CANNABIDIOL AND THE CENTRAL NERVOUS SYSTEM: TRANSLATING INTO CLINICS

A. Saviano[1#], F. Raucci[1#], M. Tallarico[2], C. De Caro[2], S. Di Martino[3], V. Nesci[2], R. Roberti[2], L. F. Iannone[2], A. L. Colia[4], S. Dimonte[4], A. Furgiuele[5], F. Marino[5], M. Ferrari[5], M. Cosentino[5], M. Bove[4], P. Tucci[4], V. Micale[3], A. Leo[2], V. Franco[6], F. Maione[1], E. Russo[2]*
on behalf of SIF Working Group Pharmacognosy, Phytotherapy and nutraceuticals

[#]Equally contributed
[1]ImmunoPharmaLab, Department of Pharmacy, School of Medicine and Surgery, University of Naples Federico II, Naples, Italy
[2]Science of Health Department, School of Medicine, University Magna Grecia of Catanzaro, Catanzaro, Italy
[3]Department of Biomedical and Biotechnological Sciences, Section of Pharmacology, University of Catania, Catania, Italy
[4]Department of Clinical and Experimental Medicine, University of Foggia, Foggia, Italy
[5]Center for Research in Medical Pharmacology and Center for Research in Neuroscience, University of Insubria, Varese, Italy
[6]Division of Clinical and Experimental Pharmacology, Department of Internal Medicine and Therapeutics, University of Pavia, IRCCS Mondino Foundation (member of the ERN EpiCARE), Pavia, Italy

E-mail: erusso@unicz.it
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SUMMARY

Cannabis plants contain more than a hundred cannabinoids of which we have only limited knowledge about their effects and exact mechanisms of action. Cannabidiol (CBD) represents one of the most studied; it was discovered at the beginning of the last century while only recently has attracted greater attention. CBD mechanism of action is still unclear and debated; in the plant only the (-) enantiomer can be found and it seems that only the (+) enantiomer is able to bind to the cannabinoid receptors although evidence of a modulation of the endocannabinoid system exists. Furthermore, several other mechanisms have been proposed which may be differently relevant for different disease spanning from ion channels to immune responses and modulation of inflammation. It is a highly lipophilic drug with a peculiar pharmacokinetic which has led to the use and proposal of different formulations and routes of administration; of note, CBD has a complex interaction with cytochromes and is at high risk of drug-drug interaction with many drugs. Nowadays, CBD has been studied for potential efficacy in several pathologies and central nervous system (CNS) diseases are the most promising with clinical studies already in the started and some formulations with CBD already authorized for the treatment of spasticity in multiple sclerosis and seizures in some specific epileptic syndromes such as Lennox-Gastaut or Dravet. This review article is summarizing the relevant studies and perspectives of CBD use in the most relevant areas of CNS disorders also including a detailed description of its proposed mechanism(s) of action, pharmacokinetic characteristics and safety profile.

Key words
Mechanism of action; pharmacokinetic; safety; depression; psychosis; epilepsy.

Impact statement
Cannabidiol represents pharmacologically a great opportunity for the study of mechanisms involved in central nervous system disorders further than being itself a therapeutic candidate against a variety of brain diseases.

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INTRODUCTION

Cannabis plant varieties contain a considerable number of active molecules of which, the most famous are mainly located in the female plant flowers within resin glands. Δ^9- tetrahydrocannabinol (Δ^9-THC) is the most characterized and studied of the family of cannabinoids, of which about 100 have been identified so far. Cannabis plants have been historically used for thousands of years to treat many different disorders including these of the central nervous system (CNS) while our knowledge has drastically increased after the second half of the last century. In the 1990's, the discovery of the endocannabinoid system represents a landmark in cannabinoid science and since then the number of studies on cannabinoids and the CNS has been growing constantly although much more is likely to be discovered. Pharmacologically, we need to distinguish three different classes of cannabinoids (1, 2): 1) endocannabinoids (*e.g.* anandamide) and the related unconventional neurotransmettitorial system including cannabinoid (CB) receptors and enzymes; 2) synthetic cannabinoids, which include CB receptors ligands (*e.g.* agonist, antagonists, partial agonists and inverse agonists) further than indirect modulators of the system acting on enzymes involved in the regulation of endocannabinoid neurotransmission; 3) phytocannabinoids, which are a wide variety of terpenophenolic derivatives which are mainly but not exclusively found in the Cannabis species of which only some are able to bind to CB receptors while some others can have pharmacological effects through other mechanisms.

This review is focused on the cannabidiol (CBD) which belongs to the class of phytocannabinoids, while it was isolated as early as 1930-40's further studies only started after 1960 (3). CBD (2-[(1R,6R)-3-methyl-6-(prop-1-en-2-yl)cyclohex-2-en-1-yl]-5-pentylbenzene-1,3-diol) is naturally occurring as the (-) enantiomer while it can also be synthesized as a racemic mixture. The (+) enantiomer and its metabolites differ from the (-) enantiomer for their ability to bind to CB receptors at the nanomolar range (see below) which should always be considered in the studies performed since different effects are obviously elicited (4, 5).

In here, we will briefly review the pharmacological properties of CBD (*i.e.* pharmacodynamic and pharmacokinetic) and then summarize the current evidence on the effects of this cannabinoid in several disorders of the CNS including preclinical and clinical available data.

PHARMACODYNAMIC

The chemistry and pharmacology of CBD, as well as its molecular targets, have been extensively studied although not completely understood (**table I**). Together with other phytocannabinoids, CBD has a chemical structure theoretically capable of binding to cannabinoid receptors and other components of the endocannabinoid system (6). CBD presents very low affinity and displays slight agonist activity on the CB_{1R} and CB_{2R}, the G protein-coupled endocannabinoid system (ECS) receptors (7). Specifically, CBD is a weak agonist of CB_{1R} (8) and it demonstrates an inverse agonism activity of the CB_{2R} (7). Nevertheless, some studies suggest the antagonist activity against CB_1 and CB_2 receptors agonists (9, 10). Notably, some studies differentiated CBD enantiomers binding to CB receptors indicating that the naturally occurring (–) enantiomer does not bind ($K_i > 10000$ nM) to CB receptors while the (+) enantiomer has a K_i of 842 nM for CB_{1R} and 203 nM for CB_{2R} (5).

Further than the debated action on CB receptors, CBD inhibits adenylyl cyclase and voltage gated calcium channels activity, while activates potassium channels and mitogen activated protein kinase (MAPK) and it has been suggested to increase mTOR pathway activity (11). This effect is highly debatable considering the more recent observation that CBD is effective in Tuberous sclerosis (characterized by hyperactivation of mTOR pathway) patients and animal models (12, 13). In fact, CBD has also been shown to inhibit apoptosis in hu-

Table I. Overview of cannabidiol molecular targets.

