 receptor, it produces the euphoria of the runner's high.

So what exactly is this "high" feeling one gets after intense aerobics? The term "euphoria" means "a profound state of well being" or "an intense state of transcendent happiness combined with an overwhelming sense of contentment; the power of enduring easily."

The opposite would be to live in despair, anxiety, depression, and give up. To reject this state of bliss because it is produced by an external agent on the

grounds that it is not natural is a false paradigm I reject, and is quite hypocritical, especially living in our culture with nonstop drug advertising on TV to "fix" countless human conditions.

Remember: Due to illness, disability, age and pain, many individuals are not capable of getting their heart rates into the target range to activate the anandamide pathway. Neurons that fire together, wire together. With use, this pathway strengthens; with disuse, the system weakens, which may lead to the depression seen in many chronic illnesses.

Cannabis then becomes their lifeline to joy and happiness. As proof, when CB1 receptors are blocked by the anti-obesity drug and CB1 antagonist, Rimonabant, people overwhelmingly reported experiencing severe depression and suicidal thoughts, and this drug that was being marketed had to be pulled in February 2006.

So when these receptors are turned on, the opposite effect takes place: the will to live.

Ingestion of cannabis is the same model for optimal health that runners experience. Imagine that!

An internal and external molecule that produces bliss akin to a mystical experience, and the first body system named after cannabis... How can cannabis ever be labeled as unnatural?

The totality of human experience can be described as a search for happiness. Isn't this the quest of life, why we toil and struggle? Aren't we all seeking a state of bliss?

It's even enshrined in the U.S. Constitution as "the pursuit of happiness." Isn't the individual the one who defines what happiness is for himself or herself?

"The time to be happy is now. The place to be happy is here. The way to be happy is to make other people happy." ~ Robert Ingersoll

10. Cannabis Hope for Inflammatory Bowel Disease

ScienceDaily (Dec. 21, 2009) — Chemicals found in cannabis could prove an effective treatment for the inflammatory bowel diseases Ulcerative Colitis and Crohn's Disease, say scientists.

Laboratory tests have shown that two compounds found in the cannabis plant -- the cannabinoids THC and cannabidiol -- interact with the body's system that controls gut function.

Crohn's Disease and Ulcerative Colitis, which affect about one in every 250 people in Northern Europe, are caused by both genetic and environmental factors. The researchers believe that a genetic susceptibility coupled with other triggers, such as diet, stress or bacterial imbalance, leads to a defective immune response.

Dr Karen Wright, Peel Trust Lecturer in Biomedicine at Lancaster University, presented her soon-to-be published work at The British Pharmacological Society's Winter Meeting in London.

She said: "The lining of the intestines provides a barrier against the contents of the gut but in people with Crohn's Disease this barrier leaks and bacteria can escape into the intestinal tissue leading to an inappropriate immune response.

"If we could find a way to restore barrier integrity in patients we may be able to curb the inflammatory immune response that causes these chronic conditions."

Dr Wright, working with colleagues at the School of Graduate Entry Medicine and Health in Derby, has shown that cells that react to cannabinoid compounds play an important role in normal gut function as well as the immune system's inflammatory response.

"The body produces its own cannabinoid molecules, called endocannabinoids, which we have shown increase the permeability of the epithelium during inflammation, implying that overproduction may be detrimental," said Dr Wright.

"However, we were able to reverse this process using plant-derived cannabinoids, which appeared to allow the epithelial cells to form tighter bonds with each other and restore the membrane barrier."

The research was carried out using cell cultures in a dish but, interestingly, when the team attempted to mimic the conditions of the gut by reducing the amount of oxygen in the cells' environment, much lower concentrations of cannabinoid were needed to produce the same effect.

Dr Wright added: "What is also encouraging is that while THC has psychoactive properties and is responsible for the 'high' people experience when using cannabis, cannabidiol, which

has also proved effective in restoring membrane integrity, does not possess such properties."

11. Treating Swine and Avian Flu With Cannabinoids

By Dr. Robert Melamede, Cannabis Science - Wednesday, April 29 2009

Dr. Robert Melamede explains how the active substances in the cannabis plant can be used to treat avian and swine flu.

The problem with current approaches to defeating Swine Flu, is we have not been able to beat the virus, because it's too good at mutating. Although it might be possible to use different antigenic targets to create a vaccine that would be moreuniversal and effective in the future, we need something now to defend against Swine Flu. We believe the solution is to change how our bodies deal with the virus. Recent discoveries about the anti-inflammatory properties of cannabinoids can provide us with new medicines, which can modify how we respond to these viruses and provide us with effective, non-toxic therapies. This paper provides a theory, with peer reviewed references, that supports the use of cannabinoids to prevent deaths associated with avian flu infections. If the lethality caused by the current swine flu can also be attributed to ARDS, then our proposal can be extended to the current problem.

1.0 Introduction: The Endocannabinoid System

Far-from-equilibrium thermodynamics as pioneered by Nobel Laureate Ilya Prigogine, provides a physical underpinning for all biological processes [1,2]. An intrinsic characteristic that emerges, and permeates all organizational levels of life, is oscillations of opposing biochemical phenomena, often linked with inflammatory anti-inflammatory processes. In the same manner that temperature in a house varies around the set point determined by a thermostat, countless interacting reactions in human biochemistry oscillate around set points that turn up or down inflammatory responses and associated free radical production [3]. Evolution has selected the endocannabinoid system as a critical modulator of inflammatory biochemical pathways [4].

Essentially, inflammation-generated free radicals may be thought of as biochemical friction, and endocannabinoids as the oil of life [5] in that they reduce this friction. From this perspective, it is easy to understand why the endocannabinoids system has life promoting activities [6], and why phytocannabinoids (plant derived cannabinoids), by virtue of their ability to

mimic endocannabinoids, have therapeutic benefits for such wide range of illnesses, including cardiovascular [7,8], neurological [9,10], immunological [11-14], skeletal [15,16], diseases and cancers [17-24]. They in fact appear to function as anti-aging compounds as indicated by the increased lifespan observed when mice were treated with THC for extended periods of time [25]. In contrast, knockout mice that lacked the CB1 receptor die prematurely and CB2 knockout mice appear to have a number of associated phenotypes relating to the immune system, cardiovascular system, nervous system, digestive system, and reproductive system [26-29].

The bird flu is one of the most critical viral diseases to threaten mankind today. Influenza viruses have already killed millions of individuals around the world. The following sections on the bird flu are a logical synthesis of existing knowledge that dramatically shows how important cannabis-based research can be for mankind, and why we have chosen influenza as an early focus of our research efforts. We feel that the evidence below sufficiently supports the possibility that cannabinoids may save millions of lives that would otherwise be lost due to influenza and HIV infections, and it would be immoral and irresponsible not to determine if our hypothesis is correct.

2.0 A Brief Introduction Into the Immune System

In order to appreciate the hypothesized life-saving possibilities offered by cannabinoids with respect to the bird flu and HIV, a limited understanding of how the immune system works is necessary. Upon infection, the infectious agent and damaged host tissue release chemical signals that serve as markers so that neutrophils, the foot soldiers of the immune system, can find their way to the invading pathogens. These specialized white blood cells bring with them a formidable array of biochemical weaponry including specialized receptors (TLRs), known as toll receptors that recognize molecular patterns on various pathogens. Bound TLRs activate neutrophils to produce highly inflammatory bacteriocidal chemicals such as hydrogen peroxide and sodium perchlorate. Additionally, neutrophils phagocytize the invaders. The neutrophils die young, lasting only a few days. The debris field is subsequently cleaned up by additional, late to arrive, phagocytic cells, monocytes and macrophage. The immune process thus far described is known as the innate immune system. We inherit it and are born with it functioning. The high levels of free radicals and other cytotoxic agents produced during the innate response create a lot of collateral damage. To overcome this damage problem, evolution has selected an additional more targeted, less inflammatory immune process known as the acquired response.

The acquired immune response takes pieces of the phagocytized pathogens and presents them on the surface of phagocytic cells in order to generate a specific response via the collaborative action of T and B cells that ideally kill pathogens and pathogen infected cells with a more specific targeted, less inflammatory response directed by B and T cell receptors.

2.1 Pathology associated with an Excessive Inflammatory Response

Today, most people in first world countries die from age-related illnesses [30]. One hundred years ago, people in the same countries died predominantly of infectious diseases. The proinflammatory arm of the human immune system has evolved to play a critical role in fighting many infectious diseases. However, the inflammatory responses and associated free radical production appear to be at the heart of age-related illnesses including neurological disorders, cardiovascular disease, autoimmune diseases, and cancers [31].

Man has changed the world in which we live in a manner that – for now – has greatly increased our lifespan. Improvements in public health, for example, have resulted in dramatic increases in the health of the human population. However, these changes have occurred too rapidly for the evolution of our immune system to keep pace with changing environmental demands. We live cleaner today, and in general appear to need lower levels of inflammation for control of most infections. Since the endocannabinoid system plays a critical role in up-regulating the antiinflammatory arm of the immune system, phytocannabinoids can play a natural role in bringing man's immune system up-to-date by reducing the levels of immune generated inflammation, i.e. resetting the inflammatory thermostat.

It is important to keep in mind that different infections elicit different types of immune responses. There is an ongoing evolutionary battle between our immune system and pathogens. While many illnesses are exacerbated by an excessive inflammatory immune response, this type of response is required to control infection of tuberculosis, Legionella pneumophila, and Leishmania. The use of cannabis for these types of infections could be lethal as indicated by animal models [32], because some types of infections actually require the pro-inflammatory response for their survival as most recent studies indicate is the case with HIV.

2.2 Avian Influenza (Bird Flu)

The Problem: The bird flu is one of the most dangerous viral diseases to threaten mankind today [33]. The main source of fear is that mutated viruses will be acquire the capacity to transfer not only from wild birds to domestic birds and then to people, from man to man [34] and result in a worldwide pandemic. North Americans may be particularly vulnerable to this threat as a result of the migratory route over Canada taken by many wild birds [35].

This danger is underscored by the recent outbreak of the avian flu on the Canadian turkey farms that resulted in the killing of thousands of birds 36. The Canadian press recently reported that Baxter International’s European facility in Austria mistakenly provided materials that were contaminated with the deadly avian H5N1 strain of influenza to a research company that subsequently sent samples to other European countries 37. When samples were injected into animals, the unexpected death that resulted led to investigations that identified the deadly strain as the problem.

This error could easily have resulted in a pandemic that would have killed millions. The magnitude of the threat posed by the avian flu to humanity was recently further emphasized by studies showing an unexpected rise in resistance to currently used antiviral medications 33. The bird flu, should it mutate to efficiently infect humans, will kill many millions in one season.

The Solution:

The lethality associated with bird flu infections in humans is very high (63%) 38. Based on animal studies, it appears that the bird flu elicits a proinflammatory immune response that is many times greater than that which results from infections by other influenza strains both in the lungs 39 and the brain 40. The apparently excessive proinflammatory immune response results in the lethal development of adult respiratory distress syndrome (ARDS) and multiple organ failure 41. We hypothesize that a life-saving down-regulation of the excessively high proinflammatory response to the bird flu may be accomplished by orally ingesting an appropriate dose of phytocannabinoids without impairing immune control of the virus (resetting the inflammatory thermostat). Smoking or vaporizing cannabis will not work, and in fact could make things worse since using the pulmonary route will promote an added degree of inflammation.

There are typically two phases to any immune response. Initially, the innate arm of the immune system responds by initiating acute inflammation and free radical-induced cell killing. This general, non-specific response is then turned down as the more targeted acquired immune response kicks in. A successful immune response is characterized by the control of infection in a manner that minimizes harm to the infected organism. This goal is difficult and complex to accomplish. Both genetic and environmental factors, as well as chance, determine the outcome of a given infection.

The immune-cell-driven functions of the innate immune system are very inflammation dependant, and as a consequence produce collateral damage to surrounding tissue. Neutrophils, monocytes and macrophage are migratory cells that travel to the site of infection and initiate an innate immune response. Ultimately, these same cell types are also responsible for the transition to the acquired response as a result of antigen uptake and presentation. Current thinking suggests that the monocytes release MCL-1 attractant protein that binds the chemokine receptor CCR2 on a novel dendritic cell subset that produces TNF and iNOS (Tip) 42, which during later stages of infection promote antigen specific T cell responses 43.

There are numerous studies that demonstrate the capacity of cannabinoids to down regulate the cascade of pro-inflammatory immune responses. Neutrophil 44 and monocyte migration in inhibited by activating CB2 receptor 45Similarly, cannabinoids reduce the response to proinflammatory chemokines and cytokines 46 including TNF 47-49. Most relevant to our proposal, the effect of THC on influenza induced lung inflammation has been examined50,51. These studies demonstrated that THC could prevent influenza induced lung epithelial cell death even though there was an increase in viral load.

In order to appreciate the significance of these findings the thermostat model is helpful. The inflammatory thermostat of Homo sapiens was set over the past hundreds of thousands of years. Humans lived short dirty lives. A strong inflammatory response was essential. Under some circumstances, such as occurs with influenza infection in a cleaner modern world, the inflammatory thermostat may be set too high. As a result, rather than protecting us, our immune system is killing us. Biology is never simple. The influenza virus is itself cytolytic and therefore destructive of respiratory epithelial cells, and our defenses are complicated 52. The question therefore becomes what kills first the virus or the immune system? The probable, but complex answer is that the outcome will depend the idiosyncratic biochemical balances of an individual, past exposures, and their genetics.

Case Study

What counts is the way in which the complicated dialog between an infectious agent and its host comes together to promote survival or death. Can an individual reduce inflammation and its lethal consequences while still controlling the viral infection? We have a limited but significant answer. Steve Kubby has inoperable, metastasized pheochromocytoma. He is the sole long-term survivor of this illness, having had it for 35 years. His only medication has been Cannabis. Recently, when he came down with serious case of the common flu, he treated himself with "homemade" cannabis extract-based lozenges instead of smoking cannabis. His symptoms were much milder than what normally occurs when he has had the flu and smoked cannabis in the past. Turning down immune modulated inflammation did not harm him and in fact appears to have been beneficial.

Today it is essential to determine if our lozenge is effective in reducing bird flu associated deaths. We feel that this work is particularly important since people are continually coming down with new variants of the influenza virus, such as the bird and swine strain currently threatening humanity. Because of the intrinsic high degree of variability that is built into the influenza virus, it’s only a matter of time before this problem becomes even more serious. We hope to provide a cost-effective, and safe solution to this threat, which could literally kill millions. It is critical that
work start as soon as possible. When the "Spanish flu" broke out in 1918, more people died from it than from World War I.

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12. Cannabinoid receptor CB1 mediates baseline and activity-induced survival of new neurons in adult hippocampal neurogenesis

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Abstract

Background

Adult neurogenesis is a particular example of brain plasticity that is partially modulated by the endocannabinoid system. Whereas the impact of synthetic cannabinoids on the neuronal progenitor cells has been described, there has been lack of information about the action of plant-derived extracts on neurogenesis. Therefore we here focused on the effects of Δ9-tetrahydrocannabinol (THC) and Cannabidiol (CBD) fed to female C57Bl/6 and Nestin-GFP-reporter mice on proliferation and maturation of neuronal progenitor cells and spatial learning performance. In addition we used cannabinoid receptor 1 (CB1) deficient mice and treatment with CB1 antagonist AM251 in Nestin-GFP-reporter mice to investigate the role of the CB1 receptor in adult neurogenesis in detail.

Results

THC and CBD differed in their effects on spatial learning and adult neurogenesis. CBD did not impair learning but increased adult neurogenesis, whereas THC reduced learning without affecting adult neurogenesis. We found the neurogenic effect of CBD to be dependent on the CB1 receptor, which is expressed over the whole dentate gyrus. Similarly, the neurogenic effect of environmental enrichment and voluntary wheel running depends on the presence of the CB1 receptor. We found that in the absence of CB1 receptors, cell proliferation was increased and neuronal differentiation reduced, which could be related to CB1 receptor mediated signaling in Doublecortin (DCX)-expressing intermediate progenitor cells.

Conclusion

CB1 affected the stages of adult neurogenesis that involve intermediate highly proliferative progenitor cells and the survival and maturation of new neurons. The pro-neurogenic effects of CBD might explain some of the positive therapeutic features of CBD-based compounds.

Background

The recreational use of cannabis is often justified by extrapolation from the unquestionable physiological role of endocannabinoids in brain function [1], and the successful and beneficial manipulation of the endocannabinoid system for medical purposes [2,3] by plant extracts from *cannabis sativa* or synthetic agonist and antagonists specific for cannabinoid receptor1 or 2 (CB1, CB2) [4,5]. The abuse of cannabis can be associated with detrimental long-term consequences, for example an increased risk of developing memory impairments [6,7].

The process of generating new neurons throughout life in the hippocampus probably plays a role in learning and memory processes [8], and impairment of adult hippocampal neurogenesis is thought to be part of the pathogenesis of neurodegenerative disorders like dementia, epilepsy and schizophrenia [9,10]. Adult neurogenesis is a particular example of brain plasticity as it involves the integration of entire cells [11,12]. Due to its physiological role in brain plasticity the endocannabinoid system might contribute to the control of adult hippocampal neurogenesis in health and disease. A number of arguments point into the direction that cannabinoids might exert some of their actions via their effects on adult neurogenesis (reviewed in [13]).

The therapeutic activities of cannabinoids include analgesia, immuno-suppression, mood stabilization, anti-emesis, bronchodilatation and neuroprotection [14]. Because of the psychotropic effects of some cannabinoids, their clinical use is limited. Cannabidiol (CBD) is the main non-psychotropic compound of the plant *cannabis sativa* and belongs to the group of exogenous cannabinoids [15]. Due to its lack of psychoactive actions, CBD represents one of the most promising candidates for clinical application [14]. CBD was shown to act anti-psychotic in Parkinson's disease and as a monotherapy in treatment-resistant schizophrenia [16,17]. The neuroprotective effects of CBD have been linked with its antioxidant activity [18]. Evidence emerges that CBD realizes some of its effect via the classical CB receptors [19].

Many constituents of the endogenous cannabinoid system like the CB1 and CB2 receptors and their endogenous ligands Anandamide (AEA) and 2-

arachidonylglycerol (2-AG) as well as the AEA-degrading enzyme fatty acid amide hydrolase (FAAH) and the 2-AG synthesizing enzyme diacylglycerol lipases are found in neuronal developmental and adult neurogenesis [20-22].

Several studies investigating the role of the cannabinoid system in adult neurogenesis found that stimulation of CB1 seemed to either increase or decrease adult neurogenesis [21,23]. For example, the synthetic agonist HU210 decreased the number of intermediate progenitor cells in one study [24], but promoted neuronal differentiation in another [25]. In other studies, CB1 receptor activation promoted precursor cell proliferation and the generation of neurospheres ex vivo, which was abrogated in CB1-deficient precursor cells, and proliferation of hippocampal precursor cells was increased in FAAH deficient mice [21,23,26]. Likewise, in adult CB1-deficient mice, neural progenitor proliferation is decreased. In addition, endocannabinoid signaling controls neural progenitor differentiation in the adult brain by promoting astroglial differentiation of newly born cells [23]. Along the same line, Rueda et al. have shown that the endocannabinoid AEA inhibited neuronal progenitor cell differentiation through attenuation of the extracellular signal regulated kinase pathway in vitro, and that adult neurogenesis in the dentate gyrus was significantly decreased by the AEA analogue methanandamide and increased by the CB1 antagonist SR141716 [27].

