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Cannabidiol: State of the art and new challenges for therapeutic applications

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ABSTRACT

Over the past years, several lines of evidence support a therapeutic potential of *Cannabis* derivatives and in particular phytocannabinoids. Δ^9-THC and cannabidiol (CBD) are the most abundant phytocannabinoids in *Cannabis* plants and therapeutic application for both compounds have been suggested. However, CBD is recently emerging as a therapeutic agent in numerous pathological conditions since devoid of the psychoactive side effects exhibited instead by Δ^9-THC. In this review, we highlight the pharmacological activities of CBD, its cannabinoid receptor-dependent and -independent action, its biological effects focusing on immunomodulation, angiogenetic properties, and modulation of neuronal and cardiovascular function. Furthermore, the therapeutic potential of cannabidiol is also highlighted, in particular in nuerological diseases and cancer.

Contents

Abbreviations: (5-HT)1A, 5-hydroxytryptamine receptor; Δ^9-THC, delta-9-tetrahydrocannabinol; 2-AG, 2-arachidonoylglycerol; A2A, adenosine receptor; AEA, anandamide; ALI, acute lung injury; Aml-1, acute myeloid leukemia; APP, amyloid precursor protein; AR, androgen receptor; Aβ, beta-amyloid; BDS, botanical drug substance; CBC, cannabichromene; CBD, cannabidiol; CBDV, cannabidivarin; CBG, cannabigerol; CCL, chemokine (C-C motif) ligand; CHO, Chinese hamster ovary; CNS, central nervous system; COX, cyclo-oxygenase; CRC, colorectal cancer; CYP, cytochromes P450; EAE, experimental autoimmune encephalomyelitis; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; EMT, epithelial mesenchymal transition; ER, endoplasmic reticulum; ERK, extracellular signal-regulated kinase; FAAH, fatty acid amide hydrolase; FAK, focal adhesion kinase; GPR55, G Protein Coupled Receptor-55; GSCs, glioma stem-like cells; GSH, glutathione; HIF-1α, hypoxia-inducible factor-1-α; HSP, heat shock proteins; ICAM-1, intercellular adhesion molecule 1; IFN, interferon; IL, interleukin; iNOS, inducible nitric oxide synthase; JNK, c-Jun N-terminal kinase; LAK, lymphokine-activated killer; LAK, lymphokine-activated killer; LGS, Lennox-Gastaut syndrome; MAPK, mitogen activated protein kinase; MCP-1, monocyte chemoattractant protein-1; MDSC, myeloid-derived suppressor cells; MHRA, medications health care products regulation agency; MIP-2, macrophage inflammatory protein-2; MMP, matrix metalloproteinases; MOG, myelin oligodendrocyte glycoprotein; MT1-MMP, membrane type 1-matrix metalloproteinase 1; NF-kB, nuclear factor-k B; NK, natural killer; NKT, natural killer T; NMDA, *N*-methyl-D-aspartate receptor; NO, nitric oxide; NREM, non-REM; PAI-1, plasminogen activator inhibitor; PARP, poly (ADP ribose) polymerase; PI3K, phosphatydilinositol-3-kinase; PPARγ, peroxisome proliferator-activated receptor; RBD, REM behaviour disorder; REM, rapid eye movement; ROS, reactive oxygen species; SOD, superoxide dismutase; STAT, signal transducer and activator of transcription; TAM, tumor-associated macrophages; TH, tyrosine hydroxylase; THCV, tetrahydrocannabivarin; TIMP-1, matrix metalloproteases-1; TNF-α, tumour necrosis factor-alpha; TRPM8, transient receptor potential melastatin type-8; TRPV, transient potential vanilloid receptor; VCAM-1, vascular cell adhesion molecule-1.

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1. Introduction

1.1. Phytocannabinoids: focus on CBD

Cannabis sativa contains hundreds of chemical entities produced by secondary metabolism including, beyond cannabinoids, terpenes and phenolic compounds, each one with potential interesting biological properties (Andre, Hausman, & Guerriero, 2016). Known cannabinoids are more than 90, even if some derive from breakdown reactions. Currently, the scientific community indicates with the term 'cannabinoid' these terpenophenols derived from *Cannabis sativa* but also synthetic compounds able to directly or indirectly act on cannabinoid receptors (Appendino, Chianese, & Taglialatela-Scafati, 2011). Delta-9-tetrahydrocannabinol (Δ^9-THC) is the main component of *Cannabis sativa* and the first cannabinoid to be discovered and studied, well known for its psychoactive effects (Russo, 2011). Among the other major phytocannabinoids isolated from the plant there are: CBD (Mechoulam & Shvo, 1963), cannabichromene (CBC) (Gaoni & Mechoulam, 1966), cannabigerol (CBG) (Gaoni & Mechoulam, 1964), cannabidivarin (CBDV) and tetrahydrocannabivarin (THCV) (Gill, Paton, & Pertwee, 1970; Vollner, Bieniek, & Korte, 1969) (Table 1). Although these compounds have similar chemical structures, they can elicit different pharmacological actions. Mainly, their pharmacological properties rely on the interaction with components of the endocannabinoid system machinery like cannabinoid receptors and enzymes of endocannabinoid synthesis and degradation. Focusing on CBD, it is well known that this compound is the second major comonent of the plant, the most prevalent in the fibre-type hemp, it is not associated with psychoactivity and does not affect motor function, memory or body temperature on its own. It displays with respect to Δ^9-THC lower CB_1 and CB_2 receptor affinity (Bisogno et al., 2001; Pertwee, 1999; Showalter, Compton, Martin, & Abood, 1996; Thomas, Gilliam, Burch, Roche, & Seltzman, 1998) and it was found to be an inverse agonist at the human CB_2 receptor, property that may contribute to its anti-inflammatory effects (Thomas et al., 2007). Beyond numerous *per se* pharmacological effects, CBD acts as an entourage molecule, reducing the collateral effects of Δ^9-THC, thus ameliorating its safety profile.

1.2. Overview of the pharmacological action of CBD

Thanks to its good safety profile and the lack of psychoactivity, CBD is undoubtedly the more interesting cannabinoid with a lot of reported pharmacological effects in several models of pathologies, ranging from inflammatory and neurodegenerative diseases, to epilepsy, autoimmune disorders like multiple sclerosis, arthritis, schizophrenia and cancer (Table 2). CBD shows lower CB_1 and CB_2 receptor affinity with respect to Δ9-THC. In the presence of Δ^9-THC, it is able to antagonize CB_1 at low nanomolar concentrations, finding that supports its regulatory properties on Δ^9-THC related adverse effects like tachycardia, anxiety, sedation and hunger in humans and rats (Murillo-Rodríguez, Millán-Aldaco, Palomero-Rivero, Mechoulam, & Drucker-Colín, 2006; Nicholson, Turner, Stone, & Robson, 2004; Russo & Guy, 2006). Indeed, both human and animal studies suggest anxiolytic properties associated with CBD. In generalized social anxiety disorders, CBD significantly decreased anxiety in patients and such effect was associated with its action on paralimbic and limbic areas as revealed by single photon emission

Table 1
Molecular structure and mechanism of action of phytocannabinoids.

