CHAPTER

93

Cannabidiol and Multiple Sclerosis

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SUMMARY POINTS

- Multiple sclerosis (MS) is the most frequent chronic neurological disease among young and middle-aged people in the northern industrialized countries.
- MS is pathologically characterized by multifocal inflammation, demyelination and neuronal injury in the central nervous system (CNS).
- The first-line disease modifying therapies for MS patients include immunomodulatory and immunosuppressive medications.
- Cannabidiol (CBD), which constitutes up to 40% of *Cannabis sativa* extract, may represent a promising agent for human therapeutic use due to the lack of psychoactive actions.
- Up to date, there is no evidence about the full binding of CBD to any known receptor site, and the molecular pharmacology of CBD has not been well defined.
- Experimental autoimmune encephalomyelitis (EAE) and Theiler's murine encephalomyelitis virus-induced demyelinating disease (TMEV-IDD) are two experimental models of MS that include classical MS hallmarks like inflammation, neuronal damage, and demyelination.
- CBD has been shown to ameliorate EAE and TMEV-IDD model symptomatology by diminishing inflammation, microglial activity, and leukocyte homing.
- Sativex is used for the treatment of symptoms of MS, particularly spasticity and neuropathic pain.

KEY FACTS OF NEUROLOGY

- Multiple sclerosis is a complex heterogeneous demyelinating autoimmune disease that primarily affects the myelin in the central nervous system (CNS).
- CNS demyelination, the pathological process in which myelin sheaths are lost, is the consequence of a direct insult targeted at the oligodendrocyte.
- Axonal degeneration is accepted as the major cause of irreversible neurological disability in multiple sclerosis (MS) patients.
- Autoantigen-specific Th17 and Th1, B cells and monocytes/macrophages have major pathological roles.
- Pharmacological treatment options approved for MS aim at limiting inflammation and decreasing relapse rate, but, no current therapies can cure the disease.

KEY FACTS OF CNS REPAIR

- Remyelination is the process in which myelin sheaths are restored to demyelinated axons restoring functional deficits.
- Remyelination involves the generation of new mature oligodendrocytes from oligodendrocyte progenitors.
- A key function of remyelination in multiple sclerosis is axon survival.
- The efficiency of remyelination is affected by age, sex, and genetic background regardless of the disease process.
- The most significant approach to enhance remyelination is to target the endogenous regenerative process.

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KEY FACTS ON THE CANNABINOID SYSTEM

- The cannabinoid system is a regulatory system identified following the study of *Cannabis sativa* derivatives involved in the homeostasis control.
- The endocannabinoid signaling system is composed of cannabinoid receptors, their endogenous ligands and the enzymes that produce and inactivate these ligands.
- There are >60 cannabinoid compounds present in extracts of *C. sativa*, and Δ^9-tetrahydrocannabinol (Δ^9-THC) is responsible for the psychoactive effects of cannabis, and for many of the potential medicinal effects.
- The nonpsychoactive cannabidiol (CBD)is the other cannabinoid derived from *C. sativa* of current medical interest in MS.
- Sativex is an oromucosal spray, constituted by an equimolecular combination of Δ^9-THC and CBD-enriched botanical extracts approved for the treatment of spasticity and pain associated to MS.

LIST OF ABBREVIATIONS

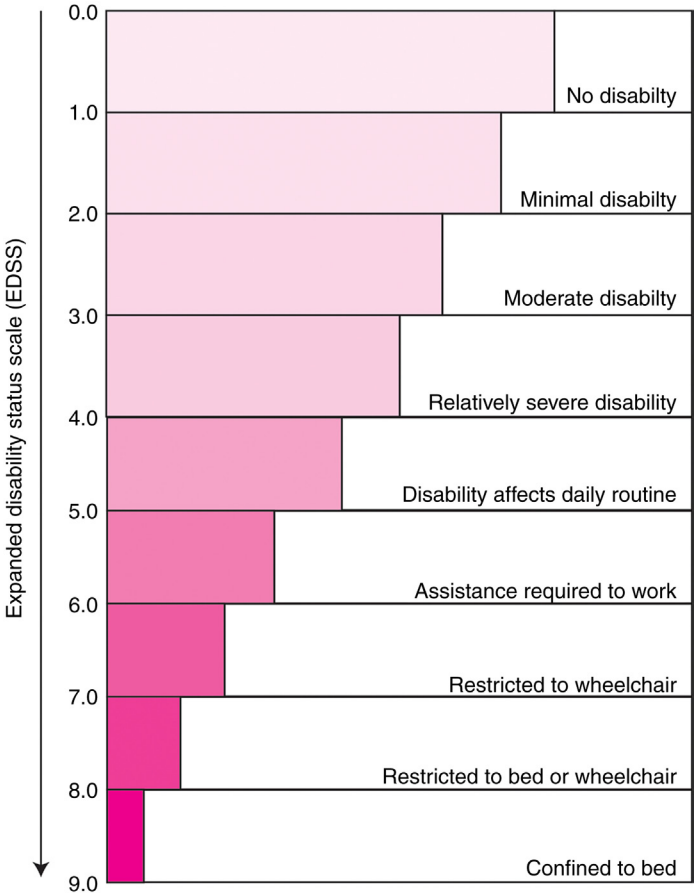
AD	Alzheimer disease
CBD	Cannabidiol
CNS	Central nervous system
EAE	Experimental autoimmune encephalomyelitis
EDSS	Expanded disability status scale
FAAH	Fatty acid amide hydrolase
GPCR	G-protein coupled receptor
IFN-β	Interferon-beta
MCA	Middle-cerebral-artery
MHC	Major histocompatibility complex
MRI	Magnetic resonance image
MS	Multiple sclerosis
PPAR	Peroxisome proliferator activated receptors
PPMS	Primary progressive multiple sclerosis
RRMS	Remittent recurrent multiple sclerosis
SPMS	Secondary progressive multiple sclerosis
THC	Tetrahydrocannabinol
TMEV-IDD	Theiler´s murine encephalomyelitis virus - induced demyelinating disease
TPVR-1	Transient potential vanilloid receptor-1

