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Rassegna

Benefits and risks associated with cannabis and cannabis derivatives use

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Abstract. *Cannabis sativa L.* has been used as a therapeutic for several centuries. Interestingly, medicinal plant extracts or synthetic cannabinoids exert their activities by binding to specific receptors similarly to endocannabinoids, produced naturally in the body by humans and animals. Although cannabinoids present an interesting therapeutic potential to control several diseases (e.g. pain, epilepsy, dementia, neurodegenerative diseases etc.), still there is insufficient evidence on their efficacy and further rigorous clinical trials are essential to clearly define their therapeutic role. Moreover, cannabinoids use is limited to a few specific indications because of their undesirable psychoactive properties and potential recreational use that determined legal restrictions in most countries by a set of undesirable problems.

Key words: cannabis, endocannabinoids, CB receptors, clinical uses of cannabis and derivatives, and efficacy, and safety

Benefici e rischi associati all'uso di cannabis e derivati della cannabis

Riassunto. La *Cannabis sativa L.* è stata impiegata a scopo terapeutico per numerosi secoli. È di notevole interesse evidenziare che gli estratti medicinali della pianta o i cannabinoidi di sintesi esplicano le loro attività legandosi agli stessi recettori a cui si legano gli endocannabinoidi, prodotti naturalmente nel corpo umano ed in quello degli animali. Sebbene i cannabinoidi abbiano evidenziato un interessante potenziale terapeutico in numerose patologie (es. dolore, epilessia, demenza, malattie neurodegenerative, etc.) non ci sono ancora prove sufficienti sulla loro efficacia e ulteriori rigorosi studi clinici sono essenziali per definire chiaramente il loro ruolo terapeutico. Inoltre, l'uso dei cannabinoidi è limitato ad alcune indicazioni specifiche a causa delle loro indesiderabili proprietà psicoattive e del loro potenziale uso ricreativo che ne ha determinato restrizioni legali nella maggior parte dei paesi.

Parole chiave: cannabis, endocannabinoidi, recettori CB, usi clinici, efficacia e sicurezza della cannabis e dei suoi derivati

Beneficios y riesgos asociados con el uso de cannabis y derivados de cannabis

Resumen. *Cannabis sativa L.* ha sido usado como un terapéutico por varias siglos. Curiosamente, concentrados de plantas medicinales o cannabinoides sintético ejerceres suyo actividades por medio de enlace a especifico receptores similarmente como endocannabinoides, producido naturalmente en el cuerpo de humanos y animales. A pesar de que cannabinoides han un interesante potencial terapéutico para controlar varias

enfermedades (p.ej. pain, epilepsia, demencia, enfermedades neurodegenerativa etc.), aún ahi esta evidencia insuficiente en su eficacia y mas riguroso estudios clínico son esencial para definir claramente su rol terapéutico Además, el uso de cannabinoides es limitado a unos pocos indicaciones especifico a causa de suyo propiedades psicoactiva indeseable y potencial uso recreacional que determinó restricciones legali en la mayoría de los países.

Palabras clave: canabis, endocannabinoides, receptores CB, usos clínico, efectividad y seguridad de canabis y de su derivados

Introduction

Cannabis sativa L. has been used as a therapeutic for many millennia. The beginnings of its use by humans are difficult to trace, because it was cultivated and consumed long before the appearance of writing (1). However, it was not until the 1960s that its main component, (-) -Δ9-tetrahydrocannabinol (Δ9-THC), was isolated and synthesized. Consequently, research in the pharmaceutical industry and academic laboratories produced a large number of new and structurally related compounds, named cannabinoids, with very potent biological properties. Later it was revealed that these compounds exerted their activities by binding to specific receptors similarly to some compounds, named endocannabinoids, produced naturally in the body by humans and animals (2).

Cannabinoids present an interesting therapeutic potential as antiemetics, analgesics, appetite stimulants, and in the treatment of multiple sclerosis, spinal cord injuries, Tourette's syndrome, epilepsy, Parkinson's disease, dystonia, dementia, glaucoma (1, 3, 4). However, still there is insufficient evidence on the efficacy of cannabis and its derivatives to control some of these diseases (5-6) and further rigorous clinical trials are essential to clearly define the therapeutic role of cannabinoids (7).

For each pathology, it remains to be determined what type of cannabinoid and what route of administration are the most suitable to maximize the beneficial effects of each preparation and minimize the incidence of undesirable reactions. The use of cannabinoids is indeed limited by a set of undesirable problems. These include addiction, diversion, cognitive impairment and increased risk for development of psychosis and psychotic symptoms (7-8).

