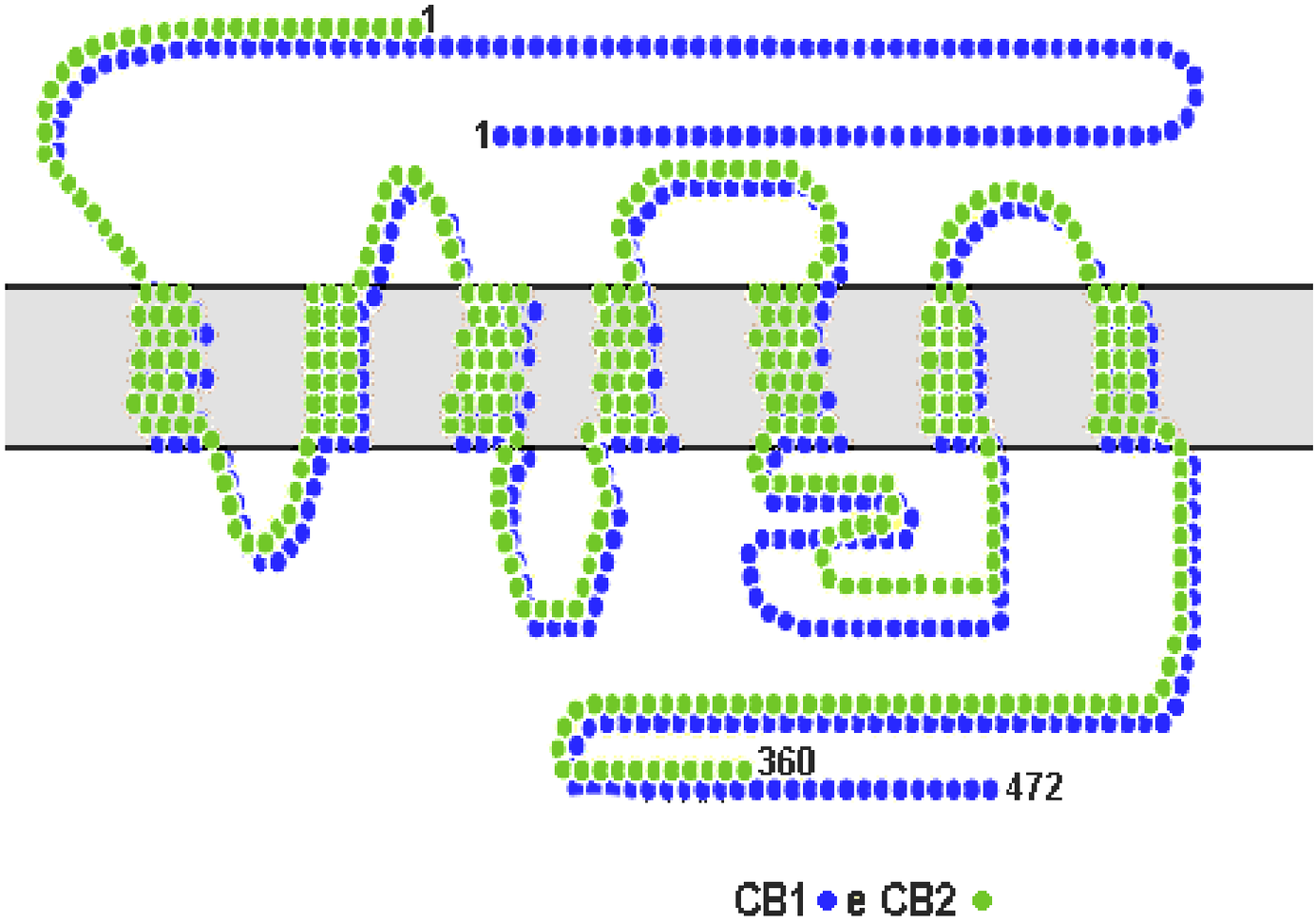

Paulo Carvalho Sá

Cannabinoids as a Therapeutical Resource

Monografia realizada no âmbito da unidade de Estágio Curricular do Mestrado Integrado em Ciências Farmacêuticas, orientada pela Professora Doutora Isabel Rita Barbosa e apresentada à Faculdade de Farmácia da Universidade de Coimbra

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Abreviaturas

CB1 – cannabinoid receptor type 1

CB2 – cannabinoid receptor type 2

THC – Δ-9-tetrahydrocannabinol

CBD – Cannabidiol

AIDS – Adquired Imuno deficiency syndrome

GPR55 – G protein coupled receptor 55

PPARα – peroxisome proliferator-activated receptor α

PPARγ – peroxisome proliferator-activated receptor γ

TRPV1 – transient receptor potential vannilloid-1

cAMP – Cyclic adenosine monophosphate

MAPK – mitogen-activated protein kinase

CNS – Central nervous system

PI3K – phosphoinositide 3-kinase

NK – natural killer

AEA – anandaminde

2-AG – 2-Arachidonoylglycerol

NAPE – *N*-arachidonoyl phosphatidylethanolamine

NAPE-PLD – N-acetylphosphatidylethanolamine-hydrolysing phospholipase D

SC – synthetic cannabinoids

FAAH – fatty acid amide hydrolase

PLC – phospholipase C

DAGL – diacylglycerol lipase

MAGL – monoacylglycerol lipase

Hsp70s – Include heat shock proteins 70

FABPs – fatty acid binding proteins

ECM – extra cellular matrix

AD – Alzheirmer's disease

Aβ – Amyloid percursor protein

MLR – Mixed Lymphocyte Reaction

Tregs – regulatory T-cells

Anti-IL-10 – anti-inflammatory cytokine IL-10

ED – Emergency department

DUID – driving under the influence dose

Resumo

A comunidade científica reconheceu a possibilidade da existência de propriedades terapêuticas dos compostos canabinóides, e desde 2014 que existe uma vasta quantidade de publicações sobre o tema. As áreas onde há a hipótese destes compostos terem propriedades terapêuticas serão: doença de Alzheimer, oncologia, distúrbios de adição, epilepsia, anti-inflamação central e analgesia.