INTRODUCTION

Multiple sclerosis (MS) is the most frequent chronic neurological disease among young adults in the northern industrialized countries. MS is pathologically characterized by multifocal inflammation, demyelination, and neuronal injury in the optic nerves, brain and spinal cord, and is considered to be an autoimmune disease with a complex pathophysiology (Compston & Coles, 2002). The pathological hallmarks of the disease result in an affectation of white matter tracts and injury to the cortical and deep gray matter, generating neurologic symptoms and disability in patients with MS (Table 93.1).

MS occurs in a female–male ratio of 3 to 1, and has an estimated prevalence of more than 2.1 million people worldwide (Niedziela, Adamczyk-Sowa, & Pierzchala, 2014). It is widely accepted that MS cause is multifactorial, including multiple genetic and environmental risk factors like risk alleles in genes for major histocompatibility complex (MHC), and interleukin-2 and 7 receptors among others (Beecham et al., 2013). Moreover, the geographic location of residence before adolescence may also be predictive of MS risk, as it has been found

TABLE 93.1 Expanded Disability Status Scale (EDSS) in Patients With MS



The Kurtzke's EDSS scores 8 functional systems and the person's ability to walk. From 0.0 to 4.0, people are able to walk without assistance. From 4.0 to 7.5, people can walk but with assistance. Mainly, point 6 on the scale represents walking with a cane, and this point is often used as an endpoint of the progression of disability. From 7.5 to 10, the main determinant of EDSS is the person's ability to transfer from wheelchair to bed and self-care. An EDSS of 10 is not included in this table because it means death of the patient due to MS.

increased rates of the disease in northern and southern latitudes compared with equatorial countries, probably linked to a vitamin D deficiency (Van der Mei et al., 2003). Additionally, the risk to MS may be influenced by the exposure to particular infectious like Epstein–Barr virus (Lassmann, Niedobitek, Aloisi, & Middeldorp, 2011) and Herpesvirus 6 (Tait & Straus, 2008).

The first-line disease modifying therapies for MS patients include immunomodulatory and immunosuppressive medications like interferon-β (IFN-β) or glatiramer acetate among others (Table 93.2), which has been shown to reduce both risk of relapse and new lesion formation on MRI scans. Paradoxically, antiinflammatory treatment might contribute to the failure of repair, as results from studies of patients with MS indicate that the inflammation blockade might trigger counter-regulatory inflammatory mechanisms used to heal the injured tissue (Martino et al., 2002), and suggesting that tissue integrity is restored by the conversion of the inflammatory response from a damaging to a repairing one (Nathan, 2002).

Evidence suggest that neuroinflammation, demyelination, and neurodegeneration may occur in parallel, the combination of antiinflammatory, oligoprotective and neuroprotective strategies arise as an emerging therapy for the symptomatic and therapeutic treatment of MS. In this line, *C. sativa* derivatives have attracted special interest regarding putative therapeutic properties, despite the fact that these compounds have always raised both ethical and practical problems for their potential abuse and unavoidable psychotropic effects. Among marijuana compounds, cannabidiol (CBD), which constitutes up to 40% of cannabis extract, may represent a promising agent for human therapeutic use as it lacks any cognitive and psychoactive actions, and has an excellent tolerability profile in humans (Mechoulam & Hanus, 2002).

A large number of clinical trials have been performed to assess the clinical efficacy of CBD in different pathologies. Most of them has been focused on Sativex compounds (GW Pharmaceuticals; Salisbury, United Kingdom), a commercially available preparation containing CBD and Δ^9-THC under four different formulations that differs in the concentration of CBD and Δ^9-THC (tetrahydrocannabinol). The oromucosal spray administration of Sativex has been agreed in 2005 for the treatment of pain and spasticity in MS (Perras, 2005). In the central nervous system (CNS), CBD has been reported to act as an antiinflammatory compound, thus being useful for neuroinflammatory disorders but also as a neuroprotective agent by normalizing glutamate homeostasis, reducing oxidative stress, and attenuating glial activation and the occurrence of local inflammatory events (reviewed by Fernandez-Ruiz et al., 2013). All these properties point out CBD as a promising therapeutic agent for the treatment of neuroinflammatory disorders like MS.