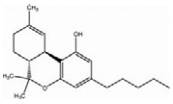
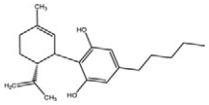
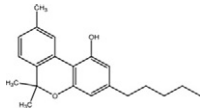
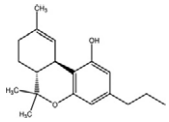
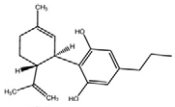
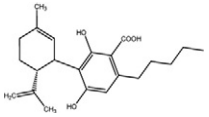
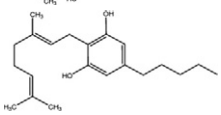
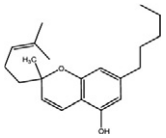
Phytocannabinoids		
Δ^9-tetrahydrocannabinol (Δ^9-THC)		Psychoactive. Most abundant in drug-type plants Partial agonist CB1 ≈ CB2
Cannabidiol (CBD)		Non psychoactive. Most abundant in fiber-type plants. Not specific antagonist of CB1 e CB2 Inhibitor of AEA uptake and metabolism
Cannabinol (CBN)		Weak CB1 agonist, partial CB2 agonist
Δ^9-tetrahydrocannabivarin		Δ9-THCV antagonizes Δ9-THC at low doses (<3 mg/kg) CB1 agonist at greater doses (10 mg/kg)
Cannabidivarin (CBDV)		Mechanism of action unknown
Cannabidiolic acid		Selective inhibitor of COX2 TRPA1 and TRPV1 agonist
Cannabigerol		TRPA1 and TRPV1 agonist CB agonist; inhibitor of AEA reuptake
Cannabichromene		TRPA1 agonist Inhibitor of AEA reuptake

Table 2
Overview of CBD pharmacological effects

Disease	Effects	Refs
Alzheimer's disease	Antinflammatory, antioxidant, antiapoptotic in *in vitro* and *in vivo* models of Aβ-evoked neuroinflammatory and neurodegenerative responses.	Hayakawa et al. (2007), Esposito et al. (2006a), Esposito et al. (2006b), Martín-Moreno et al. (2011), Scuderi et al. (2014), Cheng et al. (2014)
Parkinson's disease	Attenuation of the dopaminergic impairment *in vivo*; neuroprotection; improvement of psychiatric rating and reduction of agitation, nightmare and aggressive behaviour in patients.	Lastres-Becker et al. (2005), Zuardi et al. (2009), Chagas, Eckeli, et al. (2014)
Multiple sclerosis	Improved signs of EAE in mice, antinflammatory and immunomodulatory properties.	Buccellato et al. (2011), Kozela et al. (2011, 2015), Mecha et al. (2013), Giacoppo et al. (2015)
Epilepsy	Anticonvulsant *in vitro* and *in vivo*; reduced seizures frequency in children and adults with treatment-resistant epilepsy.	Pertwee (2008), Devinsky et al. (2016)
Huntington's disease	Neuroprotective and antioxidant in mice transgenic models; no significant clinically important differences in patients.	Iuvone et al. (2009), Sagredo et al. (2011), Consroe et al. (1991)
Hypoxia-ischemia injury	Short term neuroprotective effects; inhibition of excitotoxicity, oxidative stress and inflammation *in vitro* and in rodent models.	Pazos et al. (2012, 2013), Hayakawa et al. (2007, 2009), Valdepeñas et al. (2011)
Pain	Analgesic effect in patients with neuropathic pain resistant to other treatments.	Petzke et al. (2016), Boychuk et al. (2015)
	Attenuation of the behavioural and glial changes in animal models of schizophrenia; anti-psychotic properties on ketamine-induced symptoms.	Crippa et al. (2015), Gomes et al. (2015), Zuardi et al. (2006, 2012)
Anxiety	Reduction of muscular tension, restlessness, fatigue, problems in concentration, improvement of social interactions in rodent models of anxiety and stress; reduced social anxiety in patients.	Lemos et al. (2010), Almeida et al. (2013), Moreira et al. (2006), de Mello Schier et al. (2014), Bergamaschi et al. (2011), Marinho et al. (2015)
Depression	Anti-depressant effect in genetic rodent model of depression.	El-alfy et al. (2010), Hsiao et al. (2012), Shoval et al. (2016)
Cancer	Antiproliferative and anti-invasive actions in a large range of cancer types; induction of autophagy-mediated cancer cell death; chemopreventive effects.	Ligresti et al. (2006), McAllister et al. (2011), Shrivastava et al. (2011), Pisanti et al. (2013), Rocha et al. (2014), Ramer et al. (2014), Scott et al. (2014)
Nausea	Suppression of nausea and conditioned gaping in rats	Parker et al. (2002), Rock et al. (2008)
Inflammatory diseases	Antinflammatory properties in several in vitro and in vivo models; inhibition of inflammatory cytokines and pathways.	Ribeiro et al. (2012, 2015), Kozela et al. (2010, 2011), Mecha et al. (2012, 2013)
Rheumatoid arthritis	Inhibition of TNF-α in an animal model	Malfait et al. (2000)
Infection	Activity against methicillin-resistant *Staphylococcus aureus*	Appendino et al. (2008)
Inflammatory bowel and Chron's diseases	Inhibition of macrophage recruitment and TNF-α secretion *in vivo* and *ex vivo*; reduction in disease activity index in Chron's patients.	Sacerdote et al. (2005), De Filippis et al. (2011), Naftali et al. (2011)
Cardiovascular diseases	Reduced infarct size through anti-oxidant and anti-inflammatory properties *in vitro* and *in vivo*.	Durst et al. (2007), Booz (2011), Stanley et al. (2013)
Diabetic complications	Attenuation of fibrosis and myocardial dysfunction	Weiss et al. (2006, 2008), Rajesh et al. (2010), Kozela et al. (2010)

computed tomography (Crippa et al., 2011). Considering CBD low adverse-effect profile (Zuardi, Morais, Guimarães, & Mechoulam, 1995), it is believed a potential antipsychotic drug. In addition, it is also cosidered an interesting possible curative drug for cancer, diabetes, inflammation and neurodegenerative disorders (Izzo & Camilleri, 2009). CBD is also an anticonvulsant (Carlini & Cunha, 1981; Jones et al., 2010), neuroprotective antioxidant (Hampson, Grimaldi, Axelrod, & Wink, 1998), analgesic (Costa, Trovato, Comelli, Giagnoni, & Colleoni, 2007) and anti-nausea molecule (Parker, Mechoulam, & Schlievert, 2002). CBD is analog to capsaicin and behaves as a TRPV1 (transient potential vanilloid receptor type 1) agonist, however it does not show noxious effects (Bisogno et al., 2001).Moreover, it is able to inhibit AEA uptake and to weakly prevent its hydrolysis. Furthermore, CBD shows cytotoxicity in breast tumour cells (Ligresti et al., 2006) and is cyto-preservative for normal cells (Parolaro & Massi, 2008). CBD in an animal model of rheumatoid arthritis, is also able to antagonize tumour necrosis factor-alpha (TNF-α) (Malfait et al., 2000), increases A2A adenosine receptor signaling by inhbiting an adenosine transporter (Carrier, Auchampach, & Hillard, 2006), and prevents neural toxicity and prion accumulation (Dirikoc, Priola, Marella, Zsürger, & Chabry, 2007). In a murine model, a CBD extract demonstrated higher anti-hyperalgesic effect with respect to pure compound, with ameliorated thermal perception and reduced oxidative damage and allodynia, (Comelli, Bettoni, Colleoni, Giagnoni, & Costa, 2009). In addition, CBD showed potent effects against methicillin-resistant *Staphylococcus aureus* (Appendino et al., 2008). Agonistic properties at 5-hydroxytryptamine (5-HT)1A have been also ascribed to CBD (Russo, Burnett, Hall, & Parker, 2005), data that may underlie its anti-anxiety (Soares Vde et al., 2010) and anti-nausea properties (Rock, Limebeer, Mechoulam, Piomelli, & Parker, 2008), reduction of stroke risk (Mishima et al., 2005), and ability to ameliorate cognition in a hepatic encephalopathy murine model (Magen et al., 2009). It was also suggested the use of CBD as antidepressant, since in the forced swim test, it reduced immobility time. This effect was inhibited by WAY100635, a 5-HT1A antagonist (Zanelati, Biojone, Moreira, Guimarães, & Joca, 2010). An interesting observation about lymphopenia in rats suggests that CBD antagonism to Δ^9-THC would be mediated by inverse agonism at the CB_2 receptor (Ignatowska-Jankowska, Jankowski, Glac, & Swiergel, 2009), however, such activity has not been described in humans (Crippa, Zuardi, & Hallak, 2010). CBD has been showed to be a crucial factor in the oromucosal extract nabiximols in the treatment of tumour pain of patients unresponsive to opioids, since its absence in a high-THC extract failed to distinguish from placebo (Johnson et al., 2010). Such finding suggests synergysm of THC and CBD in eliciting higher effects than a summation of those obtained from the drugs used alone (Berenbaum, 1989).