Existe alguma controvérsia sobre estes compostos, e por isso, alguns dos artigos científicos não têm factos concordantes. Além disto, como os canabinóides são utilizados como uma droga de abuso, a maior parte dos países baniram estes compostos, tornando mais complicado adquirir informação sobre estes.

Para poder então contextualizar as propriedades terapêuticas dos canabinóides será necessário então descrever o Sistema endo-canabinóide e algumas das suas vias bioquímicas. Alguns dos compostos canabinóides com possível utilização terapêutica serão também descritos.

O principal objeto desta revisão será então analisar a hipótese da utilização de canabinóides, sintéticos ou não, como um recurso terapêutico.

Abstract

The scientific community has acknowledged the therapeutic possibilities of cannabinoids since 2014 and has started to publish a vast amount of papers on the topic. Areas where cannabinoids have the possibility of bringing new therapeutical contributes have been recognized as Alzheimer's disease therapy, oncology, epilepsy management, addiction based disorders, central anti-inflammatory action and pain management.

Controversial scientific papers regarding these compounds are recent and some have diverging results. To add to this factor, since these compounds have been used as a recreational abuse drug by a large group of people, most countries decided to ban them making it harder to acquire information on the topic.

To explain the wide range of therapeutic possibilities, the endo-cannabinoid system and some of the biochemical pathways will be focused. The types of cannabinoids that can be used as therapeutic resources will be described individually and all the areas of interest where cannabinoids have a possible future of being an effective treatment option will be described and corroborated with scientific studies.

The main objective of this revision will be to describe and to analyse the future possibilities of utilizing synthetic cannabinoids and phytocannabinoids as therapeutic resources. Some aspects to increase our understanding about the use of cannabinoids as therapeutic resources will also be provided.

1. Introduction

Cannabis has been used in medicine for thousands of years prior to achieving its current illicit substance status. It has been discovered recently that during the Egyptian Pharaoh era, *Cannabis sativa* plant was administered to patients that possessed diagnosed tumors. It was only in 1940 that this plant stopped being completely available to be used as medicine because of its potential to be used as an abuse drug. Recently some countries have been changing this plant's legal statute, allowing it to be used in therapy. Since the biggest technological advances surged after 1940, and cannabinoids were banned during most of the scientific revolution, there is a huge lack of information on this topic. Most of the information available currently is recent and controversial. Almost studies demonstrate very promising capabilities for cannabinoids to be used on diseases like cancer and Alzheimer but, the mechanisms of action for such effects are still mostly unknown.

It has been noted that cannabinoids, the active components of *Cannabis sativa*, mimic the effects of the endogenous cannabinoids, activating specific cannabinoid receptors, particularly CB1 found predominantly in the central nervous system and CB2 found predominantly in cells involved with immune function. THC, the main bioactive cannabinoid in the plant, has been available as a prescription medication approved for treatment of cancer chemotherapy-induced nausea and vomiting and anorexia associated with the AIDS wasting syndrome. Cannabinoids may be of benefit in the treatment of cancer-related pain, possibly synergistic with opioid analgesics.

This means that if this subject is studied in depth, new possibilities of treatment for some of the most prevalent diseases may be found. Unfortunately, there is also a great lack of information on long term cannabinoid use since it should not be possible because of its legal status, which means that this has to be clarified in order to provide maximum safety to cannabinoid use.

2. The endocannabinoid system

The neuromodulator system consists of cannabinoid receptors, endogenous ligands termed endocannabinoids, and some enzymes responsible for their synthesis and degradation [1]. Two main cannabinoid receptors designated CB1 and CB2 have been described and studied. THC, the main compound present on cannabis, produces most of its effects by binding itself to both of these receptors but can also bind to other nonspecific such as GPR55, PPARα and PPARγ, and transient receptor potential vannilloid-1 TRPV1 channels[2]. These facts indicate the existence of additional cannabinoid receptors. These two receptors have approximately 44 % amino acid homology [3]. Both of them have seven transmembrane domains, are coupled to G-inhibitory proteins, and are linked to signaling cascades that probably involve adenylyl cyclase and cAMP, MAP kinase, and the regulation of intracellular calcium [3]. The alteration of intracellular calcium concentration may be one of the factors responsible for motor impairment during CB1 and CB2 receptor expression.

CB1 receptors are the most abundant G protein-coupled receptors in the central nervous system, expressed in both neurons and glial cells, where they regulate important brain functions including cognition and memory, emotion, motor control, feeding, and pain perception [2]. CB1 is predominantly responsible for the psychoactive effects of THC, also is involved in regulating pain, stress responses, energy regulation and lipogenesis, and immune function. CB1 receptors are usually located at the terminals of neurons of the central and peripheral nervous system where they act as modulators of excitatory and inhibitory neurotransmission. Such CB1 receptors are also found in peripheral tissues, and play an important role in energy balance and metabolism[4].

The CB2 receptor is primarily associated with immune function and is expressed on almost all immune cells, which comprehend microglial cells within the nervous system, but its expression in the CNS is of a much smaller rate than that of CB1. Relatively low CB2 receptor expression has also recently been found in some neurons [2]. Further proof of CB2 receptor expression in neurons comes from the observation that axonal damage in one cerebellar hemisphere induced the expression of CB2 receptors in contralateral pre-cerebellar neurons; CB2 receptor agonists facilitated neuronal survival, whereas the selective PI3K inhibitor blocked CB2 receptor effects on axotomized neurons [2]. The level of CB2 expression differs among different immune cell populations, with B lymphocytes expressing the highest levels followed by macrophages, monocytes, NK cells, and polymorph nuclear cells, in that order[3]. Early studies have also concluded that the distribution of CB2 was

confined to peripheral non-neuronal sites. However, it is now accepted that this receptor is expressed by a variety of subsets of immunocompetent cells found in the CNS [3]. In addition, the CB2 has been reported to be present also on neurons[3]. In general, the immunomodulatory effects attributed to THC have been linked to activation of CB2 receptors, and the psychoactive properties of cannabis have been associated with CB1 receptor activation.