Target	CBD Effect	Bioactivity	References
CB1	antagonist	CBD decreases ROS production and inflammatory response.	(233)
	negative allosteric modulator	CBD reduces CB1 receptor signaling in HEK 293A cells	(234)
CB2	antagonist	CBD decreases ROS and TNF-α levels, reducing oxidative stress and inflammation	(233)
A1	agonist	CBD displaces antiarrhythmic effects	(21)
A2A	agonist	CBD promotes anti-inflammatory activity by reducing TNFα in mice challenged with LPS	(235)
ORs	allosteric modulator	CBD accelerates D-Ala2, N-MePhe4,Gly-ol]-encephalin dissociation from μ ORs in rat cortical membranes. A similar effect was found for δ ORs	(24)
5-HT1A	agonist	In CHO cells CBD increases G protein activity by displacing [3H]8-OH-DPAT binding	(25)
	analgesia	In a Von Frey test, WAY 100135, a selective 5-HT$_{1A}$ receptor antagonist provokes withdrawal of the CBD-mediated analgesia	(236)
	anxiolytic-like effects	CBD increases the total distance moved in a open field test in the OBX mice; it increases sucrose consumption in the sucrose preference test and glutamate release	(237)
GPR55	antagonist	In cells overexpressing GPR55, CBD is able to decreases agonist CP55940 potency during the GTPγS assay	(238)
PPARγ	agonist	CBD can ameliorate lipid and glycemic parameters in Type 2 Diabetes	(239)
	anti-inflammatory	CBD inhibits the expression of pro-inflammatory genes and inflammatory signaling and tumor cell viability	(15)
TRPV1	agonist	CBD reducing the levels of oxidative stress and the biosynthesis of an endocannabinoid lipid mediator (2-AG)	(35)
VGSCs	inhibition	CBD inhibits hNav1.1-1.7 currents (IC50 of 1.9-3.8 μmol/L)	(42)
VGCCs	inhibition	CBD inhibits human T-Type and L-type Ca^{2+} channels in rat myocytes	(40, 41)

man breast cancer cells by inhibiting the expression of cyclin D1 and mTOR pathway, and by increasing the peroxisome proliferator-activated (PPARγ) receptor expression (14). CBD is a PPARγ receptor agonist which inhibits the expression of pro-inflammatory genes and inflammatory signaling (15). CBD has also been shown to inhibit tumor cell viability and stimulates the activity of the luciferase reporter gene in HEK293 cells transiently overexpressing retinoid X receptor and PPARγ (16).

More recently, attention has turned to interactions between CBD and non-endocannabinoid G protein-coupled receptors (GPCRs) (17, 18). Specifically, the orphan GPCR 55 (GPR55) shares structural similarities in transmembrane domains 1, 2, and 3 when compared with the cannabinoid receptors, which may indicate a binding site for cannabinoids (17, 19). CBD

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is a GPR55 receptor antagonist, strongly expressed in the nervous and immune systems, as well as in other tissues (20). CBD also acts as an inverse agonist of GPR3, GPR6 and GPR12 receptors and it is associated with the reduction of the levels of molecules involved in the development of amyloid plaques in Alzheimer's disease (20).

Moreover, CBD not only elicits effects in the central nervous system (CNS), but also within the cardiovascular system. Indeed, it can exert antiarrhythmic effects, possibly interacting with adenosine A1 receptor (21). CBD is also an agonist of adenosine A_{2A} receptors (8) which could play an anti-inflammatory activity in vivo (22) by reducing TNF-α levels and preventing oxidative stress (23).

Furthermore, CBD may operate as an allosteric modulator at μ and δ opioid receptors (ORs), $G_{i/o}$ protein-coupled receptors. Kathmann and colleagues observed that CBD accelerates D-Ala2, N-MePhe4,Gly-ol]-encephalin dissociation from μ ORs in rat cortical membranes. A similar effect was found for δ ORs (24).

CBD showed a direct (25) and indirect (26) affinity for the human 5-HT_{1A} receptor: the activation of the latter is related to an antioxidant effect (27), with a reduction of the physiological response to stress in animal models (28).

CBD can also be used in inflammatory and neuropathic pain, although its effect on acute or chronic pain is still poorly understood (29). TRP channels may represent the possible targets for pain efficacy, although to date only TRPA1 and TRPV1 have been involved in CBD management of pain as reported in several preclinical models (30-32). CBD also acts on ion channel as an agonist of the TRPV1 receptor (33), which is then desensitized (34), reducing the levels of oxidative stress and the biosynthesis of an endocannabinoid lipid mediator (2-AG) (35). It also activates other vanilloid receptors (such as TRPV2 and TRPA). The affinity with these channels determines the regulation of the proliferation, secretion (36, 37) and expression (37) of pro-inflammatory cytokines. The modulation of this type of re-

ceptor-channel determines transient changes in potential and affects the redox balance and inflammation (38, 39). Finally, CBD inhibits human T-type voltage-gated calcium channels (VGCCs) (40) and it has also been reported to inhibit L-type Ca^{2+} VGCCs with an IC_{50} of 0.1 µmol/L in rat myocytes (41). Furthermore, CBD can block voltage-gated sodium channels (VGSCs) inhibiting hNav1.1-1.7 currents (IC50 of 1.9–3.8 µmol/L) (42).

PHARMACOKINETICS

CBD is characterized by high lipophilic properties that allow the compound to easily cross the blood-brain barrier. CBD pharmacokinetics in mice and rats have been established by comparing intraperitoneal (i.p.) vs oral administration. Exposure in mice after i.p. administration of 120 mg/kg of CBD was higher in plasma and brain compared with oral administration. Plasma and brain CBD maximum concentration (T_{max}) after i.p. administration was detected within 1-2 hours. After oral intake AUC_{0-6h} in the brain was 319 µg/g min compared with 1229 µg/g min after i.p. administration. Brain to plasma ratio determined using AUC_{0-6h} obtained after oral and i.p. administration was 0.84 and 0.51 respectively. Pharmacokinetics data in rats appeared to be similar for both oral and i.p. administration (43).

CBD pharmacokinetics in humans is characterized by relevant variability (44). Gastrointestinal absorption after oral administration of CBD is relatively rapid with peak plasma concentrations usually achieved between 0.5 and 6 hours (45). After single ascending doses of CBD within the 1500 to 6000 mg dose range to healthy volunteers AUC and C_{max} achieved at 3-5 h increase less than proportionally (46). CBD oral bioavailability in the fasting state has been estimated to be about 6% due to a very low solubility in the gastric lumen and a prominent presystemic elimination. In healthy subjects CBD co-administration with a high-fat/high-calorie meal resulted in an approximately 4-fold increase in AUC and 5-fold increase in

C_{max} with a lower total variability compared with the fasted state. The increase in bioavailability of CBD associated to its improved dissolution in the gastrointestinal tract with a high-fat meal is likely to be potentiated by diversion of the absorbed drug from the portal to the lymphatic system (47). Apparent volume of distribution in healthy volunteers for doses of 1500 up to 6000 mg given in the fasting state ranged from 21,000 to 43,000 L (46). These results represent an overestimation due to the assumption of a complete oral bioavailability. There is evidence from *in vitro* studies that CBD and its metabolites are > 94% bound to plasma proteins. CBD elimination is characterized by a multiphasic process with half-life values ranging from 14 to 60 h after single and multiple dosing respectively (46). CBD is predominantly eliminated by metabolism in the liver and the gut and excreted in feces as unchanged drug. CYP2C19 is the major cytochrome P450 enzyme involved in the conversion of CBD to the active metabolite 7-hydroxycannabidiol, which is further metabolized to 7-carboxy-cannabidiol by CYP3A4 (48). After multiple dosing with CBD in healthy subjects, the 7-hydroxy-cannabidiol metabolite occurs in human plasma 62% lower compared to parent drug based on AUC values (46). The uridine 5'-diphospho-glucuronosyltransferase (UGT) enzymes involved in the CBD Phase II conjugation are UGT1A7, UGT1A9, and UGT2B7 (49).