1.3. CB_1 and CB_2 receptor-dependent action of CBD

Given the low affinity of CBD for both the cannabinoid receptors, most of the pharmacological studies on CBD were directed to search CB_1 and CB_2 receptor independent action. However, some evidence suggests interaction of CBD with cannabinoid receptors at low doses. CBD is able to antagonize cannabinoid CB_1/CB_2 receptor agonists WIN55212 and CP55940 in the range of nanomolar in brain membranes of mice and in membranes of CB_2 receptor-tranfected Chinese hamster ovary (CHO) cells (Thomas et al., 2007). When CBD is administered at a concentration able to antagonize WIN55212 and CP559540, it blocks [^{35S}]GTPγS binding to mouse brain membranes, effect in part mediated by the CB_1 receptor. However, in the same assay, CBD was not less efficient to block [^{35S}]GTPγS binding to $CB_1^{-/-}$ than to wild-type mouse brain, thus suggesting a CB_1-receptor-independent component in its

inverse action. Such inhibitory effect has also been revealed in membranes of human CB_1-CHO cells (MacLennan, Reynen, Kwan, & Bonhaus, 1998; Thomas et al., 2007), whereas no block was observed in membranes of untransfected CHO cells (Thomas et al., 2007). The antagonist effect of CBD versus CP559540 and WIN55212 is consistent with previous investigations showing that CBD at the dose of 10 mM antagonizes CP55940-induced stimulation of [^{35S}] GTPγS binding to rat cerebellar membranes (Petitet, Jeantaud, Reibaud, Imperato, & Dubroeucq, 1998), that is able to antagonize WIN55212 and CP55940 in the mouse isolated vase deferens (Pertwee & Ross, 2002) and that blocks several *in vivo* responses to Δ^9-THC in mice, rats, rabbits and humans (Pertwee, 2005). A very recent study shows that CBD is a negative allosteric modulator of the CB_1 receptor (Laprairie, Bagher, Kelly, & Denovan-Wright, 2015). Allosteric modulators of this receptor have the potential to cure central nervous system and peripheral disorders and avoid the adverse effects associated with orthosteric agonism or antagonism of CB_1 receptor (Bagher, Laprairie, Kelly, & Denovan-Wright, 2016; Laprairie et al., 2015). In CB_2 receptor-CHO cell membranes, CBD inhibited [^{35S}] GTPγS binding (MacLennan et al., 1998; Thomas et al., 2007). CBD is likely to produce such antagonism to CP55940 in a non-competitive manner, by contrasting the ability of this agonist to activate CB_2 receptors (Thomas et al., 2007). More data are needed to clarify if CBD has inverse agonist effects in tissue expressing the CB_2 receptors or if it can interact with additional targets to elicit inverse effects in brain tissues and whether these interactions are additive or synergic in nature. The fact that CBD can exert CB_2 receptor inverse agonism may in part explain its known anti-inflammatory effects (Pertwee, 2005). Evidence supports that CB_2 inverse agonism can block migration of immune cells and decrease inflammation (Lunn, Reich, & Bober, 2006); indeed, CBD potently inhibits migration of macrophages, microglial cells and neutrophils (McHugh et al., 2006; Sacerdote et al., 2005; Walter et al., 2003). It is likely that other actions of CBD can contribute to reduce inflammation, or modulation of microglial cell migration might be mediated by a CBD specific receptor that has not been identified to date (Walter et al., 2003). In support of a potential activation of CB_2 receptor by CBD there is the finding that CBD-induced block of chemotaxis of macrophages can be prevented by SR144528, a CB_2 selective antagonist (Sacerdote et al., 2005). In addition, CBD potently inhibits forskolin-stimulated cyclic AMP production by human CB_2 receptor-expressing CHO cells (Gauson, Stevenson, & Thomas, 2007). Further studies may clarify which of CBD actions contribute to its anti-inflammatory effects and if they are CB receptor-dependent.

1.4. CB_1 and CB_2 receptor-independent action of CBD

Beyond CB_1 and CB_2 receptors, other targets of CBD have been identified. In particular, CBD and its (+) enantiomer can act on the transient potential vanilloid receptor type-1 (TPVR-1), exhibiting an action similar to that elicited by the natural TPVR-1 agonist capsaicin, both *in vitro* (Bisogno et al., 2001) and in an animal model of acute inflammation (Costa, Giagnoni, Franke, Trovato, & Colleoni, 2004). Furthermore, in the search for sites in charge of CBD activity, it was observed that CBD binds as an agonist to the serotonin receptor 5-HT1A and this interaction (Russo et al., 2005) enhances its activity and allosterically regulates μ and δ opioid receptors in rat cerebral cortex membrane homogenates (Kathmann, Flau, Redmer, Trankle, & Schlicker, 2006). Finally, CBD significantly antagonizes the orphan receptor GPR55 at nanomolar to micromolar concentrations (Brown, 2007).

1.5. CBD pharmacodynamics, pharmacokinetics, metabolism and toxicology

To date, the pharmacodynamics of CBD remains unclear in many aspects; however, its pharmacokinetics seems better defined. Once orally consumed, after a first-pass effect, CBD bioavailability is between 13% and 19%, thus suggesting the intravenous administration as preferable (Grotenhermen, 2003). After injection CBD, that is lipophilic, quickly diffuses and easily crosses the blood–brain barrier (BBB), while in turn its elimination is prolonged (Grotenhermen, 2003). Metabolism of CBD is regulated by biotransformation routes usually observed for phytocannabinoids (Harvey & Mechoulam, 1990; Samara, Bialer, & Harvey, 1991), although several metabolic pathways have been described in different animal species and in humans. Furthermore, CBD is subjected to multiple reactions like hydroxylation, oxidation to carboxylic acids, conjugation, epoxidation and beta-oxidation (Harvey & Mechoulam, 1990; Samara, Bialer, & Harvey, 1990a). Finally, CBD in its free state and in its glucuronide derivative is primarily excreted from urine and has a half-life of 9 h (Samara, Bialer, & Harvey, 1990b). Taking advantage of the lack of psychotropic effects, efforts tryed to define its toxicological profile. Thus, very low toxicity has been ascribed to CBD both in humans and in other species (Rosenkrantz, Fleischman, & Grant, 1981). Indeed, CBD does not show teratogenic or mutagenic activities (Rosenkrantz & Hayden, 1979). In different animal species, CBD seems to interfere with hepatic drug metabolism of some compounds (Samara, Brown, & Harvey, 1990) by inactivating cytochrome P450s (CYP450) of 3A and 2C subfamilies. Such interactions have to be considered in case of CBD co-administration with other drugs metabolized through these routes.