After the discovery of these two receptors, it was discovered that in order to activate such receptors our cells produce and release cannabinoid like endogenous molecules, the endocannabinoids, which actively regulate CB1 and CB2 receptor expression. Endocannabinoids have been found in immune cells such as monocytes, macrophages, basophils, lymphocytes, and dendritic cells[3]. Endocannabinoids can also act as neurotransmitters, they are synthesized and released by neurons, able to bind and activate membrane receptors, and are inactivated by reuptake and enzymatic degradation within the cell.

Firstly, isolated in 1992, Anandamide is a neurotransmitter and endocannabinoid. Anandamide, also known as N-Arachidonoylethanolamine or AEA, is an endogenous cannabinoid that acts as a complementary molecule fitting into the active sites of the CB1 and CB2 receptors.

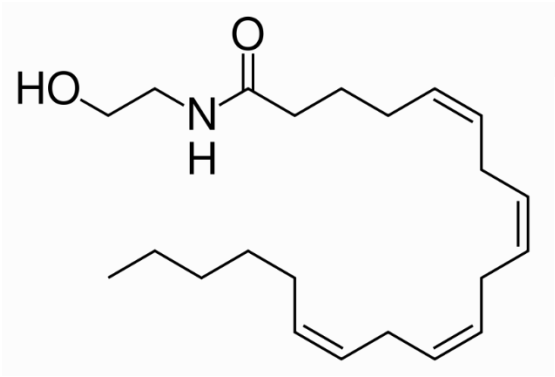
Neuronal damage may also increase the production of endocannabinoids, which can provide a defense mechanism against toxicity[2].

3. Endogenous and Exogenous Cannabinoids

3.1 Endogenous Cannabinoids

Endocannabinoids have two fundamental properties that differentiate them from other neurotransmitters: they act as retrograde messengers and they do not accumulate in the interior of synaptic vesicles[2]. For several years it has been theorized that endocannabinoid compounds are exclusively synthesized on demand to act on cells located near their site of synthesis, and then are rapidly inactivated by the action of specific degradation enzymes. However, recent studies have shown intracellular reservoirs of anandamide in places other than synaptic vesicles as in adiposomes where it is concentrated to higher levels than in the extracellular space[2].

The endocannabinoid receptors found within our bodies are usually bound by two endogenous cannabinoid like molecules; Anandamide, as referred previously, also known as *N*-arachidonoylethanolamine or AEA and 2-Arachidonoylglycerol, which is also known as 2-AG. It is theorized that these endocannabinoids are enzymatically produced and released "on demand" in a similar fashion as the eicosanoids.



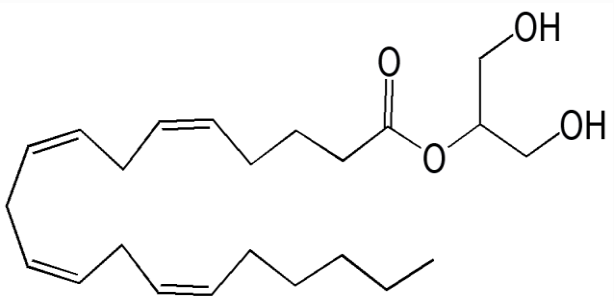


Image 1: AEA molecule CB1agonist from[5]. **Image 2:** 2-AG molecule CB1 and CB2 agonist from[5].

Anandamide's can produce its effects in either the central or peripheral nervous system. These different effects are mediated primarily by CB1 receptors in the central nervous system, and CB2 receptors in the periphery[3]. The latter are mainly involved in the immune system function. Anandamide has been shown to impair working memory in rats[3]. And it has been theorized that it may interact with human behavior such as sleep and feeding patterns. The human body synthesizes anandamide from NAPE, which is created by transferring arachidonic acid from lecithin to the free amine of cephalin through an (*N*-acyltransferase enzyme[1]. Anandamide synthesis from NAPE occurs via multiple pathways and includes enzymes such as phospholipase A2 and phospholipase C and NAPE-PLD[2]. Endogenous anandamide is found at very low levels and has a very short half-life due to the action of the enzyme FAAH, which dissociates it into free arachidonic acid and ethanolamine. Studies with piglets show that dietary levels of arachidonic acid and other essential fatty acids affect the levels of anandamide and other endocannabinoids in the brain[6]. High fat diet feeding, in mice, increases levels of anandamide in the liver and also increases lipogenesis[7].

2-AG is an ester created from the omega-6 fatty acid arachidonic acid and glycerol. It is present, with increased levels in the central nervous system, and has neuromodulator effects. It has been isolated in maternal bovine and human milk. Unlike anandamide, formation of 2-AG is calcium-dependent and is mediated by the activities PLC and DAGL[8]. 2-AG acts as a full agonist at the CB1 receptor[9]. At a concentration of 0.3 nM, 2-AG induces a rapid,

transient increase of intracellular free calcium in NG108-15 neuroblastoma and glioma cells through a CB1 receptor-dependent mechanism[9]. 2-AG is hydrolysed *in vitro* by MAGL, FAAH, and by the uncharacterized serine hydrolase enzymes ABHD6 and ABHD12[5]. There have been identified some transport proteins for 2-AG and AEA. These include Hsp70s and FABPs[10].