A pharmacokinetic study in healthy volunteers detected a bidirectional interaction between CBD and clobazam associated with elevation of the active metabolites of both drugs. In this study clobazam (5 mg b.i.d.) has been reported to increase the AUC of CBD and of the active metabolite 7-hydroxycannabidiol by about 30% and 50% respectively and a clinical relevant increase of N-desmethylclobazam (the active metabolite of clobazam) of 3.4-fold for both C_{max} and AUC, possibly mediated by CYP2C19 inhibition (48). This interaction can be associated not only to improved seizure control but also to a greater burden of clobazam-related adverse effects (50). Adverse effects may be reduced by clobazam dosage reduction after initiation of CBD treatment (51, 52). No increase in N-desmethylclobazam exposure has been detected when CBD is added in patients treated with the combination of clobazam and stiripentol (53). Furthermore, CBD has a complex interaction with CYP enzymes mostly inhibiting and sometime inducing their activity, several interactions with other drugs can be predicted (54).

PHARMACOGENETICS

Pharmacogenetics (PGx) is a branch of pharmacology that studies the relationship between inter-individual variations in DNA sequence and the response to drugs (EMEA/CPMP/3070/01). The goal of PGx is to identify genetic predictors of treatment response, thus enabling safer and more effective pharmacotherapies (55). CBD shows a high inter-individual pharmacological variability, which is expected to impact on clinical response (46, 49). Thus, the identification of genetic variants in genes that encode for protein involved in CBD pharmacodynamics and pharmacokinetics should be a priority to explain, at least in part, the great variability observed in the response to this drug.

Several genetic variants, in particular Single Nucleotide Polymorphisms (SNPs, *i.e.* DNA sequence variations occurring when individual nucleotides differs between paired chromosomes), have been described in genes coding for CBD targets. For instance, SNPs with functional consequences in both *CNR1* and *CNR2* genes (coding for CB1 and CB2 respectively) have been correlated with cannabis dependence (56), eating behaviour (57), panic disorder (58), and metabolic syndrome (59). Also in the Transient Receptor Potential (TRP) V family genes, several functional allelic variants occur. For example, SNPs in TRPV1 and TRPV3 have been associated with acute pain (60) and with the effects of some experimental analgesics (61). Finally, genetic variants in peroxisome proliferator-activated receptor (PPAR) γ gene

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have been suggested as promising target for precision medicine in Type 2 diabetes mellitus (62, 63). So far, however, no studies exist investigating the role of such genetic variants in the effects of CBD. Nevertheless, clinical trials of cannabinoids are currently ongoing, such as those examining the effects of genetic variant in catechol-O-methyl-transferase (COMT) gene on the effects of CBD (64, 65).

Compared to the paucity of PGx studies about CBD targets, more evidence exists concerning the PGx of genes involved in CBD pharmacokinetics. CBD absorption and distribution are influenced by P-glycoprotein (P-gp), an efflux protein encoded by ABCB 1 gene (66) and several SNPs are found in both the coding and the regulatory regions of the gene. Among these, in particular three SNPs (rs2032582, rs1045642, and rs1128503) are known to modify P-gp expression and activity, and in turn the pharmacokinetics of many drugs. Considering the role of P-gp in CBD disposition, it is likely that P-gp genetic variants affect CBD kinetics, however no studies have been so far performed (67). CBD biotransformation occurs as a result of both phase I and phase II drug-metabolism reactions. The enzymes involved in CBD phase I reactions include cytochrome P450 (CYP450) superfamily enzymes, and in particular CYP3A4 and CYP2C9 (68). To date, 60 polymorphic alleles have been described in the CYP2C9 gene, in Caucasian populations the most frequent being CYP2C9*2, and CYP2C9*3, which lead to decreased enzyme activity and poor metabolizer phenotype (69). As for the CYP3A4, 26 polymorphic alleles have been characterized, CYP3A4*2, CYP3A4*11, CYP3A4*12, CYP3A4*17 being the most common in Caucasian population and resulting in reduced enzyme activity (70). Although *in vitro* studies on CYP450 enzymes activity underline the potential contribution of these enzyme in the metabolism of CBD (68), no information is available on the effect of SNPs in genes coding for CYP450 family on CBD pharmacokinetics in humans. The UDP-glucuronosyltransferase (UGT) enzyme family is involved in phase II metabolism of CBD (68), and in particular UGT1A9, UGT2B7, and UGT2 B17. For the UGT1A9 gene, three polymorphic alleles: UGT1A9 *3, *4, and UGT1A9 *5 show high frequency in the Caucasian population and lead to the reduction or suppression of the enzymatic activity (71). However, CBD glucuronidation has a minor role in overall elimination of the drug (72), therefore genetic variants in UGT enzymes are unlikely to affect CBD PK to a major extent.

In summary, genetic variants with functional relevance are well known in several genes involved in CBD pharmacodynamics as well as pharmacokinetics, supporting CBD as a major candidate for clinical PGx studies. Clarifying CBD PGx will be a significant step towards reduction of CBD PD/PK variability and improvement of its clinical exploitation.

PAIN - CENTRAL MECHANISMS OF ANALGESIA

Three main classes of drugs nowadays are used for pain management: opioids, gabapentinoids and non-steroidal anti-inflammatory agents. These drugs are widely used in clinical practice but their use is associated with dose-limiting side effects, such as tolerance, abuse liability and gastrointestinal toxicity. Thereby, although important progresses about the mechanisms underlying pain, there is still the need for safer and more effective analgesic therapies (73). During the last decades, great interest for pain therapy has been received by cannabis plant (*e.g.*, *Cannabis sativa*) derived molecules. Δ⁹-THC is the primary psychoactive compound of cannabis that has a broad spectrum antinociceptive effect in animal models through its action on CB receptors (74). CBD also possesses analgesic efficacy by a not yet completely clarified mechanism of action (75). As above mentioned, recent developments reported that CBD poorly competes with cannabinoids ligands at the orthosteric site of CB receptors (76). Pharmacological studies explained this phenomenon through nega-