2. Biological effects of CBD

2.1. CBD and the immune system: from pre-clinical data to clinical practice

CBD has been largely characterized for its action on the immune compartment and the observed properties led it to be tested in several *in vitro* and *in vivo* disease models of inflammation. Although the effects can be different with its concentration or magnitude or type of immune stimulus (Karmaus, Wagner, Harkema, Kaminski, & Kaplan, 2013), overall CBD has been shown to exert immunosuppression through various other receptors in addidtion to the canonical CB_1 and CB_2. First, in a murine model of lipopolysaccharide (LPS)-induced acute lung injury (ALI), CBD by enhancing the endogenous adenosine signaling, mainly through the inhibition of its uptake, potently reduced the inflammatory lung response in an adenosine A2A receptor-dependent manner (Ribeiro et al., 2012). Later evidence by the same group showed that CBD was also able to decrease total lung resistance and elastance, neutrophils, macrophages and lymphocytes migration into the lungs, mieloperoxidase activity in tissue and the production of both pro-inflammatory cytokines tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and chemokines monocyte chemoattractant protein-1 (MCP-1) and macrophage inflammatory protein-2 (MIP-2) in the bronchoalveolar lavage supernatant (Ribeiro et al., 2015). Of course, the resulting clear improvement of lung function suggests the possible beneficial effects of CBD in the treatment of inflammatory lung diseases.

Various *in vitro* and *in vivo* evidence also support a role for CBD in inflammatory degenerative diseases. First because CBD had a strong ability to inhibit the production of inflammatory cytokines, including IL-1β, IL-6, and interferon-β (IFN-β), in LPS-stimulated murine microglial cells (Kozela et al., 2010). Indeed microglia act as primary responding cells for pathogen infection and injury, but a prolonged or excessive activation may result in pathological forms of inflammations that contribute to the progression of neurodegenerative (Parkinson's and Alzheimer's diseases, multiple sclerosis and HIV-associated dementia) and neoplastic diseases (Saijo & Glass, 2011). Also in this case the effects are not mediated *via* CB_1, CB_2, nor abn-CBD-sensitive receptors, while it has been documented its ability to decrease the activity of the nuclear factor-k B (NF-kB) signaling pathway and that of signal transducer and activator of transcription 1 (STAT1) transcription factor, key player in IFN-β-dependent proinflammatory processes (Kozela et al., 2010). In a viral model of multiple sclerosis, in addition to the attenuation of microglia activation, CBD also decreased the transmigration of blood leukocytes by downregulating the expression of vascular cell adhesion molecule-

1 (VCAM-1, vascular cell adhesion molecule-1) and chemokines (chemokine C-C motif ligand 2 (CCL2) and 5 (CCL5)), partially through Adenosine A2A receptors (Mecha et al., 2013). As expected, these actions resulted in an amelioration of motor deficits (Mecha et al., 2013), as well as of the severity of the clinical signs of autoimmune encephalomyelitis (EAE) in myelin oligodendrocyte glycoprotein (MOG)-injected mice, where CBD primarly suppressed microglial activity and the proliferation of encephalitogenic T cells (Kozela et al., 2011). In this context, its additional neuroprotective effects, which included the up-regulation of a number of anti-oxidative genes (e.g. those of glutathione synthesis) in CBD exposed microglial cells (Juknat et al., 2012) and protection of oligodendrocyte progenitor cells from inflammation-induced apoptosis (Mecha et al., 2012), may significantly enhance its anti-inflammatory beneficial properties and account for the alleviation of MS (multiple sclerosis) pathology for which it is already used as therapeutic agent in combination with Δ^9-THC (e.g., Sativex®).

Moreover, by inhibiting adenosine uptake, which in turn activates A2A receptors in the retinal microglial cells, CBD exerted an anti-inflammatory effect in the retina, as evidenced by decreased TNF-α secretion after LPS treatment (Liou et al., 2008). These findings should help develop novel CBD-based therapeutics to treat retinal inflammatory disorders (uveitis, age-related macular degeneration, diabetes, and glaucoma).

The reduction of intestinal inflammation through the control of neuroimmune axis exerted by CBD also configures this molecule as a promising drug for the therapy of inflammatory bowel disease, especially Chrohn's disease. More in detail, Sacerdote et al. documented the CBD modulation of IL-12 and IL-10 secretion in mouse peritoneal macrophages and showed that CBD decreased the formyl-methionyl-leucyl-phenylalanine-induced chemotaxis of macrophages in a CB2-dependent manner (Sacerdote et al., 2005). But more interestingly, CBD demonstrated the capability to mediate a strong inhibition of recruitment of mast cells and macrophages in the intestine of LPS-treated mice as well as a significant reduction of TNF-α secretion in *ex vivo* cultured human derived intestinal biopsies from patients with ulcerative colitis, achieving a reduction of intestinal damage principally mediated by peroxisome proliferator activated receptor-γ (PPAR-γ) receptor pathway (De Filippis et al., 2011). These observations may explain the significant reduction in disease activity index, as assessed by the Harvey Bradshaw index for Crohn's disease, in a first retrospective observational study in a cohort of 30 patients treated with Δ^9-THC and CBD (Naftali, Lev, Yablecovitch, Half, & Konikoff, 2011). Lymphocytes constitute another key target of the immunomodulatory action of CBD. Specifically, CBD exhibited a generalized suppressive effect on T-cell functional activities, *via* (a) inducing CD11b(+)Gr-11(+) myeloid-derived suppressor cells (MDSC) (Hegde, Nagarkatti, & Nagarkatti, 2011; Hegde, Singh, Nagarkatti, & Nagarkatti, 2015), (b) inducing a caspase 8-dependent apoptosis (Lee et al., 2008; Wu et al., 2008), (c) inhibiting their proliferative potential (Kozela et al., 2011), (d) reducing cytokine secretion including IL-17, a key autoimmune factor (Kozela et al., 2013, 2016), (e) inducing anergy (Kozela et al., 2015), and (f) hampering antigen presentation and promoting T cell exhaustion/tolerance (Kozela et al., 2016). These findings underpin other mechanisms by which CBD exerts its anti-inflammatory action as well as explain the beneficial role of CBD in pathological memory T cells and in autoimmune diseases. However while a lot of evidence in pre-clinical disease models make CBD an attractive therapeutic tool for the attenuation and treatment of inflammatory conditions, little is known about its effects on physiological immune response. Indeed it is tempt to speculate that, through the same mechanisms and cellular targets, this natural compound may also dampen host defence against invading pathogens (e.g. helminth parasites and infectious agents). For example, activated microglial cells shown to exacerbate inflammation, first are important in the removal of cellular debris and fighting infection, so care should be taken when translating data to human clinical practice and a more precisely understanding of its possible negative consequences on healthy counterpart is mandatory.

But interestingly, while CBD exerted a multimodal inhibition of T cell compartment, in a recent study in mice, assessing the impact of repeated, systemic administration of CBD on peripheral blood lymphocyte cell subsets distribution, it selectively increased the total number of Natural Killer T (NKT) cells and the percentage of NKT and NK cells (Ignatowska-Jankowska et al., 2009). These findings fit well with the increased production of IL-12, a well known NK-stimulatory cytokine, by CBD-treated murine macrophages (Sacerdote et al., 2005). To our knowledge, these are some of few reports concerning the ability of CBD to stimulate immune responses. Indeed NK cells are thought to play an important role in non-specific antiviral response and cancer immune surveillance (Ali et al., 2014; Childs & Carlsten, 2015; Ciaglia et al., 2014). Concerning this last aspect, the antitumor effect of CBD, that will be discussed below, might be in part related to the boosting of cytotoxic activity of NK cells both in direct but more interestingly, in indirect immunogenic manner. Indeed, by inducing upregulation of intercellular adhesion molecule-1 (ICAM-1) on primary lung cancer cells, CBD demonstrated the surprising ability to enhance susceptibility of cancer cells to lymphokine-activated killer (LAK) cells (Haustein, Ramer, Linnebacher, Manda, & Hinz, 2014). The new role of CBD in immunological antitumor responses was also corroborated by the strong inhibition of the recruitment of tumor-associated macrophages (TAM) in stroma and secondary lung metastases of primary breast cancers, primarly by lowering levels of granulocyte-macrophage colony-stimulating factor (GM-CSF) and CCL-3 cytokines which are crucial for TAM recruitment and activation (Elbaz et al., 2015). These last interesting observations enhance our understanding of the effects of CBD on the immune system and highlight its novel mechanisms of tumor suppression by tumor microenvironment modulation.