CBD PHARMACOLOGY AND MECHANISMS OF ACTION

Isolated in across the 1930s and 1940s from *C. sativa*, the structure and configuration of CBD was fully elucidated by Mechoulam et al. in the 1960s (Mechoulam & Shvo, 1963; Mechoulam, Shani, Edery, & Grunfeld, 1970). The first study focusing on CBD pharmacology was published in 1981, regarding hypnotic and anticonvulsant properties (Carlini & Cunha, 1981). Along this time, several studies have been done to identify the mechanisms through which CBD exerts its actions. Up to date, there is no evidence about the full binding of CBD to any known receptor site. The molecular pharmacology of CBD has not been well defined yet, and little is known about a possible CBD-receptor-mediated signaling pathway. It is known that many of the CBD effects are associated with both central and peripheral actions, and numerous studies has been reported trying to elucidate some of its mechanisms of action (Table 93.3), that are detailed later in the chapter.

CBD is a Potent Antioxidant

Pioneer works by Hampson, Grimaldi, Axelrod, and Wink (1998) showed that CBD has antioxidant properties that have been confirmed along several publications. The plant cannabinoids, being monophenols, monophenolic esthers (like THC), or resorcinols (like CBD) are likewise potent antioxidants. Hampson et al. (1998) demonstrated that CBD exerted a potent antioxidant activity that resulted more protective than either ascorbate (vitamin C) or α-tocopherol (vitamin E) against glutamate-mediated neurotoxicity. These observations suggest that CBD may be a potential therapeutic agent for the treatment of oxidative neurological disorders like cerebral ischemia, and for diseases that course with oxidative damage.

CBD Potentiates the Endocannabinoid Signaling System

In the immature damaged brain, CBD has shown direct activity binding to the CB2 receptor, and an indirect activity through an inhibitory effect on the mechanisms of inactivation of endocannabinoids (transporter, FAAH enzyme) (Bisogno et al., 2001; De Filippis et al., 2008). In this line, the enhancement of the endocannabinoid tone may mediate some of the antiinflammatory and neuroprotective effects of CBD.

CBD Binds With Low Affinity to Both CB1 and CB2 Cannabinoid Receptors

At concentrations in the micromolar range, CBD shows weak ability to remove ^{3}[H]CP55940, a not selective ligand for CB receptors from both CB1 and

TABLE 93.2 Currently Available MS Treatments

	Treatment	Year of FDA approval	Type	Route of administration	Implications	Mechanism of action	Side effects
Acute	Methylprednisolone (Solu-Medrol)	1959	Glucocorticoid	Intravenous (3–5 days)	Acute relapse	Stabilizing the BBB, decreasing proinflammatory cytokines, and inducing T cell apoptosis	Disturbance of taste, facial flushing, insomnia, psychiatric disturbance, exacerbation of acne, transient hyperglycemia, and hypertension
	ACTH (H.P. Acthar)	1978	Hormone	Intramuscular or subcutaneous (5–15 days)	Acute relapse	Stimulates the adrenal cortex gland to secrete cortisol, corticosterone, and aldosterone	Vomiting, change in appetite, diarrhea, constipation, restlessness, difficulty sleeping, sweating
	Plasma exchange	/	/	Intravenous	Acute relapse	Exchange of immunoglobulins	Hypocalcemia, hypovolemia, and anaphylactoid reactions
Chronic	IFNβ-1a (Avon ex)	1996	Cytokine (low dose)	Intramuscular injection	RRMS first line	Suppresses the proliferation of myelin-basic protein-specific T cells. Reduces the production of proinflammatory cytokines, and induces antiinflammatory cytokines. Effects on the endothelial cells of the BBB	Production of NAb (IFNβ1A less than IFNβ1B) Influenza-like symptoms. Headache, injection site reaction, asthenia, lymphopenia, depression, hepatic injury, congestive heart failure, anaphylactic shock, and pain
	IFNβ-1a (Rebif)	2002	Cytokine (high dose)	Subcutaneous injection			
	IFNβ-1b (Betaseron)	1993	Cytokine (high dose)	Subcutaneous injection			
	Glatiramer acetate (Copaxone)	1996	Synthetic polymer of random sequences of 4 aa (L-tyr, L-glut L-Ala, L-Lys)	Subcutaneous injection	RRMS first line	Unknown, possible binding to the major MHC-II competing with the other MS putative Ag	Injection site reactions, tightness, anxiety, dyspnea, palpitation, vasodilation
	Mitoxantrone (Novantrone)	2000	Antineoplastic drug	Intravenous infusion	RRMS SPMS	Inhibit B cell, T cell, macrophage proliferation. Impair antigen presentation, and the secretion of IFN-γ, TNFα, and IL-2	Cardiotoxicity, myelogenous leukemia, gonadal dysfunction
	Natalizumab (Tysabri)	2006	Humanized anti-VLA-4 monoclonal antibody	Intravenous infusion	RRMS first line	Targets the α4-chain of α4β1 integrin decreasing the accumulation of activated leukocytes within the CNS	Headache, fatigue, arthralgia, urinary tract infection, lower respiratory infections, gastroenteritis, vaginitis, diarrhea, hypersensitivity reactions, hepatotoxicity, PML
	Dalfampridine (Ampyra)	2010	4-aminopyridine (4-AP)	Oral capsule	Chronic progressive	Potassium channel blocker, potent calcium activator	Dizziness, nervousness, nausea urinary tract infection
	Fingolimod (Gilenya)	2010	Sphingosine-1-phosphate (SIP) receptor modulator	Oral capsule	RRMS second line	SIP receptor antagonist in lymphocytes. It blocks the ability of lymphocytes to leave lymph nodes	Headache, infections, bradycardia bradyarrhythmias, macular edema
	Teriflunomide (Aubagio)	2012	Drug	Oral capsule	RRMS	Binds to DHODH protein inhibiting pyrimidine synthesis in proliferating cells such as T and B lymphocytes (immunomodulatory)	Dyspnea, diarrhea, nausea, alopecia, hepatotoxicity, acute renal failure, hypertension, leukopenia
	Dimethyl fumarate (Tecfidera, BG12)	2013	α,β-unsaturated ester	Oral capsule	RRMS first line	Reduce transendothelial migration of activated leukocytes through the BBB. Neuroprotective effects via activation of antioxidative pathways	Gastrointestinal disorders, lymphopenia, flushing, pruritus, rash, erythema