Precursor cell proliferation is a relatively non-specific measure of neurogenesis and not identical to the net production of new neurons. Progenitor cell proliferation is, for example, increased after epileptic seizures without necessarily leading to functional neurogenesis [28]. The incorporation of the progenitor cell into the neuronal network is impaired after seizures despite a high proliferation rate [29]. In the study by Jin et al. only BrdU incorporation was measured without further phenotyping the labeled cells and only cell proliferation was directly addressed [30]. However, they reported increased cell proliferation after treatment with CB1 antagonists SR141716 and AM251, which is in line with the findings by Rueda at al. [27]. In addition, they showed that SR141716 enhances cell proliferation via the vanillin receptor 1 [30]. The absence of the CB1 receptor resulted again in decreased proliferation.

The confusion that emerges when comparing the studies could be explained by differences in the study design, compounds used, sex of the animals, duration of application, and the readout parameters for "adult neurogenesis". Moreover, in the context of adult neurogenesis, only synthetic compounds interacting with the endogenous cannabinoid system have been investigated so far. It has been speculated that also plant-derived cannabinoids might have an impact on

neurogenesis, but no data exist to date. The sole exception is a brief study reporting no effects on cell proliferation in general [31].

In our study we therefore first examined the effects on adult neurogenesis in female C57Bl/6 mice by pharmaceutical extracts enriched with Δ9-tetrahydrocannabinol (THC) or Cannabidiol (CBD) directly derived from the plant *cannabis sativa*. Since THC's and CBD's mode of actions are partly CB1-dependent [19,32], we then looked at the time course (including proliferation and net-neurogenesis) of the maturation process of neuronal precursor cells in CB1-/- C57Bl/6 female mice and the impact of the CB1 antagonist AM251.

Results

Chronic THC treatment impairs spatial learning

Either THC- or CBD-enriched or control (CTR) diet was fed to female C57Bl/6 or Nestin-GFP-reporter mice. The food intake and the weight gain over the period of 6 weeks were similar in all the treatment groups (see additional file 1, Additional file 2). To examine the impact of chronic THC vs. CBD treatment on spatial memory, we tested the three experimental groups (THC, CBD, CTR) in the Morris water maze (MWM). The task in the MWM is to navigate to a hidden platform using spatial cues in the room. As shown in figure 1A, THC mice were slower in finding the hidden platform over the whole acquisition period (repeated measures ANOVA, $F_{2,20} = 3.49$; $p = 0.0014$). In addition, THC mice showed a significantly impaired performance during the reversal learning (with the hidden platform at a new position) with regard to both latency (THC: 36.13 +/- 10.94, CTR: 16.88 +/- 4.21, $p = 0.002$, ANOVA, $F_{2,20} = 3.49$, Fig. 1A) and distance to platform (THC: 49.4 ± 6.76, CTR: 31.93 ± 2.94, $p = 0.002$, ANOVA, $F_{2,20} = 3.49$; Fig. 1B). All three groups performed at the same level when the platform was made visible on day 6 to test for possible visual impairments and the general ability to perform the task (Fig. 1A, B). The impaired learning performance of the THC-treated mice was also reflected by the shorter time the animals spent in the old target quadrant and target zone during the probe trial at day 4 (Fig. 1C). A rotarod test to assess general locomotor functions and fitness was performed on day 7. The THC group performed better than CTR (CTR 138.47 ± 25.844 s, THC group 180.82 ± 26.17 s; $p = 0.0046$, ANOVA, $F_{2,16} = 3.634$; Fig. 1C), whereas CBD performed at control level. Therefore, the decreased performance of THC mice in the water maze could not be attributed to a reduced general fitness.

Figure 1.

Spatial learning was impaired after THC treatment. C57Bl/6 female mice were either fed with food supplemented with THC-rich or CBD-rich plant extracts or a control diet. Spatial memory was tested after 6 weeks of treatment. THC mice were slower in finding the hidden platform over the whole acquisition period and during the reversal learning, with regard to both latency (A) and distance (B) to platform. All three groups performed at the same level when the platform was made visible on day 6 to test for possible visual impairments and the general ability to perform the task. A Rotarod test to measure general locomotor function and fitness was performed on day 7. The THC group performed better than CTR (C); * $p \leq 0.05$.

Additional file 1. Weight gain and food intake. The two graphs show the food intake and weight gain (g) during the whole period of the experiment of 6 weeks. In the beginning of the experimental period, some variances could be seen in the food intake between the groups at certain days. At 6 weeks, when the analysis started, all groups reached a similar level of food intake and weight in average.

Format: JPEG Size: 25KB Download file OPEN DATA

Additional file 2. Weight gain and food intake. The two graphs show the food intake and weight gain (g) during the whole period of the experiment of 6 weeks. In the beginning of the experimental period, some variances could be seen in the food intake between the groups at certain days. At 6 weeks, when the analysis started, all groups reached a similar level of food intake and weight in average.

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CBD mice showed some (statistically not significant, $p = 0.124$; $F_{2,20} = 3.49$; Fig. 1A, B) impairment during acquisition. During the probe trial (Fig. 1C) and the reversal they performed very similar to CTR.

Chronic THC treatment decreases adult neurogenesis

We have previously reported that adult hippocampal neurogenesis can be linked to aspects of the acquisition phase during water maze learning [33] with a particular contribution to reversal performance [34,35]. We did not find a specific reversal phenotype in the present study but nevertheless asked whether a decrease in adult neurogenesis, possibly matching the observed alterations in water maze performance, might be found after THC or CBD treatment.

We found that chronic THC application reduced precursor cell proliferation in the DG (THC vs. CTR: 2018 ± 96; 2515 ± 180; n = 5; $p = 0.037$, Fig. 2A) without affecting cell survival or net neurogenesis. In contrast, however, despite

decreasing proliferation, CBD increased cell survival (proliferation: CBD vs. CTR: 2083 ± 102 vs. 2515 ± 180; n = 5; p = 0.0358; survival: CBD vs. CTR: 756 ± 28 vs.180 ± 21; n = 5; p = 0.0012; Fig. 2A).

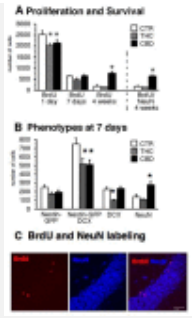

Figure 2.

CBD treatment enhanced adult neurogenesis. BrdU cells reflect the population of proliferating cells at a given period of time. Proliferation was measured 24 h after BrdU injection, while survival was measured 4 weeks after BrdU injection. NeuN/BrdU double positive cells at 4 weeks after BrdU injection are the neurons that were generated and survived during the period of 4 weeks. THC treatment for 6 weeks reduced cell proliferation without affecting neuronal survival. In contrast, CBD treatment decreased proliferation as well, but increased neuronal cell survival seen at 4 weeks after BrdU injection. (A). Animals expressing Nestin, an early marker of neuronal maturation, under a GFP promotor were also fed with THC-rich, CBD-rich, or control (CTR) diet for 6 weeks. The animals were assessed at 7 days after BrdU injection to evaluate the early stages of neurogenesis. In both THC and CBD groups we found a minimal reduction in the number of BrdU-labeled type-1/2a cells (Nestin-GFP-positive, DCX-negative) but a significant reduction on the level of the type-2b (Nestin-GFP-positive, DCX-positive). In THC the number of DCX-positive/Nestin-GFP-negative cells was also reduced. There was a significant increase in the production of BrdU/NeuN-positive cells in the CBD group (B); * $p \leq 0.05$. In (C) we show a representative micrograph of BrdU labeled cells (red) within the granule cell layer labeled with NeuN (blue) of the dentate gyrus. The section is one out of nine throughout one hippocampus of a female C57Bl/6 animal that received 3 BrdU injections 4 weeks prior to analysis. The section is 40 μm thick and the scale bar is 50 μm.

In both, THC and CBD groups we found a minimal reduction in the number of BrdU-labeled type-1/2a cells, i.e. Nestin-GFP-positive, Doublecortin (DCX)-negative cells, but a significant reduction at the level of the type-2b cells (Nestin-GFP-positive, DCX-positive; THC vs. CTR: 502 ± 83 vs. 748 ± 78; n = 5; p = 0.028; CBD vs. CTR: 507 ± 62 vs. 748 ± 78; n = 5; p = 0.032; Fig. 2B). In THC the number of DCX-positive/Nestin-GFP-negative cells was also reduced (THC vs. CTR: 113 ± 19 vs. 249 ± 26; n = 5; p = 0.004; Fig. 2B), possibly indicating that THC might not increase net neurogenesis but nevertheless accelerate the transition through the DCX-positive stage. For an overview of the maturation stages see below. A representative BrdU/NeuN staining is shown in figure 2C.

There was, however, a significant increase in the production of BrdU/NeuN-positive cells in the CBD group (CBD vs. CTR: 297 ± 32 vs.146 ± 23; n = 5; p = 0.001; Fig. 2A, B), suggesting that in this case an accelerated transition through the DCX stage might result in more neurons, an effect absent in the case of THC.

Taken together THC treated mice showed reduced water maze performance, albeit not the presumably neurogenesis-related reversal impairment, and reduced adult neurogenesis, whereas CBD mice did not. On the other hand our results pointed to a positive effect of CBD on adult neurogenesis that we now intended to explore further.

CBD effects on adult neurogenesis are absent in CB1 -/- mice

We next asked whether these CBD-mediated effects on BrdU incorporation might be mediated by the CB1 receptor, which is highly expressed in the dentate gyrus and fed CBD to CB1-/- mice and their wild type litter mates in parallel for 6 weeks. We found that the increase in BrdU cell survival induced by CBD was abolished in CB1-/- mice (Fig. 3; WT/CBD vs. CB1-/-/CBD: 716 ± 83 vs.152 ± 28; n = 5; p = 0.001). CB1-/- mice showed a decrease in the number of BrdU cells. This decrease in CB1-/- has already been described in the literature. But we could here show a similar effect of CB1-/- at the survival time point at 4 weeks after BrdU application (CB1-/- vs. WT: 180 ± 15 vs.368 ± 21; n = 5; p = 0.002).

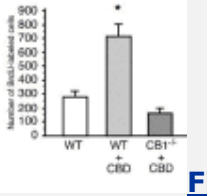

Figure 3.

CBD effect was absent in CB1-/- mice. We fed additional wild type and $CB1^{-/-}$ mice with CBD for 6 weeks. We found that the increase in cell survival induced by CBD was abolished in $CB1^{-/-}$ mice; * $p \leq 0.05$.

CB1 is expressed during the DCX-stage of adult hippocampal neurogenesis

Given this relevance of the CB1 receptor for the observed adult neurogenesis phenotype we next asked, which cells would express the CB1 receptor in the course of adult neurogenesis. We used hippocampal sections from untreated female Nestin-GFP-reporter mice. Based on these mice we have previously proposed a model of neuronal development in the adult hippocampus [36-38]. From a Nestin-GFP-positive radial glia-like putative stem cell (type-1)

development proceeds over a population of highly proliferative Nestin-GFP-positive intermediate progenitor cells with glial properties, but lacking the radial morphology (type-2a) and a similarly proliferative progenitor cell which is still Nestin-GFP-positive but also expresses DCX (as well as, for example, Prox1 and NeuroD1) and thus shows signs of neuronal determination (type-2b). Migratory, neuroblast-like type-3 cells are DCX-positive but do not express Nestin-GFP anymore. They show limited proliferation. After this stage, cells go through a postmitotic maturation stage, during which the new neurons extend their neurites and which is associated with the transient expression of Calretinin [37,39] and of the lasting postmitotic neuronal marker NeuN.

Based on this model of adult hippocampal neurogenesis and the sequence of cellular morphology and marker expression, we found CB1-receptor expression spread over the entire dentate gyrus and the whole course of neurogenesis (Fig. 4A). However, it appeared that comparatively less staining was observed in the population of Nestin-positive type 1 cells (Fig. 4B) and type 2a cells. However, there was a tendency towards a stronger signal in cells expressing DCX (type 2b/3 cells; Fig. 4C, D), postmitotic new neurons that express Calretinin (Fig. 4E, F) and NeuN-positive mature new neurons (Fig. 4G, H). This implies that CB1 expression would increase with the degree of differentiation from type-2b cells onwards.

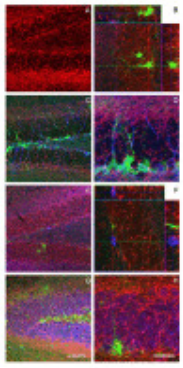

Figure 4.

Cannabinoid receptor 1 immunoreactivity in the dentate gyrus. We here show representative photomicrographs of immunofluorescent staining of 40 μm thick mouse brain sections. CB1 immunoreactivity appears in red, Nestin-GFP in green, DCX, Calretinin (CR) and NeuN in Blue. The confocal scanning photomicrograph, 1 μm thickness, 40× magnification, shows that the CB1 receptor (red) is highly expressed in the dentate gyrus (A). The three-dimensional reconstruction of z-series of 8 confocal scanning photomicrograph (1.5 mm each) in shown in (B). The co-localization with Nestin-GFP (green) was found in some of cells with rounded morphology, less in the radial glia-like type-1 cells as shown in detail using orthogonal projections. The projection of 11 (C) and 13 (D) confocal scanning micrographs (1,5 μm thickness) reveals that DCX-positive cells (blue) show co-localization with the CB1 receptor (red).

The 113× magnification shows in more detail the expression of CB1 receptor in DCX-positive cells (D). The confocal scanning photomicrograph, 1 μm thickness, magnification 40× shows a co-localization of Calretinin-positive staining with CB1 receptor (E). The three-dimensional reconstruction of z-series of 6 confocal scanning photomicrographs (1.5 mm each), 113× magnification show that Calretinin-positive cells (blue) are surrounded by CB1 receptor (red). The three-dimensional reconstruction of z-series of 13 (G) and 9 (H) confocal scanning photomicrographs (1.5 μm each) show immuno-reactivity to CB1 (red) in NeuN (blue) cells. The 113× magnification shows the co-localization in more detail (H). The representative scale bar is in G (50 mm) for A, C, E, and G; it is in H (20 mm) for B, D, F, and H.

CB1 mRNA expression is induced by activity

We subjected adult untreated female C57Bl/6 mice to either voluntary wheel running (RUN) or enriched environment (ENR). One group was housed in conventional cages (CTR). Type-2 cells are highly regulated cells *in vivo* and are influenced by behavioral activity. We have previously shown that both environmental enrichment and voluntary physical activity induce adult hippocampal neurogenesis while having differential effects on the type-2 progenitor cells [40]. We here confirmed this observation at the level of Nestin-mRNA expression, showing that RUN but not ENR increased Nestin mRNA (Fig. 5C CTR vs. ENR: 8 ± 2.2 vs. 6 ± 1.7; n = 6; p = 0.214; CTR vs. RUN: 8 ± 2.2 vs.13 ± 5.1; n = 6; p = 0.041). These findings are consistent with the counts of BrdU positive cells (Fig. 5A CTR vs. ENR: 1184 ± 81 vs.1462 ± 35; n = 5; p = 0.051; CTR vs. RUN 1184 ± 81 vs. 2197 ± 94; n = 5; p = 0.002). In the same samples, we found that both RUN and ENR increased the expression of CB1 receptor mRNA in the hippocampus (Fig. 5C; CTR vs. ENR: 4 ± 1.8 vs.10.5 ± 0.8; n = 6; p = 0.0001; CTR vs. RUN: 4 ± 1.8 vs. 12.5 ± 1.6; n = 6; p = 0.0001). This supported our result from immunohistochemistry with regard to the expression of CB1 on neuronal progenitor cells. We next wanted to know, whether CB1 receptor expression would also be necessary to elicit the effects of RUN and ENR on adult neurogenesis

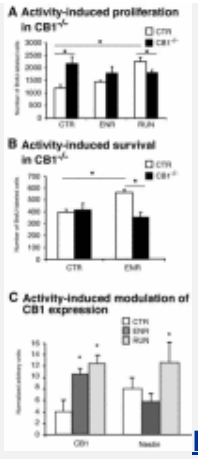

Figure 5.

Activity induced effects. Running is known to enhance proliferation of cells in the dentate gyrus while enriched environment has a stronger effect on neuronal survival. We found that while RUN increased cell proliferation in WT mice, this was not the case in CB1$^{-/-}$ mice. CB1$^{-/-}$ mice showed an increased baseline proliferation (A). In CB1$^{-/-}$ ENR-induced increase in cell survival was abolished. (B) Both housing conditions resulted in an increase of CB1-mRNA in the hippocampus of wild type mice. The early neuronal marker Nestin was only increased in the RUN paradigm (C); * $p \leq 0.05$.

Activity-induced neurogenesis is absent in CB1$^{-/-}$ mice

We next subjected female CB1$^{-/-}$ mice and their littermates to voluntary wheel running (CB1$^{-/-}$/RUN and WT/RUN) for 10 consecutive days. We found that voluntary wheel running did not increase cell proliferation in CB1$^{-/-}$ mice as it did in WT mice. CB1$^{-/-}$ mice however, showed an increased baseline proliferation consistent with the findings presented in figure 6A (Fig. 5A; WT/CTR vs. CB1$^{-/-}$/CTR: 1184 ± 81 vs. 2251 ± 118; n = 5; p = 0.002; WT/RUN vs. CB1$^{-/-}$/RUN: 2197 ± 74 vs. 1772 ± 68; n = 5; p = 0.018). ENR primarily affects cell survival. In CB1$^{-/-}$ mice the ENR-induced increase in cell survival was abolished (Fig. 5B; WT/ENR vs. CB1$^{-/-}$/ENR: 553 ± 14 vs. 362 ± 42; n = 5; p = 0.023). Taken together both results suggest that CB1-mediated mechanisms play an important role in mediating the behavior-induced regulation of adult hippocampal neurogenesis.

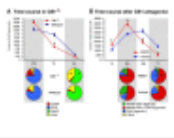

Figure 6.

Effects of CB1 absence on neuronal maturation. By using animals at different points of time after BrdU injection a detailed time course of neuronal maturation has been established. At 24 h after BrdU the number of BrdU-positive cells was increased in the mutant mice but at 4 weeks the number of BrdU-positive cells was reduced compared to the controls. The percentage of NeuN-positive cells was also reduced resulting in a net reduction of adult neurogenesis in the mutants. When we looked at the 24 h time point we found a relative reduction in the number of proliferative DCX-positive cells At an intermediate 7d time point, the increased proliferation in CB1$^{-/-}$ animals had yielded to a strong reduction in BrdU positive cells compared to controls (A). To investigate early stages of neuronal maturation, we used Nestin-GFP-reporter mice and injected the CB1 antagonist AM251. At 1 h after BrdU application counts of BrdU-positive cells reflect S-phase entry. AM251-treatment increased the number of BrdU-positive cells compared to vehicle controls. At 24 h the numbers had roughly doubled, reflecting a completed cell cycle. Phenotypic analysis revealed that this increase was largely accounted by type-2b cells and later DCX-positive cells, whereas type-2a was even reduced. At 48 h control values for BrdU began to be higher than in the AM251-treated mice, leading to an almost two-fold reduction in AM251-treated mice at 7d (B); * $p \leq 0.05$.