2.2. Roles of CBD in the nervous system

A lot of collected evidence highlights that CBD operates on brain signaling systems and that this action is at the basis of its therapeutic effects in nervous system pathologies (Borgelt, Franson, Nussbaum, & Wang, 2013). Pre-clinical research shows that CBD has neuroprotective, antioxidant, analgesic, anti-psychotic and anti-anxiety properties, not acting through the CB_1 receptor but interacting with other targets that may be relevant in neurologic disorders (Zlebnik & Cheer, 2016). Several reports show how CBD can preserve neuronal structure and function, in cell cultures as well as in animal models of numerous neurodegenerative diseases, including Alzheimer's and Parkinson's diseases (Martín-Moreno et al., 2011), stroke and multiple sclerosis (García-Arencibia et al., 2007; Pryce, Riddall, Selwood, Giovannoni, & Baker, 2014).

Recent studies reported below, highlight that CBD is able to cope with oxidative stress, mitochondrial dysfunction, inflammatory changes, excitotoxicity, iron accumulation, and protein aggregation, all features of neurodegeneration. An *in vitro* study reported a beneficial effect of CBD in reducing infarct size in stroke models and against epithelial barrier damage in human brain microvascular endothelial cell and astrocyte co-cultures models. CBD produced neuroprotection in ischaemic stroke, through a mechanism mediated by PPAR-γ and 5-HT1A receptors (Hind, England, & O'Sullivan, 2016). Moreover, in support of this finding, an *in vivo* study demonstrated that CBD has short-term neuroprotective effects in the immature brain following hypoxia-ischemia injury. As a matter of fact, in Newborn Wistar rats that underwent to hypoxia-ischemia injury, CBD modulated brain excitotoxicity, oxidative stress and inflammation (Pazos et al., 2012). Such effect was dependent on the involvement of both CB_2 and 5HT(1A) receptors, as subsequently confirmed also in Human Embryonic Kidney 293 cells (Pazos et al., 2013). Another mechanism of neuroprotection exerted by CBD is mediated by the up-regulation of the mRNA levels for Cu–Zn superoxide dismutase, an important enzyme in endogenous defenses against oxidative stress (García-Arencibia

et al., 2007). Ryan, Drysdale, Lafourcade, Pertwee, & Platt, 2009, characterized the mechanisms by which CBD regulates Ca^{2+} homeostasis and mediates neuroprotection in neuronal preparations, underlining that CBD may be beneficial in preventing apoptotic signaling via a restoration of Ca^{2+} homeostasis. CBD has been proposed to restore the damage caused by iron loading in rats, that affects mitochondrial dynamics, possibly triggering synaptic loss and apoptotic cell death (Da Silva et al., 2014). Finally, Santos et al., 2015, using as experimental model the PC12 cell line, derived from a pheochromocytoma of the rat adrenal medulla, suggested that CBD, with his neurorestorative potential independent of NGF, might help neuroprotection against the neurotoxin MPP+; indeed, CBD increased cell viability, differentiation, and the expression of axonal (GAP-43), synaptophysin and synapsin I proteins.

The scientific evidence showing that CBD has an analgesic effect and the explanation of how it fulfills this function is not yet clear and probably, the same effect can be attributed to the anxiolytic properties of CBD that may have an influence on the behavior of pain. Randomized controlled studies show that cannabinoids administration in the management of pain is marginally superior to placebo in terms of efficacy and inferior in terms of tolerability (Petzke, Enax-Krumova, & Häuser, 2016). Furthermore the drug-drug interactions profile is poorly documented; certainly CYP450 2C9 and 3A4 are involved in the metabolism of CBD, which implies possible interactions with CYP450 inhibitors and inducers, such as anti-convulsivants and HIV protease inhibitors, prescribed in patients with neuropathic pain. Nevertheless, *Cannabis*-based medicinal extracts used in different populations of chronic non-malignant neuropathic pain patients may supply effective analgesia in conditions that are refractory to other treatments (Boychuk, Goddard, Mauro, & Orellana, 2015).

To date, there are few small-scale clinical trials in which patients with psychotic symptoms have been treated with CBD. Since most patients with schizophrenia are not always good responders to currently available pharmacological treatments, CBD, through the inhibition of anandamide reuptake and thanks to its multiple effects on the nervous system, could be used for the prevention and treatment of psychosis (Crippa, Hallak, Abílio, de Lacerda, & Zuardi, 2015). Also, an increasing number of data has linked schizophrenia with neuroinflammatory conditions and it has been reported that the treatment with CBD (30 and 60 mg/kg) attenuates the behavioral and glial changes observed in an animal model of schizophrenia based on the *N*-methyl-D-aspartate (NMDA) receptor hypofunction (Gomes et al., 2015). This could be an objective first signal of the therapeutic potential of CBD for patients affected by schizophrenia. The antipsychotic effect of CBD is possibly mediated by the activity patterns in key brain regions implicated in the pathophysiology of schizophrenia opposite to Δ^9-THC, such as the hippocampus, striatum and prefrontal cortex, as demonstrated previously by the fMRI results (Iseger & Bossong, 2015; Zuardi et al., 2012). The evidence for the antipsychotic-like properties of CBD was supported by the results of two studies on healthy volunteers using perception of binocular depth inversion and ketamine-induced psychotic symptoms (Zuardi, Crippa, Hallak, Moreira, & Guimarães, 2006). However, large randomized clinical trials would be fundamental to fully disclose the therapeutic potential of CBD for patients with schizophrenia and other forms of psychosis (Schubart et al., 2014). Until now, CBD has shown therapeutic efficacy in a range of animal models of anxiety and stress, reducing muscular tension, restlessness, fatigue, problems in concentration, both behavioural and physiological (e.g., heart rate) measures of stress and anxiety (Lemos, Resstel, & Guimarães, 2010). Considering that spontaneously hypertensive rats present a deficit in social interaction and hyperlocomotion, and that when they are treated with typical and atypical antipsychotics their symptom ameliorated, the effects of CBD on social interaction presented by control animals Wistar and spontaneously hypertensive rats were also investigated (Almeida et al., 2013). A low dose of CBD (1 mg/kg) increased passive and total social interaction of rats. Nevertheless, the deficit in social interaction and the hyperlocomotion were not altered by CBD at any dose tested. The results of this study in hypertensive rats do not support an antipsychotic property of cannabidiol on symptoms-like behaviors in the context of hypertension, but reinforce the anxiolytic profile of this compound in normal conditions. Moreira, Aguiar, & Guimarães, 2006, tested the effects of CBD in the Vogel test, a widely used animal model of anxiety, also examining if they are dependent on benzodiazepine-receptor activation. CBD induced an anti-conflict effect not mediated by benzodiazepine receptors or by non-specific drug interference on nociceptive threshold or water consumption, strengthening the hypothesis of its anxiolytic properties. Other similar experiments that have exploited animal models through a variety of texts usually used to study anxiety disorders, such as the forced swimming test, the elevated plus maze and the Vogel conflict test, suggested that CBD exhibits anti-anxiety and antidepressant effects. Such effects do not depend on the activation of CB_1 and CB_2 receptors, whereas a good interaction between CBD and the 5-HT1A neuro-receptor has been observed (de Mello Schier et al., 2014). Furthermore, CBD has shown efficacy in small human laboratory and clinical trials. CBD reduced anxiety, cognitive impairment and discomfort in speech performance in patients with social anxiety subjected to a stressful public speaking task (Bergamaschi et al., 2011). Pre-treatment with CBD also significantly decreased alert in anticipatory speech. In the same year, Crippa et al., 2011, investigated brain mechanisms in patients with generalized social anxiety disorder using functional neuroimaging; the study suggested that CBD reduces anxiety and its activity is correlated to its effects in paralimbic and limbic brain areas. Such effects in the limbic brain areas depend on the nature of the animal model, being influenced by previous stressful experiences and mediated by facilitation of 5HT1A receptors neurotransmission, although the precise mechanism remains to be elucidated (Campos et al., 2013; Marinho, Vila-Verde, Fogaça, & Guimarães, 2015).