Acute and chronic treatments available for MS patients (reviewed in Damal, Stoker, & Foley, 2013; Kantarci, Pirko, & Rodriguez, 2014). PML, progressive multifocal leukoencephalopathy; ACTH, adrenocorticotropic hormone; DHODH, dihydroorotate dehydrogenase BBB, blood–brain barrier. Intravenous immunoglobulins (IVIG) and cyclophosphamide for acute relapses are not covered in this table.

TABLE 93.3 CBD Molecular Mechanism of Actions and Effects

Targets	Mechanisms	Affinity/dose	Effect
Receptors and channels			
CB1/CB2	Antagonist	KB 79 nM (CB1); 138 nM (CB2)	Antagonizes cannabinoid induce antispasmodic effect
CB2	Inverse agonist	EC50: 503 nM	Antiinflammatory effect
GPR55	Antagonist	IC50: 350 nM	Antagonistic activity in human osteoclasts
5-HT3A ligand-gated channel	Allosteric inhibition	IC50: 0.6 μM	Modulation of nociception and emesis
TRPM8 cation channel	Antagonist	EC50: 80–140 nM; IC50: 60 nM	Analgesic effects
TRPA1 cation channel	Agonist	EC50: 110 nM; IC50: 160 nM	Analgesic effects
PPARγ nuclear receptor	Agonist	IC50: ±5 μM	Vasorelaxation antiinflammatory effect
T-type Ca^{2+} channel	Inhibitor	IC50: ±1 μM	Nociceptive and antiepileptic effects, sleep regulation
TRPV1 cation channel	Agonist	EC50: 1 μM; IC50: 0.6 μM	Antipsychotic and analgesic effects
TRPV2 cation channel	Agonist	EC50: 1.25 μM; IC50: 4.5 μM	Antiinflammatory/analgesic/antinociceptive effect
5-HT1A receptor	Agonist	80% displacement at 16 μM	Antiischemic and anxiolitic properties. Neuroprotective
μ and δ opioid receptors	Allosteric modulation	EC50 4.38 (μ); 4.10 (δ)	Potentially enhance the effects of opiates
α1 and α1β glycine ligand-gated channels	Positive allosteric modulation	EC50: 12.3 μM (α1); 18.1 (α1β)	Role in chronic pain after inflammation or nerve injury
Abnormal-CBD receptor	Antagonist	Effect at 1 μM	Attenuates the vasodilator response to anandamide
Targets	**Mechanisms**	**Affinity/dose**	**Effect**
Enzymes			
CYP1A1	Competitive inhibitor	IC50: 0.41 μM	Might lead to interaction of drugs or toxicants metabolized by CYP enzymes. Possible adverse effects or intoxication
CYP1A2 and CYP1B1	Competitive inhibitor	IC50: 3.8 μM (A2); 5.96 μM (B1)	
CYP2B6	Inhibitor	K_i: 0.694 μM	
CYP2D6	Competitive inhibitor	IC50: 6.65 μM	
CYP3A5	Inhibitor	IC50: 1.65 μM	
CYP2A6	Inhibitor	K_i: 55 μM	
CYP3A4 and CYP3A7	Inhibitor	IC50: 11.7 μM (A4)23–31 μM (A7)	
FAAH	Inhibitor	IC50: 15.2 μM	Increment of AEA tone. Analgesic, antiinflammatory effects
Mg^{2+} -ATPase	Inhibitor	Effect at 50 μM	Anticonvulsant effect
AANAT	Inhibitor	65% reduction at 10 μM	Reduces melatonin biosynthesis
5-Lipoxygenase	Inhibitor	IC50: 73.73 μM	Antimitotic effect in glioma cells
15-Lipoxygenase	Inhibitor	IC50: 2.56 μM	Involved in atherosclerosis
Phospholipase A2	Activator/inhibitor	EC50: 6.4 μM(+)134 μM(−)	Antiinflammatory effects
Indoleamine-2,3-dioxygenase	Inhibitor	IC50: 2.8 μM/mL	Enhance of tryptophan and therefore serotonin. Improvement of mood disturbances

(*Continued*)

TABLE 93.3 CBD Molecular Mechanism of Actions and Effects (*cont.*)