Proliferation is increased but neurogenesis is reduced in CB1$^{-/-}$ mice

We now returned to evaluation of CB1 effects during the course of adult neurogenesis and how the affected neuronal maturation stages are influenced via the cannabinoid-mediated pathway. We therefore conducted a time-course study in female untreated CB1$^{-/-}$ mice compared to littermate controls (WT). At 24 h after BrdU the number of BrdU-positive cells was higher in the mutant mice (WT vs. CB1$^{-/-}$: 2175 ± 32 vs. 2489 ± 27; n = 5; p = 0.0042; Fig. 6A) but was lower at 4 weeks after BrdU (WT vs. CB1$^{-/-}$: 368 ± 21 vs.180 ± 15; n = 5; p = 0.0024; Fig. 6A). The percentage of NeuN-positive cells was also lower at 4 weeks after BrdU resulting in a net reduction of adult neurogenesis in the mutants (WT vs. CB1$^{-/-}$: 82% vs. 66%; n = 5; p = 0.048; Fig. 6A). When we looked at 24 h we found a relative reduction in the number of proliferative DCX-positive cells in the knock out animals (WT vs. CB1$^{-/-}$: 81% vs. 75%; n = 5; p = 0.063; Fig. 6A). The contribution of type-2 cells to the increase in proliferation in CB1$^{-/-}$ mice could not be further elucidated. GFAP-positive cells largely accounted for the initial increase in proliferation (WT vs. CB1$^{-/-}$: 6% vs. 20%; n = 5; p = 0.001; Fig. 6A). Additional studies will have to investigate to what degree these GFAP-positive cells include the radial glia-like type-1 cells. At an intermediate point in time at 7 days after BrdU injection, the proliferation in CB1$^{-/-}$ animals was strongly reduced compared to controls, leading to the later reduction in adult net neurogenesis at 4 weeks after BrdU injection (WT vs. CB1$^{-/-}$: 1683 ± 65 vs. 836 ± 19; n = 5; p = 0.002; Fig. 6A).

CB1 receptor antagonist AM251 induces cell proliferation of DCX-positive precursor cells and reduces further differentiation

These observations suggested that CB1-activity stimulates neurogenesis in particular from type-2 cells onward, especially affecting the DCX-positive populations by accelerating development and promoting survival. To further study this shift from reduction to stimulation, which is already apparent in the time-course depicted in Fig. 6A, we conducted another experiment in untreated adult female Nestin-GFP-reporter mice to identify effects of CB1 antagonist AM251 on the intermediate precursor cell stages.

At 1 h after BrdU application counts of BrdU-positive cells reflect S-phase entry. As expected, AM251-treatment increased the number of BrdU-positive cells compared to vehicle controls (vehicle vs. AM251: 1142 ± 28 vs. 1674 ± 27; n = 5; p = 0.038; Fig. 6B). At 24 h the numbers had roughly doubled, reflecting a completed cell cycle. The relative difference between the groups was maintained at this point in time, confirming that CB1 receptor activity reduced

cell proliferation from preventing S-phase entry onward (vehicle vs. AM251: 2810 ± 35 vs. 4048 ± 120; n = 5; p = 0.0042; Fig. 6B). Phenotypic analysis revealed that this increase was largely accounted by type-2b cells, whereas type-2a was even reduced (vehicle vs. AM251: type-2b 58% vs. 77%; n = 5; p = 0.032; type-2a 27% vs. 12%; n = 5; p = 0.024; Fig. 6B).

At 48 h AM251 values for BrdU began to be lower than in the vehicle-treated mice (vehicle vs. AM251: 3182 ± 62 vs. 2035 ± 78; n = 5; p = 0.028; Fig. 6B), leading to an almost two-fold reduction in AM251-treated mice at 7d (Fig. 6B; vehicle vs. AM251: 1627 ± 18 vs. 567 ± 15; n = 5; p = 0.023). Most likely the BrdU positive cells in later maturation stages (depicted as "other") mainly account for the decrease (vehicle vs. AM251: other 3% vs. 0.5%; n = 5; p = 0.002; Fig. 6B).

Discussion

In this study we have found substantial differences between THC and CBD treatment, supporting the previously reported disruption of memory formation by THC [41]. We did not find a suggestive association between CBD-mediated CB1 activity, learning performance in the water maze, and adult hippocampal neurogenesis. THC impaired cognitive and enhanced locomotor function but had no effect on neurogenesis, when given chronically. The learning phenotype in the Morris water maze did not correlate with the neurogenesis phenotype. Taken together, both THC and CBD effects on this type of hippocampus-dependent function cannot be linked to adult neurogenesis in a straightforward way. This discrepancy between functional and cellular hippocampal features had not yet been shown for THC or CBD, but the phenomenon of divergence between learning paradigms and neurogenesis is known from other studies (reviewed in [42]). When neurogenesis was blocked by focal x-radiation the mice that had been exposed to an enriched environment still performed better in the Morris water maze than the mice housed in standard cages. Since enriched environment enhances the survival of newly generated neurons, the investigators claimed separate effects of the enriched environment on neurogenesis and on spatial learning [43]. Other groups showed that hippocampal irradiation immediately before the test had no effect, while irradiation days before the test impaired long-term memory in the water maze, indicative of a critical time window [44].

We showed that CBD increased neurogenesis at the survival stage 4 weeks after BrdU injection. Similar to the neuronal survival effect of CBD it has been reported, that the synthetic non-selective cannabinoid agonist WIN-55,212-2

restored the physiologically decreased levels of adult neurogenesis in aged rats [45]. In the current study we have studied young adult mice, but it might be worthwhile to investigate in further studies the CBD effect in aged animals. CBD is known to be beneficial in schizophrenia or schizophrenic-like behaviour [17], where patients show a decrease in hippocampal volume and neurogenesis might be impaired [17,46]. CBD has some antipsychotic properties [47]. Moreover, smoking some strains of cannabis containing relatively more CBD, in addition to THC, appears to be more protective against the psychotic symptoms induced by THC alone [48].

A study by Boucher et al. has shown that THC impaired spatial memory and reversal learning, even in animals that received a THC pretreatment, indicating that although tolerance to the effects of THC on neuronal activity in the prefrontal cortex was reported, cannabinoid-induced memory impairment in these animals persisted [7]. Although we could only test our animals at one time point, including the information of tolerance resistance to THC from the reference mentioned above makes us confident that no acute or tolerance effects were present during our testing phase.

Taken together, the findings suggest diverse effects of the cannabinoid system on memory and cellular plasticity. These effects cannot be plainly categorized into impairing or enhancing effects of cannabinoid activation or deactivation [49]. The same might be true for the finding that THC increased the performance in the rotarod test. CB1 activation in the cerebellum by intra-cerebellar THC injection led to locomotor deficits [50]. Moreover, stimulation of cerebellar CB1 receptors with the agonists CP55,940 and HU-210 impaired rotarod performance [51]. THC injected intraperitoneally on the other hand failed to provoke motor coordination disturbances in wild type B6/CBA mice [52]. The route of administration seems to be a key difference between this one and the other studies. CB1 receptors were activated in all relevant brain regions and the local concentration of THC in a given brain structure was lower than when administered directly into the cerebellum [52]. In our study the mice took up the THC via the food, which led to improved rotarod performance. In the light of therapeutically targeting locomotor dysfunction with cannabinoids this finding might be notable. The therapeutic potential of the cannabinoids was also investigated in neurological diseases such as multiple sclerosis, Gilles de La Tourette syndrome, Parkinson and Huntington disease that all include locomotor disabilities [53]. Although the efficacy was not always clearly established, the undesirable effects observed were generally mild and well tolerated [54]. The drugs used to treat symptoms of multiple sclerosis (Sativex, contains THC and CBD) failed to change the neuropathological hallmarks of the disease. Patients

reported only minor changes in memory loss, while improvements in locomotor and spasticity and neuropathic pain were dominant [55]. Experiments with THC and CBD in different concentrations might help to unravel the complex pattern of such treatments and should, as our results suggest, include measures of adult neurogenesis.

The neurogenic effect of CBD was not found in $CB1^{-/-}$ animals. Although CBD has low affinity to CB1 and its effects are often mediated via non-CB receptors (e.g. the vanilloid receptor) at least three other studies support that cannabidiol effects were CB1 receptor-dependent [19,56,57]. It might not be the only or usual mode of action, but with regard to enhanced adult hippocampal neurogenesis, CBD at least partially acts through the CB1 receptor. This result prompted us to investigate CB1 dependent regulation of neurogenesis utilizing CB1 receptor knock out animals as well as the CB1 receptor antagonist AM251 in Nestin-GFP-reporter mice.

In female $CB1^{-/-}$ mice on a C57BL/6 background we found increased proliferation 24 h after BrdU injection and decreased net-neurogenesis (7 days and 4 weeks after BrdU injection). Jin and colleagues reported impaired progenitor cell proliferation in $CB1^{-/-}$ mice but found contradicting increases with pharmacological CB1 antagonists SR141716A and AM251 [30,58]. We did not observed such discrepancy. The time point of analysis in the Jin et al. study was 3 days after BrdU injection. Thus, they might not have detected the most acute effects. Using the same compound AM251 on wild type mice, we got different results at 7 days after BrdU injection. Although we observed an increase of BrdU-labeled cells at 1 h and 24 h, which would be in line with the findings by Jin et al., we found a decrease in BrdU-labeled cells at 48 h and 7 days. When phenotyped, DCX-positive cells accounted for the increase in proliferation at early time points, but at late time points fewer DCX- and more Nestin-positive cells were present indicating that maturation was impaired at the DCX-stage. This supports our data, that CB1 stimulation or blockage had different effects on neuronal progenitor proliferation and differentiation or maturation. The same pro-proliferative effect of AM251 at 24 h after BrdU injection have also been observed by Hill et al. in rats [59].

One notable difference between the study of Jin et al. and other studies (including ours) was their use of a $CB1^{-/-}$ strain bred onto the CD1 background [60]. We have previously shown that CD1 show a very unusual pattern of baseline adult neurogenesis. Despite lower levels of proliferation compared to C57BL/6 they actually achieve high levels of net neurogenesis since survival exceeds any other strain investigated so far [61]. Another difference between

the studies might have been the use of male vs. female mice since a recent report demonstrated differences in CB1 receptor abundance in the hippocampus between female and male mice [62]. Unfortunately Jin et al. did not report the gender examined in their studies. On the other hand, receptor abundance per se does not allow strong conclusions about receptor activity.

In addition, we show that the time point of measurement is critical when assessing the effects of the antagonists. Our findings imply, that CB1 receptor activity would increase proliferation of type-1/2a, reduce proliferation of type-2b/3 but accelerate maturation from these cells and lead to a net reduction of adult neurogenesis. Consequently, the increase observed in the Jin et al. study after 3 days of AM251 in parallel to 3 days of BrdU is likely to actually reflect a mix of increases and decreases, which can only be untangled with a different BrdU injection protocol and a distinction of the different precursor cell types.

CB1 receptors are expressed in the course of neuronal development but they are present on all precursor cells, beginning with the radial glia-like type-1 cells [23]. CB1 expression appears to increase with differentiation, an observation that has also been made in embryonic cortical development [63]. Together with our previous data on wild type mice [40] these data indicate that CB1 is expressed by cells that are primarily affected by activity-dependent regulation of adult hippocampal neurogenesis. We could consequently show that this type of regulation is impaired, if the CB1 receptor is absent. Keeney at al. have shown that the CB1 antagonist Rimonabant (SR141716) decreased running activity in C57Bl/6 female mice when injected for 9 consecutive days at the peak of running [64]. The situation in the knock out animal in our study is different, since CB1 is absent constitutively and not only at the peak of running like in the Keeney et al. study. It is also notable that SR141716 has different effects on neurogenesis than the absence of CB1 [30]. We measured running performance as the distance run per day for 10 consecutive days. As long as running the same distance is indicative of a similar stimulus for neurogenesis, the conditions should have been the same for $CB1^{-/-}$ and wild type mice. In the hippocampus of wild type mice we found an upregulation of CB1 receptor mRNA in the ENR and RUN mice along with an increase in Nestin mRNA only in the RUN paradigm. This is in line with studies reporting an increase in density of CB1 receptors in the hippocampus after voluntary wheel running. When AM251 was administered, activity-induced neurogenesis was impaired [65]. This result also supports our findings that activity-induced neurogenesis is absent in $CB1^{-/-}$ mice. In contrast, a study using male mice that ran over a period of 6 weeks, $CB1^{-/-}$ animals covered less distance but showed greater numbers of DCX-expressing cells in the dentate gyrus indicating that a running-phenotype can

be discovered after a prolonged running period [66]. Another study reported that CB1 receptor sensitivity in the striatum increased after voluntary wheel running [67]. In the light of therapeutic interventions targeting the cannabinoid system, increasing the receptor by simple running might be of interest.

The putative contribution of new neurons to hippocampal function has recently become increasingly clearer. Neurogenesis and specific aspects of learning (temporal separation, contextual integration, flexibility of relearning, and integration of novelty) [34,68,69] have been linked and a role in affective behavior has been described [70,71]. Jiang and colleagues have suggested that the CB1-mediated effects of HU210 on adult neurogenesis might have anxiolytic and anti-depressant-like consequences [25]. It might thus be that the cannabinoid-dependent regulation of adult neurogenesis is more relevant for the emotional than for the cognitive aspects of hippocampal function.

Conclusions

In this study we have shown that (1) exogenous cannabinoids THC and CBD differ in their effects on spatial learning and adult neurogenesis. (2) CBD did not impair learning but increased adult neurogenesis despite (3) a CBD-induced reduction in cell proliferation. We found (4) the pro-neurogenic effect of CBD to be dependent on the CB1 receptor, which (5) shows a widespread expression over the entire dentate gyrus, including the neuronal precursor cells. Similarly, (6) the pro-neurogenic effect of environmental enrichment and voluntary wheel running depended on the presence of the CB1 receptor. Along the same line, (7) voluntary wheel running increased CB1 receptor mRNA in the hippocampus. We observed that (8) in the absence of CB1 receptors, cell proliferation was increased and neuronal differentiation reduced.

Although it has been reported that CBD binds with a low affinity to the CB1 receptor, its mode of action on neurogenesis seems to involve the CB1 receptor since CBD had no effect on $CB1^{-/-}$ animals. This prompted us to investigate the CB1 dependent regulation of neurogenesis using a genetic model and an antagonist. Taken together, our results indicate that the CB1 receptor appears to play an important role in modulating adult hippocampal neurogenesis. More specifically, CB1 affects the stages of adult neurogenesis that involve intermediate highly proliferative progenitor cells (type-2 and type-3 cells) and the survival and maturation of the new neurons. While these results are mostly in line with previous results on CB1 function in adult neurogenesis (reviewed in [13]), they also go beyond what was known since our data elucidate the time-course of this action and reveal a contribution of CB1 to activity-dependent

regulation. Although others and we found CB1 receptor expression on precursor cells, the effects on cannabinoids on neurogenesis might still be indirect as well.

Materials and methods

Animals

The generation of CB1-/- mice on a C57Bl/6 background has been described elsewhere [72]. The animals were kindly provided by Roland Martin, National Institutes of Health, Bethesda. The control group consisted of age-matched littermates. Since we carried out heterozygote breeding, we genotyped the progeny by PCR using the following primers

3'AAGAACGAGATCAGCAGCCTCTGTT5'; 3'GGATTCAGAATCATGAAGCACTCCA5'.

The experiments measuring the early stages of neurogenesis were performed in transgenic mice expressing the green fluorescent protein (GFP) driven by regulatory elements of the Nestin gene, Nestin-GFP mice [73].

All the animals were held in the same room with a consistent 12-hour-light-dark-cycle and were fed with the standard or supplemented food and water *ad libitum*. To estimate the daily food intake, animals and food were weighted every 3rd day for the whole period of the experiment (see additional file 1, 2).

All applicable local and federal regulations on animal welfare were followed. The animal protocol was approved by "Landesamt für Arbeitsschutz, Gesundheitsschutz und technische Sicherheit Berlin (LaGetSi)".

Experimental design

Thirty female CB1$^{-/-}$ and their littermates (WT) mice were randomly assigned to either enriched (CB1$^{-/-}$/ENR; WT/ENR) or standard housing (CB1$^{-/-}$/CTR; WT/CTR) or standard cages that were equipped with a running wheel (CB1$^{-/-}$/RUN; WT/RUN). RUN-assigned animals had unlimited access to the running wheel (Tecniplast, Hohenpeißenberg, Germany) for 10 days. The enriched housing in which the animals lived for 4 weeks was similar to our previous studies [74]. Briefly, it consisted of a spacious cage of approximately 80 × 80 cm floor area, complemented with a re-arrangeable system of tubes, a cardboard box house and a crawling ball. The ENR and CTR animals were housed in groups of 5, in the RUN cages 2 animals lived together. To evaluate CB1 mRNA expression changes by activity, we subjected additional 5 female C57Bl/6 to either experimental condition (RUN, ENR, CTR).

In a different set of experiments, 30 female wild-type mice were housed in standard cages and fed with either a diet supplemented with THC or CBD or

without any drug (CTR) for 6 weeks (chronic treatment). Ten CB1 knockout mice were fed with either a diet supplemented with CBD or standard food.

After treatment 10 animals of each group were tested in the Morris water maze for spatial memory performance and in the rotarod for locomotor functions. All remaining mice received daily intraperitoneal injections of Bromdesoxyuridin BrdU (50 μg/kg body weight, Sigma) for either 1 day or 5 consecutive days and were killed either 24 hours (proliferation) or 4 weeks (survival) after the last BrdU injection. At the starting point of all experiments the age was 6-8 weeks. The behavioral testing occurred 6 weeks later so that the animals were at least 12 weeks old when the testing started and 16 weeks when the survival time point of adult neurogenesis was assessed.

To analyze the chronic impact of the cannabinoids on the early stages of neuronal development, we utilized transgenic animals where the Nestin promotor is linked with an eGFP-construct emitting green fluorescence [73]. Twenty Nestin-GFP female animals were fed for 6 weeks with a THC-rich or CBD-rich or standard diet (see below). The antagonist AM251 compared to vehicle injections (Torisolve, Tocris) was used on 10 Nestin-GFP-reporter mice to evaluate the impact of CB1 at the early stages of neurogenesis, as described previously [36]. In parallel, five female $CB1^{-/-}$ and their littermates (WT) each received BrdU for either 1 day or 5 consecutive days and were killed either 24 hours (proliferation), 7 days or 4 weeks (survival) after the last BrdU injection

Cannabinoid and antagonist treatment

THC-rich or CBD-rich plant extract was kindly provided by GW-Pharmaceuticals, UK. The plant extracts were incorporated into a standard diet by ResearchDiet, USA at the concentration of either 41.2% for active THC or 38.8% for active CBD. The diets were colour -labelled for easier handling.

CB1 antagonist AM251 (Tocris) was injected intraperitoneally at 0.25 mg/kg in Tocrisolve (0.5 μg/μl in 100 μl per animal).