tive allosteric modulation of the cannabinoid receptors (77). CBD interacts with various orphan GPCRs (GPR), it is antagonist for GPR55 (78) and CBD is also an inverse agonist for GPR3, GPR6 and GPR12 that are implicated in neuropathic pain development (79). Different studies also suggested that CBD binds to other Gi-coupled receptors, such as opioid receptors, μ-opioid receptor (MOR) and δ-opioid receptor (DOR) with functional high-affinity for dopamine. A recent computational study revealed that the dopamine receptor D3 is a novel predicted target for CBD action (80). Moreover, the physiological effects of CBD are due to high activity for ionotropic receptors such as transient receptor potential (TRP) channels, a group of cationic ion channels, localized on the plasma membrane of numerous animal cell types. CBD can activate TRPA1, TRPV1, TRPV2 and TRPV4 (38, 81); these channels have been implicated in inflammation and chronic pain. It was also shown that CBD is also able to bind intracellular transporters of endocannabinoids enhancing endocannabinoids action through inhibition of anandamide uptake (82). It was also demonstrated that CBD modulates serotoninergic transmission, in neuropathic pain rat model, repeated CBD injections reduced mechanical allodynia, anxiety-like behavior and normalized 5-HT activity (83). In another experiment where neuropathic pain was induced by paclitaxel administration, CBD exerted a positive effect against mechanical and thermal allodynia in mice and prevented mechanical sensitivity, this effect was reversed by the 5HT1a receptor antagonist (WAY100635) but not by CB1 or CB2 receptor antagonists (84). Several studies reported the analgesic and anti-inflammatory effects of CBD in pain models. In preclinical studies, CBD has been demonstrated to exert analgesic effects, reducing hyperalgesia and mechanical/thermal allodynia. It was shown that in a model of L5 spinal nerve ligation, CBD treatment suppressed chronic neuropathic pain, this effect was correlated with cannabinoid potentiation of the α3 GlyRs but not with their binding affinity for CB receptors (85). Li *et al*. also demonstrated that CBD may be an anti-inflammatory agent attenuating the production of pro-inflammatory cytokine and chemokine, in a model of spinal cord injury (86). In a model of induced inflammation, CBD reduced serum levels of pro-inflammatory factors interleukin 6 (IL-6) and tumor necrosis factor α (TNFα) and increased the levels of the anti-inflammatory cytokine interleukin 10 (IL-10) (87). Britch et al. demonstrated that CBD treatment had minimal effects on inflammatory pain but significantly reduced interleukin 1β (IL-1β), IL-10, interferon γ (IFN-γ) levels and increased IL-6 levels (88). These preclinical data are accompanied by preliminary clinical trials, in fact a transdermal CBD-containing gel in patients with peripheral neuropathic pain mitigated pain, as well as cold and itchy sensations (89). Overall, CBD represents a promising drug for the treatment of pain and further research is warranted.

PSYCHOSIS

Literature evidence has shown an increased tone of ECS in patients with psychosis, independently from pharmacological therapy and inversely associated with symptoms' severity, suggesting the modulation of this system as a novel therapeutic target (90). Despite the several studies linking cannabis use and psychosis, reviewed elsewhere (91), potential anti-psychotic properties of CBD have emerged from preclinical and clinical studies (92). To date, numerous molecular mechanisms have been hypothesized to explain how CBD exerts its antipsychotic-like effect, but they are not fully clarified (93).

Besides the ECS potential effects, CBD can modulate the dopaminergic transmission in the mesolimbic system through the regulation of both dopamine release in the nucleus accumbens and ventral tegmental area neuronal substrates (94). CBD seems to have a partial agonist activity on dopamine D2 receptors, similarly to aripiprazole (95) and reverses dopamine D3 receptor mRNA overexpression in

prefrontal cortex, hippocampus and nucleus accumbens of rodent models, although contrasting results on D3 receptors' role have been observed in human studies (96). Dopamine release in mesolimbic system and associated behaviors can be modulated through a 5-HT$_{1A}$-dependent mechanism, which is also responsible for the CBD counteracting effects on haloperidol-induced catalepsy (97).

Differently from traditional antipsychotics, molecular pathways of glycogen synthase kinase-3 (GSK-3), protein kinase B (Akt) and β-catenin do not seem to be involved in CBD effects on mesolimbic system. Indeed, via 5-HT$_{1A}$ receptors, CBD down-regulates the phosphorylation of GSK-3 and Akt, bypassing β-catenin substrates, whereas phosphorylation of both mTOR and its downstream effector p70S6K is up-regulated, without increased signaling in the Wingless/Integrated (Wnt) pathway (94). Moreover, CBD activates TRPV1 receptor pathways, facilitating glutamate pre-synaptic release and enhancing neurogenesis through the ECS (92). Finally, CBD exerts itself anti-inflammatory properties and could interfere with glial cell function (98).

Preclinical data have been mainly provided by rodent models in which the administration of dopamine agonists or NMDA-receptor antagonists simulated specific psychotic symptoms, and to a lesser extent by genetic models of schizophrenia (99). In these studies, CBD has exerted effects on positive, negative and cognitive symptoms.

In the milestone study by Zuardi and colleagues, CBD reduced stereotyped behaviors induced by apomorphine in rats, without motor side effects (100). Furthermore, CBD reduced the hyper-locomotor activity induced by the administration of D-amphetamine or ketamine, and similarly to the atypical antipsychotic clozapine, it was not associated with catalepsy (101). Likewise, chronic but not acute CBD administration decreased dexamphetamine-induced hyperlocomotion in C57BL/6JArc mice (102).

Intra-accumbens administration of CBD attenuated the sensorimotor gating deficits induced by amphetamine in mice and rats submitted to the pre-pulse inhibition (PPI) test (103, 104). Moreover, in a spontaneously hypertensive rat strain (an animal model of schizophrenia), intra-peritoneal CBD prevented both hyperlocomotion and deficits in PPI (105). CBD attenuated also hyper-locomotion, social interaction and cognitive impairments induced in rodents by the NMDA receptor antagonist MK-801 (106, 107).

In schizophrenia genetic models, CBD showed effects only on social interaction deficits in neuregulin 1 mutant mice (108), whereas in pre-natal infection models CBD improved social interaction, recognition, and working memory in rats and reduced hyper-locomotion in mice (109, 110).

Recently, both schizophrenia-like cognitive deficits and transcriptional changes in prefrontal cortex induced by 10 days of ketamine injection in rats have been reverted following a 6-days treatment with CBD at 7.5 mg/kg (111). In well validated neurodevelopmental animal models of schizoaffective disorders (*i.e.* the poly I:C model, the MAM model, the THC model and the Spontaneous Hypertensive Rat "SHR" strain) CBD treatment during earlier periods of development (peripubertal/adolescence) was able to prevent the development of molecular and behavioral abnormalities at adulthood (105, 110-113).

In humans, after the first suggestion of potential benefits of CBD in case reports (114, 115), few clinical trials have been performed to assess CBD antipsychotic effects in patients with a confirmed diagnosis of schizophrenia or schizophreniform psychosis.

Following a single dose of CBD (300 mg or 600 mg), no differences have been observed in cognitive performances in a small group of patients compared with placebo (116).

In a phase II, double-blinded, randomized controlled trial performed by Leweke and colleagues, CBD monotherapy (up to 800 mg/day) for 4-weeks resulted as effective as amisulpride in alleviating both positive and negative schizophrenia symptoms, with a supe-

rior tolerability profile. Moreover, likely due to the CBD-mediated inhibition of the fatty acid amide hydrolase (FAAH), higher serum levels of anandamide were observed in the CBD group, which were significantly associated with clinical improvement (117).

The other two randomized controlled trials evaluated the CBD add-on therapy compared with placebo; no significant difference was observed on cognitive function and psychotic symptoms after 6-weeks of CBD treatment (600 mg/day) (118), whereas an improvement on schizophrenia' positive symptoms was reported after 6-weeks of CBD 1000 mg/day (119). Interestingly, neuroimaging studies suggest that CBD may exert a modulatory effect on neural substrates underlying learning and memory impairment in patients at first episode of psychosis (120), and normalize mediotemporal and prefrontal dysfunction and mediotemporal-striatal functional connectivity in patients with established psychosis (121).

In conclusion, accumulating evidence suggests CBD as a potential well-tolerated antipsychotic, with a unique mechanism of action which involves molecular pathways different from traditional antipsychotics (122). However, evidence on CBD efficacy in clinical trials is mixed, suggesting a relatively good efficacy only on positive symptoms and requiring large, placebo-controlled trials.