Pharmacognosy

Cannabis sativa L. (Cannabaceae) is an annual herbaceous species originating from Central Asia, which has been used as a source of textile fiber and as a therapeutic for centuries (9). There are several species of cannabis. The most relevant are *Cannabis sativa, Cannabis indica and Cannabis ruderalis. Cannabis sativa*, the largest variety, grows in both tropical and temperate climates. The two main preparations derived from cannabis are marijuana and hashish.

The Cannabis plant and its products consist of an enormous variety of chemicals: terpenes, phenolic compounds and a group of C21 or C22 (for the carboxylated forms) terpenophenolic compounds grouped under the name of cannabinoids or more recently phytocannabinoids (9). More than 100 different cannabinoids have been reported in the literature (4), although some of these are breakdown products. Phytocannabinoids are synthesized in glandular trichomes present mainly on female flowers and accumulated in the secretory cavity of these glands, which largely occur both in female flowers and in most aerial parts of the plants (9).

Cannabis is one of the oldest psychotropic drugs known to humanity (1) and the major psychoactive ingredient is delta-9-tetrahydrocannabinol, commonly known as THC (Δ9-THC). Other cannabinoids include delta-8-tetrahydrocannabinol (Δ8-THC), cannabinol (CBN), cannabidiol (CBD), cannabicyclol (CBL), cannabichromene (CBC) and cannabigerol (CBG), present in small quantities and without significant psychotropic effects compared to Δ9-THC (10). However, they may have an impact on the product overall effect.

Currently the two main cannabinoids of interest are Δ9-THC and CBD. The psychoactive effects of

Δ9-THC are mainly attributable to its action at cannabinoid receptors. On the contrary, cannabidiol is the most abundant nonpsychoactive phytocannabinoid contained in cannabis and it has low affinity for cannabinoid receptors (2).

In cannabis, the content of phytocannabinoids, and especially of Δ9-THC, can vary greatly depending on the strain, cultivating factors, and the method of preparation, rendering it extremely difficult to conduct reproducible and consistent pharmacological studies and to accurately assess dosage and therapeutic efficacy of medical cannabis.

Cannabinoid receptors

Cannabinoids exert their actions by binding to specific receptors: the CB1 cannabinoid receptors, discovered by Devane and colleagues (1988) (11), then cloned by Matsuda and colleagues (1990) (12) and the CB2 cannabinoid receptors, identified by Munro and colleagues (1993) (13). Both cannabinoid receptors are part of the G-protein coupled class and their activation results in inhibition of adenylate cyclase activity (14). CB1 receptors are mainly localized at the synaptic terminals of central and peripheral neurons (14). One consequence of their activation is to decrease calcium entry through voltage-dependent calcium channels decreasing neurotransmitter release (15).

Some of the main roles of CB1 receptors are pain and sensory perception, attention, emotion, cognition, memory, mood, movement control, food intake, and autonomic and endocrine functions (2, 16). The cellular localization of CB1 receptors in the mammalian central nervous system (CNS) was first described by *in vitro* autoradiography of receptor binding and *in situ* hybridization of CB1 mRNA. These studies show enrichment of CB1 receptors in the hippocampus, basal ganglia, cerebellum, pyriform and cerebral cortices (17), consistent with regions associated with the psychomotor effects of cannabis. Lower levels were found in hypothalamus and spinal cord. CB1 receptor binding was almost absent from the respiratory centers of the brainstem, consistent with the clinical observation of the low lethality of cannabis overdose. Interestingly, in humans, CB1 receptors are highly expressed in amygdala and cingulate cortex compared with rat or monkey and may explain interspecies differences in the behavioral effects of cannabinoids. In addition to the CNS, CB1 receptors are widely expressed in the peripheral nervous system, both on sensory nerve fibers and in the autonomic nervous system. CB1 receptors are also found in moderate levels in the testis. CB1 receptors have also been found in peripheral tissues involved in the regulation of energy homeostasis such as intestine, liver, adipose tissues, and skeletal muscle (18).

CB1 receptors are also expressed in some immune cells, but their level of expression is considerably lower than that of CB2 receptors (14).