Targets	Mechanisms	Affinity/dose	Effect
Glutathione peroxidase	Activator	Effect at 10 and 25 μM	ROS production and caspase activation in tumor cells
Glutathion reductase	Activator		
Superoxide dismutase	Inhibitor	Effect at 100 μM	Generates ROS and induces cell toxicity in tumor cells
Catalase	Inhibitor		
NAD(P)H-quinone reductase	Inhibitor		
Progesterone 17α-hydrolase	Inhibitor	Effect at 1 M	Interaction with steroid metabolism. Inhibits the testosterone synthesis in rat testis
Testosterone 6β-hydrolase	Inhibitor		
Testosterone 16α-hydrolase	Inhibitor		
Targets	**Mechanisms**	**Affinity/dose**	**Effect**
Transporters and cellular uptake			
Adenosine uptake	Competitive inhibitor	IC50: 120 nM	Increment of adenosine tone antiinflammatory effect
Intracellular Ca^{2+} uptake	Inhibition	Effect at 1 μM	Neuroprotective and antiepileptic properties
Anandamide reuptake	Inhibitor	IC50: 28 μM	Increment of AEA tone
Choline uptake	Inhibitor	EC50: 1.6 pM	Increment of Ach tone
P-glycoprotein (drug efflux transporter)	Inhibitor	IC50: 39.6 μM	Potentially influence the absorption and disposition of compounds that are P-gp substrates

Molecular targets of CBD, including cannabinoid and noncannabinoid receptors, enzymes, transporters, and cellular uptake proteins (reviewed in Izzo, Borrelli, Capasso, Di Marzo, & Mechoulam, 2009; Mechoulam, Petersa, Murillo-Rodriguez, & Hanus, 2007; Hill, Williams, Whalley, & Stephens, 2012). CYP, Cytochrome P450 enzymes; AANAT, arylalkylamine *N*-acetyltransferase; FAAH, fatty acid amide hydrolase; AEA, *N*-arachidonoylethanolamide; Ach, acetylcholine; ROS, reactive oxygen species.

CB2 receptor sites (Thomas, Gilliam, Burch, Roche, & Seltzman, 1998).

CBD Antagonizes CB1 and CB2 Receptor Agonists, and Can Act as an Inverse Agonist of CB2 Receptor

CBD attenuates the effects of WIN55212 and CP55940 (CB1 agonists) at prejunctional sites in mouse vas deferens (Pertwee, Ross, Craib, & Thomas, 2002). Moreover, at concentration values in low nanomolar range, CBD could work as an inverse agonist of CB2 receptor, as it has been demonstrated in whole-brain membranes and membranes from CHO cells transfected with human CB2 receptors (Thomas et al., 2007). This mechanism may explain some of the pharmacological effects of CBD such as its antiinflammatory properties.

CBD Enhances Adenosine Signaling

CBD binds to the equilibrative nucleoside-transporter-1 with a K_i value below 250 nM, which in turn led to the increase of extracellular adenosine (Carrier, Auchampach, & Hillard, 2006). Neuroprotective effects of CBD in hypoxic–ischemic brain damage also involve adenosine A_2 receptors (Castillo, Tolon, Fernandez-Ruiz, Romero, & Martinez-Orgado, 2010). Moreover, CBD diminishes inflammation in acute models of injury (Ribeiro et al., 2012) and in a viral model of MS through adenosine A_2 receptors (Mecha et al., 2013b).

CBD Interacts With the Transient Potential Vanilloid Receptor Type-1 (TPVR-1), and With 5-HT1$_A$ Serotonin Receptor

Both CBD and its (+) enantiomer interact (EC50 estimated between 3.2 and 3.5 μM) with TPVR-1 receptor, with a maximal effect that is similar to the natural agonist capsaicin. These effects have been confirmed both in vitro (Bisogno et al., 2001) and in a model of acute inflammation in rats (Costa, Giagnoni, Franke, Trovato, & Colleoni, 2004).

In relation to 5HT1$_A$ serotonin receptors, Russo, Burnett, Hall, and Parker (2005) demonstrated that CBD displaces the agonist ([^{3}H]-8-OH-DPAT) from the human cloned receptor in a concentration/dependent manner, and that it increases [^{35}S]GTPgS binding in this G-protein-coupled receptor (GPCR) system decreasing cAMP concentration at similar apparent levels of receptor occupancy. In addition, CBD significantly reduces the infarct

volume induced by middle-cerebral-artery (MCA) occlusion (Mishima et al., 2005), exerting a neuroprotective effect that was inhibited by the serotonin 5-HT1$_A$ receptor antagonist WAY100135, but not by capsazepine, a vanilloid-receptor antagonist.

CBD Allosterically Modulates μ and δ Opioid Receptors

Data from Kathmann, Flau, Redmer, Tränkle, and Schlicker (2006) show that, on rat cerebral cortex membrane homogenates, CBD accelerated the dissociation of both the μ opioid receptor agonist [^{3}H]DAMGO induced by naloxone, and the δ opioid receptor agonist [^{3}H] naltrindole induced by naltrindole. Interestingly, this property was shared by THC, but not by the CB1 cannabinoid-receptor antagonist rimonabant. As this modulation of opioid receptors occurs at very high levels of CBD, it cannot be expected to contribute markedly to the phytocannabinoid actions in vivo.