ANXIETY AND SLEEP

Anxiety is an adaptive, emotional response that naturally occurs because of a perceived threat. Anxiety becomes maladaptive when it occurs excessively or inappropriately in the absence of relevant threatening stimuli (123). However, recent studies suggest that the variation between adaptive and maladaptive anxiety responses is modulated by regions of the limbic system-primarily the amygdala and key neurotransmitters, such as dopamine (DA), norepinephrine (NE), γ-aminobutyric acid (GABA), and serotonin (5-HT) (124).

The ECS is a promising therapeutic target for anxiolytic drug development owing to its purported role in modulating synaptic plasticity and neuronal activity involved in anxiety response. According to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5), these include, but are not limited to generalized anxiety disorder (GAD), panic disorder (PD), social anxiety disorder (SAD), and specific phobia (SP) (125,126).

Many clinical studies are assessing the impact of CBD on feelings of anxiety. Unfortunately, these studies have very small sample sizes. All the studies use single-dose CBD and the chronic impact of the drug cannot be determined (127). As such, only generalizations about using CBD prophylaxis before or after an anxiety-provoking event can be made (99). From clinical studies of CBD on anxiety symptoms, only two relevant completed RCTs were identified (93). In a double-blind trial was investigated the efficacy of an acute administration of CBD in 24 never-treated individuals with SAD through a simulation public speaking test (SPST). Compared with placebo, pre-treatment with CDB 600 mg significantly reduced anxiety, cognitive impairment, and discomfort in speech performance and significantly decreased alert in their anticipatory speech (128). In a double-blind, placebo-controlled, crossover study, 10 patients with SAD and not additional psychiatric diseases showed a significant decrease in anxiety score levels after an oral dose of CBD 400 mg, compared to placebo (129). Recently retrospective chart review of subjects with different anxiety disorders indicated that concomitant treatment with psychiatric medications and 25-75 mg of CBD attenuated the levels of anxiety (130).

In addition, shreds of evidence suggest that CBD could have a role in the treatment of sleep disorders (SDs). Notably, a study revealed that Sativex extract formulation (containing ∼ 2 mg doses of THC and CBD) improved sleep in patients with pain-related sleep disorders (131), while a recent controlled clinical study indicated that CBD-dominant cannabis (100 mg)

increased subjective sleepiness. Researchers remarked that CBD alone was not able to influence sleepiness, indicating that the effects of the CBD-dominant cannabis were correlated to the small quantities of THC in cannabis preparation (132).

Unfortunately, the results of these trials are inconsistent, and it is unclear whether patients taking CBD before non-public speaking anxiety-provoking events is an effective strategy. The variances in CBD doses, manufacturers, routes of administration, durations between CBD dosing and stressor, total evaluative times, anxiety rating scales, and stressors can all introduce heterogeneity, as can the small sample sizes employed (133, 134). Moreover, based on the data and findings discussed, it is clear that more investigations on CBD and sleep are required (135).

DEPRESSION

Among the over 100 phytocannabinoids isolated from *Cannabis sativa*, CBD is the most promising non-psychotropic component for its potential antidepressant properties. Its efficacy has been assessed in several experimental models (136, 137); CBD treatment elicited rapid and sustained antidepressant-like effects in genetic models of depression characterized by a number of behavioral and physiological endophenotypes similar to those observed in major depressive disorder (MDD), such as the Flinders Resistent/Flinders Sensitive and Wistar Kyoto rats in the forced swim test (FST) (138). The antidepressant-like effect of CBD in the FST has been further confirmed in other studies and it seems to be related to enhanced serotonin brain levels (139), changes in synaptic plasticity mediated by BDNF-TrkB pathway activation in the medial prefrontal cortex (mPFC) (140), the modulation of DNA methylation levels and DNA methyltransferase (DNMT) activity in the PFC and hippocampus (141) or to change in synaptosomal AMPAR expression at level of posterior basal lateral amygdala-ventral CA1 innervation (142). However, the pharmacological mechanism of antidepressant-like

effects of CBD is complex and involves several targets. More specifically, the ECS (specifically the CB1, CB2 and TRPV1 receptors, and the FAAH enzyme), the serotonergic system (specifically the 5-HT1A receptor) seems to play a pivotal role, as well as CBD may modulate the opioid and the adenosine signaling, or it may act as PPARγ agonist, GABAA positive allosteric modulator and iNOS or NF-κB inhibitor (143). Interestingly, CBD was able to reverse the immobility time both in experimental type-1 diabetic rats (144) and in lipopolysaccharide exposed mice, this latter a well-validated neuroinflammatory model of depression, paralleled by a reduction of different inflammatory markers (*i.e.*, IL-6, NF-κB, KYN) in the brain and in the periphery (145), which usually have been found increased in MDD patients (146). Although the potential therapeutic effects of cannabinoids on depressive disorders have been debated for long time, there are still few randomized data to support CBD's antidepressant effect in human and no clinical trial in depression has been published so far. It has been described that oral CBD treatment (200 mg/day for 10 weeks) significantly decreased depressive and psychotic symptoms as well as it improved cognitive performance in Cannabis users, protecting hippocampal subregions by the Cannabis harmful effects (147, 148).

In an online survey, CBD users reported that it was very effective for the treatment of depressive state (149). However, an ongoing double-blind, randomized, placebo-controlled clinical trial is evaluating the effects of CBD as an Adjunctive Treatment for Bipolar Depression (150). Overall, these recent preclinical studies further support the potential antidepressant effects of CBD, which must be confirmed by larger clinical trials.

CBD AND SUBSTANCE ABUSE DISORDER

According to DSM-5, the current edition of the standard classification of mental disorders used worldwide for clinical, research, and health policy purposes, substance use disorder

occurs when at least two of the following criteria are met: hazardous use, social/interpersonal problems related to use, giving up important activities because of substance use, withdrawal, tolerance, used larger amounts/longer, repeated attempts to quit/control use, much time spent using, physical/psychological problems related to use, activities given up to use, craving. Specific disorders are grouped as follows: alcohol, caffeine, cannabis, hallucinogen related disorders, inhalant related disorders, opioid related disorders, sedative, hypnotic, or anxiolytic disorders, stimulant related disorders, tobacco related disorders, other (or unknown) substance related disorders. DSM-5 considers gambling disorder as the sole condition in a new category on behavioral addictions, and suggests criteria for Internet use disorder, calling for further research about the topic (151).

In the neurobiological mechanisms involved in substance use disorder, the endocannabinoid system is increasingly regarded as a key player, profoundly affecting rewarding and motivational activities (152, 153), and several studies support this system as a promising target for treatment of various disorders related to substance abuse (153, 154). Due to the lack of psychotropic effects and to the excellent safety profile even at high doses, CBD has been increasingly considered as a suitable candidate therapeutic for substance use disorder. Several well written reviews have recently summarized preclinical and clinical evidence supporting CBD use in the treatments of disorders related to opioids, cannabis, alcohol, nicotine, and stimulants (152, 155-160).