3.2 Synthetic Cannabinoids

Synthetic cannabinoids interact with CB1 and CB2 cannabinoid receptors and create cannabimimetic effects similar to THC , the primary psychoactive compound present in cannabis[11]. SC were developed as research tools to explore the endocannabinoid system and as potential therapeutic resources[12]. Most SC use comes from abuse. Between 2011 and 2012 there were identified 9 SC epidemiological studies, none population or community-based and 2 worldwide surveys of self-selected convenience samples. Contrary to the permitted use, they are per orally consumed as a replacement for marijuana to get "high" and are found in a variety of herbal incense mixtures. In almost all cases, detailed information on the physicochemical and pharmacological properties of the synthetic compounds present in these spice preparations are not satisfactory since there were no regulatory entities involved.

The main types of synthetic cannabinoid receptor agonists can be divided into the following major chemical classes:

a) Classical cannabinoids

Compounds isolated from the plant *Cannabis sativa* or synthetic analogues of these compounds belong to this category; HU-210, Δ9-THC, Δ8-THC and desacetyl-L-nantradol are synthetic cannabinoids which behave as CB1/CB2 receptor agonists (but they lack CB1/CB2 selectivity). Other CB2-selective agonists that have been synthesized by structurally modifying THC molecule are JWH-133, JWH-139, HU-308, L-759633 and L-759656 which were effective in nanomolar range[13].

b) No classical Cannabinoids

These are a group of AC-bicyclic and ACD-tricyclic cannabinoid analogue molecules. Furthermore bi-cyclic analog, CP55940, an important cannabinoid agonist has similar affinity

for CB1 and CB2 receptors. Also, it is highly potent *in vivo*. CP55244 and CP47497 are other cannabinoids that fall in this category[14].

c) Aminoalkylindoles

A group of aminoalkylindoles with cannabimimetic properties. R-(+)-WIN55212 is the most well studied compound in this series. It exhibits high affinity for both cannabinoid receptors, but it is more selective for CB2. It has similar pharmacological effects like THC *in vivo*. JWH-015 and L-768242 also show more affinity towards CB2 than R-(+)-WIN55212[14].

d) Eicosanoids

Anandamide, an endogenous cannabinoid ligand was originally found in mammalian brain and acts similarly to THC. Methanandamide is anandamide's R-(+)-isomer and is nine times more CB1 specific than the S-(+)-isomer. Another molecule that belongs to this category is 2-arachidonoylglycerol, another well studied endocannabinoid has both CB1 and CB2 affinity. Other compounds that belong to this group are arachidonyl-2-chloroethylamide (ACEA) and arachidonylcyclopropylamide (ACPA)[14].

e) Other Cannabinoids

Representing diarylpyrazole compounds, which function as antagonists to cannabinoid receptors. SR141716A is a strong CB1 antagonist and SR144528 is a CB2 antagonist. AM251 and AM281 are analogs of SR141716A which block CB1 receptor-mediated effects[14].

4. Therapeutic Applications of Cannabinoids

4.1 Cannabinoids and Cancer

Cannabinoids are clinically dispensed for anti-palliative effects which include the inhibition of nausea and emesis which are correlated with chemo- or radiotherapy, appetite stimulation, pain relief, mood elevation and sleep induction in cancer patients. Recent studies opened a promising possibility for cannabinoids to be used as anti-cancer agents. By showing anti-proliferative and anti-angiogenic effects *in vitro* as well as *in vivo,* in a large variety of cancer models by specifically targeting tumor cells. Synthetic THC (Marinol, Dronabinol) and its

derivative nabilone (Cesamet), as well as Sativex an oral spray containing both THC and CBD (canabidiol), have been approved in several countries to reduce nausea and cancer-related pain in cancer patients undergoing chemotherapy[14].

4.1.1 Cannabinoid receptor mediated signaling in cell and growth regulation

CB1 and CB2 receptor activation is responsible for proliferation, motility, invasion, adhesion and apoptosis of cancer cells both *in vitro* and *in vivo*. CB1/2 receptor expression leads to various events like modification of Ca2+ and K+ channels, modulation of adenyl cyclase and cAMP levels in most tissues and models, regulation of the MAPKs, extracellular signal regulated kinase-1 and -2 (ERK1/2)[14].

One of the most important aspects of an effective anti-tumor drug is its ability to inhibit proliferation and replication of cancer cells. Cancer cells proliferate rapidly in uncontrolled manner. Also, these cells escape death mechanisms under which a normal cell undergoes, such as apoptosis. Apoptosis, a programmed cell death mechanism, involves the activation of caspase dependent and sometimes independent pathways. Cannabinoids have been proved to be selective anti-proliferative and apoptotic drugs[14]. These anti-proliferative effects act on breast, prostate, lung, skin, pancreatic, bone, oral, neck cancers, and even on lymphoma and thyroid carcinoma. On most of these cases cannabinoids interact with tumoral cells only, and inhibit their growth by blocking the tumoral growth mechanism which vary between the types of the tumor. They do this by promoting G1 cell cycle arrest on some cases, such as melanoma, and they also induce apoptosis on tumoral cells leaving the undifferentiated cells unharmed. Most of these mechanisms are CB1 and CB2 dependent but there have been cases, such as pancreatic cancer, where the cancer cell apoptosis were not CB1 and CB2 dependent[14].