Immunohistochemistry

Animals were deeply anesthetized with ketamine and perfused transcardially with cold 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. The brains were dissected from the skulls and were postfixed overnight. Before sectioning from a dry-ice-cooled copper block on a sliding microtome (Leica, Bensheim), the hemispheres were transferred to 30% sucrose in 0.1 M phosphate buffer, pH 7.4, until they had sunk. Hemispheres were cut in the coronal plane in 40 μm thick sections and cryo-protected. The level of generation of new cells was determined by the *in vivo* injection of BrdU, which incorporates during the S-

phase into the cell and thus labelled proliferating cells. BrdU labelled cells in the subgranular zone of the dentate gyrus in the hippocampus were quantified as described previously [69]. Briefly all labelled cells per dentate gyrus were counted in every 6th section containing the hippocampus. For each animal 9 sections have been counted (both sides). The number was multiplied by six to estimate the total cell number per brain. For BrdU staining, DNA was denatured in 2N HCL for 30 minutes at 37°C. Free-floating sections were than rinsed in 0.1 M borate buffer, pH 8.5, and thoroughly washed in tris-buffered saline (TBS), pH 7.4. To block endogenous peroxidase reactions, sections were pre-treated with 0.6% H_2O_2. The rat-anti-mouse-BrdU antibody (Harlan Seralab) was diluted 1:500 in TBS supplemented with 0.1% TritonX-100, 0.1% Tween 20 and 3% donkey serum (TBS-plus) and the sections were incubated overnight at 4°C. After rinsing the sections in TBS and a blocking step in TBS-plus, an incubation step with the biotinylated secondary antibody (donkey-anti-rat, Vector) diluted 1:500 in TBS-plus followed. ABC reagent (Vectastain Elite, Vector Laboratories) was applied for 1 h at a concentration of 9 μl/ml for each reagent. Diaminobenzidine (DAB, Sigma) was used as a chromogen at the concentration of 0.25 mg/ml in TBS with 0.01% H_2O_2 and 0.04% nickelchloride followed by rinsing with tap water and TBS. The sections were mounted on slides and coverslipped with Neomount. To phenotype the proliferating cells, we used triple staining for BrdU and a combination of maturation markers as applicable. A total of randomly selected 50 BrdU-positive cells per animal were phenotyped. Knowing the absolute number of BrdU cells in a given brain, we were able to convert the percentage of cells expressing one of the maturation markers and BrdU into the absolute number of cells per phenotype in the whole brain. All counting were done blinded by the same researcher as described previously [38,69]. The primary antibodies were applied in the following concentrations: BrdU (1:500, Harlan Seralab), anti-Doublecortin (DCX, 1:200, Santa Cruz Biotechnologies), anti-Calretinin (1:250, Santa Cruz), anti-GFP (to visualize Nestin, 1:500, Swant), anti-NeuN (1:100, Chemicon), anti-GFAP (1:250, Chemicon), anti-CB1 (1:250, LifeBioscience). Secondary antibodies were anti-goat, anti-rabbit, anti-mouse, and anti-rat (1:250, Jackson Laboratories) directly coupled to a fluorochrome for confocal analysis. To test for statistical significant differences ($p = 0.05$) between two groups, we used the non-parametrical Mann-Whitney-U-test.

RNA isolation and RT-PCR

RNA was isolated with an RNeasy mini isolation kit according to the manufactures instructions (Qiagen, Hilden, Germany). CB1 and Nestin content in 1 μg RNA per sample was measured using the QuantiFast SYBR Green RT-PCR Kit according to the manufactures instructions (Qiagen, Hilden, Germany).

We used the following primer pairs generated with primer3 software: CB1: forward CTGGTTCTGATCCTGGTGGT, reverse TGTCTCAGGTCCTTGCTCCT; Nestin: forward TTGAGGCCTCCAGAAGAAGA, reverse GCCATCTGCTCCTCTTTCAC. The RNA amount was normalized to the housekeeping gene GAPDH. Statistical analysis has been done using the non-parametrical Mann-Whitney-U test between two groups. PCR was performed using an OPTICON II (BioRad, Munic, Germany).

Behavioral Tests

The Morris water maze (MWM) test is widely used to test rodents for spatial memory performance [75]. We followed the protocol revised by Wolfer and Lipp [76]. Six trials of training, each maximally lasting 2 minutes, were given each day. Latencies to reach the platform and swim paths were recorded with an automatic video tracking system (Ethovision, Noldus, Utrecht, Netherlands).

Animals were exposed to the MWM that contained an escape platform submerged 1 cm below the water line. The platform was kept at a constant location within the pool during the first 3 days of training. On the morning of the 4^{th} day the escape platform was placed in the quadrant opposite to the first target quadrant to start the reversal learning task for two more days. The first trial of the reversal period was analyzed as "probe trial". To control for parameters that are not hippocampus-dependent such as vision impairments, the task was afterwards repeated with a visible platform. To evaluate learning of the spatial location of the platform, latencies to reach the platform (in seconds) and total length of swim path (in pixels converted to cm) were compared between trials. Additionally, the time spent in the target quadrant on the probe trials was used as an indicator of targeted searching for the platform. During the reversal learning, time spent in quadrant 1 (location of the platform during initial training) versus quadrant 3 (location of the platform during reversal training) was measured.

To analyze performance in the MWM test, we performed a repeated measure ANOVA test of the daily means. Analysis of the differences between the groups in the parameters escape latency, and distance moved per day, using the Fisher post-hoc-test, if applicable.

To test general locomotor functions and fitness of the animals, a rotarod was used. The mice were placed on a slowly rotating rod (20 rpm) and a stopwatch was started. The rod accelerated with 20 rpm. When the mice overbalanced and touched the ground, the stopwatch stopped automatically. Each animal performed 4 trials.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

SAW has designed the study, carried out the in vivo experiments, did statistical analysis and drafted the manuscript

ABS participated in the design of the study and did the behavioral analysis

KF and PLG carried out the CB1 staining and prepared Fig. 4

ST revised the manuscript

AM carried out confocal analysis

TPW participated in the draft of the manuscript and carried out the immunoassays

GRR carried out cell culture work (data not shown)

AM carried out the RNA analysis

OU worked on the manuscript

GK supervised the experimental design, prepared figures and substantially contributed to the drafting, writing and revision of the manuscript

All authors read and approved the final manuscript.

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13. Marijuana Improves Fertility in Tobacco Smokers

http://news.softpedia.com/news/Marijuana-Improves-Fertility-in-Tobacco-Smokers-41535.shtml

A new compound imitating cannabinoids from cannabis drugs may improve the fertility of tobacco smokers. Two-thirds of the male tobacco smokers will exhibit a small or a significant decrease in fertility, some with serious loss, characterized by low sperm count and low percentage sperm motility.

"Nicotine addiction is quite powerful. The best solution is to stop smoking and then wean yourself off of all nicotine products. But for smokers who can't quit, the in vitro use of AM-1346 may significantly improve their fertilizing capacity." explained Lani Burkman, associate professor in the departments of gynecology/obstetrics and urology and head of the Section on Andrology in the University of Buffalo School of Medicine and Biomedical Sciences.

The sperm from male smokers exposed to a synthetic chemical called AM-1346 - a synthetic version of a natural cannabinoid found in the human body and cannabis - doubled its fertilizing capacity. The same team previously showed that fertilizing ability of the sperm is altered by nicotine, whether in vitro, or through long-term tobacco use.

In the new study, nine selected smokers were assessed for sperm fertilizing potential checking the binding ability on the outside cover of a human egg, "zona pellucida".

4 men had a high number of sperm attaching to the egg (normal fertilizing potential, Group I), while 5 other smokers had sperm with poor egg binding (poor fertilizing potential, Group II). The researchers sought how poor fertilizing capacity from smokers could be improved. They looked at the potential interaction between two chemical systems that control sperm. "Human sperm carry the cholinergic receptor, which responds to the neurotransmitter acetylcholine," explained Burkman. "Nicotine mimics acetylcholine and binds to the cholinergic receptor."

The second chemical system involves cannabinoid receptors, which respond to cannabis (like marijuana and hash), as well as natural cannabinoids from the body. "Research from other scientists indicates that the cholinergic system and the cannabinoid system naturally regulate human sperm and help prepare them for fertilizing an egg," she said. "This natural regulation is out of balance for the majority of smokers when sperm are continuously

exposed to nicotine."

"We think there is an important communication between the cannabinoid and cholinergic receptor systems in human sperm," said Burkman. "In 22 Hemizona tests, we showed that the response to AM-1346 depended on the initial fertility of the tobacco smoker, and if his semen showed poor quality, meaning low sperm count and low percentage motility."

The sperm from Group II individuals was exposed to AM-1346 for several hours and then retested. All cases resulted an increase of sperm binding to the egg varying from 133 % to 330 % (a 201 % mean). "In contrast," said Burkman, "samples from Group I (normal fertility, normal semen quality) reacted in the opposite manner. This two-way, or biphasic, response is common for cannabinoid action. With Group I, the drug AM-1346 caused a substantial decrease in sperm binding to the egg for eight out of nine samples.

"This opposite response must be studied further," Burkman said. "It might be tied to early-versus-late steps in fertilization, where it is expected that one process is slowed down while another process is stimulated."

"It does appear that sperm functioning in tobacco smokers with low fertility and low semen quality is quite different when compared to smokers with higher fertility and good semen quality. Nicotine appears to change the sperm membranes and sperm receptors. It also raises the question of why sperm from some smokers is protected from the effects of tobacco and nicotine."

14. Cannabis improves symptoms of ADHD Case report

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Abstract
Attention-deficit/hyperactivity disorder (ADHD) is characterized by attention deficits and an altered activation level. The purpose of this case investigation was to highlight that people with ADHD can benefit in some cases from the consumption of THC.

A 28-year old male, who showed improper behaviour and appeared to be very maladjusted and inattentive while sober, appeared to be completely inconspicuous while having a very high blood plasma level of delta-9- tetrahydrocannabinol (THC).

Performance tests, which were conducted with the test batteries ART2020 and TAP provided sufficient and partly over-averaged results in driving related performance.

Thus, it has to be considered, that in the case of ADHD, THC can have atypical effects and can even lead to an enhanced driving related performance.

Keywords: ADHD, cannabis, performance, driving

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Introduction

Assessing the performance or impairment of cannabis users is generally problematic as there is no stringent proof of a linear dose-effect relationship between the concentration of delta-9-tetrahydrocannabinol (THC) in blood and THC-induced impairment. The cause of the absence of such a relationship has not been identified. In this context it is rarely considered that the missing correlation may be due in part to a conceivable positive effect of cannabis on the behaviour and performance of individuals.

Recently, Adriani et al. [1] gave evidence that cannabinoid agonists reduce hyperactivity in a spontaneously hypertensive rat strain, which is regarded as a validated animal model for attention deficiency hyperactivity disorder (ADHD). There was also a significantly better treatment retention of cocaine dependent patients with comorbid ADHD among moderate users of cannabis compared to abstainers or heavy users [2]. ADHD was long considered a disorder limited to children and adolescents. It has now been proven that ADHD symptoms may persist into adulthood [3,4].

Individuals suffering from ADHD characteristically have an increased drive to move around and are unable to calm down. They are lacking in directed planning of their actions and the ability to assess the impact of their decisions. Their ability to organize day-to-day activities is reduced, they usually have a poor short-term memory, are forgetful and tend to work in a chaotic and inefficient way. Emotionally, they are prone to impulsive outburst, excessiveness and instability [5,6].

This present case study describes a male, 28 years of age, who was diagnosed with attention deficit hyperactivity disorder (ADHD), and whose response to THC suggests that such a positive effect may exist. Considering that the subject applied for the reinstallation of his driving licence gives particular significance to psycho-physical performance deficits caused by ADHD. Numerous studies have shown that various performance functions, such as divided attention, selective attention, long-term attention and vigilance are impaired [7].

Case Description

The subject had a record of several violations of the German drug control law. He also had a record of numerous violations of traffic laws, including speeding, running of a red traffic light and driving under the influence of cannabis during which a high THC concentration in blood had been detected. Seven years ago, the subject had been diagnosed with ADHD (ICD 10 F90.0) for the first time, and that diagnosis had been assessed repeatedly and independently since by several psychiatric units.

There was some evidence from his carer that typical symptoms were already present in childhood, they were, however, not properly recorded. Comorbidities such as addiction, including cannabis, or personality disorders were absent. He had been treated over a period of about 12 months through a combination of methylphenidate (Ritalin®, 20-30 milligram/day) and behaviour therapy. Being not sufficiently efficacious, the medication was stopped. A subsequent certificate by a specialist for general medicine suggests that ADHD symptoms were much improved under cannabis and that dronabinol (THC) had been prescribed, even though ADHD isnot indicated for this drug.
Prior to the first contact the subject had been advised not to consume any medicinal or recreational drug. During that first visit he showed grossly conspicuous behaviour. His attitude was pushy, demanding and lacking distance. He expressed impatience, for example by drumming his fingers on the table. He also constantly shifted position, folded arms behind his head or leaned over the table in front of him. He was not open to discussing the potential impairment of driving skills caused by cannabis consumption. As the conversation continued and he was informed of the preconditions for a positive assessment of his suitability to operate a vehicle, his behaviour became even more conspicuous and aggressive. Finally, he got up, grabbed the table, leaned forward and shouted that he needed a driving license and that he needed cannabis. Overall he showed behaviour typical of persons who suffer from ADHD.

During this visit, an appropriate performance of the tests was impossible. He was then offered to undergo, at a later time, a test of the impact of dronabinol on performance. During this appointment he appeared fundamentally changed and was not disturbed at all. He stated that he had stopped smoking cannabis, was taking dronabinol on a regular basis and that he had consumed it just two hours ago. He appeared calm, but not sedated, organized and restrained. Unlike during the first meeting he was able to accept and discuss arguments. When trying to make clear that THC was indispensable for his quality of life he became more engaged but without losing restraint.Rather, he was understanding of the position of the expert and indicated that the path to get back his driver license may be long but that he was willing to undertake it.

His behaviour, motor function, mood and consciousness did not give any indications of a prior use of a psychoactive substance. The tests of performance functions that are relevant to driving skills involved the four subtests of ART2020, a computer-controlled test system, which is commonly used to assess driving performance.

These subtests evaluate complex reactions (RST3), sustained attention (Q1), directed attention (LL3) and visual surveying and perception (TT15). In addition the functions of "vigilance" and "divided attention" were tested with the attention test module (TAP). The results of these tests (see Fig. 1) showed that the subject met, in all of the functions tested by ART2020, not only minimum criteria but that he achieved average or, in some areas, even above-average results.

In the very demanding tests for "vigilance" and "divided attention" categories he also showed average performance. ADHD or acute effects of THC by themselves would usually impair performance particularly in these tests. A blood sample was taken after completion of the tests.

It showed a very high concentration of THC (71 ng/mL serum), of the psychoactive metabolite 11-hydroxy-THC (30 ng/mL serum) and of the main nonpsychoactive metabolite 11-nor-delta-9-carboxy-THC (251 ng/mL serum). Such levels indicate recent as well as frequent consumption of THC-containing matters, and the analyte pattern also suggests smoking. Detection of cannabinol in hair (5.3 ng/mg) along with THC (3 ng/mg) gives evidence that the medication could not have been the only source of the THC. Only much later did the subject, who had been arrested for a drug offence a few days after the second visit, report that he had not consumed pharmaceutical dronabinol products but instead smoked cannabis just before the tests, since it was much less costly.

Conclusions

The present case report suggests that individuals suffering from ADHD, a dysfunction with a symptomatic change in activity levels, may - in some cases – benefit from cannabis treatment in that it appears to regulate activation to a level which may be considered optimum for performance. There was evidence, that the consumption of cannabis had a positive impact on performance, behaviour and mental state of the subject. The present observation corroborates previous data of Müller-Vahl et al. [8] suggesting that in patients suffering from Tourette syndrome, treatment with THC causes no cognitive defects.

Gilles de la Tourette syndrome is a neurobehavioral disorder associated with motor and vocal tics as well as behavioural and cognitive problems. The authors also hypothesized that the effects of cannabinoids in patients may be different from those in healthy users suggesting an involvement of the central cannabinoid receptor systems in the pathology of the disorder. The same conclusion may be drawn from previous studies [1, 2] and the present case report, although more information on these atypical effects should be provided and the underlying mechanisms are still to be elucidated.

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15. US: Is Marijuana A Valuable Treatment For Autism?

URL: http://www.mapinc.org/drugnews/v05.n1275.a08.html
Newshawk: Beth
Votes: 1
Pubdate: Sun, 01 May 2005
Source: Mothering (US)
Section: Issue 124, May/June 2004

Contact: letters@mothering.com
Website: http://mothering.com/
Details: http://www.mapinc.org/media/3358
Author: Bernard Rimland, PhD
Note: Rimland, the father of an adult autistic son, is the founder of the Autism Society of America and the Director of the Autism Research Institute. He was chief consultant on autism for the 1988 film Rain Man.
Bookmark: http://www.mapinc.org/mmj.htm (Cannabis - Medicinal)
Bookmark: http://www.mapinc.org/women.htm (Women)
Related: Cannabis and the Law
Related: Common Treatments For Hyperemesis Gravidarum
Related: Medical **Marijuana:** A Surprising Solution To Severe Morning Sickness
Related: Marijuana Use during Pregnancy

IS MARIJUANA A VALUABLE TREATMENT FOR AUTISM?

I am not an advocate for drugs, either legal or illicit. I have never smoked and I don't

care at all for alcohol. I agree with Oliver Wendell Holmes: "I firmly believe that if the whole materia medica could be sunk to the bottom of the sea it would be all the better for mankind and all the worse for the fishes."

Recently, our newsletter, Autism Research Review International, published a letter from a father in New Jersey whose teenage autistic son had become extremely assaultive, sending members of his family to the hospital and requiring police intervention on a number of occasions. Like that New Jersey father, thousands of parents are dealing with autistic children who are so out of control, and so violent to themselves and others, that they can make their own lives and those of their families hellish.

We then heard from a mother in Florida whose very large autistic son had changed from a sweet, loving boy to a teenager who flew into unpredictable rages that "were usually associated with self-injury, aggression and property damage. At times I had to lock myself in the bathroom; otherwise he would attack me. We gave him many medications, but nothing worked."

A friend suggested a solution: a brownie with marijuana baked into it. "Soon after he ate the brownie," she said, "my son's anxiety disappeared, and his sweet, loving behavior returned. He shows no signs of being under the influence of a drug. He now receives one marijuana brownie and several doses of Marinol, which contains the active ingredient in marijuana, each day. This has clearly saved my child's life and my family's life."

Some severe behavioral problems in autistic children have improved remarkably when the child is given a treatment of high-dose vitamin B6 and magnesium, which has been proven to be safe and effective in more than 20 research studies. But in many cases that treatment does not work. Drugs such as risperidone (Risperdal) are often used to control severe behavior problems in autistic individuals, but they have a large range of highly toxic effects. It seems to me that if one is going to need to use drugs because the safe nutritional supplements do not work, one ought to consider a relatively safe drug such as marijuana, if research bears out the good results that a number of parents have reported.

While medical marijuana is not a drug to be administered lightly, compare its side effects to the known effects of Risperdal, which include massive weight gain, a dramatically increased risk of diabetes, an elevated risk of deadly heart problems, and a host of other major and minor problems. Other psychotropic drugs are no safer, causing symptoms ranging from debilitating tardive dyskinesia to life-threatening malignant hyperthermia or sudden cardiac arrest. Of all drugs, the psychotropic drugs are among the least useful and most dangerous; in comparison, the benefit/risk profile of medical marijuana seems fairly benign. Moreover, the reports we are seeing from parents indicate that medical marijuana often works when no other treatments, drug or non-drug, have helped. Among the comments received:

"I know it's not the end-all answer, but it's been the best answer for the longest time for us in [comparison] to all the other medications. I cannot tell you how many months we would go on a medication wondering if it was doing anything, anything at all. Here we can see the difference in 30 to 60 minutes."

"My son (who is almost nine years old) has been on medications to address his severe autistic behaviors. None of the medications has ever made a difference, except for making his behaviors worse. A few months ago we tried the prescription drug Marinol and noticed a drop in the severe episodes, no fits and little to no aggression toward his teacher and family members on a daily basis. A few weeks ago we started him on cannabis and stopped the Marinol. He has been in a much better mood and is much easier to keep on task in the classroom now. He still has days when he gets angry and moody, but we can adjust the dose to help him through those days. I feel much more comfortable administering cannabis than something like Risperdal."

Medical marijuana is not legal in most states. Information on whether or not medical marijuana can be legally prescribed in your state is available on the Internet at (http://www.mpp.org) www.mpp.org. Additional information can be found at (http://www.maps.org/mmj) www.maps.org/mmj, www.NORML.org, and (http://www.druglibrary.org) www.druglibrary.org.