In comparison, CB2 receptors seem to be located especially in cells and tissues associated with the immune system (14) where they regulate cytokine release and are involved in inflammatory and pain responses (2, 16). Both *in situ* hybridization studies and autoradiographic studies suggest expression of CB2 receptors in multiple lymphoid organs. Cannabinoid CB2 receptor mRNA is found in spleen, thymus, tonsils, bone marrow, pancreas (14). Although early studies suggested that cannabinoid CB2 receptors are absent in the brain, several studies using *in situ* hybridization, radioligand binding assays and RT-PCR detected CB2 mRNA and receptor binding in retina, cortex, striatum, hippocampus, amygdala and brainstem. Interestingly, mRNA for CB2 has been identified also in neonatal rat brain cortical microglia maintained in vitro at levels that exceed those for CB1. In addition, recently, Zhang et al., (2016) (19) reported the expression of CB2 receptors in ventral tegmental area neurons suggesting that they modulate dopaminergic activities and cocaine self-administration behavior in rats. Moreover, a significant increase in CB2 receptor levels has been found in microglia surrounding senile plaques of post-mortem Alzheimer's disease (AD) brains suggesting these receptors as a therapeutic target against AD.

Endocannabinoid system

The existence of the receptors implied that endogenous substances in the brain normally bind to them and studies on several lipid fractions collected from

rat brain led to the isolation of arachidonoyl ethanolamide (anandamide; AEA), 2-arachidonoyl glycerol (2-AG) (20) which bind more or less equally well to CB1 and CB2 receptors, and 2-arachidonyl glyceryl ether (noladin) which is CB1 selective. AEA behaves as a partial cannabinoid receptor agonist with less CB2 than CB1 efficacy. In addition AEA can also activate type 1 vanilloid receptor (called transient receptor potential vanilloid type1, TRPV1), which is the target for capsaicin, the pungent ingredient in hot peppers.

Moreover, since 2002, evidence has accumulated that endocannabinoids/endocannabinoid-like compounds (but also phytocannabinoids and synthetic cannabinoid ligands) bind to and activate the peroxisome proliferator-activated receptors (PPARs).

The endocannabinoids that have been identified to date are all analogues of arachidonic acid (AA).

Endocannabinoids are synthesised and released by neurons on demand, functioning as neurotransmitters or neuromodulators (2). There is also evidence that endocannabinoids serve as retrograde synaptic messengers.

Following their release, the effects of at least some endocannabinoids are thought to be rapidly terminated by cellular uptake and intracellular enzymatic hydrolysis (21). In particular, the effects of AEA depend on its extracellular concentration, which is controlled by cellular uptake via an AEA membrane transporter (AMT) followed by intracellular hydrolysis to AA and ethanolamine by fatty acid amide hydrolase (FAAH). Conversely, a key factor in AEA synthesis is represented by the N-acyl-phosphatidylethanolamine- hydrolyzing phospholipase-D (NAPE-PLD). Moreover, monoacylglycerol lipase (MGL) is the major degradative enzyme for 2-arachidonoylglyerol (2-AG) in the brain.

Endocannabinoids along with the proteins that bind, transport, synthesize, and degrade these lipids constitute what is now generally known as the "endocannabinoid system" (22).

Prominent FAAH expression is reported in the cerebellum, hippocampus, neocortex, olfactory bulb and amygdala in which it is present in processes of major output neurons that are postsynaptic to processes containing CB1 receptors suggesting presynaptic retrograde regulation of transmitter release by endocannabinoids. Interestingly, AEA and 2-AG effects involve presynaptic modulation of ion channels that result in inhibiting glutamate or γ-aminobutiric acid (GABA) release and modulation of protein kinases and gene transcription. Reduction of glutamate and GABA release contributes to short-term synaptic plasticity, while the reduction of glutamate release inhibits excitotoxicity following ischemia. Evidence implicates 2-AG rather than AEA in plasticity while both AEA and 2-AG are involved in neuroprotection (23).

The effects on synaptic plasticity and neuroprotection appear to depend on retrograde transmission in which postsynaptic dendrites release an endocannabinoid that binds to presynaptic CB1 receptors to reduce transmitter release (15).

FAAH expression is also reported in areas concerned with nociceptive transmission (periaqueductal grey, thalamus, and the spinal cord) and dorsal root ganglion. Interestingly, Nucci and colleagues (2007) (24) reported that in rat retina, ischemic insult followed by reperfusion resulted in enhanced FAAH activity and protein expression paralleled by a significant decrease in the endogenous AEA tone, whereas the AEA-membrane transporter or the AEA-synthase NAPE-PLD were not affected. Systemic administration of a specific FAAH inhibitor (e.g., URB597) reduced enzyme activity and minimized the retinal damage observed in ischemic-reperfused samples (24). MGL, though it has a somewhat similar distribution as FAAH, is localized presynaptically rather than postsynaptically, with a near complementary distribution of MGL and FAAH immunoreactivities in rat hippocampus, cerebellum and amygdala; with MGL in presynaptic and FAAH in postsynaptic processes. The presynaptic contacts contained CB1R-immunoreactivity. These data are consistent with the presence of MGL presynaptically as well as the identity of 2-AG as a retrograde transmitter at excitatory synapses. Interestingly, recent data demonstrate that MGL in astrocytes is an important regulator of 2-AG levels, AA availability, and neuroinflammation.