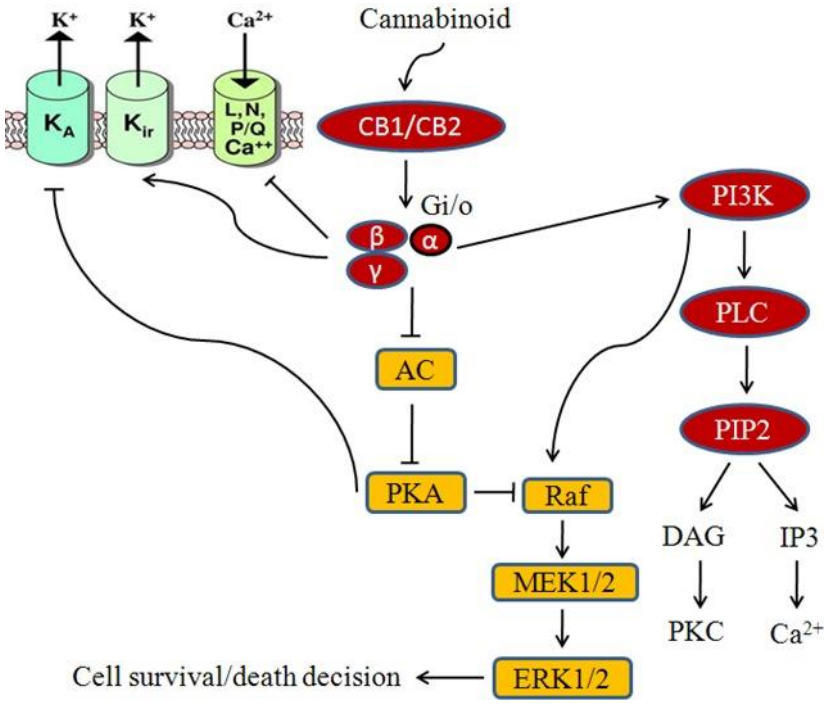


Image 3: "Cannabinoid Mediated signaling in Cancer Cells" from[14].

4.1.2 Clinical use of cannabinoids in cancer

Cannabinoids exert a direct anti-proliferative effect wich can be CB1 and CB2 receptor dependent or not. They have been shown to be anti-migratory and anti-invasive and inhibit Matrix Metalo Proteinases which usually degrade the ECM, thus affecting metastasis of cancer to the distant organs. Also, cannabinoids modulate other major processes in our body like energy metabolism, inflammation making it a priority to further understand such mechanisms. Depending on their source, cannabinoids regulate different signalling pathways and modulate different tumor cell types. It is important to understand which of the cannabinoid receptors are expressed and activated in each tumor because each receptor follows a different signalling mechanism. Furthermore, endocannabinoids- AEA and 2-AG are broken down into secondary metabolites like prostaglandin E2 and epoxyeicosatetraenoic acid which enhance tumor growth and metastasis in diverse cancer types[14].

In addition, cannabinoids are more specific to cancer cells than normal cells, but the administration of single cannabinoids produces limited relief compared to the administration of crude extract of plant containing multiple cannabinoids, terpenes and flavonoids. The combination of cannabinoids with other chemotherapeutic drugs might provide a potent clinical outcome, but further research will be needed before such options are a reality[14].

4.2 Cannabinoids and Alzheimer's disease

Alzheimer's disease is very hard to effectively treat nowadays because the available therapy options are not effective. This means that most of the treatments for AD are only ways to manage the disease's progression. This creates an emerging need to research and develop new therapeutic weapons in order to fully treat AD. During the last few years, the endogenous cannabinoid system has emerged as a potential therapeutic approach to treat Alzheimer. Several findings indicate that the activation of both CB1 and CB2 receptors by natural or synthetic agonists, at non-psychoactive doses, have beneficial effects in Alzheimer experimental models by lowering the harmful β-amyloid peptide action and tau phosphorylation, as well as by promoting the brain's intrinsic repair mechanisms[2].

Alzheimer is an age-dependent neurodegenerative process distinct from normal aging and characterized morphologically by the presence of senile plaques, which are mainly composed by different species of fibrillar β-amyloid produced by the cleavage of the Aβ precursor protein due to β- and γ-secretases, and by the presence of neurofibrillary tangles, mostly composed of various isoforms of hyper-phosphorylated and nitrated tau protein[2]. Recent studies have shown that Aβ deposits acts as a seed of new Aβ production and deposition under appropriate conditions and that abnormal tau promotes the production and deposition of hyper-phosphorylated tau, under determinate experimental conditions[15]. Therefore, Aβ and hyper-phosphorylated tau enhance the progression of the pathological process in an exponential way once these abnormal proteins have been accumulated in the brain[2]. The disease progression from the early stages of the neurodegenerative process to the symptomatic stages may take decades, also, once the cognitive impairment and dementia appear the disease progression rate increases. This means that AD is a relatively well-tolerated degenerative process during a long period of time, but it may have devastating effects once some thresholds are crossed[2]. These facts demonstrated the need for treatments that act on selective targets during the silent period of the disease, aimed at curing or retarding disease progression toward dementia[2].

Recently, it has been demonstrated that endocannabinoid signalling modulates the main pathological processes occurring during the silent period of the neurodegenerative process, including protein misfolding, neuroinflammation, excitotoxicity, mitochondrial dysfunction, and oxidative stress[2].