CBD Affects Nuclear Receptors of the Peroxisome Proliferator-Activated Receptors (PPAR-g), and Antagonizes the Orphan Receptor GPR55

Recently, it has been discovered the ability of different endocannabinoids and phytocannabiniods, including CBD, to display an extra-cannabinoid receptor binding activity by the interaction with peroxisome proliferator-activated receptors (PPARs) (O'Sullivan & Kendall, 2010). PPARs belong to the family of nuclear hormone receptors, and have been reported to control the expression of genes related to inflammatory responses. Esposito et al. (2011) reported that the blockade of PPARγ in a rat model of Alzheimer disease (AD), blunted CBD effects on reactive gliosis and subsequently on β-amyloid-induced neurotoxicity. Moreover, and due to the interaction of CBD with PPARγ, this cannabinoid was observed to stimulate hippocampal neurogenesis.

Additionally, CBD displays GPR55 antagonistic activity in human osteoclasts (Whyte et al., 2009), and has no GPR55 agonistic activity when assayed in β-arrestin recruitment and calcium mobilization assays (Yin et al., 2009; Kapur et al., 2009), demonstrating that CBD can act as an antagonist of the orphan receptor GPR55.

CBD TOXICITY AND PHARMACOKINETIC

In view of the potential therapeutic use of CBD, and encouraged by the lack of its undesired psychotropic effects, several studies have been performed to determine the toxicological profile of this phytocannabinoid.

Very low toxicity of CBD has been found both in human and in other species, with an LD50 of 212 mg/kg when intravenously injected into rhesus monkey (Rosenkrantz, Fleischman, & Grant, 1981). The oral toxicity of CBD had not been clearly established, but Rosenkrantz et al. (1981) showed that an oral dose of 20–50 times larger than the intravenous route of CBD is required to initiate severe intoxication. Additionally, CBD does not cause relevant CNS alterations, and does not display mutagenic or teratogenic activities (Dalterio, Steger, Mayfield, & Bartke, 1984; Matsuyama & Fu, 1981).

In regards of the pharmacokinetic of CBD, reviewed by Grotenhermen (2003), once orally given and due to a marked first-pass effect, CBD bioavailability ranges between values of 13% and 19%, while the systemic bioavailability of inhaled CBD has a range of 11–45% making for this reason the intravenously administration route preferable. With plasma pattern similar to that of THC, after its administration CBD is rapidly distributed, and due to its lipophilic nature can easily pass the blood–brain barrier. The biotransformation routes for CBD are those typically observed for phytocannabinoids, with multiple hydroxilations, oxidations to carboxylic acids, β-oxidation, conjugation and epoxidation (Harvey & Mechoulam, 1990; Samara, Bialer, & Harvey, 1990a). This turns into a prolonged elimination of CBD, with a terminal half-life of about 9 h, being preferentially excreted in the urine as free and its glucuronide compound (Samara, Bialer, & Harvey, 1990b).

CBD AND ANIMAL MODELS OF MS

The use of animal models to study a complex disease like MS has allowed not only to a better understanding about the pathophysiology of the human disease, but also to the development of preclinical testing of disease therapies. There are two main animal models to study MS: experimental autoimmune encephalomyelitis (EAE), induced by immunization against myelin, and experimental viral infection like the one induced with Theiler's murine encephalomyelitis virus (TMEV) in susceptible mice (reviewed in Mecha, Carrillo-Salinas, Mestre, Feliu, & Guaza, 2013a). Both animal models include classical MS hallmarks like inflammation, neuronal damage and demyelination, and it has been described the ability of cannabinoids to exhibit therapeutic potential using these two models.

The enhanced leukocyte trafficking is a key feature in MS, and there are available therapies designed to target CNS inflammation in its early events, such as natalizumab (Tysabri), which interferes with the homing of immune cells to the CNS and is currently prescribed for the treatment of relapsing remitting MS (Krumbholz, Derfuss, Hohlfeld, & Meinl, 2012). In this line, the treatment with cannabinoid agonists has been shown to be effective in the leukocyte rolling and adhesion to endothelial cells in

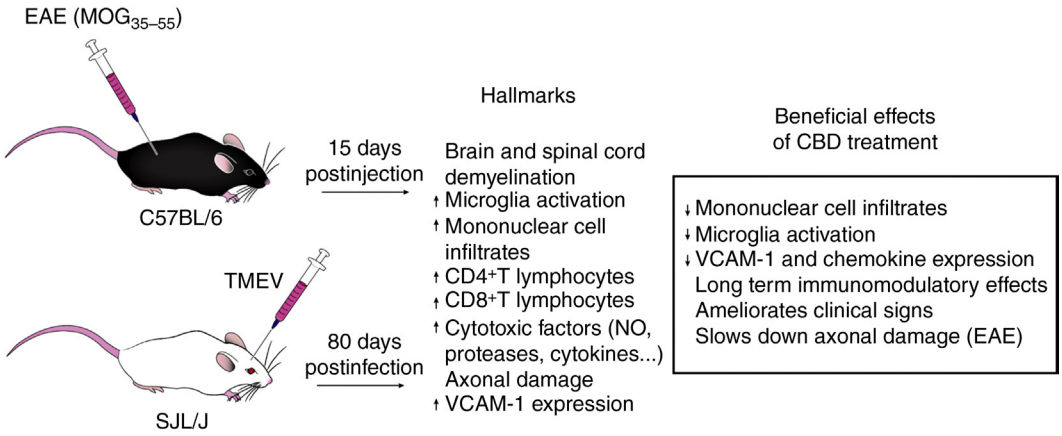


FIGURE 93.1 CBD and animal models of MS. Principal hallmarks of the two mouse models of MS, and beneficial effects of CBD (dpi, days postimmunization in the case of EAE; and days postinfection in TMEV-induced demyelinating disease).