It is important to keep in mind the distinction between legalizing marijuana for medical uses, which has been done in some states, and "recreational" drug use, which is illegal throughout the US. Judging from the evidence in hand, I believe legalization of medical use is justified. Legalizing marijuana for nonmedical use, as has been done for tobacco and alcohol, is quite another issue.

Even if medical marijuana can be legally prescribed in your state, doctors are likely to be very reluctant to help you obtain it. You might be able to obtain information or help from local AIDS awareness and advocacy groups, which have been in the forefront of making medical marijuana available to the public.

Again, I stress that I am strongly opposed to drugs in general, and consider them a last resort, to be employed only when safer and more efficacious treatments fail. But while I am not "pro-drug," I am very much "pro--safe and effective treatment," especially in cases where an autistic individual's behaviors are dangerous or destructive. Early evidence suggests that, in such cases, medical marijuana can be a beneficial treatment, as well as being less harmful than the drugs doctors routinely prescribe.

A two-page letter provided to the Autism Research Institute (ARI) by a parent, providing additional information about medical marijuana and a list of more than 20 websites on the topic, is available on request. Fax ARI at 619-563-6840 619-563-6840, or send a self-addressed, stamped envelope to Autism Research Institute, 4182

Adams Avenue, San Diego, CA 92116. Specify that you would like information about Marinol.

16. THE SAM PROJECT: James D.

The American Association for Medical Cannabis
http://www.letfreedomgrow.com/articles/james_d.htm

This is the story of the Sam Project, but probably not the last word.

I have a very large, teenage autistic son. James D. is extremely anxious most of the time. Over time, James developed frequent and unpredictable rages. These rages increased in intensity and frequency, encompassing property destruction, aggression, some SIBs, and a number of police visits. Big and pissed, size does matter! James reached the point of severe anxiety and explosive rage 24/7. Life with our son became close to impossible.

For myself, I spent a lot time locked behind a solid core door. Later I bought pepper spray and finally a stun gun. The pepper spray was completely ineffective. James never noticed it, he never even coughed. That is perhaps a good way to describe these rages, that pepper spray had no impact whatsoever.

One of my friends, also the mother of an autistic child, who calls me from time to time, later told me that my voice sounded so stressed and different. I did not sound like myself. One night, she called and said that she and her husband were driving over to see us for a few minutes. She gave me some cupcakes and told me to give one to James when life was tough, and if he needed it, give him another.

Snap your fingers, a miracle happened for us! No more rage, reduced anxiety, no constant deafening noise and no house rocking and rolling. Those cupcakes had marijuana baked into them. This marijuana was left over from a dying wolf dog named Sam. Sam was the family pet, suffering with a brain tumor. My friend eased her dying dog by putting marijuana into his food. The cupcakes were made with left overs after Sam's dying. So, really, Sam saved my son's life, and our family's life.

My son now uses 2 1/2 mg of Marinol up to four times a day, and one brownie up to four times a day. We try to keep the dose to a minimum, because many days he is able to get by on less medication. When he has not had enough medication we have Los Tormiento, a storm. We are able to recover now, but in the past this was not the case.

We are in the process of obtaining permission for medical marijuana use in our

state. James has three doctors, two of them specialists, and a Ph.D. involved with his medication decisions, so we are not alone. We also have an attorney involved. However, with or without state sanction, this is a very difficult road to travel for old parents who are no longer able to locate a drug dealer! The cost of my son's medication is prohibitive. But when we run out, we remember why we are willing pay the price.

James D. has no discernible side effects from the marijuana, and that cannot be said about previous medications we have tried. Most of the drugs used for behavioral control with the developmentally disabled are riddled with side effects, whether an SSRI (luvox, celexa, paxil), or one of the anti-psychotic/tranquilizers (Haldol, Risperdal, Thorazine); sometimes Ritalin and blood pressure medications are added. Frequently a real cocktail of drugs is the only effective approach. Side effects are hair raising and heartbreaking. I know a mother who was told by her pediatrician to check and see that her son was still breathing every once in a while. My son lives with severe anxiety and a panic monster so vicious and so strong that when it attacks, all he can do is lash out at an invisible nemesis as it gradually drives him crazy. He doesn't understand. Only the most powerful drugs MAY have a chance of diminishing the attacks, if they don't kill him first.

So, my friend, our benefactor, who helped us at the worst possible time, tells me my voice is so different now, I laugh easily again. She says my husband and I seem to be more lighthearted together, too. When we are not afraid of the legal ramifications of our solution, we do indeed laugh at the irony of our situation. We cannot believe the twists and turns our lives have taken.

More on the saga of the 8-year-old boy who uses marijuana, who was also featured in the recent "48 Hours" report:

May 31, 2002 -- Youth on marijuana pills needs fewer

Category: Local News
Created: 12:23:07 PM on 6/3/02
Publication: Mountain Democrat (Placerville, CA)
Publication Date: 5/31/02
Page and Section: 1 A
Body: By MEGAN MARSHACK Staff writer

The mother of the previously violent 8-year-old boy now using marijuana as a medical aid to ameliorate his rages is sweet-voiced and articulate in a telephone interview.

She emphasizes her son's use of cannabis, recommended by a physician, comes after years of trial and failure of conventional psychotropic medications, diet, holistic medicine, behavior modification and other therapies.

The mother, who is not being named for reasons of confidentiality, says she was working at the Rocklin school district as a teacher's aide when students in her class investigated the pros and cons of medical marijuana under Proposition 215, the Compassionate Use Act, approved by state voters in 1996.

In helping her students do research, she "stumbled on the idea" of a way to help her son.

"They knew he was in a residential home," she says. "Yeah," she quotes the students, "You've got to do this."

The boy had failed at placement at a special education school for conduct disorder and emotionally-disturbed children.

The mother quit her job in February 2001 and was being paid by Placer County for taking care of her son, along with social service workers, around the clock.

"By May 15, he was out of control. It was a horrible nightmare," she said.

"I'm not about breaking laws, I wanted to do the best for my child," she said.

The boy was facing a lock-down type of psychiatric placement.

She consulted the director of Wo/Men's Alliance for Medical Marijuana and an Oakland pediatrician who also had experience in the use of medical marijuana.

The doctor said, "What do you have to lose? It's far less toxic than any medication," according to the mother.

She said her son is the first documented case study for children using cannabis medicinally.

Initially, the boy ate portions of muffins that had been prepared with marijuana.

"Within half an hour, actually 35 minutes, of the first dose, it was a miracle," she said.

But the boy couldn't stand the taste, even when dressed up with whipped

cream or sprinkles or other treats. So the boy's mother and grandmother began to pack his daily doses of marijuana in capsules.

"Another pill was nothing," the mother says after the boy's history of medication.

"We grind up the marijuana in a coffee grinder, sift it, put it on the skillet for an hour with butter and water to cook it, then we spread it out in a big lasagna type pan and bake it in the oven to dry it back out to a powder so that we can put it into capsules," according to the mother's Web site.

"Each pill contains 0.36 gram of marijuana," the mother said. Up until his first anniversary on the medication, the boy took three of them in the morning, two at 1 p.m. and three in the evening before bed, the mother said. That's a total of 2.88 grams per day. There are 28.5 grams to an ounce.

"Now he's down to one capsule before bed," the mother said.

When the mother remarried and moved to El Dorado County, her son's new school had to tell her they could not medicate the boy during the school day. She had to drive 26 miles round trip to deliver the boy's capsules which had to be administered off campus.

School staff did not meet the criteria to possess and dispense marijuana as primary caregivers under Prop. 215. State law forbids dispensing medication without a prescription. Physicians can only "recommend" marijuana -- not formally prescribe it. And, of course, marijuana remains illegal under federal law.

The Oakland physician who first recommended the marijuana comes to El Dorado County to examine the boy. But the mother wants to find a local pediatrician for regular medical checkups and emergencies.

The breakthrough day was May 21, 2001, a year ago. In the first six weeks, the mother said, "He's sleeping, no violence, no different than a normal kid." At 9 months old the child had "uncontrollable fits, rage, tantrums," the mother said. Later he was compulsive about food on his plate and his mother had to have plastic dishes because the boy would destroy them. Washing up could take from three to four hours.

"He couldn't get them clean enough," she said. In 1997 through 1998 the boy did not sleep more than two hours a night, she said, keeping her awake to watch him as well.

In 1999, the boy had three separate admissions to a psychiatric hospital.

"The doctor said she sanctioned no more medications, because they did not help him," the mother said.

She says the boy consistently hit, bit and kicked her.

A sadly typical story happened on Mother's Day, 1999.

"I had taken (the boy) to church right down the street. He was horrible. I went to the grocery store because I wanted to bake something for myself and I had to do a take-down in the store. The (shopping) cart went over and he took a big chunk out of my hand," she said.

This year on Mother's Day, she said the boy was grounded, but just "for 8-year-old stuff, for sassing. There's no violence in our home."

The mother said her blended family is very supportive of the marijuana treatment. "We all know some day it might quit working. We just live day to day," she said. As far as marijuana's illegality under federal law she said she would ask officials, "What would you do with him? He's living life. He's not a drugged-out child. What would you do with him?"

Megan Marshack can be contacted via e-mail at mmarshack@mtdemocrat.net

17. Study Confirms That Cannabis Is Beneficial for Multiple Sclerosis

ScienceDaily (Dec. 4, 2009)

Cannabis can reduce spasticity in multiple sclerosis (MS) patients. A systematic review, published in the open access journal *BMC Neurology*, found that five out six randomized controlled trials reported a reduction in spasticity and an improvement in mobility.

Shaheen Lakhan and Marie Rowland from the Global Neuroscience Initiative Foundation, Los Angeles, USA, searched for trials evaluating the cannabis extracts delta9-tetrahydrocannabinol (THC) and cannabidiol (CBD). According to Lakhan, "We found evidence that combined THC and CBD extracts may provide therapeutic benefit for MS spasticity symptoms."

Spasticity, involuntary muscle tension or contraction, is a common symptom of MS. Many existing therapies for this symptom are ineffective, difficult to obtain, or associated with intolerable side effects. In this study, reported incidence of side effects from cannabis, such as intoxication, varied greatly depending on the amount of cannabis needed to effectively limit spasticity, but the researchers note that side effects were also seen in the placebo groups. They add, "Considering the distress and limitations spasticity brings to individuals with MS, it is important to carefully weigh the potential for side effects with the potential for symptom relief ."

Lakhan concludes, "The therapeutic potential of cannabinoids in MS is comprehensive and should be given considerable attention."

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Story Source:

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Journal Reference:

1. Shaheen E Lakhan and Marie Rowland. **Whole plant cannabis extracts in the treatment of spasticity in multiple sclerosis: a systematic review**. *BMC Neurology*, (in press) [link]

18. Cannabis truly helps multiple sclerosis sufferers

New Scientist Magazine

16:37 10 September 2004 by **Anna Gosline, Exeter**

http://www.newscientist.com/article/dn6387-cannabis-truly-helps-multiple-sclerosis-sufferers.html

Cannabis may loosen the stiff and spastic muscles of multiple sclerosis sufferers, and not just their minds, a follow-up study has found.

The results contradict findings from the first phase of the study, where improvements seemed to be largely due to "good moods".

"There does seem to be evidence of some benefit from cannabis in the longer term that we didn't anticipate in the short term study," says John Zajicek, at Peninsula Medical School in Exeter, UK, and one of the research team.

In 2003, Zajicek and his colleagues published results on the largest study to date of cannabinoids and MS. The trial included 630 advanced-stage MS patients who took either cannabinoid compounds or a placebo for 15 weeks.

Compared with those on placebos, patients who received active compounds said they both felt less pain and less muscle spasticity - the spasms characteristic of this neurodegenerative disease.

Good guess

But physiotherapists using standard evaluations were unable to corroborate the patients' claims of improved mobility or muscle stiffness.

The results were further complicated because about two thirds of the patients who received cannabis compounds, such as D9-tetrahydrocannabinol (THC), guessed they had not received a placebo, due to the drugs effect on their mind.

The knowledge that they were receiving an active compound, along with the mood-altering effects of THC, may have explained why subjects reported improvements.

"If you've got a drug that elevates mood and makes people feel better, how can you be sure that it's really affecting their underlying disease and their symptoms?" asks Zajicek.

Marked improvement

When the short-term study ended, however, the researchers gave all subjects the opportunity to continue their treatment for a full year. The team wanted to extend the study to gather information on the safety of long-term cannabinoid use.

More than 500 patients agreed to stay on their original treatment. One group took pills of D9-tetrahydrocannabinol (THC), the active ingredient in cannabis. The second group received natural cannabis extract, and the third group took a placebo.

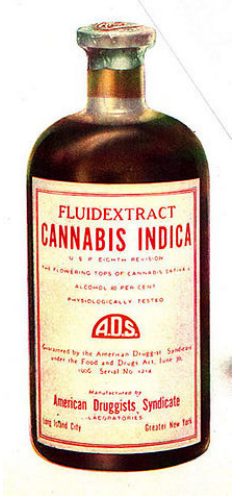
At the end of the 12 month period, the patients were evaluated again using the same measures as in the first study. But this time, physiotherapists saw a marked improvement for subjects on active drugs. They had reduced muscle spasticity and an improved overall score for their level of disability.

Zajicek is cautious about the implications of the study as it was not specifically designed to test the efficacy of drugs over 12 months. But the results do support animal research that shows cannabinoids may slow nerve cell death and protect against damage.

The findings were presented at the British Association for the Advancement of Science Festival, in Exeter, UK.

19. Medical cannabis

From Wikipedia, the free encyclopedia



Cannabis Indica fluid extract, American Druggists Syndicate, pre-1937.

Vaporizer with flexible drawtube

Medical cannabis (also referred to as **medical marijuana**) is the use of cannabis and its constituent cannabinoids such as THC as a physician-recommended form of medicine or herbal therapy. The *Cannabis* plant from which the cannabis drug is derived has a long history of medicinal use, with evidence dating back to 2,737 BC.[1]

Although the extent of the medicinal value of cannabis has been disputed, and despite the opposition to research and use put forward by most national governments, it does have several well-documented beneficial effects.[2][3][4][5] Among these are: the amelioration of nausea and vomiting, stimulation of hunger in chemotherapy and AIDS patients, lowered intraocular eye pressure (shown to be effective for treating glaucoma), as well as gastrointestinal illness. Its effectiveness as an analgesic has been suggested (and disputed), as well.

There are several methods for administration of dosage, including vaporizing or smoking dried buds, smoking, drinking, or eating extracts, and taking capsules. The comparable efficacy of these methods was the subject of an investigative study[5] conducted by the National Institutes of Health.

Synthetic cannabinoids are available as prescription drugs in some countries. Examples include Marinol, available in the United States and Canada, and Cesamet, available in Canada, Mexico, the United Kingdom, and also in the United States.

While cannabis for recreational use is illegal in all parts of the world, though decriminalized in some, its use as a medicine is legal in a number of territories, including Canada, Austria, Germany, the Netherlands, Spain, Israel, Italy, Finland, and Portugal. In the United States, federal law outlaws all cannabis use, while permission for medical cannabis varies among states. Distribution is usually done within a framework defined by local laws. Medical cannabis remains a controversial issue worldwide.

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Clinical applications

"Victoria", the United States' first legal medical marijuana plant grown by The Wo/Men's Alliance for Medical Marijuana.[*citation needed*]

In a 2002 review of medical literature, medical cannabis was shown to have established effects in the treatment of nausea, vomiting, premenstrual syndrome, unintentional weight loss, insomnia, and lack of appetite. Other "relatively well-confirmed" effects were in the treatment of "spasticity, painful conditions, especially neurogenic pain, movement disorders, asthma, [and] glaucoma".[6]

Preliminary findings indicate that cannabis-based drugs could prove useful in treating inflammatory bowel disease, migraines, fibromyalgia, and related conditions.[7]

Medical cannabis has also been found to relieve certain symptoms of multiple sclerosis[8] and spinal cord injuries[9][10][11] by exhibiting antispasmodic and muscle-relaxant properties as well as stimulating appetite.

Other studies have shown cannabis or cannabinoids may be useful in treating alcohol abuse,[12] amyotrophic lateral sclerosis,[13][14] collagen-induced arthritis,[15] asthma,[16] atherosclerosis,[17] bipolar disorder,[18][19] colorectal cancer,[20] HIV-Associated Sensory Neuropathy[21] depression,[22][23][24][25] dystonia,[26] epilepsy,[27][28] digestive diseases,[29] gliomas,[30][31] hepatitis C,[32] Huntington's disease,[33] leukemia,[34] skin tumors,[35] methicillin-resistant *Staphylococcus aureus* (MRSA),[36] Parkinson's disease,[37] pruritus,[38][39] posttraumatic stress disorder (PTSD),[40] sickle-cell disease,[41] sleep apnea,[42] and anorexia nervosa.[43] Controlled research on treating Tourette syndrome with a synthetic version of tetrahydrocannabinol (brand name Marinol), the main psychoactive chemical found in cannabis, showed the patients taking Marinol had a beneficial response without serious adverse effects;[44][45] other studies have shown that cannabis "has no effects on tics and increases the individuals inner tension".[46] Case reports found that marijuana helped reduce tics, but validation of these results requires longer, controlled studies on larger samples.[47][48]

Recent studies

Alzheimer's disease

Research done by the Scripps Research Institute in California shows that the active ingredient in marijuana, THC, prevents the formation of deposits in the brain associated with Alzheimer's disease. THC was found to prevent an enzyme called acetylcholinesterase from accelerating the formation of "Alzheimer plaques" in the brain more effectively than commercially

marketed drugs. THC is also more effective at blocking clumps of protein that can inhibit memory and cognition in Alzheimer's patients, as reported in Molecular Pharmaceutics.[49]

Lung cancer and chronic obstructive pulmonary disease

One of the surprising research results from the last decade has been the finding that smoking cannabis does not increase the risk of developing lung cancer or chronic obstructive pulmonary disease (COPD) among people who do not smoke tobacco, and may indeed confer a mildly protective effect. Beginning in 2001, multiple research teams began to report results showing that smoking cannabis does not, by itself, increase the risk of lung cancer, and this result is now well-established. Many studies did report a strongly synergistic effect, however, between tobacco use and smoking cannabis such that tobacco smokers who also smoked cannabis dramatically increased their already very high risk of developing lung cancer or chronic obstructive pulmonary disease by as much as 300%. Some of these research results follow below:

- In 2006, Hashibe, Morgenstern, Cui, Tashkin, *et al.* presented the results from a study involving 2,240 subjects that showed non-tobacco users who smoked marijuana did not exhibit an increased incidence of lung cancer or head-and-neck malignancies. These results were supported even among very long-term, very heavy users of marijuana.[50]

  Tashkin, a pulmonologist who has studied marijuana for 30 years, said, "It's possible that tetrahydrocannabinol (THC) in marijuana smoke may encourage apoptosis, or programmed cell death, causing cells to die off before they have a chance to undergo malignant transformation". He further commented that "We hypothesized that there would be a positive association between marijuana use and lung cancer, and that the association would be more positive with heavier use. What we found instead was no association at all, and even a suggestion of some protective effect."[*unreliable medical source?*][51][*unreliable medical source?*][52]

- Researchers from the University of British Columbia presented a study at the American Thoracic Society 2007 International Conference showing that smoking marijuana and tobacco together more than tripled the risk of developing COPD over just smoking tobacco alone.[*unreliable medical source?*][53] Similar findings were released in April 2009 by the Vancouver Burden of Obstructive Lung Disease Research Group. The study reported that smoking both tobacco and marijuana synergistically increased the risk of respiratory symptoms and COPD. Smoking only marijuana, however, was not associated with an increased risk of respiratory symptoms of COPD.[*unreliable medical source?*][54][55] In a related commentary, pulmonary researcher Donald