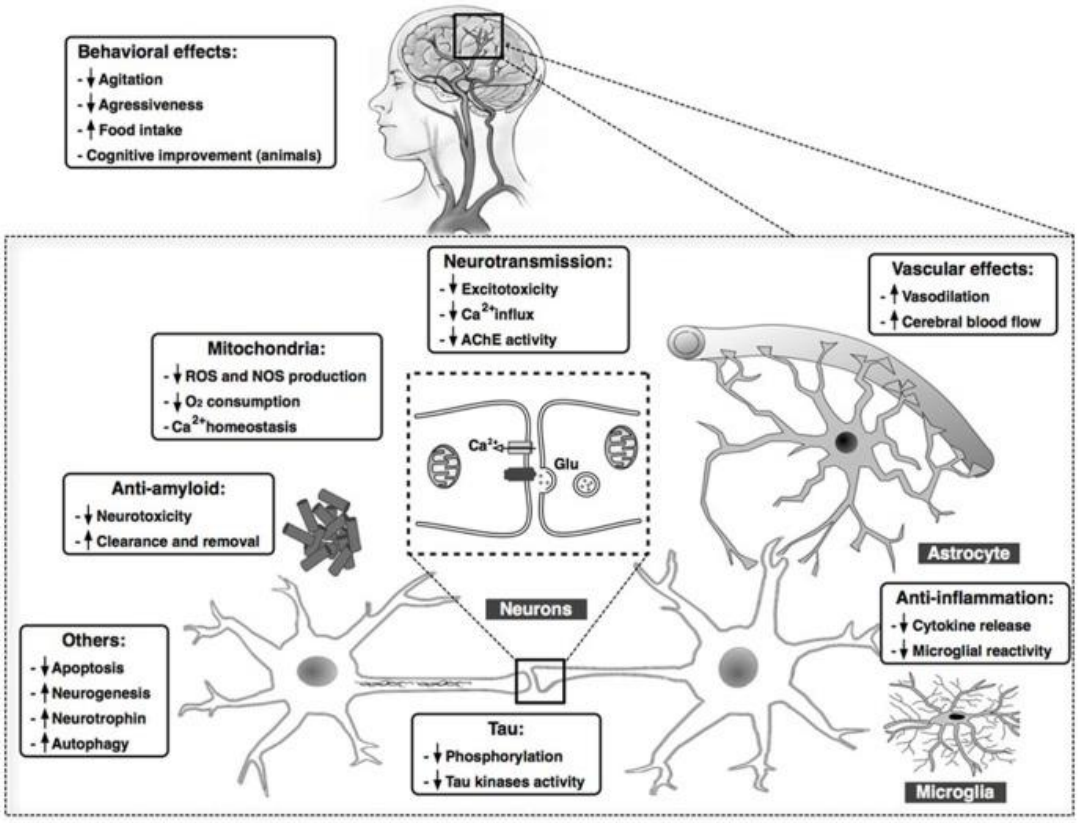


Image 4: "Summary of the main findings demonstrating beneficial effects of cannabinoid compounds in AD models" from[2].

4.2.1 The endogenous cannabinoid system in AD brains

The analysis of human *post-mortem* samples revealed some alterations in the ECS composition and signalling in AD brains, although the occurrence of such alterations in the pathophysiology of the disease remains unknown. Some authors have reported a significant reduction in the CB1 levels in cortical areas and in neurons distant from senile plaques[16], while others have described no changes in the expression, distribution, or availability of CB1 receptors in cortex and hippocampus in AD or have failed to dissociate CB1 receptor expression changes from normal aging. Since cannabinoids are a new potential treatment to AD such discrepancies should be rectified. In contrast, there is no controversy regarding the significant increase of CB2 expression in AD brains, mainly corresponding to receptors expressed on microglia surrounding senile plaques[2]. Interestingly, expression levels of CB2 receptors correlates with Aβ42 levels and senile plaque deposition, although not with cognitive status, suggesting that such pathogenic events enhance CB2 receptor expression[16].

Some studies targeted other components of ECS in AD human samples. The first study analysing endocannabinoid levels reported no differences between AD patients and healthy controls in the plasmatic concentrations of AEA and 2-AG[2]. However, a recent lipidomic study in *post-mortem* brain samples showed lower AEA levels in midfrontal and temporal cortices in AD compared to control subjects, which is inversely correlated with the neurotoxic brain Aβ42 peptide levels and cognitive deficiencies observed in these patients, suggesting a contribution for Aβ42-dependent AEA impairment to cognitive dysfunction[2]. In addition, some variations have were encountered in the contents and/or activity of the enzymes related to endocannabinoid synthesis and degradation in AD brains. Which means, the endocannabinoid metabolizing enzyme FAAH is up-regulated in AD both neuritic plaque-associated glia and also in peripheral blood mononuclear cells[2]; this could participate in the increase of AEA degradation in the vicinity of the senile plaque. Such FAAH overexpression may have at least two negative consequences in disease progression, such as, neuronal AEA availability limitation and increase of pro-inflammatory molecules induced by AEA metabolites such as arachidonic acid[2]. A different study revealed altered 2-AG signalling during late stages of AD due to the combination of impaired MAGL recruitment and augmented DAGL levels, which promote synapse silencing in AD[13].

4.2.2 Clinical and preclinical evidence of therapeutic properties of cannabinoids in AD

Most of the evidence accumulated sustaining the potential therapeutic utility of cannabinoids in AD has been gathered by using cellular and animal models that mimic a variety of AD-related alterations. However, the scarce clinical data available also supports the beneficial effects of cannabinoid compounds for managing some behavioural symptoms related to AD[2]. Only a few clinical trials and one case report are available on the topic so far. In all of the cases an analogue of Δ9-THC (nabilone or dronabinol) was tested. Interestingly, one clinical trial including 15 AD patients resulted in a decreased severity of altered behaviour and an increase in the body weight in AD patients, who were previously not accepting food, after 6 weeks of dronabinol treatment. Side effects associated with cannabinoid administration were limited to euphoria, somnolence, and tiredness, but these did not require discontinuation of therapy. In addition, two other studies including eight patients with dementia concluded the reduction in night-time agitation and behavioural disturbances, without adverse effects during the trial period with dronabinol[2]. In agreement with these observations, the use of the cannabinoid receptor agonist nabilone correlated with instant and major improvements in

the severe agitation and aggressiveness exhibited by an advanced AD patient who was refusing anti-psychotic and anxiolytic medications. In spite of the low number of patients included in these trials and the fact that none of them objectively evaluated cognitive or neurodegenerative markers, the positive behavioural results represent valuable, although limited, information, considering that no major side effects were reported. However, the revision in 2009 of the Cochrane Dementia and Cognitive Improvement Group Specialized Register encountered no evidences of cannabinoid effectiveness in the improvement of behaviour and other parameters of dementia, and suggested that specific trials are required to assess the effectiveness of cannabinoids in the possible treatment of dementia[2].