EAE (Ni et al., 2004), as well as in the progression of the disease in TMEV infected mice through the regulation of adhesion molecules both in vivo (Mestre et al., 2009) and in vitro (Mestre et al., 2011). The immunoregulatory capacity of cannabinoids in EAE has also been reported (Cabranes et al., 2005; Maresz et al., 2007).

Regarding the effects of CBD in the animal models of MS (Fig. 93.1), only two studies have been published showing the promising therapeutic effect of this *C. sativa* derivative compound. In this line, CBD has been shown to ameliorate EAE symptomatology by diminishing inflammation, microglial activity and leukocyte homing in the spinal cord of immunized animals (Kozela et al., 2011). Lately, Mecha et al. showed in 2013 that CBD also exerts beneficial effects in TMEV-IDD induced demyelinating disease by decreasing the expression of adhesion molecules, the homing of leukocytes to the CNS, and the neuroinflammation in the acute phase of the TMEV-IDD model with long lasting-effects on the clinical course of the disease.

CBD AND MS: PERSPECTIVES

MS, one of the most common autoimmune neurodegenerative diseases, remains with an unclear trigger and still missing therapeutic solutions. Difficulties in clinical trials in patients with MS are due to the diverse and heterogeneous population varying in terms of disease type, severity, variable progression/time course, and with regard to the wide range of presenting symptoms. Beside the currently available MS treatments described in the introduction, there are other potential compounds in clinical phase that involves the immune system as a therapeutic approach (Table 93.4).

Extensive preclinical findings have reinforced the notion deduced from the anecdotal observations that cannabis derivatives may have a role in relieving symptoms in MS patients. The clinical trials focused in Sativex efficacy in the treatment of symptoms of MS, particularly spasticity and neuropathic pain. Beneficial results in placebo-controlled trials were obtained when Sativex was administered as an add-on therapy in these indications, supporting the view that Sativex is efficacious and well tolerated for the treatment of these symptoms (Collin, Davies, Mutiboko, & Ratcliffe, 2007; Wade, Collin, Stott, & Duncombe, 2010; Novotna et al., 2011). Additional trials confirmed that the CBD/THC combination reduce sleep disturbance in patients with MS-related neuropathic pain and that this treatment is mostly well tolerated (Iskedjian, Bereza, Gordon, Piwko, & Einarson, 2007; Rog, Nurmikko, & Young, 2007). Current THC analogs or Sativex trials in MS for symptomatology control have, so far, failed to demonstrate a relevant reduction of relapse, indicative of immunosupressive properties in humans (Zajicek et al., 2005). Evidence from preclinical observations underlines the interest of cannabinoids as neuroprotective agents besides their abilities as immunomodulators. However, there is a lack of preclinical and clinical studies evaluating the Sativex use in progressive MS (primary or secondary) disease as a disease modifying drug. In addition, there is a lack of clinical trials relating the administration of oral CBD alone in MS patients, besides the suggestive profile of CBD as immunomodulator, antioxidant, neuroprotective, and oligoprotective agent as we described above.

Because of the interest of CBD alone as therapeutic agent in other neurodegenerative pathologies (Table 93.5) such as Huntington disease as well as in very diverse CNS pathologies that include epilepsy, schizophrenia or even affective disorders, there are studies administering CBD-alone at different doses to healthy volunteers by oral or IV route (Zhornitsky & Potvin, 2012). On the basis of CBD effects on experimental models of MS and on CNS cell types (Fig. 93.2) future studies should investigate clinical applications of oral CBD for MS, RRMS, and PPMS/SPMS, and should also administer CBD to

TABLE 93.4 MS Therapy Pipeline

Name	Type	Route of administration	Pending indications	Phase	Mechanism of action
Laquinimod	Derivative of linomide	Oral	RRMS	III	Induces antiinflammatory cytokine profile in humans. Inhibits Th1 and Th2 leukocyte migration into de CNS.
Rituximab (Zituxam)	Chimeric human/mouse anti-CD20 monoclonal antibody	Intravenous	RRMS (approved by FDA for lymphoma and arthritis rheumatoid)	III	Depletes $CD20^{+}$B lymphocytes via cell-mediated and complement-dependent cytotoxic effects, and promotes apoptosis of these cells in the peripheral circulation
Daclizumab (Zenapax)	Humanized anti-CD25 monoclonal antibody	Subcutaneous	RRMS	III	Targets the α subunit of IL2R CD25 on activated T lymphocytes. Blocking the CD25 downregulates the proliferation of B and T lymphocytes via reduction the secretion of proinflammatory cytokines. Production of CD56 positive natural killer cells with regulatory function
Alemtuzumab (Campath-1H)	Humanized anti-CD52 monoclonal antibody	Intravenous	RRMS (approved by FDA for leukemia; better than Rebif)	III	Targets the molecule CD52, expressed on the surface of mature lymphocytes, monocytes, and macrophages. It depletes these cells via complement-mediated lysis antibody-dependent cell toxicity and apoptosis. Induces the production of neurotrophic factors by the reconstituted autoreactive T lymphocytes
Ocrelizumab	Humanized anti-CD20 monoclonal antibody	Intravenous	RRMS	II	Depletes $CD20^{+}$B lymphocytes

Perspectives for new treatments for MS patients, including the phase of the clinical trial and the expected mechanism of action (reviewed in Minagar, 2013).