Tashkin wrote, "…we can be close to concluding that marijuana smoking by itself does not lead to COPD".[56]

- One of the principal constituents of cannabis, THC, has been found to reduce tumor growth in common lung cancer by 50 percent and to significantly reduce the ability of the cancer to spread, say researchers at Harvard University, who tested the chemical in both *in vitro* lab studies and in mouse studies. The researchers suggest that THC might be used in a targeted fashion to treat lung cancer.[*unreliable medical source?*][57]

Breast cancer

According to a 2007 study at the California Pacific Medical Center Research Institute, cannabidiol (CBD) may stop breast cancer from spreading throughout the body.[58] These researchers believe their discovery may provide a non-toxic alternative to chemotherapy while achieving the same results minus the painful and unpleasant side effects. The research team says that CBD works by blocking the activity of a gene called Id-1, which is believed to be responsible for a process called metastasis, which is the aggressive spread of cancer cells away from the original tumor site.[58]

HIV/AIDS

Investigators at Columbia University published clinical trial data in 2007 showing that HIV/AIDS patients who inhaled cannabis four times daily experienced substantial increases in food intake with little evidence of discomfort and no impairment of cognitive performance. They concluded that smoked marijuana has a clear medical benefit in HIV-positive patients.[59][60] In another study in 2008, researchers at the University of California, San Diego School of Medicine found that marijuana significantly reduces HIV-related neuropathic pain when added to a patient's already-prescribed pain management regimen and may be an "effective option for pain relief" in those whose pain is not controlled with current medications. Mood disturbance, physical disability, and quality of life all improved significantly during study treatment.[61] Despite management with opioids and other pain modifying therapies, neuropathic pain continues to reduce the quality of life and daily functioning in HIV-infected individuals. Cannabinoid receptors in the central and peripheral nervous systems have been shown to modulate pain perception. No serious adverse effects were reported, according to the study published by the American Academy of Neurology.[62] A study examining the effectiveness of different drugs for HIV associated neuropathic pain found that smoked Cannabis was one of only three drugs that showed evidence of efficacy.[63]

Brain cancer

A study by Complutense University of Madrid found the chemicals in marijuana promotes the death of brain cancer cells by essentially helping them feed upon themselves in a process called autophagy. The research team discovered that cannabinoids such as THC had anticancer effects in mice with human brain cancer cells and in people with brain tumors. When mice with the human brain cancer cells received the THC, the tumor shrank. Using electron microscopes to analyze brain tissue taken both before and after a 26- to 30-day THC treatment regimen, the researchers found that THC eliminated cancer cells while leaving healthy cells intact.[64] The patients did not have any toxic effects from the treatment; previous studies of THC for the treatment of cancer have also found the therapy to be well tolerated. However, the mechanisms which promote THC's tumor cell–killing action are unknown.[64]

Opioid dependence

Injections of THC eliminate dependence on opiates in stressed rats, according to a research team at the *Laboratory for Physiopathology of Diseases of the Central Nervous System* (France) in the journal *Neuropsychopharmacology*.[65] Deprived of their mothers at birth, rats become hypersensitive to the rewarding effect of morphine and heroin (substances belonging to the opiate family), and rapidly become dependent. When these rats were administered THC, they no longer developed typical morphine-dependent behavior. In the striatum, a region of the brain involved in drug dependence, the production of endogenous enkephalins was restored under THC, whereas it diminished in rats stressed from birth which had not received THC. Researchers believe the findings could lead to therapeutic alternatives to existing substitution treatments.[65]

In humans, drug treatment subjects who use cannabis intermittently are found to be more likely to adhere to treatment for opioid dependence.[66] Historically, similar findings were reported by Clendinning, who in 1843 utilized cannabis substitution for the treatment of alcoholism and opium addiction[*unreliable medical source?*][67] and Birch, in 1889, who reported a success in treating opiate and chloral addiction with cannabis.[68]

Spasticity in multiple sclerosis

A review of six randomized controlled trials of a combination of THC and CBD extracts for the treatment of MS related muscle spasticity reported, "Although there was variation in the outcome measures reported in these studies, a trend of reduced spasticity in treated patients was noted." The authors postulated that "cannabinoids may provide neuroprotective and anti-inflammatory benefits in MS."[69]

Medicinal compounds

Cannabis contains over 300 compounds. At least 66 of these are cannabinoids,[70][71] which are the basis for medical and scientific use of cannabis. This presents the research problem of isolating the effect of specific compounds and taking account of the interaction of these compounds.[*unreliable medical source?*][72] Cannabinoids can serve as appetite stimulants, antiemetics, antispasmodics, and have some analgesic effects.[73] Five important cannabinoids found in the cannabis plant are tetrahydrocannabinol, cannabidiol, cannabinol, β-caryophyllene, and cannabigerol.

Tetrahydrocannabinol

Main article: Tetrahydrocannabinol

Tetrahydrocannabinol (THC) is the primary compound responsible for the psychoactive effects of cannabis. The compound is a mild analgesic, and cellular research has shown the compound has antioxidant activity.[74] THC is believed to interfere with parts of the brain normally controlled by the endogenous cannabinoid neurotransmitter, anandamide.[75][76] Anandamide is believed to play a role in pain sensation, memory, and sleep.

Cannabidiol

Main article: Cannabidiol

Cannabidiol (CBD), is a major constituent of medical cannabis. CBD represents up to 40% of extracts of the medical cannabis plant.[77] Cannabidiol relieves convulsion, inflammation, anxiety, cough and congestion, nausea, and inhibits cancer cell growth.[78] Recent studies have shown cannabidiol to be as effective as atypical antipsychotics in treating schizophrenia.[79] Because cannabidiol relieves the aforementioned symptoms, cannabis strains with a high amount of CBD would be ideal for people with multiple sclerosis, frequent anxiety attacks and Tourette syndrome.[80][*unreliable medical source?*][81][*unreliable medical source?*][82]

Cannabinol

Main article: Cannabinol

Cannabinol (CBN) is a therapeutic cannabinoid found in *Cannabis sativa* and *Cannabis indica*.[83] It is also produced as a metabolite, or a breakdown product, of tetrahydrocannabinol (THC).[84] CBN acts as a weak agonist of the CB_1 and CB_2 receptors, with lower affinity in comparison to THC.[85][86]

β-Caryophyllene

Main article: Caryophyllene

Part of the mechanism by which medical cannabis has been shown to reduce tissue inflammation is via the compound β-caryophyllene.[87] A cannabinoid receptor called CB2 plays a vital part in reducing inflammation in humans and other animals.[87] β-Caryophyllene has been shown to be a selective activator of the CB2 receptor.[87] β-Caryophyllene is especially concentrated in cannabis essential oil, which contains about 12–35% β-caryophyllene.[87]

Cannabigerol

Main article: Cannabigerol

Like cannabidiol, cannabigerol is not psychoactive but has been shown to lower blood pressure in rates greater than cannabinol.[*unreliable medical source?*][88]

- Tetrahydrocannabinol (THC).
- Cannabidiol (CBD) is known to relieve convulsion, aiding those with diseases such as multiple sclerosis.
- Cannabinol (CBN).
- β-Caryophyllene has important anti-inflammatory properties.
- Cannabigerol.

Pharmacologic THC and THC derivatives

In the USA, the FDA has approved two cannabinoids for use as medical therapies: dronabinol (Marinol) and nabilone. These medicines are taken orally.

These medications are usually used when first line treatments for nausea and vomiting associated with cancer chemotherapy fail to work. In extremely high doses and in rare cases "psychotomimetic" side effects are possible. The other commonly-used antiemetic drugs are not associated with these side effects.

The prescription drug Sativex, an extract of cannabis administered as a sublingual spray, has been approved in Canada for the adjunctive treatment (use along side other medicines) of both multiple sclerosis and cancer related pain.[89][90] This medication may be legally imported into the United Kingdom and Spain on prescription.[91] William Notcutt is one of the chief researchers that has developed Sativex, and he has been working with GW and founder Geoffrey Guy since the company's inception in 1998. Notcutt states that the use of MS as the disease to study "had everything to do with politics."[92]

Medication	Approval	Country	Licensed indications	Cost
Nabilone	1985	USA, Canada	Nausea of cancer chemotherapy that has failed to respond adequately to other antiemetics	$4000.00 U.S. for a year's supply (in Canada)[93]
Marinol	1985	USA Canada (1992)	Nausea and vomiting associated with cancer chemotherapy in patients who have failed to respond adequately to conventional treatments	$652 U.S. for 30 doses @ 10 mg online[94]
	1992	USA	Anorexia associated with AIDS–related weight loss[95]	
Sativex	1995	Canada	Adjunctive treatment for the symptomatic relief of neuropathic pain in multiple sclerosis in adults	$9,351 Canadian per year[96]
	1997	Canada	Pain due to cancer	

Criticism

One of the major criticisms of cannabis as medicine is opposition to smoking as a method of consumption. However, smoking is no longer necessary due to the development of safer methods. Today, medicinal marijuana patients can use vaporizers, where the essential marijuana compounds are extracted and inhaled. This is somewhat similar to steaming vegetables to avoid cancerous by-products that are produced at higher temperatures. In addition, edible marijuana, which is produced in various baked goods, is also available, and has demonstrated longer lasting effects.

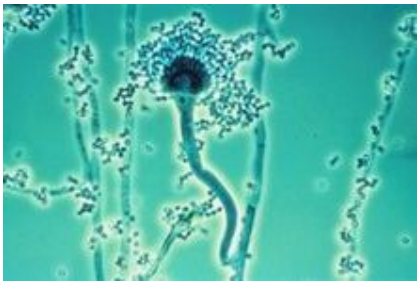
The United States Food and Drug Administration (FDA) issued an advisory against *smoked* medical marijuana stating that, "marijuana has a high potential for abuse, has no currently accepted medical use in treatment in the United States, and has a lack of accepted safety for use under medical supervision. Furthermore, there is currently sound evidence that smoked marijuana is harmful."[97]

The Institute of Medicine, run by the United States National Academy of Sciences, conducted a comprehensive study in 1999 to assess the potential health benefits of cannabis and its constituent cannabinoids. The study concluded that smoking cannabis is not recommended for the treatment of any disease condition, but did conclude that nausea, appetite loss, pain and anxiety can all be mitigated by marijuana. While the study expressed reservations about smoked marijuana due to the health risks associated with smoking, the study team concluded that until another mode of ingestion was perfected that could provide the same relief as smoked marijuana, there was no alternative. In addition, the study pointed out the inherent difficulty in marketing a non-patentable herb. Pharmaceutical companies will not substantially profit unless there is a patent. For those reasons, the Institute of Medicine concluded that there is little future in smoked cannabis as a medically approved medication. The report also concluded that for certain patients, such as the terminally ill or those with debilitating symptoms, the long-term risks are not of great concern.[98][99]

Marinol was less effective than the steroid megestrol in helping cancer patients regain lost appetites.[100] A phase III study found no difference in effects of an oral cannabis extract or THC on appetite and quality of life (QOL) in patients with cancer-related anorexia-cachexia syndrome (CACS) to placebo.[101]

"Citing the dangers of marijuana and the lack of clinical research supporting its medicinal value" the American Society of Addiction Medicine in March 2011 issued a white paper recommending a halt to using marijuana as a medicine in U.S. states where it has been declared legal.[102][103]

Harm reduction



Aspergillus fumigatus

The harm caused by smoking can be minimized or eliminated by the use of a vaporizer[104] or ingesting the drug in an edible form. This risk is also thought to be decreased by processing the cannabis leaves into hemp oil.[*unreliable medical source?*][105]

Vaporizers are devices that heat the active constituents to a temperature below the ignition point of the cannabis, so that their vapors can be inhaled. Combustion of plant material is avoided, thus preventing the formation of carcinogens such as polyaromatic hydrocarbons, benzene and carbon monoxide. A pilot study led by Donald Abrams of UC San Francisco showed that vaporizers eliminate the release of irritants and toxic compounds, while delivering equivalent amounts of THC into the bloodstream.[106]

In order to kill microorganisms, especially the molds *A. fumigatus*, *A. flavus* and *A. niger*, Levitz and Diamond suggested baking marijuana at 150 °C (302 °F) for five minutes. They also found that tetrahydrocannabinol (THC) was not degraded by this process.[107]

Organizational positions

A number of medical organizations have endorsed reclassification of marijuana to allow for further study. These include, but are not limited to, the following:

- The American Medical Association[108][109]
- The American College of Physicians – America's second largest physicians group[110]
- Leukemia & Lymphoma Society – America's second largest cancer charity[111]
- American Academy of Family Physicians opposes the use of marijuana except under medical supervision[112]

History

The use of cannabis, at least as fiber, has been shown to go back at least 10,000 years in Taiwan. "Dà má" (Pinyin pronunciation) is the Chinese expression for cannabis, the first character meaning "big" and the second character meaning "hemp."

Ancient China and Taiwan

Cannabis, called *má* 麻 or *dàmá* 大麻 (with "big; great") in Chinese, was used in Taiwan for fiber starting about 10,000 years ago.[113] The botanist Li

Hui-Lin wrote that in China, "The use of Cannabis in medicine was probably a very early development. Since ancient men used hemp seed as food, it was quite natural for them to also discover the medicinal properties of the plant."[114] The oldest Chinese pharmacopeia, the (ca. 100 CE) *Shennong Bencaojing* 神農本草經 ("Shennong's Materia Medica Classic"), describes *dama* "cannabis".

The flowers when they burst (when the pollen is scattered) are called 麻黃 [*mafen*] or 麻勃 [*mabo*]. The best time for gathering is the 7th day of the 7th month. The seeds are gathered in the 9th month. The seeds which have entered the soil are injurious to man. It grows in [Taishan] (in [Shandong] …). The flowers, the fruit (seed) and the leaves are officinal. The leaves and the fruit are said to be poisonous, but not the flowers and the kernels of the seeds.[115]

Cannabis is one of the 50 "fundamental" herbs in traditional Chinese medicine,[116] and is prescribed to treat diverse indications.

Every part of the hemp plant is used in medicine; the dried flowers (勃), the achenia (黃), the seeds (麻仁), the oil (麻油), the leaves, the stalk, the root, and the juice. The flowers are recommended in the 120 different forms of (風 *feng*) disease, in menstrual disorders, and in wounds. The achenia, which are considered to be poisonous, stimulate the nervous system, and if used in excess, will produce hallucinations and staggering gait. They are prescribed in nervous disorders, especially those marked by local anaesthesia. The seeds, by which is meant the white kernels of the achenia, are used for a great variety of affections, and are considered to be tonic, demulcent, alterative, laxative, emmenagogue, diuretic, anthelmintic, and corrective. They are made into a congee by boiling with water, mixed with wine by a particular process, made into pills, and beaten into a paste. A very common mode of exhibition, however, is by simply eating the kernels. It is said that their continued use renders the flesh firm and prevents old age. They are prescribed internally in fluxes, post-partum difficulties, aconite poisoning, vermillion poisoning, constipation, and obstinate vomiting. Externally they are used for eruptions, ulcers, favus, wounds, and falling of the hair. The oil is used for falling hair, sulfur poisoning, and dryness of the throat. The leaves are considered to be poisonous, and the freshly expressed juice is used as an anthelmintic, in scorpion stings, to stop the hair from falling out and to prevent it from turning grey. They are especially thought to have antiperiodic properties. The stalk, or its bark, is considered to be diuretic, and is used with other drugs in gravel. The juice of the root is used for similar purposes, and is

also thought to have a beneficial action in retained placenta and post-partum hemorrhage. An infusion of hemp (for the preparation of which no directions are given) is used as a demulcent drink for quenching thirst and relieving fluxes.[117]

In the early 3rd century CE, Hua Tuo was the first person known to use cannabis as an anesthetic. He reduced the plant to powder and mixed it with wine for administration.[118]

Ancient Egypt

The Ebers Papyrus (ca. 1,550 BC) from Ancient Egypt describes medical marijuana.[119] Other ancient Egyptian papyri that mention medical marijuana are the Ramesseum III Papyrus (1700 BC), the Berlin Papyrus (1300 BC) and the Chester Beatty Medical Papyrus VI (1300 BC).[120] The ancient Egyptians even used hemp (cannabis) in suppositories for relieving the pain of hemorrhoids.[121] The egyptologist Lise Manniche notes the reference to "plant medical marijuana" in several Egyptian texts, one of which dates back to the eighteenth century BCE[122]

Ancient India

Surviving texts from ancient India confirm that cannabis' psychoactive properties were recognized, and doctors used it for a variety of illnesses and ailments. These included insomnia, headaches, a whole host of gastrointestinal disorders, and pain: cannabis was frequently used to relieve the pain of childbirth.[123]

Cannabis sativa from *Vienna Dioscurides*, 512 AD

Ancient Greece

The Ancient Greeks used cannabis not only for human medicine, but also in veterinary medicine to dress wounds and sores on their horses.[124]

In humans, dried leaves of cannabis were used to treat nose bleeds, and cannabis seeds were used to expel tapeworms.[124] The most frequently described use of cannabis in humans was to steep green seeds of cannabis in either water or wine, later taking the seeds out and using the warm extract to treat inflammation and pain resulting from obstruction of the ear.[124]

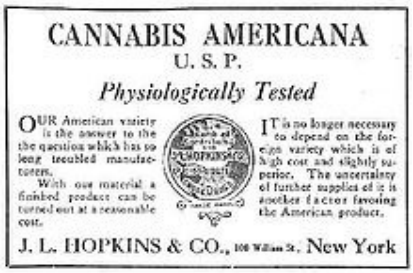
In the 5th century BC Herodotus, a Greek historian, described how the Scythians of the Middle East used cannabis in steam baths.[124]

Medieval Islamic world

In the medieval Islamic world, Arabic physicians made use of the diuretic, antiemetic, antiepileptic, anti-inflammatory, pain killing and antipyretic properties of *Cannabis sativa*, and used it extensively as medication from the 8th to 18th centuries.[125]

Modern history

An Irish physician, William Brooke O'Shaughnessy, is credited with introducing the therapeutic use of cannabis to Western medicine. He was Assistant-Surgeon and Professor of Chemistry at the Medical College of Calcutta, and conducted a cannabis experiment in the 1830s, first testing his preparations on animals, then administering them to patients in order to help treat muscle spasms, stomach cramps or general pain.[126]


CANNABIS AMERICANA
U. S. P.
Physiologically Tested
OUR American variety is the answer to the the question which has so long troubled manufacturers.
With our material a finished product can be turned out at a reasonable cost.
IT is no longer necessary to depend on the foreign variety which is of high cost and slightly superior. The uncertainty of further supplies of it is another factor favoring the American product.
J. L. HOPKINS & CO., 100 William St. New York

An advertisement for cannabis americana distributed by a pharmacist in New York in 1917.

Cannabis as a medicine became common throughout much of the Western world by the 19th century. It was used as the primary pain reliever until the invention of aspirin.[127] Modern medical and scientific inquiry began with doctors like O'Shaughnessy and Moreau de Tours, who used it to treat melancholia and migraines, and as a sleeping aid, analgesic and anticonvulsant.