5. Cannabinoids and immune system modulation

Almost all immune cells have present CB1 and CB2 receptors meaning that the endocannabinoid system can alter the immune system function. Much attention has been focused on CB2 receptor not only because of its expression in cells and tissues of the immune system[3], but also because of its intricate involvement in immune function and because its activation is largely devoid of psychotropic effects. The level of CB2 expression varies among different immune cell populations, with B lymphocytes expressing the highest levels followed by macrophages, monocytes, NK cells, and polymorphonuclear cells, in that order[3]. Early studies concluded that the distribution of CB2 was confined to peripheral non-neuronal sites, however, it is now clear that this receptor is expressed by a variety of subsets of immunocompetent cells found in the CNS. In addition, CB2 has been reported to also be present on neurons[3]. In general, most of the immunomodulatory effects attributed to THC have been linked to activation of CB2. Accordingly, it is proposed that selective CB2 agonists can act as therapeutic agents for treatment of autoimmune diseases and for reduction of graft rejection with decreased incidence of side effects. The potential selection of CB2 agonists to reduce graft rejection is particularly relevant in the report by Robinson et al. that explores the mechanism by which agonists selective for CB2, such as O-1966, inhibit the MLR[3], an in vitro paradigm used to correlate organ graft rejection mediated predominantly through effects on T-lymphocytes. These investigators observed augmented percentage of Tregs in MLR cultures using mouse spleen cells. Furthermore, pre-treatment with an antibody to the anti-IL-10 resulted in a partial reversal of the inhibition of proliferation and blocked the increase of Tregs. Their results support the argument that CB2-selective agonists may represent useful therapeutic resources to prolong graft survival in transplant patients. While a large amount of data from *in vitro* studies and animal models

indicates that the immunomodulatory activity of THC and CB2 agonists can lead to decreased resistance to infectious agents, a comparable correlation in humans has yet to be demonstrated.

It has been suggested that 2-AG is the known functionally-relevant endocannabinoid for CB2[8]. AEA also has been associated with modulation of immune function. However, whether this linkage involves activation of a cannabinoid receptor is still uncertain. The immunomodulatory activity mediated by endocannabinoids can occur in an autocrine and paracrine fashion, impacting the functionality of immune cells in a localized environment. Furthermore, such mediated action may have a short extent because of the rapid degradation of endocannabinoids in the intracellular[3].

It is now apparent that immune cells present within the CNS contain a constitutive endocannabinoid system[3]. Thus, it appears that the immediate effective action of endocannabinoids on immune function happens at localized sites in the periphery and CNS. It is speculated that, in this context, endocannabinoids play an important role in controlling the overall normal function of the immune homeostatic balance within the host. There is also compelling evidence that the endocannabinoids may provide protective activity, particularly in the brain. However, the basis for the neuroprotection mediated by these endogenous cannabinoids is still rather poorly defined[3].

6. Long term cannabinoid use

Recreational SC intake arose strongly in the 2000's and many adverse effects were reported. Acute SC intoxication can lead to ED presentation and hospitalization, requiring supportive care, benzodiazepines, and fluids. While most patients were released within 24 h of admission, severe adverse effects such as cardiotoxicity, acute kidney injury, and psychosis resulted in hospitalization for as long as 2 weeks. Deaths directly linked to SC use were quite rare. Some chronic SC users experienced withdrawal symptoms when they stopped drug intake. SC consumption has become widespread, even with law enforcement and regulatory control measures. Epidemiological data suggest that the majority of SC users are young adults who perceive SC as safer than non-cannabinoid illicit drugs and a favourable cannabis alternative inducing cannabis-like "high" while avoiding detection by standard drug screening. However, data suggests that many SC users prefer cannabis over SC due to the drugs' negative effects. SC are readily accessible, sold under several names and packaging with smoking as the most common route of administration. Most SC smokers are men from

13–59 years old, many with a history of polydrug use such as cannabis, alcohol, and nicotine. Most SC have greater binding affinity to CB1 receptors than THC, suggesting a possible mechanism for the severity of acute clinical reactions that result in ED presentation. However, SC intrinsic activity data are very limited, with very few direct comparisons to THC, making it premature to draw any conclusions about mechanisms[17].

7. Synthetic Cannabinoid dependence

The three following cases collected from literature are illustrative:

A 20-year-old man who smoked "Spice Gold" 3 g/day for 8 months, was hospitalized about 1.5 days after last use with a severe withdrawal syndrome, including increased craving, restlessness, nightmares, tachycardia (maximum heart rate 125 bpm), hypertension (180/90), nausea, sweating, and muscle spasms. The syndrome resolved within one week with symptomatic treatment[17].

A 22-year-old woman smoking 3 g/day SC attended the ED complaining of severe anxiety, "vivid" dreams, headache, cramping of extremities, "sweats and chills", anorexia, and craving 6 days after last use. She was discharged within 3 h of receiving IV saline and 2 mg lorazepam[17].

A 20-year-old man, with a history of smoking "Mr. Nice Guy" for 18 months, ceased smoking 6 days prior to ED presentation for headache, chest pain, profuse sweating, and body tremors. Prior to his ED visit, he attempted to alleviate symptoms by smoking cannabis, which was ineffective, but taking his roommate's quetiapine provided relief. Benzodiazepines did not alleviate his symptoms, and after admission, subsequent hydroxyzine and diphenhydramine administrations also were unsuccessful. The patient's symptoms subsided after the physician administered 50 mg quetiapine, and he was released with an unspecified quetiapine dose[17].

8. Synthetic Cannabinoid Mortality

Until 2014 only 4 fatalities associated with SC intake were identified; MAM2201 (dose and route of administration unknown) was linked to the death of a 59-year-old Japanese man who was found dead at home with MAM2201 detected in his femoral blood (1.24 μg/L),

brain, body organs, and adipose tissues. Because there were no signs of physical injury and the deceased was assumed healthy, MAM2201 intoxication was considered cause of death[17].