The last five years studies suggest that CBD may be beneficial for the treatment of clinical depression and other conditions characterized by prominent anhedonia. An antidepressant-like activity of CBD that demonstrates endogenous hormonal and behavioral abnormalities that emulate many of those found in symptom-presenting depressive patients was reported in mice (El-alfy et al., 2010). CBD increased exploration of the novel object and locomotion, indicating an improvement in the characteristically low motivation of these rats to explore. These findings extend the anti-depressant effect of CBD, shown for the first time in a genetic animal model of depression (Shoval et al., 2016). Moreover, CBD has been reported to reduce physiological rapid eye movement (REM) sleep and non-REM (NREM) sleep in normal rats, in addition to generating its anxiolytic effect. CBD efficiently stops anxiety-induced REM sleep suppression, but has little effect on the alteration of NREM sleep, possibly due to its anxiolytic effect, rather than through a direct regulation of sleep mechanisms. This is an important result for patients with post-traumatic stress disorder that often complain of having sleep disturbances, such as REM sleep abnormality and insomnia (Hsiao, Yi, Li, & Chang, 2012).

Future clinical trials involving patients with different anxiety disorders are required, especially of obsessive-compulsive, panic, post-traumatic stress and social anxiety disorders in order to definitely claim the therapeutic effectiveness of CBD in nervous system pathologies.

2.3. Cardiovascular effects of CBD

Several studies in recent years, have demonstrated that the cardiovascular system is a potential therapeutic target for cannabinoids (Randall, Harris, Kendall, & Ralevic, 2002). The vascular effects of cannabinoids affect various cardiovascular diseases including heart failure, atherosclerosis, hypertension and ischemic/reperfusion injury (Bátkai

& Pacher, 2009; Montecucco & Di Marzo, 2012; Stanley et al., 2013). These actions are mediated by CB receptors that, at the central level, inhibiting the release of neurotransmitters, act on sympathetic outflow, which controls the cardiovascular function. Most of these studies have focused on endo- and phytocannabinoids (Bátkai & Pacher, 2009). Only in the last years, the beneficial effects of CBD in the cardiovascular system are becoming clear (Durst et al., 2007). A synthetic CBD analog, Abn-CBD, has been shown to cause vasorelaxation in various types of isolated arteries (Begg et al., 2003; Ho & Hiley, 2003; Singla, Sachdeva, & Mehta, 2012). Despite the conflicting results often obtained with Abn-CBD, these findings prompt the hypothesis that hyperpolarization induced by Abn-CBD employs calcium-activated potassium channels and is dependent on the presence of the endothelium. Similarly, it has been proved that CBD causes endothelium-dependent vasorelaxation in human mesenteric arteries with the involvement of CB_1 receptor activation (Parmar & Ho, 2010; Stanley, Hind, Tufarelli, & O'Sullivan, 2015), that is expressed on both vascular smooth muscle and endothelial cells. Despite CBD binds CB_1 receptors with low affinity, recently it was proposed that CBD is able to modulate the degradation of endocannabinoids (Stanley et al., 2015) through the inhibition of fatty acid amide hydrolase (FAAH) activity (Fantozzi et al., 2003). However, CBD also activates and induces physiologic effects mediated by PPAR-γ (De Filippis et al., 2011; Pertwee et al., 2007; Wheal, Cipriano, Fowler, Randall, & O'Sullivan, 2014). In particular, it has been demonstrated that CBD is capable of mediating a time-dependent vasorelaxation in rat isolated aortae that was inhibited by PPAR-γ-antagonists (Stanley et al., 2015).

Another proposed vascular effect of CBD includes the release of vasorelaxant mediators as nitric oxide (NO) and/or actions mediated by vascular enzymes as well as cycloxygenase (COX) and superoxide dismutase (SOD) (Takeda et al., 2011; Wheal et al., 2014). In vitro experiments on isolated femoral arteries of Zucker diabetic fatty rats showed that exposure to CBD increases the ability of arteries to relax via an enhanced release of the COX1- and COX2–derived vasodilator products. In addition, these studies demonstrated the CBD-mediated enhancement of SOD activity (O'Sullivan, Kendall, & Randall, 2006; Wheal et al., 2014). Indeed, CBD-induced vasodilation is also due to reductions of reactive oxygen species (ROS) promoted by SOD radical scavenger activity (Hwang et al., 2005; O'Sullivan et al., 2006; O'Sullivan, Sun, Bennett, Randall, & Kendall, 2009; O'Sullivan, Tarling, Bennett, Kendall, & Randall, 2005). In general, these findings have indicated that vasorelaxation depends on various signalling pathways influenced by mediators derived from endothelial cells and local regulators. In this context, studies using retinal arterioles, provide an optimum approach to understand the role of endothelium (Su, Kelly, Cringle, & Yu, 2015). Indeed, retinal vessels preparations represent CNS vasculature that is autoregulated and it is not influenced by autonomic innervations (MacIntyre et al., 2014). Other experiments conducted using Abn-CBD on rat retinal arterioles both endothelial intact and endothelial denuded, demonstrated that the vasoactive responses to CBD are endothelium dependent and highly dependent on the vascular tone (Su et al., 2015).

Although the mechanism still remains to be defined, increasing evidence has shown that different sites from CB_1 and CB_2 receptors mediate the vasodilator effect of cannabinoids. The involvement of non-CB1/CB2 receptors sensitive to CBD has been particularly noted in the peripheral vasculature where CBD analog induces vasodilator effects demonstrated in animal genetically lacking CB_1 and CB_2 receptors (Stanley, Hind, & O'Sullivan, 2013; Zakrzeska et al., 2010). Based on these data, it was concluded that abn-CBD is a selective agonist of a putative novel CB receptor located on endothelial cells called endothelial cannabinoid receptor (eCB) (Bondarenko, 2014). About this, in earlier studies it was suggested that CBD could act as partial agonist of eCB receptor but at concentration of 5–10 μM it antagonized contractile responses (Offertáler et al., 2003; Pertwee, Ross, Craib, & Thomas, 2002).

In addition, subsequent findings have proposed a different mechanism of action of CBD in rat mesentery and rat and human pulmonary arteries as well as in rat aortae, where it induced vasorelaxation in a PPARγ-mediated way, confirming the role of PPARγ in several biological actions of CBD (Hwang et al., 2005; O' Sullivan et al., 2009). Despite this, in the presence of 0-1918, an antagonist of eCB receptor, the effect of CBD appeared unaltered, suggesting that eCB is not a selective CBD endothelial target.

Collectively, experimental observations are at the moment controversial and suggest that CBD acts through multiple target sites to influence cardiovascular system function. The discovery of antioxidative and anti-inflammatory actions mediated by CBD, have suggested its therapeutic utility in various conditions based on inflammation and oxidative stress (Booz, 2011; Rajesh et al., 2007) including diabetic complications and other cardiovascular diseases, through its action on inflammation, fibrosis and oxidative/nitrosative stress (Durst et al., 2007; Rajesh et al., 2010).