CBD has a complex pharmacology (**table I**), and it is presently yet to be established which of the many molecular targets are relevant for the effects of CBD on the various types of substance use disorder. Nevertheless, CBD modulates brain activity level across limbic regions during emotional processing tasks (161), and it has been shown to affect mesolimbic dopamine activity (162), and to attenuate substance-induced dysregulation of the mesolim-

bic circuitry (104). Available literature about the effects of CBD on various fear and drug memory processes shows that CBD affects the acquisition and expression of drug memories, may have anti-relapse properties in opiate addiction, and also possibly affects drug memory extinction (163).

As a proxy for the current clinical interest in CBD as a therapeutic for substance use disorder, we searched ClinicalTrials.gov, the largest clinical trials public database, run by the United States National Library of Medicine at the National Institutes of Health, for registrations of clinical trials of CBD in any kind of disorders related to substance use. On 11th April 2020, we retrieved 24 trials (50% completed, 25% recruiting, 17% not yet recruiting, 1 withdrawn and 1 with unknown status). CBD was studied for cannabis/marijuana use disorder in 37.5% of the cases, for opioid addiction/dependence withdrawal in 33%, for alcohol use disorder in 17%. One trial studied CBD in cocaine craving/dependence, and 2 did not specify which kind of drug addiction was under study. For comparison, a recent study retrieving published articles about CBD as treatment for substance use disorders (164) found 207 papers, including: 51% for cannabis, 28% for hallucinogens, 8% for alcohol, 4% for opioids, 3% for tobacco, 3% for inhalants, 1.5% for sedatives, and 1% for amphetamines. The authors also systematically assessed the outcome measures, surrogate endpoints, and biomarkers in selected studies, concluding that in recent years more substance use disorders are considered (in particular, alcohol and cocaine use disorders), more prolonged efficacy trials are performed, with improved methodology, including predictive biomarkers of efficacy related to the endocannabinoid system, the monoamine system, or the immune system (164).

Finally, since the DSM-5 considers gambling disorder as a behavioral addiction included among substance use disorders (151), it should be mentioned that recent studies on the neurobiology of gambling disorder (165) indicate among the key players both CB_1 and CB_2 re-

ceptors, as well as 5-HT$_{1A}$, all of which are targets for CBD (Table 1). Preclinical evidence in rodents indicates that some CB ligands as well as some modulators of the endocannabinoid system exert complex effects on gambling choice behaviors (166, 167). No medications have been approved so far for gambling disorder, and in view of its activity in other substance use disorders and of its excellent tolerability in humans, CBD should be possibly considered as a straightforward drug candidate in clinical studies in association to current psychological and behavioral treatments.

AUTISM SPECTRUM DISORDERS AND NEURODEVELOPMENTAL DISORDERS

Autism spectrum disorder (ASD) is a neurodevelopmental disorder with a prevalence of four times higher in boys than in girls (168). Core symptoms of ASD include disrupted sociability and consequent social withdrawal, restricted or repetitive behaviors and sensory abnormalities, associated with cognitive deficits, intellectual disability, and language delay (169). Together with autism, other neurodevelopmental disorders are included in the large family of ASD, such as Fragile X syndrome (FXS), characterized by severe behavioral alterations, including hyperactivity, impulsivity and anxiety, together with poor language development and seizures (170); Asperger's syndrome, defined by difficulties in social interactions, verbal and non-verbal communication with stereotyped and limited interests (171); Tourette syndrome, characterized by the persistence of unwanted, brief, repetitive, non-rhythmic motor movements, and one or more vocal/phonic tic (172). Moreover, ASD symptomatology overlap with different neurodevelopmental disorders, such as Rett syndrome, identified by severe cognitive and physical disabilities (173), and attention deficit/hyperactivity disorder (ADHD) (174). Indeed, ASD patients are frequently affected by comorbidities (175), the most common of which are sleep disorders, psychosis, anxiety, mood and cognitive

disorders and epilepsy (176). Actually, no effective treatment for ASD is currently available and the patients often fail to respond to conventional treatments. Thus, alternative treatment approaches are advancing, such as the utilization of *Cannabis sativa* and its derivatives. Among them, CBD is gaining increasing interest, also considering that alterations in ECS and immune dysfunctions might contribute to the onset of ASD (177). In literature, there are no current data regarding preclinical studies with CBD. Concerning clinical studies, the published data are limited. Indeed, ethical and legal issues, due to the vulnerability of pediatric population, resulted in a restricted use of cannabinoids and in a small number of patients enrolled. In particular, pure CBD has not been used, but has been orally administrated as CBD-enriched cannabis extract oils with other phytocannabinoid molecules (such as THC), in a CBD/THC ratio of 20:1 (178-181), or 75:1 (182). The subjects enrolled in clinical trials were ASD patients at different developmental stages (age range of 4-22 years), mainly boys, with an average of 94 patients/study. Interestingly, not all patients completed the clinical trials and this could be correlated to the CBD dose used. The mean daily dose of CBD was between 5 mg/kg and 10 mg/kg/day (178-181), while only one study from Barchel et al. used a higher CBD dose (16 mg/kg/day) (178). Indeed, in this case, the mean duration of the trial for each patient was lower than the others (2 months versus 4-10 months), suggesting that a higher CBD dose might influence negatively the conclusion of the study. Concerning the side effects reported, the most frequent were sleep disturbances, restlessness, sleepiness, irritability and loss or increase of appetite, but these effects could be partially due to the synergic actions of other medications, taken as a therapeutic regimen by patients, in association with CBD treatment. Despite these adverse effects, immediate improvements in the patients' behavior were observed followed by an increase in patients' autonomy, motor and cognitive performances, as

well as, communication and social interaction improvements. The benefits of CBD treatment included also the reduction in the concomitant use of other medications (178-180) and an increased quality of life for the whole family consequent to the reduction of disruptive behavior in ASD patients (179). However, there are various methodological limitations reported in all the studies. In particular, results were based on subjective reports of the patients' parents or caregivers; hence, one important limitation is represented by the unavailability of an objective scale for the symptom changes, together with the lack of control groups and pharmacokinetic data. Moreover, it would be useful to clarify whether CBD treatment benefits are due to CBD effects *per se* or to the entourage effects of cannabinoid molecules present in the cannabis oil extracts used in these studies. Regarding the ongoing studies, only one clinical trial uses 98% pure CDB (183), whose results are not yet available. Although CBD efficacy in treating ASD symptoms needs to be further confirmed through more specific preclinical studies and multicenter clinical trials, the studies examined in this paragraph suggest that early treatment with CBD might be a promising therapy for ASD and related neurodevelopmental disorders.