By the time the United States banned cannabis in a federal law, the 1937 Marijuana Tax Act, the plant was no longer extremely popular.[128][*citation needed*] Skepticism about cannabis arose in response to the bill.[*citation needed*] The situation was exacerbated by the stereotypes promoted by the media, that the drug was used primarily by Mexican and African immigrants.[128]

Later in the century, researchers investigating methods of detecting cannabis intoxication discovered that smoking the drug reduced intraocular pressure.[129] In 1973 physician Tod H. Mikuriya reignited the debate concerning cannabis as medicine when he published "Marijuana Medical Papers". High intraocular pressure causes blindness in glaucoma patients, so he hypothesized that using the drug could prevent blindness in patients. Many Vietnam War veterans also found that the drug prevented muscle spasms caused by spinal injuries suffered in battle.[130] Later medical use focused primarily on its role in preventing the wasting syndromes and chronic loss of appetite associated with chemotherapy and AIDS, along with a variety of rare muscular and skeletal disorders.

Later, in the 1970s, a synthetic version of THC was produced and approved for use in the United States as the drug Marinol. It was delivered as a capsule, to be swallowed. Patients complained that the violent nausea associated with chemotherapy made swallowing capsules difficult. Further, along with ingested cannabis, capsules are harder to dose-titrate accurately than smoked cannabis because their onset of action is so much slower. Smoking has remained the route of choice for many patients because its onset of action provides almost immediate relief from symptoms and because that fast onset greatly simplifies titration. For these reasons, and because of the difficulties arising from the way cannabinoids are metabolized after being ingested, oral dosing is probably the least satisfactory route for cannabis administration.[131] Relatedly, some studies have indicated that at least some of the beneficial effects that cannabis can provide may derive from synergy among the multiplicity of cannabinoids and other chemicals present in the dried plant material.[132] Such synergy is, by definition, impossible with respect to the use of single-cannabinoid drugs like Marinol.

During the 1970s and 1980s, six U.S. states' health departments performed studies on the use of medical cannabis. These are widely considered some of the most useful and pioneering studies on the subject.[*citation needed*] Voters in eight states showed their support for cannabis prescriptions or recommendations given by physicians between 1996 and 1999, including Alaska, Arizona, California, Colorado, Maine, Michigan, Nevada, Oregon, and Washington, going against policies of the federal government.[133]

Cannabis female flowers closeup with trichomes (white). These plant parts contain the highest concentration of medicinal compounds.

In May 2001, "The Chronic Cannabis Use in the Compassionate Investigational New Drug Program: An Examination of Benefits and Adverse Effects of Legal Clinical Cannabis" (Russo, Mathre, Byrne et al.) was completed. This three-day examination of major body functions of four of the five living US federal cannabis patients found "mild pulmonary changes" in two patients.[134]

On October 7, 2003, a patent entitled "Cannabinoids as Antioxidants and Neuroprotectants" http://www.patentstorm.us/patents/6630507/fulltext.html (#6,630,507) was awarded to the United States Department of Health and Human Services, based on research done at the National Institute of Mental Health (NIMH), and the National Institute of Neurological Disorders and Stroke (NINDS). This patent claims that cannabinoids are "useful in the treatment and prophylaxis of wide variety of oxidation associated diseases, such as ischemic, age-related, inflammatory and autoimmune diseases. The cannabinoids are found to have particular application as neuroprotectants, for example in limiting neurological damage following ischemic insults, such as stroke and trauma, or in the treatment of neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease and HIV dementia."[135]

National and international regulations

European laws on cannabis possession (small amount). Data are from multiple sources detailed on the full source list
Main article: Legal and medical status of cannabis

Cannabis is in Schedule IV of the United Nations´ Single Convention on Narcotic Drugs, making it subject to special restrictions. Article 2 provides for the following, in reference to Schedule IV drugs:[136]

A Party shall, if in its opinion the prevailing conditions in its country render it the most appropriate means of protecting the public health and welfare, prohibit the production, manufacture, export and import of, trade in, possession or use of any

such drug except for amounts which may be necessary for medical and scientific research only, including clinical trials therewith to be conducted under or subject to the direct supervision and control of the Party.

The convention thus allows countries to outlaw cannabis for all non-research purposes but lets nations choose to allow medical and scientific purposes if they believe total prohibition is not the most appropriate means of protecting health and welfare. The convention requires that states that permit the production or use of medical cannabis must operate a licensing system for all cultivators,manufacturers and distributors and ensure that the total cannabis market of the state shall not exceed that required "for medical and scientific purposes."[136]

Austria

In Austria both Δ^9-THC and pharmaceutical preparations containing Δ^9-THC are listed in annex V of the Narcotics Decree (*Suchtgiftverordnung*).[137] Compendial formulations are manufactured upon prescription according to the German *Neues Rezeptur-Formularium*.[138][139]

On July 9, 2008, the Austrian Parliament approved cannabis cultivation for scientific and medical uses.[140] Cannabis cultivation is controlled by the Austrian Agency for Health and Food Safety (*Österreichische Agentur für Gesundheit und Ernährungssicherheit, AGES*).[141]

Canada

In Canada, the regulation on access to marijuana for medical purposes, established by Health Canada in July 2001, defines two categories of patients eligible for access to medical cannabis. Category 1 covers any symptoms treated within the context of providing compassionate end-of-life care or the symptoms associated with medical conditions listed below:

- severe pain and/or persistent muscle spasms from multiple sclerosis, from a spinal cord injury, from spinal cord disease,
- severe pain, cachexia, anorexia, weight loss, and/or severe nausea from cancer or HIV/AIDS infection,
- severe pain from severe forms of arthritis, or
- seizures from epilepsy.

Category 2 is for applicants who have debilitating symptom(s) of medical condition(s), other than those described in Category 1. The application of eligible patients must be supported by a medical practitioner.[142]

The cannabis distributed by Health Canada is provided under the brand CannaMed by the company Prairie Plant Systems Inc. In 2006, 420 kg of

CannaMed cannabis was sold, representing an increase of 80% over the previous year.[143] However, patients complain of the single strain selection as well as low potency, providing a pre-ground product put through a wood chipper (which deteriorates rapidly) as well as gamma irradation and foul taste and smell.[144]

It is also legal for patients approved by Health Canada to grow their own cannabis for personal consumption, and it's possible to obtain a production license as a person designated by a patient. Designated producers were permitted to grow a cannabis supply for only a single patient, however. That regulation and related restrictions on supply were found unconstitutional by the Federal Court of Canada in January, 2008. The court found that these regulations did not allow a sufficient legal supply of medical cannabis, and thus forced many patients to purchase their medicine from unauthorized, black market sources. This was the eighth time in the previous ten years that the courts ruled against Health Canada's regulations restricting the supply of the medicine.[145]

In May, 2009, Health Canada revised their earlier regulations to permit licensed, designated producers to grow cannabis for a maximum of two patients. The move was called a "mockery" of the court's intention by lawyer Ron Marzel, who represented plaintiffs in the successful challenge in Federal Court to Health Canada's previously-existing rules. Marzel has announced plans to ask the court to overturn all prohibitions on cannabis use if Health Canada refuses to create regulations that will allow an adequate legal supply for use by medically-authorized patients.[145]

Germany

In Germany dronabinol was rescheduled 1994 from annex I to annex II of the Narcotics Law (*Betäubungsmittelgesetz*) in order to ease research; in 1998 dronabinol was rescheduled from annex II to annex III and since then has been available by prescription,[146] whereas Δ^9-THC is still listed in annex I.[147] Manufacturing instructions for dronabinol containing compendial formulations are described in the *Neues Rezeptur-Formularium*.[139]

Spain

In Spain, since the late 1990s and early 2000s, medical cannabis underwent a process of progressive decriminalization and legalisation. The parliament of the region of Catalonia is the first in Spain have voted unanimously in 2001 legalizing medical marijuana, it is quickly followed by parliaments of Aragon and the Balearic Islands.[*citation needed*] The Spanish Penal Code prohibits the sale of cannabis but it does not prohibit consumption. Until early 2000, the Penal Code did not distinguish between

therapeutic use of cannabis and recreational use, however, several court decisions show that this distinction is increasingly taken into account by the judges. From 2006, the sale of seed is legalized,[citation needed] the sale and public consumption remains illegal, and private cultivation and use are permitted.[148][149]

Several studies have been conducted to study the effects of cannabis on patients suffering from diseases like cancer, AIDS, multiple sclerosis, seizures or asthma. This research was conducted by various Spanish agencies at the Universidad Complutense de Madrid headed by Manuel Guzman, the hospital of La Laguna in Tenerife led neurosurgeon Luis González Feria or the University of Barcelona.[citation needed]

Several cannabis consumption clubs and user associations have been established throughout Spain. These clubs, the first of which was created in 1991, are non-profit associations who grow cannabis and sell it at cost to its members. The legal status of these clubs is uncertain: in 1997, four members of the first club, the Barcelona Ramón Santos Association of Cannabis Studies, were sentenced to 4 months in prison and a 3000 euro fine, while at about the same time, the court of Bilbao ruled that another club was not in violation of the law. The Andalusian regional government also commissioned a study by criminal law professors on the "Therapeutic use of cannabis and the creation of establishments of acquisition and consumption. The study concluded that such clubs are legal as long as they distribute only to a restricted list of legal adults, provide only the amount of drugs necessary for immediate consumption, and not earn a profit. The Andalusian government never formally accepted these guidelines and the legal situation of the clubs remains insecure. In 2006 and 2007, members of these clubs were acquitted in trial for possession and sale of cannabis and the police were ordered to return seized crops.[149]

United Kingdom

In the United Kingdom, if you are arrested or taken to court for possession of cannabis, you are asked if there are any mitigating factors to explain why it is in your possession. It is unknown whether this is solely a formality, or if an excuse of medical usage has ever been used successfully to reduce the penalty issued. However, in the United Kingdom, possession of small quantities of cannabis does not usually warrant an arrest or court appearance (street cautions or fines are often given out instead). Under UK law, certain cannabinoids are permitted medically,[150] but these are strictly controlled with many provisos under the Misuse of drugs act 1971 (in the 1985 amendments). The British Medical Associations official stance is "users of cannabis for medical purposes should be aware of the risks,

should enroll for clinical trials, and should talk to their doctors about new alternative treatments; but we do not advise them to stop."[150]

United States

Main article: Medical cannabis in the United States

In the United States federal level of government, cannabis *per se* has been made criminal by implementation of the Controlled Substances Act which classifies marijuana as a Schedule I drug, the strictest classification on par with heroin, LSD and Ecstasy, and the Supreme Court ruled in 2005 that the Commerce Clause of the U.S. Constitution allowed the government to ban the use of cannabis, including medical use. The United States Food and Drug Administration states "marijuana has a high potential for abuse, has no currently accepted medical use in treatment in the United States, and has a lack of accepted safety for use under medical supervision".[97][151]

Sixteen states have legalized medical marijuana: Alaska,[152] Arizona,[153] California,[154] Colorado,[155] Hawaii,[156] Maine,[157] Michigan,[158] Montana,[159] Nevada,[160] New Jersey,[161] New Mexico,[162] Oregon,[163] Rhode Island,[164] Vermont,[165] Virginia,[166] and Washington;[167] Maryland allows for reduced penalties if cannabis use has a medical basis.[168] California, Colorado, New Mexico, Maine, Rhode Island, Montana, and Michigan are currently the only states to utilize dispensaries to sell medical cannabis. California's medical marijuana industry took in about $2 billion a year and generated $100 million in state sales taxes during 2008[169] with an estimated 2,100 dispensaries, co-operatives, wellness clinics and taxi delivery services in the sector colloquially known as “cannabusiness”.[170]

On 19 October 2009 the US Deputy Attorney General issued a US Department of Justice memorandum to "All United States Attorneys" providing clarification and guidance to federal prosecutors in US States that have enacted laws authorizing the medical use of marijuana. The document is intended solely as "a guide to the exercise of investigative and prosecutorial discretion and as guidance on resource allocation and federal priorities." The US Deputy Attorney General David W. Ogden provided seven criteria, the application of which acts as a guideline to prosecutors and federal agents to ascertain whether a patients use, or their caregivers provision, of medical marijuana "represents part of a recommended treatment regiment consistent with applicable state law", and recommends against prosecuting patients using medical cannabis products according to state laws. Not applying those criteria, the Dep. Attorney General Ogden concludes, would likely be "an inefficient use of limited federal resources". The memorandum does not change any laws. Sale of cannabis remains illegal under federal law. The U.S. Food and Drug Administration's position,

that marijuana has no accepted value in the treatment of any disease in the United States, has also remained the same.[171]

The Health and Human Services Division of the federal government holds a patent for medical marijuana. The patent, "Cannabinoids as antioxidants and neuroprotectants", issued October 2003[135] reads: "Cannabinoids have been found to have antioxidant properties, unrelated to NMDA receptor antagonism. This new found property makes cannabinoids useful in the treatment and prophylaxis of wide variety of oxidation associated diseases, such as ischemic, age-related, inflammatory and autoimmune diseases. The cannabinoids are found to have particular application as neuroprotectants, for example in limiting neurological damage following ischemic insults, such as stroke and trauma, or in the treatment of neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease and HIV dementia…"[172]

See also

- Legality of cannabis by country
- Multidisciplinary Association for Psychedelic Studies
- Tilden's Extract
- Chinese herbology

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External links

- Medical cannabis at the Open Directory Project, links to websites about medical cannabis.
- The Center for Medicinal Cannabis Research of the University of California.
- Links to the authorization forms patients need in each U.S. state that allows the use of medical cannabis.
- A patient advocacy group that protects rights of medical marijuana patients throughout North America.
- Bibliography on the use of medical cannabis in recent history *Advances in the History of Psychology*, York University.
- The Forbidden Medicine, an independent site operated by Harvard Medical School faculty members James Bakalar and Lester Grinspoon.

20. Cannabis as a Substitute for Alcohol

By Tod Mikuriya, MD

SUMMARY
Ninety-two Northern Californians using cannabis as an alternative to alcohol obtained letters of approval from the author. Their records were reviewed to determine characteristics of the cohort and efficacy of the treatment —defined as reduced harm to the patient. All patients reported benefit, indicating that for at least a subset of alcoholics, cannabis use is associated with reduced drinking. The cost of alcoholism to individual patients and society- at-large warrants testing of the cannabis-substitution approach and study of the drug-of-choice phenomenon.

KEYWORDS
Addiction, alcohol, alcoholism, cannabis, depression, drug-of-choice, harm reduction, marijuana, pain, substitution.

INTRODUCTION
Physicians who treat alcoholics are familiar with the cycle from drunkenness and disinhibition to withdrawal, drying out, and apology for behavioral lapses, accompanied over time by illness and debility as the patient careens from one crisis to another. (Tamert and Mendelsohn 1969)
“Harm reduction” is a treatment approach that seeks to minimize the occurrence of drug/alcohol addiction and its impacts on the addict/alcoholic and society at large. A harm-reduction approach to alcoholism adopted by 92 of my patients in Northern California involved the substitution of cannabis —with its relatively benign side-effect profile— as their intoxicant of choice.
No clinical trials of the efficacy of cannabis as a subtitute for alcohol are reported in

the literature, and there are no papers directly on point prior to my own account (Mikuriya 1970) of a patient who used cannabis consciously and successfully to reduce her problematic drinking.
There are ample references, however, to the use of cannabis as a substitute for opiates (Birch 1889) and as a treatment for delirium tremens (Clendinning 1843, Moreau 1845), which were among the first uses to which it was put by European physicians. Birch described a patient weaned off alcohol by use of opiates, who then became addicted and was weaned off opiates by use of cannabis. "Ability to take food returned. He began to sleep well; his pulse exhibited some volume; and after three weeks he was able to take a turn on the verandah with the aid of a stick. After six weeks he spoke of returning to his post, and I never saw him again."

Birch feared that cannabis itself might be addictive, and recommended against revealing to patients the effective ingredient in their elixir. "Upon one point I would insist —the necessity of concealing the name of the remedial drug from the patient, lest in his endeavor to escape from one form of vice he should fall into another, which can be indulged with facility in any Indian bazaar." This stern warning may have undercut interest in the apparently successful two-stage treatment he was describing.

At the turn of the 19th century in the United States, cannabis was listed as a treatment for delirium tremens in standard medical texts (Edes 1887, Potter 1895) and manuals (Lilly 1898, Merck 1899, Parke Davis 1909).

Since delirium tremens signifies advanced alcoholism, we can adduce that patients who were prescribed cannabis and used it on a longterm basis were making a successful substitution.

By 1941, due to prohibition, cannabis was no longer a treatment option, but attempts to identify and synthesize its active ingredients continued (Loewe 1950). A synthetic THC called pyrahexyl was made available to clinical researchers, and one paper from the postwar period reports its successful use in easing the withdrawal symptoms of 59 out of 70 alcoholics. (Thompson and Proctor 1953).

In 1970 the author reported (op cit) on Mrs. A., a 49-year-old female patient whose drinking had become problematic. The patient had observed that when she smoked marijuana socially, on week-ends, she decreased her alcoholic intake. She was instructed to substitute cannabis any time she felt the urge to drink. This regimen helped her to reduce her alcohol intake to zero. The paper concluded, "It would appear that for selected alcoholics the substitution of smoked cannabis for alcohol may be of marked rehabilitative value. Certainly cannabis is not a panacea, but it warrants further clinical trial in selected cases of alcoholism."

The warranted research could not be carried out under conditions of prohibition, but in private practice and communications with colleagues I encountered more patients like Mrs. A. and generalized that somewhere in the experience of certain alcoholics,

cannabis use is discovered to overcome pain and depression —target conditions for which alcohol is originally used— but without the disinhibited emotions or the physiologic damage. By substituting cannabis for alcohol, they can reduce the harm their intoxication causes themselves and others.

Although the increasing use of marijuana starting in the late '60s had renewed interest its medical properties —including possible use as an alternative to alcohol (Scher 1971)— meaningful research was blocked until the 1990s, when the establishment of "buyers clubs" in California created a potential database of patients who were using cannabis to treat a wide range of conditions. The medical marijuana initiative passed by voters in 1996 mandated that prospective patients get a doctor's approval in order to treat a given condition with cannabis —resulting in an estimated 30,000 physician approvals as of May 2002. (Gieringer 2002) As this goes to press a year later, the estimate stands at abut 50,000.

In a review of my records in the spring of 2002 by Jerry Mandel, PhD, 92 patients were identified as using cannabis to treat alcohol abuse and related problems. This paper describes characteristics of that cohort and the results of their efforts to substitute cannabis for alcohol.

METHODOLOGY

Identifying Alcoholism

The initial consultation (20 minutes) provided multiple opportunities to identify alcoholism as a problem for which treatment with cannabis might be appropriate. The intake form asked patients to state their reason for contacting the doctor, and enabled them to prioritize their present illnesses and describe the course of treatment to date. The form also asked patients to identify any non-prescribed psychoactive drugs they were taking (including alcohol), and invited remarks. A specific question concerned injuries incurred "while or after consuming alcohol." My reading of patients' medical records provided an additional opportunity to identify alcohol abuse, as did the taking of a verbal history.

Evaluating Efficacy

At follow-up visits (typically at 12-month intervals) patients were asked to list the conditions they had been treating with cannabis and to evaluate their status as "stable," "improved," or "worse." Patients were asked to evaluate the efficacy of cannabis (five choices from "very effective to "ineffectual") and to describe any adverse events. Patients were also asked to describe any changes in their "living and employment situation," and if so, to elaborate. The question about use of non-prescribed psychoactive drugs, including alcohol, was repeated. Comparison of responses in a given patient's initial and follow-up questionnaires enabled us to assess the utility of cannabis as an alternative to alcohol.

Patient Background

Gieringer (op cit) notes that "Many patients who find marijuana helpful for otherwise

intractable complaints report that their physicians are fearful of recommending it, either because of ignorance about medical cannabis, or because they fear federal punishment or other sanctions. This is especially true in regions where the use of marijuana is less familiar and accepted." The patients whose records form the basis for this study were all seen in ad hoc settings arranged by local cannabis clubs —72 in rural counties of Northern California, 4 in San Francisco. They form a special but not unique subset, having intentionally sought out a physician whose clinical use of cannabis—and confidence in its versatility and relative safety— was extensive and well known in their communities.