TABLE 93.5 Potential Beneficial Effects of CBD in Humans

Ischemia

Huntington disease

Parkinson disease

Multiple sclerosis

Alzheimer disease

Prion diseases

Psychiatric disorders
- Obsessive compulsive behavior
- Depression
- Schizophrenia
- Anxiety
- Psychosis

Epilepsy

Cancer

Diabetes

Rheumatoid arthritis

Amyotrophic lateral sclerosis

Different diseases in which the treatment with CBD can be therapeutic based on the effects on mouse models (reviewed in Fernandez-Ruiz et al., 2013 and Pertwee, 2012).

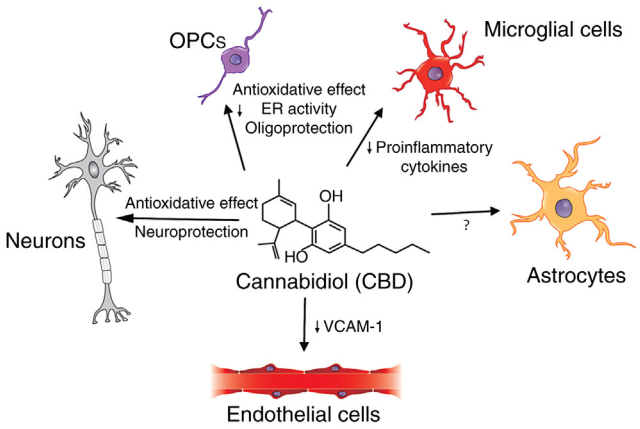


FIGURE 93.2 **Cellular targets of CBD.** Neurons, oligodendrocytes, microglial and endothelial cells can be targets of the effects of CBD in the CNS. The actions on astrocyte cells have not yet been determined.

patients for prolonged period of time in order to simulate the chronic condition of this disease. Among *C. sativa* derived compounds, CBD which lacks any unwanted psychotropic effect may represent a promising agent with the highest prospect for therapeutic use but this hypothesis needs to be proved.

MINI-DICTIONARY

Autoimmune disease An abnormal immune response of the body to tissues and molecules normally present in the body. It compromises the production of autoantibodies from the B-cells of the immune system. The treatment of autoimmunity includes immunosuppresion, to decrease the immune "self" response.
Cannabidiol One of at least 60 active cannabinoids identified in *Cannabis sativa,* counting for up to 40% of the plant's extract, considered to have a wider scope of medical applications than THC with no psychoactive effects.
Demyelination Damage to the myelin sheath that covers nerve fibers in brain and spinal cord. This provokes that nerve impulses slow or even stop, causing neurological alterations.
EAE model An animal model of CNS autoimmune inflammation that courses with inflammation and demyelination, and is used as an experimental model for the human inflammatory demyelinating disease MS. Mainly induced by inoculation with spinal cord homogenates or myelin proteins or peptides, that results in distinct models of disease course depending of the genetic background of mice.
Endocannabinoid system Lipid system of intercellular communication constituted by endogenous ligands, receptors, and the machinery of synthesis and degradation enzymes. It´s involved in a variety of physiological processes including memory, mood, pain-sensation, and appetite. Cannabinoids extracted from *C. sativa* use this endogenous system to exert their central and peripheral actions.
Multiple Sclerosis Chronic neurological disease that is pathologically characterized by multifocal inflammation, demyelination, and neuronal injury in optic nerves, brain, and spinal cord. Mainly considered as an autoimmune disease.
Neurodegeneration A condition that result in progressive loss of neuronal structure and function with neuronal death. Some neurodegenerative disorders include Parkinson, Alzheimer, and Huntington disease.
Neuroinflammation Inflammation of the central or peripheral nervous system. It can be initiated in response to infection, autoimmunity, traumatic brain injury or toxic metabolites among others. Neuroinflammation compromises microglial cells, the resident innate immune cells of the CNS, and circulating peripheral immune cells that infiltrate through the blood brain barrier.
Oligodendrocyte Myelinating cell of the CNS. Myelin acts as an insulator of axonal segments and is a prerequisite for the high velocity of nerve conduction.
Sativex Cannabinoid medicine for the treatment of spasticity due to MS which is also in development for cancer pain and neuropathic pain. Commercialized by GW Pharmaceuticals, the preparation containing CBD and Δ^9-THC is available under four different formulations that differ in the concentration of CBD and Δ^9-THC.
TMEV-IDD model An animal model of primary chronic-progressive disease that courses with inflammation, demyelination, and neurodegeneration in both brain and spinal cord, used as an experimental model for the human disease MS. Induced by the intracerebral inoculation of different strains of the Theiler's virus in susceptible mice.

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