Clinical use of cannabis and cannabis derivatives

From the discovery of the endocannabinoid signaling system several studies started to investigate the endocannabinoid physiopathological roles in central and

peripheral nervous system. It was soon clear that this system has a crucial role in the homeostasis of several physiological processes and then its up or down regulation can underlie several diseases, including emesis, obesity, metabolic disorders, hepatic diseases, pain, inflammation, and neurological and neuropsychiatric disorders (2, 4). Thus endocannabinoids, synthetic agonists, antagonists, FAAH and MGL inhibitors are in the market but much of them are currently under investigation in different experimental models or in clinical trials for the treatment of all these disorders.

The clinical use of cannabis and cannabis derivatives is mainly indicated for the treatment of severe or chronic pain associated with various disorders, such as multiple sclerosis, cancer, and rheumatoid arthritis (2, 4). Other indications include the increase in appetite and reduced nausea in patients affected by AIDS/HIV and cancer, treatment of muscle spasms, and sleep disorders (1, 2, 4).

The main limitation of medical cannabis preparations resides in the psychotropic side effects associated with Δ9-THC content. Δ9-THC acts a partial agonist at both CB1 and CB2 receptors. For this reason, several research strategies started to limit the action of cannabinoid ligands at a central level, including use of synthetic ligands, peripherally restricted cannabinoid receptor ligands, CB2 receptor selective ligands, and allosteric modulators.

In particular, nabilone, a synthetic analog of Δ9-THC and dronabinol, the synthetic levorotatory enantiomer of Δ9-THC, nonselective CB1/CB2 agonists, are licensed in some countries for the suppression of nausea and vomiting produced by chemotherapy (25). Dronabinol is also licensed for the treatment of anorexia and weight loss in patients affected by AIDS/HIV (25). Rimonabant was the first CB1 receptor antagonist/inverse agonist to be licensed for the treatment of obesity but, psychiatric side effects, potentially due to its inverse agonist effect at central CB1 receptors, resulted in its removal from the market (26). Sativex, a cannabis-derived formulation containing Δ9-THC together with the non-psychoactive cannabinoid, cannabidiol, is licensed for the relief of spasm in multiple sclerosis and as adjunctive treatment for symptomatic relief of pain (e.g. neuropathic pain in adult patients with multiple sclerosis) (25).

Recently, the research is moving towards accessing the therapeutic potential of CBD, in particular on its antiepileptic (25) and antinausea properties. Interestingly, data from a trial of cannabidiol found benefit in treatment-resistant pediatric epilepsy as a Dravet syndrome. The antioxidant and anti-inflammatory properties of CBD have led to investigation of cannabinoids in neurodegenerative disorders including Huntington's disease, Parkinson's disease and neonatal hypoxia-ischaemia.

The activation of CB2 receptors by selective ligands is devoid of psychotropic effects and it seems to have a potential therapeutic application in pain, cancer and peripheral or neurodegenerative disorders that involve inflammation (16). Conversely to nabilone and dronabinol, ajulemic acid (AJA), a synthetic analog of Δ 9-THC-11-oic acid, the major metabolite of Δ9-THC, shows selective activity at CB2 receptors and displays promising antiinflammatory properties. AJA is currently in clinical trials for the treatment of four chronic inflammatory diseases, such as cystic fibrosis, systemic sclerosis, dermatomyosites and systemic lupus erythematosus (24).

However, early clinical trials with CB2 agonists, especially in models of pain have been discouraging (16). This is probably due to a non-high selectivity for CB2 receptors by available agonists and also to the discrepancy between experimental models of pain and patients enrolled in clinical trials (16). A potential therapeutic strategy is also represented by the allosteric modulation of cannabinoid receptors to limit the possible adverse effect of drug overdosing. However, current available CB1 allosteric ligands such as pregnenolone have demonstrated poor target selectivity and psychochemical properties *in vivo*.