A majority of the patients identified themselves as blue-collar workers: carpenter (5), construction (3), laborer (3), waitress (3), truck driver (3), fisherman (3), heavy equipment operator (3), painter (2), contractor (2) cook (2), welder (2), logger (2), timber faller, seaman, hardwood floor installer, bartender, building supplies, house caretaker, ranch hand, concrete pump operator, cable installer, silversmith, stone mason, boatwright, auto detailer, tree service handyman cashier, nurseryman, glazier, gold miner, carpet layer, carpenter's apprentice, landscaper, river guide, screenprinter, glassblower.

Eleven were unemployed or didn't list an occupation; four were disabled, two retired, and two patients defined themselves as mothers. Others were in sales (5), musicians (5), clerical workers (3), paralegal, teacher, actor, actress, artist, sound engineer, computer technician.

Eighty-two of the patients were men.

Patients' ages ranged from 20 to 69. Twenty-nine were in their twenties; 16 in their thirties; 24 in their forties; 20 in their fifties; three in their sixties.

Exactly half —46 patients— had taken some college courses, but only four had college degrees. Five did not complete high school.

Thirteen were veterans, all branches of the Armed Forces being represented.

All but six—five native-Americans, one African-American— were Caucasian.

Slightly more than half (49) reported being raised by at least one addict/lcoholic parent.

Prioritizing Alcoholism

Fifty-seven of the patients identified alcoholism or cirrhosis of the liver as their primary medical problem. Secondary problems reported by this group were Depression (15), Pain (14), Arthritis (7), PTSD (6), Insomnia (6), Cramps (4) Hepatitis C (4) Anxiety (3), Stress (2), gastritis, and ADHD.

Thirty-one patients identified themselves as alcohol abusers, but reported other problems as primary: Pain (12), Depression (8), Headache (4), Bipolar Disorder (2) Anxiety (2), Arthrtitis (2), Asthma (2) Spinal Cord Injury/Disease (2), Paraplegia, PTSD, Crushed skull, Aneurysms aggravated by stress, ADHD, Multiple broken bones.

Eighteen patients reported having been injured while or after drinking heavily. Fourteen had incurred legal problems or been ordered into rehab programs.

Cannabis Use/
Awareness of Medicinal Effect

Patients were asked when they started using cannabis and when they realized it exerted a medicinal effect.
Three reported first using at age 9 or younger; 61 between ages 10 and 19; nine began using in their 20s; three in their 30s; six in their 40s; two at age 50; and one at age 65.
Twenty-four patients reported realizing immediately upon using cannabis that it exerted a beneficial medical effect. Some of their responses still seem to reflect their relief at the time.
• "In 1980 I had quit drinking for a month. My niece asked me if I ever tried marijuana to calm me down. So I tried it and it worked like a miracle."
• "Helped pain very much! Helped sleep —excellent."

Thirty-five patients answered ambiguously with respect to time —"When realized preferred to alcohol," for example, or, "when I smoked when suffering."
Seven reported becoming aware of medical effect within a year of using cannabis. Ten became aware within one to five years.
Three became aware of medical effect 12-15 years after first using. Ten became aware between 20 and 30 years after first using. All but one of these patients had resumed using cannabis after years of abstinence.

Efficacy
As could be expected among patients seeking physician approval to treat alcoholism with cannabis, all reported that they'd found it "very effective" (41) or "effective" (38).
Efficacy was inferred from other responses on seven questionnaires. Two patients did not make follow-up visits.
Nine patients reported that they practiced total abstinence from alcohol and attributed their success to cannabis. Their years in sobriety: 19, 18, 16, 10, 7, 6, 4 (2), and 2.
Twenty-nine patients reported a return of symptoms when cannabis was discontinued. Typical comments:
• "I quit using cannabis while I was in the army and my drinking doubled. I was also involved in several violent incidents due to alcohol."

Use of Other Drugs
Patients were asked to list other drugs —prescribed, over-the-counter, and herbal— that they were currently using or had used in the past to treat their illnesses. Most common of the prescription drugs were SSRIs (31), opiates (23) NSAIDs (18) disulfaram (15) and Ritalin (8).

Delivery Systems

Seventy-eight patients smoked joints —the average amount being one joint a day (assuming 3.5 joints per 1/8 ounce of high-quality marijuana).

All were strongly advised that smoking involves an assault on the lungs, and that vaporization is a safer method of inhaling cannabinoids.

Twelve patients reported using a pipe, and three owned vaporizers. All were strongly advised that smoking involves an assault on the lungs, and that vaporization is a safer method of inhaling cannabinoids.

OBSERVATIONS

Alcoholic Parents

That a slight majority patients (51) reported being raised by at least one alcoholic parent was not surprising. The children of alcoholics enter adulthood with two strikes. They have endured direct emotional abuse and/or abandonment by parent(s); and they lack role models for coping with uncomfortable feelings other than by inebriation. It is to be expected that many, when encountering problems early in life, are treated with, or seek out, mind-altering drugs.

Cannabis for Analgesia

The large number of patients using cannabis for pain relief (28) reflects the high percentage of blue-collar workers who suffer musculoskeletal injury during their careers. As expressed by a carpenter, “Nobody gets to age 40 in my business without a bad back.” Nurses who must lift gurneys, farmworkers, desk-bound clerical workers, and many others are also prone to chronic back and neck pain.
Fights and accidents — vehicular, sports- and job-related— also create chronic pain patients, many of whom self-medicate with alcohol.
Eighteen patients reported having been injured while or after drinking heavily. This comment by Jamie R., a 26-year-old truck driver, describes a typical chain-reaction of alcohol-induced trouble: “Injured in a fight after consuming alcohol, resulted in staph infection of right knuckle, minor surgery and four days in hospital.” Injuries suffered while drunk add to pain and the need for relief by alcohol …or a less destructive alternative.

A total of 26 patients reported using cannabis for both pain relief and as an alternative to alcohol. Mike G., a 47-year old landscaper who was run over by a vehicle at age 5, requiring multiple surgeries and leaving him with pins in his right ankle, first used cannabis at age 16 and appreciated its benign side-effect profile: “Given pain pills for my right ankle, I got too drowsy. Smoked herb to relieve pain.” And when he had to discontinue cannabis use, “was unable to ease pain in ankle without herb, and drink when unable to have cannabis to smoke.”

Cannabis for Mood Disorders

Twenty-three patients reported using cannabis to treat depression —39 if the category

is expanded to include anxiety, stress, and PTSD— and their comments frequently touched on the negative synergies between mood disorders and alcoholism.

• Wendy S., a 44-year-old paralegal, suffering from depression, alcoholism, and PMS noted simply, "Alcohol causes more depression." When she does not have access to cannabis, "Alcohol consumpion increases and so does depression." At her initial visit she reported consuming 5-10 drinks/day. At a follow-up (16 months) she had reduced her consumption to week-ends.

• Albert G., a 33-year-old river guide (and decorated Army vet) put it this way: "I have had a problem with violence and alcohol for a long time and I have a rap sheet to prove it. None of the problems occurred while using cannabis. Not only does cannabis prevent my violent tendencies, but it also helps keep me from drinking." On his follow-up visit (12 months) Albert reported improved communication with family members and fewer problems relating to other people. His alcohol consumption had decreased from 36 drinks/week to zero (one month of sobriety).

• Carol G. presented initially at age 35 as homeless and unemployed, suffering "severe depression. Anxiety. Pain." Her problem with alcohol was inferred from her response concerning non-medical-psychoactive drug use: "I drink and smoke too much — started when I couldn't get marijuana."

Carol had shyly requested a recommendation for cannabis from a Humboldt County physician but, as she recounted, "I'm paranoid and local Drs are scared, too. They gave me paxil & stop smoking pamphlet."

At a follow-up visit (14 months) Carol reported a change in circumstance: "Now have a room. But am on G.R. and am paying too much." She was still using alcohol "a little. I'm doing good dealing with not drinking. Being able to medicate with cannabis has helped a lot." Eighteen months later the pattern hadn't changed: "Alcohol several times/week. Depends on if I have cannabis, stress still triggers."

Fewer Adverse Effects

Patients made negative comments with respect to the efficacy of their prescribed analgesics and anti-depressants (22), side-effects (26), and cost (11) —not surprising, perhaps, in a cohort seeking an herbal alternative.

• Lance B. presented as a 41-year-old alcoholic also suffering from arthritis, pain from knee- and ankle surgeries, and depression, for which he had been prescribed Librium, Valium, Buspar, Welbutrin, Effexor, Zoloft, and Depakote over the years; "No help!," he wrote bluntly. On his return visit (one year) he reported "few relapses" and that he was able to take some classes.

• The dulling effects of Vicodin and other opiates were mentioned by seven patients. As Harvey B. put it, "When I can get Vicodin it helps the pain but I don't like being that dopey." Clarence S., whose skull was badly damaged in an accident, also appreciated the pain relief provided by opiates, but asserted that opiates "make me paranoid and mean."

• Alex A., who was diagnosed with ADHD in ninth grade, touches on some recurring themes in describing the treatment of his primary illness: "I was prescribed Ritalin and Zoloft. The Ritalin helped me concentrate slightly but caused me to be up all night. The Zoloft made me sick to my stomach and never relieved my stress or depression. I have never been prescribed anything for my insomnia but I usually have to drink some liquor to get to sleep. I think that is a bad thing as I have now begun to drink excessive amounts of whisky, which has really started to affect my stomach." Alex first used cannabis at age 19 and became aware of benefits immediately. "I found myself running to the refrigerator and then sleeping better than I had for years." At age 21 he fears permanent damage. "From drinking (I believe) my stomach has been altered, along with my appetite… I cannot really eat that much and feel malnourished and weaker than a 21-year-old should. My joints ache constantly and I am not as strong as I used to be. I also fear that I will become or am an alcoholic and I do not want to see myself turn into my dad."
At his follow-up visit (12 months) Alex reported cannabis to be "very effective." He was employed, "not partying," doing well socially, and trying to give up cigarettes.

Drug Interactions
No negative interactions between cannabis and other drugs were reported. Several patients (3) indicated that cannabis had a welcome amplifying effect on the efficacy of prescription and OTC medications. "I hurt a lot more without cannabis and can't function as well," reported Liz J. "It seems to relax me so the medicines work better and faster. Additionally, cannabis is natural, and all these other drugs —Vicodin, Soma, Aleve, Librium, Baclofen, have lots of side effects."

As cannabis comes into wider use in California and elsewhere, it is important that its interactions with other medications be studied and publicized.

As cannabis comes into wider use in California and elsewhere, it is important that its interactions with other medications be studied and publicized. Cannabis may also have an amplifying effect on alcohol, enabling some patients to achieve a desired level of inhibition-reduction or euphoria while drinking significantly less.

Defining Success
The harm-reduction approach to alcoholism is based on the recognition that for some patients, total abstinence has been an unattainable goal. Success is not defined as the achievement of perpetual sobriety. A treatment may be deemed helpful if it enables a patient to reduce the frequency and quantity of alcohol consumption; if drunken episodes and/or blackouts are reduced; if success in the workplace can be achieved; if specific problems induced by alcohol (suspended driver's license, for example) can be resolved; if ineffective or toxic drugs can be avoided.

As noted, all of the patients in this study were seeking physician's approval to use cannabis medicinally —a built-in bias that explains the very high level of efficacy reported. However, the majority were using cannabis for other conditions as well, and would have qualified for an approval letter whether or not they reported efficacy with

respect to alcoholism. Although medicinal use of cannabis by alcoholics can be dismissed as "just one drug replacing another," lives mediated by cannabis and alcohol tend to run very different courses. Even if use is daily, cannabis replacing alcohol (or other addictive, toxic drugs) reduces harm because of its relatively benign side-effect profile. Cannabis is not associated with car crashes; it does not damage the liver, the esophagus, the spleen, the digestive tract. The chronic alcohol-inebriation-withdrawal cycle ceases with successful cannabis substitution. Sleep and appetite are restored, ability to focus and concentrate is enhanced, energy and activity levels are improved, pain and muscle spasms are relieved. Family and social relationships can be sustained as pursuit of long-term goals ends the cycle of crisis and apology.

Carl S., a 42 year old journeyman carpenter, is a success story from a harm-reduction perspective. At his initial visit he defined his problem as "intermittent explosive disorder," for which he had been prescribed Lithium. Although drinking eight beers/day, he reported "Cannabis has allowed me to just drink beer when I used to blackout drink vodka and tequila." By the time of a follow-up visit (12 months), Carl had been sober for four months. He also reported "anger outbreaks less severe, able to complete projects," and, poignantly, "paranoia is now mostly realism." He plans to put his technical skill to use in designing a vaporizer.

The Doctor-Patient Relationship

As a certified addictionologist I have supervised both inpatient and outpatient treatment for thousands of patients since 1969. In the traditional alcoholism medical-treatment model, the physician is an authority figure to a patient whose life has spun out of control. The patient enters under coercive circumstances, frequently under court order, with physiologies in toxic disarray. Transference dynamics cast the physician into a parental role, producing the usual parent-child conflicts. After detoxification when cognition has returned from the confusional state of withdrawal, the patient leaves —usually with powers of denial intact. Follow-up outpatient treatment is oriented to AA and/or pharmacologic substitutes.

Treating alcoholism by cannabis substitution creates a different doctor-patient relationship. Patients seek out the physician to confer legitimacy on what they are doing or are about to do. My most important service is to end their criminal status — Aeschalapian protection from the criminal justice system— which often brings an expression of relief. An alliance is created that promotes candor and trust. The physician is permitted to act as a coach —an enabler in a positive sense.

As enumerated by patients, the benefits can be profound: self-respect is enhanced; family and community relationships improve; a sense of social alienation diminishes. A recurrent theme at follow-up visits is the developing sense of freedom as cannabis use replaces the intoxication-withdrawal-recovery cycle —freedom to look into the future and plan instead of being mired in a dysfunctional past and present; freedom from crisis and distraction, making possible pursuit of long-term goals that include family and community.

Re: Alcoholics Anonymous

Although nine patients made voluntary reference to attending 12-step meetings (three presently, six in the past), it is likely that many more actually tried the 12-step program —but the question was not posed on the intake form. A future study should examine the relationship between cannabis-only users and Alcoholics Anonymous. At AA meetings, cannabis use is considered a violation of sobriety. This puts cannabis-only users in a bind. Those who attend meetings can't practice the "rigorous honesty" that AA considers essential to recovery; and those who avoid meetings are denied support and encouragement that might help them to stay off alcohol. Support-group meetings at which cannabis-using alcoholics are welcome would be a positive development.

• Frank R., first seen at age 29, was diagnosed as an alcoholic in 1987 and began attending AA meetings, which he found helpful although he could not achieve sustained sobriety. In 1998, after realizing that cannabis reduced his cravings for alcohol, he received approval to use it. At a follow-up in November '99 he reported, "Have stopped drinking for the first time in many years. I have not taken a drink of alcohol in 14 months. I attribute some credit for this to daily use of cannabis. My life has improved with this treatment."

Frank R. was seen again in April '01 and reported, "I continue to maintain sobriety regarding alcohol. Have not had a drink for 2 1/2 years. I drank alcohol heavy for about 10 years, and had difficulty stopping drinking and staying stopped until I began this treatment. Pain symptoms from back spasms/scoliosis also better."

Factors in Drug of Choice

British psychiatrist G. Morris Carstairs spent 1951 in a large village in northern India and reported on the two highest castes, Rajput and Brahmin, and their traditional intoxicants of choice —alcohol and cannabis, respectively. The Rajputs were the warriors and governors; they consumed a potent distilled alcohol called daru. The Brahmins were the religious leaders; they were vegetarians and drank a cannabis infusion called bhang.

"By virtue of their role as warriors, the Rajputs were accorded certain privileged relaxations of the orthodox Hindu rules," writes Carstairs, "in particular, those prohibiting the use of force, the taking of life, the eating of meat and drinking of wine." The Rajputs viewed the daru-inspired release of emotions —notably sexual and aggressive impulses— as admirable. Rajput lore, as shared with Carstairs, glorified sexual and military conquests.

The priestly Brahmins, on the other hand, "were quite unanimous in reviling daru and all those who indulged in it. They described it as foul, polluting, carnal and destructive to that spark of Godhead which every man carries within him." Bhang, a Brahmin told Carstairs, "gives good bhakti." He defined bhakti as "emptying the mind of all worldly distractions and thinking only of God."The Brahmin emphasis on self-denial includes "the avoidance of anger and or any other unseemly expression of personal feelings; abstinence from meat and alcohol is a prime essential." Carstairs's stated goal was to understand how the Brahmins could rationalize intoxicant use. He concluded:

"There are alternative ways of dealing with sexual and aggressive impulses besides repressing them and then 'blowing them off' in abreactive drinking bouts in which the superego is temporary dissolved in alcohol. The way which the Brahmins have selected consists in a playing down of all interpersonal relationships in obedience to a common, impersonal set of rules of Right Behavior. Not only feelings but also appetites are played down, as impediments to the one supreme end of union with God... Whereas the Rajput in his drinking bout knows that he is taking a holiday from his sober concerns, the Brahmin thinks of his intoxication with bhang as a flight not from but toward a more profound contact with reality."

Two aspects of Carstair's report resonate strongly with my own observations:
• The disinhibition achieved via alcohol is the Rajput kind —a flight from reality, becoming "blotto"— whereas the disinhibition achieved via cannabis is the result of focused or amplified contemplation.
• "Drug of choice" is strongly influenced by social and cultural factors, and, once determined, becomes a defining element of individual self-image, i.e., possible but not easy to change in adulthood.
Prohibition of marijuana, the intense advertising of alcohol, and its widespread availability encourage the adoption of alcohol as a drug of choice among U.S. adolescents.
It is likely that legal access to cannabis would result in fewer young adults adopting alcohol as their drug of choice, with positive consequences for the public health and countless individuals.

Ring Lardner, Jr., on Cannabis as a Substitute for Alcohol

Screenwriter Ring Lardner, Jr. won an Oscar in 1938 for "Woman of the Year" and another in 1970 for "M*A*S*H." His memoir "I'd Hate Myself in the Morning" (which takes its title from his line to the House Un-American Activities Committee) includes this description of his colleagues Ian Hunter and Waldo Salt.

"Ian, too, had an alcohol problem —one that, unlike mine, increased in severity to the point of debilitation. During the period when we had to come up with an episode for a half-hour television program every week, there were times when I had to perform the task by myself. On occasion, he would pull himself together and make a big effort to match what I had done single-handed. Eventually, though, he came to the conclusion that he would have to give up drinking for good. And he proceeded to do just that, first by enlisting in Alcoholics Anonymous, as he went cold turkey, then, to fortify his abstinence, by substituting marijuana for alcohol. It happened that a friend of ours, the blacklisted writer Waldo Salt, had made the same medicinal switchover. Since Ian and Waldo also shared a love of drawing, they could pool the cost of a model and spend an evening indulging in pot and art. Neither of them drank again, as far as I know.
"Some years earlier, when the film community was still disproportionately Jewish, my good friend Paul Jarrico announced a discovery. He had been wondering why a small grup of his fellow screenwriters —Ian, Dalton Trumbo, Hugo Butler, Michael Wilson,

and I— were such a close, cozy group. What bound us together, Paul reported, was the fact that we were all gentiles. ‘Nonsense,’ Ian declared, ‘It’s that we’re all drunks.’ Instantly, I knew he was right. It was by far the stronger bond.”