NEURODEGENERATIVE DISORDERS

There is a long history of people with neurodegenerative diseases like Multiple sclerosis (MS), Parkinson's disease (PD), Huntington's Disease (HD) using cannabis as a self-medication. In 2005, for the first time, a standardized mixture of THC and CBD (nabiximols) has been approved in Canada, and later in other countries, to treat spasticity and pain in patients with MS. Nabiximols (USAN name, Sativex™) is a combination of THC 27 mg/ml and CBD 25 mg/ml (from Cannabis sativa L. extract) available as a mouth spray. It is indicated "*as treatment for symptom improvement in adult patients with moderate to severe spasticity due to MS who have not responded adequately to other an-*

ti-spasticity medication and who demonstrate clinically significant improvement in spasticity related symptoms during an initial trial of therapy". The reported adverse reactions are usually mild to moderate. Recently, the SAVANT study (a double-blind, placebo controlled randomized clinical trial) confirmed that add-on nabiximols offered a significant improvement of resistant MS spasticity compared with first-line antispasticity medication alone (184). Nabiximols use in the treatment of MS-related spasticity is supported by robust data but for other cannabis-based medicinal products the evidence is limited. Moreover, there was limited evidence on the effects of a change in spasticity on quality of life and for conditions other than multiple sclerosis. In a double-blind, randomized phase II trial, nabiximols has been used to treat spasticity in patients affected by motor neuron disease (MND). The spasticity, a key symptom of both MND and MS, improved in patients who received the cannabinoid treatment (185). Moreover, nabiximols decreased patients' reported pain levels. However, more research is needed to explore the clinical, cost effectiveness and the neuroprotective effect of cannabinoids in slowing disease progression. Beyond MS, clinical data to support the medicinal benefits of CBD on PD and HD comes from small, low-quality studies. In a randomized, double-blind, placebo-controlled trial on anxiety signs in 24 PD patients, CBD decreased anxiety and tremor amplitude (186). In an in vitro model of PD (human neuroblastoma cell line SH-SY5Y), CBD counteracted the loss of cell viability caused by MPP^+ by the activation of ERK and AKT/mTOR pathways (11). No significant results were found to support CBD use in HD.

EPILEPSY

CBD is the only cannabinoid drug to date with proved antiseizure activity in proper randomized placebo-controlled trials. Several preclinical studies have been focused on the determination of cannabinoids and more specifically

CBD efficacy (187) which has led to the first preparation of highly purified, plant-derived CBD being approved by the US Food and Drug Administration (FDA) in June 2018 (188), for the treatment of Dravet (DS) and Lennox-Gastaut (LGS) syndromes; two severe, rare, childhood-onset, epileptic encephalopathies characterized by multiple type of seizures and cognitive impairment.

In 2019, the European Medicine Agency (EMA) granted the approval of CBD as adjunctive treatment for DS and LGS in combination with clobazam (CLB) (189), due to the results of pivotal trials. Recently, FDA also approved the same cannabinoid drug for the treatment of seizures related to tuberous sclerosis complex (TSC) (188), a rare, autosomal dominant disease (mutation of *TSC1* and *TSC2* genes) causing benign tumors in several organs and different seizure types.

Nowadays, CBD's efficacy was demonstrated in five placebo-controlled pivotal trials, two conducted in LGS (190, 191), two in DS (53, 192), and one in epilepsy associated with TSC (13). Furthermore, numerous expanded access use studies as well as open label extension of RTCs have been published so far (193-198) and numerous comprehensive reviews and metanalysis investigating the clinical profile of CBD as anti-seizure medications are available (44, 49, 199-205). The existing data suggest a beneficial response with CBD oil-based solution in patients across a broad range of epilepsy disorders and etiologies. Notably, the overall tolerability profile of purified CBD was favorable throughout the different epileptic conditions and overlapped with patients enrolled in randomized controlled trials (RTCs) (44).

All daily doses tested (10 and 20 mg/kg in DS and LGS and 25 and 50 mg/kg in TSC) have resulted in a significant frequency reduction in convulsive seizures associated with DS, drop attacks in LGS, and focal and generalized seizures in TSC. Indeed, a metanalysis of randomized clinical trials involving 550 patients with LGS and DS reported a reduction in all-types seizure frequency (at least 50%) occurred in 37.2% of the patients in the CBD 20 mg group and 21.2% of the placebo participants (RR 1.76, 95% CI 1.07-2.88; p = 0.025) (200). Long-term data (up to 96 weeks) endorsed the extended treatment efficacy, with a median monthly major seizure reduction of 50% and a reduction of 44% for all seizures in 152 patients with DS and LGS (195, 206).

Recently, a systematic review summarized the currently available data beyond DS and LGS, evidencing the highest-quality evidence available for TSC (201). In the one RCT performed enrolling patients with TSC, CBD at both 25 and 50 mg/kg/die doses produced a significantly greater reduction in focal and generalized seizure frequency and total seizure frequency (13). A 40% responder rate (at least 50% reduction) was reported in approximatively 40% of patients, a data overlapping with prior expanded access study of CBD (50 mg/kg/day of maximum dose). Several patients (about 56%) treated with CBD in clinical trials were receiving CLB in comedication, leading to concerns on the actual improvement mediated by CBD and the antiseizure activity due to potential pharmacokinetic interactions with CLB (elevated *N*-desmethylclobazam concentration in plasma), as above discussed (207).

Interestingly, already in the first open-label trial on add-on CBD in patients with heterogeneous childhood-onset drug resistant epilepsy, a higher responder rate was evidenced in patients co-treated with CLB and this latter resulted to be the only independent predictor of frequency reduction (194). However, randomized clinical trials post-hoc analysis and other studies investigating different outcomes in patients treated or not with CLB, highlighted that CBD exerts therapeutic effects in patients with epilepsy that are independent of its interaction with CLB (207-209). These findings are in agreement with metanalysis (208, 210) (pooling seizure outcomes from different syndromes) and open-label trials (209), although intrinsic limitations of the study design, with no control groups, should be considered in interpreting results. Nevertheless, subgroup anal-

ysis has also methodological limitations, lack in randomization with CLB on/off patients and not powered to assess differences. Besides, in the TSC randomized trial, the 27% of patients received CLB as concurrent treatment, and no subgroup analysis was reported (13, 44).

Finally, apart from the above-mentioned epilepsy syndromes, CBD was administered in several other epileptic syndromes, such as CDKL5 deficiency disorder, Sturge-Weber syndrome, Doose syndrome, Aicardi syndrome, febrile infection-related epilepsy syndrome, and infantile spasm (206). Two systematic reviews have included 19 and 30 non-randomized studies respectively, comprising open-label interventional studies, case studies, retrospective chart reviews and self-report surveys (211, 212). Although the promising results, the inclusion of heterogenous studies and their limitations did not allow consistent conclusions on the efficacy of treatment.

Randomized clinical trials need to be performed to evaluate CBD efficacy in epileptic syndromes beyond DS, LGS and TSC and the direct CBD antiseizure activity in patients with or without CLB in co-treatment. Further studies are also required to address pharmacokinetics in pediatric patients and to improve the few data on the relation between plasma CBD concentrations and clinical response (213).

DEMENTIA AND ALZHEIMER DISEASE

As the leading cause of dementia, Alzheimer's disease (AD) is mainly characterized by extracellular deposits of amyloid β plaques and intracellular neurofibrillary tangles and by a reduction of choline acetyltransferase activity (214).

A number of studies has examined the effect of acute CBD treatment on cognition, showing its beneficial role, compared to THC, in healthy controls and cannabis users (215-219). 134 users were classified into high- and low-CBD cannabis groups and were assessed for memory and psychotomimetic signs using Recognition Memory, Prose Recall, and Source

Memory tests. Individuals who consumed high-CBD cannabis revealed significantly better recognition memory than people who used cannabis containing low CBD (216). Furthermore, in everyday cannabis users, the addition of CBD (200 mg/day) for ten weeks provoked an improvement in attentional switching, verbal learning, and memory functions (219). In contrast, CBD administration did not promote cognitive capacities in patients with neurological diseases (220).