Based on published earlier studies cannabinoids appeared to be cardioprotective and able to attenuate myocardial impairment and also to prevent ischaemia-reperfusion damage (Bátkai & Parker, 2009; Durst et al., 2007; Fouad, Albuali, Al-Mulhim, & Jresat, 2013; Walsh, Hepburn, Kane, & Wainwright, 2010). However, these studies provided conflicting results about CBD effects in both *in vitro* and *in vivo* models. Durst et al. showed that CBD causes decreased infarct size in an *in vivo* rat model of ischemia and reperfusion associated with a reduction of the inflammatory infiltration in infarct zones. Indeed, its effects were not direct but mediated by the anti-inflammatory properties of CBD. Moreover, it was observed that CBD has not significant effect on infarct size in the *in vitro* isolated rat heart model (Durst et al., 2007; Stanley et al., 2013).

In line with this results, it was reported that CBD exerts anti-inflammatory effects also in the development of alterations associated with diabetic cardiomyopathy (Rajesh et al., 2010). Different studies *in vivo* demonstrated that CBD treatment prevents and attenuates some complications derived from cardiomyocytes exposed to high glucose concentrations (Asbun & Villarreal, 2006; Westermann et al., 2009). In a mouse model of type I diabetic cardiomyopathy, CBD attenuates cardiac fibrosis myocardial dysfunction and the activation of inflammatory signalling pathways (Booz, 2011; Pacher, 2007; Rajesh et al., 2010; Weiss et al., 2006). The above-mentioned beneficial effects of CBD derive by its capacity to decrease ROS and nitrogen species generation and its potent inhibitory action on NF-kB pro-inflammatory pathway (El-Remessy et al., 2006; Kozela et al., 2010; Weiss et al., 2008). The same protective action of CBD was reported in cerebral ischemia where it reduces the accumulation of neutrophils and decreases the activity of mieloperoxidase (Hayakawa et al., 2007, 2009). In addition, recent studies highlighted the therapeutic relevance of CBD in the inflammatory responses associated to LPS in the mouse brain. Valdepenas et al., have demonstrated the vascular-stabilizing effects of CBD that was associated to LPS-induced changes in vessels diameter and permeability such as leukocyte margination (Valdepeñas et al., 2011). These actions are mediated by potent immunosuppressive in vivo effect of CBD that decreases the production of TNF-α and other cytokines and COX-2 expression (Pan et al., 2009; Rajesh et al., 2010). As regard the direct effect of CBD in the cardiac muscle, few studies have investigated its role in the regulation of contractility. Earlier findings reported bradycardic and negative ionotropic actions of CBD in *in vitro* models (Nahas, Morishima, & Desoize, 1977; Smiley, Karler, & Turkanis, 1976). More recently, it has been suggested that CBD acts on rat ventricular myocytes inhibiting Ca^{2+} channel and its signalling, thus causing in this way the negative ionotropic effect previously reported (Ali et al., 2015).

Overall, these results suggest that although the beneficial effects of CBD in cardiovascular pathologies additional studies are required to well understand its potential contribute in the clinical setting, since ad hoc clinical trials are still lacking.

3. Therapeutic potential of CBD

3.1. Neurological disorders as effective targets of CBD

Nowadays, neurodegenerative disorders are among the main causes of death in the developed countries. Loss of neurons characterizes these diseases and is responsible of the decline in cognitive and motor activity. Although environmental toxins and mutant genes seem to be involved in these diseases, their mechanism is still unsolved. Currently, inflammation is recognized as a critical factor among several neurodegenerative diseases and causes progressive nature of neurodegeneration. To date, few therapies are available and researchers have to find new therapeutic approaches. In this context CBD, may be a very promising drug, since it lacks undesired psychotropic effects. The well-known antioxidant, anti-inflammatory, and neuroprotective effects of CBD have prompted scientists to establish its efficacy in models of neurodegenerative diseases. CBD effects in main neurodegenerative diseases, Alzheimer's and Parkinson's diseases, multiple sclerosis and Huntington's disease are below more thoroughly highlighted and discussed.

3.2. CBD and Alzheimer disease

Alzheimer is a form of dementia in which deposits of “senile” plaques are enriched of activated microglia. The consequent inflammation and oxidative stress is a crucial event in the Alzheimer's pathophysiology (Candore et al., 2010). These plaques are constituted of deposits of the beta-amyloid peptide (Aβ) that form monomers, oligomers and fibrils. Some data show that oligomers of Aβ are the most neurotoxic aggregates and are chemotactic agents for microglial cells and stimulate oxidative stress (Heurtaux et al., 2010; Tamagno, Bardini, Guglielmotto, Danni, & Tabaton, 2006). Activated microglia produces pro-inflammatory cytokines able to further increase Aβ production by neural cells (Sastre, Klockgether, & Heneka, 2006). Additionally, an inflammatory condition blocks the ability of microglia to phagocyte fibrillar Aβ (Lee & Landreth, 2010).

To date the pivotal role of Aβ in inducing neuronal damage and mediating neuroinflammation in AD is well established. Promising data have been obtained about the control of toxicity induced by β-amyloid; however, it is not completely established if Aβ plaque depositions detected in post-mortem brain of AD patients, represent the cause or the result of the pathology. Numerous studies explored CBD action on neurotoxicity. In these experiments, CBD protected differentiated pheocromocytoma PC12 cells from the damaging action of Aβ peptide, via a combination of its antioxidant, anti-apoptotic and anti-inflammatory properties (Esposito, De Filippis, Carnuccio, et al., 2006; Hayakawa et al., 2007). Survival of cultured neurons and attenuation of Aβ-induced molecular changes can be ascribed to CBD antioxidant effects (Iuvone et al., 2004) via mechanisms not displayed by classic antioxidant drugs (Esposito, De Filippis, Carnuccio, et al., 2006). In fact, CBD weakened Aβ-induced GSK-3β activation that has a crucial role in the WNT/β-catenin pathway, so being able to prevent tau protein hyperphosphorylation and the following neurofibrillary tangle formation (Esposito, De Filippis, Maiuri, et al., 2006). CBD also reduced p38 mitogen activated protein kinase (MAPK) phosphorylation, so preventing NF-κB translocation into the nucleus and the consequent transcription of pro-inflammatory genes like inducible nitric oxide synthase (iNOS) (Esposito, De Filippis, Maiuri, et al., 2006). CBD exhibited beneficial effects also in a murine model of neuroinflammation induced by Aβ (1–42) fragment. In this model, CBD blocked reactive gliosis by reducing glia activation and the production of pro-inflammatory mediators (Esposito et al., 2007). Furthermore, in rat primary microglia and in N13 microglial cells, CBD reduced ATP-induced enhancement of intracellular calcium, through the involvement of cannabinoid and likely A(2A) adenosine receptors. In the same study, CBD administered for 3 weeks in Aβ-injected mice, increased cytokine gene expression and counteracted cognitive deficit. Overall, CBD modulates *in vitro* the function of microglial cells and elicits benefic effects in mice (Martín-Moreno et al., 2011). Recently, it was demonstrated that CBD induces the ubiquitination of amyloid precursor protein (APP) that reduces APP full length proteins in SHSY5Y(APP+) neurons with the following reduction of Aβ production. Indeed, CBD enhanced SHSY5Y(APP+) neuron survival, by decreasing apoptosis. These effects of CBD were dependent on the selective stimulation of PPAR-γ (Scuderi, Steardo, & Esposito, 2014). In another study investigated the effects of CBD were examined in cognitive impairments associated with Alzheimer. In particular, chronic CBD treatment reversed alterations in social recognition in APPxPS1 transgenic mice without affecting anxiety-related behaviors (Cheng et al., 2014). In the same model the preventive properties of long-term CBD treatment were evaluated. The prevented social recognition impairment was not accompanied by modifications in oxidative damage or amyloid load. Moreover, an effect of CBD on dietary phytosterol retention, cholesterol, and neuroinflammation was described (Cheng et al., 2014). Overall, these results highlight the importance of CBD as a pharmacological tool, that lacking psychoactivity is able to mitigate Aβ-evoked neuroinflammatory and neurodegenerative responses.