It is necessary to highlight that only a limited number of clinical trials have been conducted so far in order to accurately assess the efficacy and safety of medical cannabis (7, 25). Moreover, a recent meta-analysis found that cannabinoids were associated with only modest benefits for chemotherapy-related nausea and vomiting, small and inconsistent benefits for pain and spasticity, and inconclusive benefits for other indications such as improvement of appetite and weight, reduction in tic severity, and improvement of mood or sleep (27). In addition, cannabinoids were associated

with an increased risk of short-term adverse effects included dizziness, drowsiness, confusion, hallucination, dry mouth, nausea, vomiting, fatigue, somnolence, etc (27). More recently, other studies reported a limited evidence to support the use of cannabinoids in patients with rheumatoid arthritis (5) and fibromyalgia (6).

Risks associated with cannabis and cannabis derivatives use

Cannabis has a long history of medicinal use, however, unpleasant adverse effects may limit their use. In particular, cannabinoids and cannabis have acute and long-term adverse effects (7). Short-term cannabis use impairs cognition (28). In particular are affected verbal learning, memory, and attention, however, psychomotor impairment also occurs (28). These deficits arise from impairments identified in brain aereas involved in cognition such as hyppocampal, prefrontal and subcortical networks. Driving after cannabis use is associated with an increased risk of accidents and marijuana is the illicit drug most frequently reported in connection with impaired driving and accidents, including fatal accidents. Conversely, long-term cannabis use may lead to dependence. Approximately 180 million people currently use cannabis worldwide. According to the Eureopean Monitoring Centre for Drugs and Drug Addiction cannabis is the most commonly used illicit drug in Europe. An estimated 83.9 million Europeans used cannabis in the last year (51.5 million males and 32.4 million females) (29).

Cannabis dependence is more common with earlier age of initiation and higher levels of use. Sudden cessation of regular heavy cannabis use is associated with a distinctive withdrawal syndrome characterized by irritability, depression, anxiety, sleep problems, restlessness, decreased appetite/weight loss, cravings, and a range of physical symptoms (e.g., stomach pain, shakiness/tremors, sweating, fever, chills, or headache) (30). Persistent cannabis use results also in long-lasting cognitive impairments and cessation of use did not fully restore neuropsychological decline. Early marijuana use is associated with impaired school performance and an increased risk of dropping out of school (31). Heavy marijuana use has been linked to lower income, greater need for socioeconomic assistance, unemployment, criminal behavior, and lower satisfaction with life. Long-term cannabis use is also associated with an increased risk of mental illness (31). In particular, psychosis is one of the most serious among the adverse effects associated with cannabis use and higher levels of cannabis are associated with greater risk of psychosis (8).

Epidemiologic data indicate a causal effect of cannabis in anticipating, triggering, or exacerbating psychosis in vulnerable individuals and in worsening the course and outcome of the illness in those who continue to use the substance (8). Regular cannabis use is also associated with an increased risk of anxiety and depression but causality has not been established. Furthermore, recently, a temporal relationship between the use of cannabis derivatives and stroke in young people has been described. Long-term cannabis may lead to respiratory risk and possibly cancer although the evidence is weak and inconsistent. Several studies indicated cannabis use during pregnancy may compromise certain pregnancy outcomes such as fetal growth; however, Chabarria and colleagues (2016) (32) reported that marijuana exposure alone is not associated with significant perinatal adverse outcomes and co-use with cigarette smoking rendered increased risk over either alone.

Conclusion

Although *Cannabis sativa* has been used as a therapeutic for several centuries, the clinical use of medicinal plant extracts or synthetic cannabinoids have been largely empirical and limited to a few specific indications because of their undesirable psychoactive properties and potential recreational use that determined legal restrictions in most countries. The limitations of use of medical cannabis include also non-psychological long-term side effects. Therefore, it is necessary to educate patients and the public about the serious mental and physical health risks associated with cannabis use and abuse.

Clinical evidence supports cannabis and cannabinoids use only in a limitated number of diseases, but there is significant community pressure for use be-

yond these conditions and legalization and liberalization initiatives continue to spread. However, since the withdrawal from the market of the first cannabinoid targeting synthetic compound, rimonabant, there has been a paucity of translational pharmacology concerning the cannabinoid system. In fact, although cannabinoid receptors control a variety of physiological functions, the complexity of endocannabinoid signalling makes it difficult to develop compounds with defined pharmacology, accurate dosing, minimal adverse effects and optimal efficacy. In addition, in cannabis preparations it may be that therapeutic benefits are effected by the mixture of compounds rather than by the isolated cannabinoid. Moreover, the composition and bioavailability of cannabis vary across preparations of the substance and routes of administration. Therefore, further clinical trials, well-designed, carefully executed and powered for efficacy, are essential to clearly define the therapeutic role of cannabinoids.

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