These clinical data (218, 221) and encouraging results from pre-clinical studies, showing neuroprotective effects and enhanced social recognition and spatial memory after acute and chronic CBD treatment in AD (222-226), could have significant implication for the therapy of neurodegenerative diseases, principally AD. Even though the cellular and non-human primate models have shown positive effects for neurodegeneration, the precise molecular mechanism is not well understood, and there are not clinical investigations currently assessing the effects of CBD in subjects with AD.

On this perspective, further studies are needed to determine the dose-dependency and time-dependency of potential treatment of CBD to exert a neuroprotective effect and to understand the subsequent ceasing neurodegeneration and neuronal repair. Extensive randomized, controlled clinical trials are required to validate that findings translate to human patients.

SAFETY AND TOXICITY

Based on the World Health Organization's report, CBD emerged as a potential candidate in a wide-variety of clinical contexts due to its comparatively favorable therapeutic index, lack of undesirable psychoactive properties, low toxicity and low abuse potential (227). However, CBD is not a biologically inert compound and due to its complex pharmacokinetic and pharmacodynamic profile it is not devoid of adverse effects (AEs) (228). CBD appears to have little influence on vital signs

(*e.g.* heart rate, blood pressure and respiratory depression) while, with regard to effects on reproductive system, including developmental toxicity and teratogenicity, these have been seen only in preclinical studies, but with higher doses than used in humans (229,230). In randomized, double-blind, placebo-controlled multicenter trials with exposure to CBD doses of 5, 10, and 20 mg/kg/day, the most common AEs reported were somnolence/sedation, decreased appetite, gastrointestinal disturbances/diarrhea, weight changes, fatigue, behavioral changes (*e.g.*, irritability, agitation, aggression), skin rashes, and nausea. Uncommon or rare AEs included thrombocytopenia, respiratory infections, and alteration of liver enzymes (46, 53, 190, 194, 230-232) (see **table II**). Although reported AEs were mostly mild to moderate, 8-14% of the CBD-treated patients' withdrawal from the study, compared with less

Table II. Table Adverse Reactions in Patients Treated with CBD in Controlled Trials of LGS, DS and TSC.

	LGS/DS				TSC	
	CBD (mg/kg/day)			Placebo	CBD (mg/kg/day)	Placebo
	5	10	20		25	
	N = 10	N = 75	N = 238	N = 227	N = 75	N = 76
Hepatic Disorders						
Transaminases increased	10%	8%	16%	3%	25%	0
Gastrointestinal Disorders						
Decreased appetite	0%	16%	22%	5%	20%	12%
Weight decreased	0%	3%	5%	1%	7%	0%
Abdominal pain, distension, discomfort	0%	3%	3%	1%		
Gastroenteritis	10%	0%	4%	1%	8%	7%
Diarrhea	0%	9%	20%	9%	31%	25%
Nervous System Disorders						
Somnolence	40%	23%	25%	8%	13%	9%
Fatigue, malaise, asthenia	0%	11%	12%	4%	5%	1%
Sedation		3%	6%	1%		
Lethargy, disorientation, depressed level of consciousness	20%	4%	8%	2%		
Irritability, agitation	0%	9%	5%	2%		
Aggression, anger	0%	3%	5%	< 1%		
Insomnia, sleep disturbance, abnormal dreams	10%	11%	5%	4%		
Drooling, salivary hypersecretion	0%	1%	4%	< 1%		
Gait disturbance, difficulty walking,	0%	2%	3%	< 1%	9%	5%

Continue

Table II. Table Adverse Reactions in Patients Treated with CBD in Controlled Trials of LGS, DS and TSC.

	LGS/DS				TSC	
	CBD (mg/kg/day)			Placebo	CBD (mg/kg/day)	Placebo
	5	10	20		25	
	N = 10	N = 75	N = 238	N = 227	N = 75	N = 76
Infections						
Infection, all	40%	41%	40%	31%		
Infection, other		25%	21%	24%		
Infection, viral	20%	7%	11%	6%		
Pneumonia	0%	8%	5%	1%	4%	1%
Infection, fungal	0%	1%	3%	0%		
Other						
Rash	10%	7%	13%	3%	8%	4%
Respiratory failure, disorder, hypoxemia	0%	3%	3%	1%		
Pyrexia	0%	3%	0%	0%	19%	8%
Hematological changes						
Anemia					7%	1%
Platelet count decreased					5%	1%
Eosinophil count increased					5%	0%

Based on data reported in the U.S. prescribing information.

than 2% of placebo-treated group. The higher incidence of serious adverse events, was observed in the pediatric patients with rare forms of epilepsy (199). This may be related to the high doses of CBD ($\geq$ 20 mg/kg or more) taken, or to concomitant use of other antiseizure medications. In patients with DS and LGS, the frequency of somnolence and sedation, was 34% at 20 mg/kg/day compared with 27% at 10 mg/kg/day and 11% on placebo. Somnolence and sedation were twice as common in patients co-medicated with clobazam (46% vs. 16%), probably due to the increase in serum norclobazam metabolite levels (53, 190). The elevated transaminase concentrations, defined as elevation of three times or more the upper limit of normal, were reported in 17% of CBD-treated patients 20 mg/kg/day compared

with 1% of those taking 10 mg/kg/day. These effects were dose-related and potentiated by co-administration of the antiseizure medications including clobazam and valproate. Specifically, elevated liver function values were seen in 31% of patients co-medicated with both valproic acid and clobazam, in 21% of those co-medicated with valproic acid (without clobazam), in 4% of those co-medicated with clobazam (without valproic acid), compared to 3% of patients who was taking neither drug in co-administration. Furthermore, dosages greater than 25 mg/kg/day were associated with higher incidence of hepatotoxicity and a major risk of treatment withdrawal. In a study of TSC patients receiving CBD doses of 25-50 mg/kg, the AEs average increase about $\geq$ 10% than on placebo has been estimated (13)

(see **table II**). However, the safety profile observed was consistent with findings from previous studies, with no new safety risks identified (13). Furthermore, to date, no symptoms suggestive of abuse potential or physical dependence were observed in any of the preclinical or clinical studies carried out with CBD. In summary, the available data suggest that CBD is well tolerated and has relatively few serious adverse effects, however adequate medical oversight is needed to monitor and manage the side effects, potential drug-drug interactions and the proper dose, given the broad therapeutic index.

CONCLUSIONS

CBD and CBD based products have attracted great scientific and public attention in the last 20 years and pharmaceutical development has led to the authorization of two specific products (one pure CBD and one as a mixture with THC) for the treatment of some specific types of epileptic syndromes and spasticity in MS patients. As summarized above, CBD is currently undergoing preclinical and clinical evaluation for the treatment of a variety of CNS disorders while its mechanism of action has only partly been elucidated. Overall, this phytocannabinoid is very promising and the hope is its validation in many CNS diseases while the study of other cannabinoids is also extremely important and may add more to clinical practice obtaining the best out of this fascinating plant. Indeed, clinical efficacy has to follow standard procedures through well-designed randomized clinical trials and unfortunately not standardized or well-studied CBD containing products are commercially available on the market promising undemonstrated efficacy. As above mentioned, CBD and other cannabinoids are at high risk of drug-drug interaction and not controlled products may expose self-medicating people to harms and side effects. In conclusion, natural products still represent a great resource for scientific and clinical advancements with CBD being a valuable example considering its already proven efficacy in some cases but also for the identification of novel therapeutic targets which may be considered for further development.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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