In Sweden, a 17-year-old man was found alone outside (6–8° C ambient temperature), dead from hypothermia and acute SC intoxication. Prior to the man's death, his friend reported smoking a foil of herb with the deceased. The friend took two whiffs, became light-headed and felt numbness in his hands. The friend went indoors afterwards, while the deceased continued smoking outside. JWH-210 was found in post-mortem femoral blood (12.3 μg/L)[17].

A 23-year-old man died from self-inflicted injuries sustained during a violent severe psychosis episode after smoking AM2201. Prior to his death, a family member heard "stomping noises" for 30 min coming from his room. The man was eventually encountered dead on the floor with multiple injuries, including a fatal stab wound to his neck. A bag of "Mad Hatter" incense, smoke pipe, and a bag of white pills (labeled "ZAN-X") were found in his room. AM2201 (12.0 μg/L) was identified in post-mortem heart blood. No other drugs were found. AM2201 also was detected in the "Mad Hatter" incense" and pipe residue. Traces of JWH-073 also were detected. "ZAN-X" did not contain any illicit or prescription drugs[17].

Reported SC blood concentrations in these cases were 1.2–12.3 μg/L, comparing to impaired driver concentrations of 0.1-28 μg/L. This overlap between lethal and DUID SC concentrations hamper the identification of a fatal SC concentration. Other factors also could have contributed to death, such as undetected additional SC and/or other drugs of abuse, dose and route of administration, individual variation in SC metabolism, and lack of drug tolerance. Withdrawal symptoms similar to those following chronic and frequent cannabis intake were observed in chronic SC smokers after at least 1 week of abstinence[17].

9. Discussion

After such a diverse state of the art, Cannabinoids, either synthetic, natural or endogenous demonstrate to clearly have therapeutic possibilities since they interact and even regulate physiological systems. The endocannabinoid system should be systematically studied since it can present a new perspective of the normal, defensive and active operation of the human cells. Cannabinoid research should also be further advanced since it will help with the understanding the cannabinoid system inter-active and respective implications in the human bio computer and metabolism. Synthetic cannabinoid compounds should be more studied and have their pharmacological properties verified since CB1 and CB2 agonist properties can

be very useful as a therapeutic weapon. Especially if there is receptor selectivity which is present on some of these molecules.

One of the most interesting areas where cannabinoids have a great potential of being a new generation of therapeutic resources is cancer. Even though it is already used in some cases as described, very few of its effects have been fully explained. One of the major breaks on developing new cannabinoid based molecules for this pathology is the fact that there are countless types of tumors, tumor cells heterogeneity and even the surrounding tissues of the tumoral stroma can alter cannabinoid bioavailability. Even with such barriers that still need to be overcome, some cannabinoid molecules are already in use in some countries, as a palliative measure but, since some of the cannabinoids present on such preparations have the ability to selective target tumoral cells and to destroy them, they may be helping with the therapeutic progression more than it appears. Before cannabinoids can be used in clinical trials, it is necessary to acquire more knowledge on several issues such as anti-tumorigenic and anti-metastatic mechanisms as well as which type of cancer patient populations would be more responsive to cannabinoid based therapies. Understanding the exact signalling by which cannabinoids function may be the path that will lead to targeted clinical approach. Also, the difference in cellular response to cannabinoids in different cancer types might be due to the effect of the tumor environment which varies greatly and can interfere with the cannabinoid availability and pharmacodynamics within the tumor. The property of affecting multiple signalling pathways might unlock the possibility of developing cannabinoids that selectively obstruct a particular pathway, thus opening avenues for specific targeted treatments. This could be very well the future of chemotherapy since cannabinoids have the potential ability to target and destroy cancer cells while leaving the host cells unharmed.

As for cannabinoid use to treat Alzheimer's disease, there is also a good possibility to being an effective treatment, not just a progression manager for the disease. The fact that CB1 and CB2 receptor expression have a role on the disease's progression, and promote neuroprotective effects, gives the opportunity to possibly treat AD if more research is made on the topic since most of the authors have divergent points of view. Research should be made to clarify and unify the knowledge on such matter in order to create opportunities for actual cannabinoid research on AD, but for that the endocannabinoid system needs to be fully described and understood.

As for the actual acceptance of cannabinoids as a mainstream therapeutic resource some facts need concern; *cannabis* sativa *plant* has been used by human beings as a therapeutically

and recreational drug for millennia without reasonable danger. Of course it is a possible dangerous molecule as presented in the state of the art by creating dependence and withdrawal on the long term use, making it necessary research further before actually inserting it in the market. There have also been reported deaths from synthetic cannabinoid use, which also means that such compounds need to be more regulated. Cannabinoids can be safe to use as a therapeutically resource but, if misused they can be very dangerous since the endocannabinoid system is far more complex than it seems, and interacts with inflammation cascades meaning that the more knowledge we acquire on such effects, more possibilities for therapeutic purposes will arise; as well as the safety profile of the cannabinoids will be further explained.

10. Conclusion

Cannabinoids like all substances can be our friends or our foes and, only the way we use them will determine the effect. Pharmacology studies and molecules have been made and tested through tentative and error method. This has been only option to follow due to technology limits but, since the human genome has been mapped more than 10 years ago the new approach to pharmacology should be based on Pharmacogenomics.

1. Pharmacological safety achieved this way, will direct personalization of the therapy with adverse reactions and side effects made irrelevant.
2. Endocannabinoid systems will most likely emphasize the inter-individual differences which means that it is imperative to adapt the therapy to the individual and not to the population. This means that in order to fully understand the human endocannabinoid system and synthetic cannabinoid models of action, a pharmacogenomics approach supporting cannabinoid based medicines will comprehend more than one compound with possibly different effects on different individuals.
3. Cannabinoids may be a very strong and effective therapeutic resource, but only if we actively try to make them such. The age of "penicillin based discoveries" is over, we can now truly understand the human body chemical and physical reactions, and so what are we waiting for?

11. References

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