3.3. CBD in Parkinson treatment

Parkinson's disease is a motor neurodegenerative disorder, in which the main feature is a progressive death of nigrostriatal dopaminergic neurons, resulting in bradykinesia, rigidity and tremor as major motor abnormalities (Sethi, 2002).

So far, no treatment has been shown to cure Parkinson and none has been approved to slow or reverse the neurodegenerative process of the disease. Oral substitution of striatal dopamine deficiency with a dopamine precursor, levodopa, has been the gold standard of its treatment since the 1960s. Levodopa, administered in conjunction with the peripheral amino acid decarboxylase inhibitor, remains the most potent symptomatic therapy for motor disability. However, chronic use of levodopa commonly results in the development of long-term motor complications, including wearing off and dyskinesia, which compromise the long-term success of levodopa therapy (Kianirad & Simuni, 2016). There is a lot of evidence of a role of CBD in neuroprotection and neuropsychiatric disorders (Campos, Fogaça, Sonego, & Guimarães, 2016); whereby, clarify a role of CBD in Parkinson disease will be interesting to develop a new pharmacological approach in its therapy. Experimental evidence produced in the last years is reported below.

A neuroprotective effect exerted by CBD in an animal model of Parkinson was found. In these animals, 6-hydroxydopamine injections reduced, 2 weeks post-injection, dopamine contents and tyrosine hydroxylase (TH) activity in the caudate-putamen, and TH-mRNA levels in the substantia nigra. Daily administration of CBD (3 mg/kg), during these two weeks post-lesion, attenuated the dopaminergic impairment, also causing a complete recovery of the control values in some cases, without inducing tolerance (Lastres-Becker, Molina-Holgado, Ramos, Mechoulam, & Fernández-Ruiz, 2005).

In a successive study, the same research group demonstrated that CBD was able to recover 6-hydroxydopamine-induced dopamine depletion only when it was administered immediately after the lesion and that its neuroprotective effect was related to a reduction of oxidative stress (García-Arencibia et al., 2007).

Only few trials were conducted on Parkinson’s disease patients. Zuardi et al. reported a study on 6 patients, treated with levodopa. The subjects received CBD in addition to their usual therapy and significant improvements in total scores of Brief Psychiatric Rating Scale were observed; moreover, the treatment also significantly decreased psychotic symptoms (Zuardi et al., 2009).

Recently, Chagas et al. assessed the effect of CBD on REM sleep behaviour disorder (RBD) in a case report with only 4 PD patients. Three patients received CBD 75 mg/day and one received CBD 300 mg/day for 6 weeks; as result, a reduction or the complete absence of episodes

of agitation, kicking, nightmare or aggressive behaviour was observed with both the CBD doses. Regarding symptoms after drug discontinuation, RBD complex movements returned with the same frequency and intensity of baseline after the treatment was interrupted (Chagas, Eckeli, et al., 2014).

Finally, a double-blind trial was conducted on 21 patients, divided into 3 groups with seven participants each. Patients received placebo or doses of CBD (75 mg/day or 300 mg/day) for 6 weeks. Significant improvements in measures of well-being of Parkinson's disease patients treated with CBD 300 mg/day, compared to the group that received placebo, were found; no statistically significant differences concerning the motor symptoms were highlighted. However, studies involving larger samples and with systematic assessment of specific symptoms of Parkinson's disease are necessary in order to provide stronger conclusions regarding the pharmacological potential of CBD (Chagas, Zuardi, et al., 2014).

3.4. CBD for the control of chronic inflammation and spasticity in multiple sclerosis

Multiple sclerosis is a chronic neuroinflammatory disease with unknown etiology and variable clinical evolution. It is an immune-mediated disorder of the central nervous system (CNS) characterized by the destruction of myelin sheath that surrounds the axons (Trapp & Nave, 2008). Several hypotheses have been proposed to explain the physiopathology of multiple sclerosis as genetic predisposition, viral infections, autoimmune mechanisms (Sturm, Gurevitz, & Turner, 2014). Multiple sclerosis results in recurrent episodes of neurological dysfunction and accumulation of irreversible disability (Noseworthy, Lucchinetti, Rodriguez, & Weinshenker, 2000). The capability of Cannabis and its derivatives to control pain, tremor, disturbed bladder function and spasticity, justify their potential use in multiple sclerosis (Murnion, 2015). An accurate examination of literature, reveals that there are a lot of studies that testify the use of Cannabis-based medications in MS patients, but all these studies, concern the utilization of Δ^9-THC alone or Δ^9-THC in combination with CBD (Sativex® Δ^9-THC:CBD oromucosal spray). A number of difficulties exist in evaluating published data on CBD use for multiple sclerosis. Currently, clinical trials acting to test the safety and efficacy of CBD as mono-therapy in patients with multiple sclerosis do not exist. However, given the broad effects of CBD as an anti-inflammatory, neuroprotective, immune-modulator agent and furthermore considering its lack of psychoactive activity, that represents the principal limit for cannabinoids in clinical use, the employment of CBD is attracting growing attention as a novel safe and effective potential therapeutic alternative in MS patients. The acute and chronic treatment with the CBD-rich extract has been reported to be unable to induce changes of neurological signs in chronic relapsing EAE, a murine model of multiple sclerosis (Buccellato et al., 2011). However, CBD is able to improve signs of EAE in mice and this effect seems to be due to the CBD capability to suppress the microglial activity and T-cell proliferation (Kozela et al., 2011). The anti-inflammatory and immunomodulatory properties of CBD in the CNS have been demonstrated. CBD decreases the transmigration of blood leukocytes by downregulating the expression of VCAM-1, chemokines (CCL2 and CCL5) and the pro-inflammatory cytokine IL-1β, as well as by attenuating the activation of microglia in an *in vivo* viral model of multiple sclerosis (Mecha et al., 2013). CBD is able to induce regulatory T cells that have the property to promote anergy in encephalitogenic T cells in cocolture with antigen presenting cells stimulated with myelin oligodendrocyte glycoprotein (MOG)35-55 (Kozela et al., 2015). In addition, CBD determines an up-regulation of epidermal growth factor receptor 2 (EGFR2) mRNA transcription in stimulated TOG cells. In a recent study, the capability to induce apoptosis of purified CBD intraperitoneal administration in an experimental model of MS was evaluated. Immunohistochemical studies and western blot experiments of apoptotic markers reveal that CBD inhibited Fas pathway activation, extracellular signal-regulated kinase (ERK) p42/44 and cleaved caspase-3. CBD is also able to interfere with p53-p21 axis activation. Furthermore, a new formulation of purified CBD (98%) in cream as a topical treatment in an experimental model of EAE exerts neuroprotective effects against EAE. In particular, it is able to diminish clinical disease score, by recovering from paralysis of hind limbs and improving histological score typical of the disease in spinal cord tissues. Topical administration of 1% CBD-cream was also able to affect the inflammatory molecules expression's pattern, reducing release of CD4 and CD8α T cells, the expression of main pro-inflammatory cytokines, oxidative stress and apoptotic molecules (Giacoppo et al., 2015). Taken together, all these data suggest a significant therapeutic potential of this compound for the treatment of multiple sclerosis, in which the inflammatory component plays a key role for the onset and progression of the pathology.

3.5. CBD for the management of epilepsy

The term “epilepsy” refers to a complex group of neurological disorders, characterized by recurrent epileptic seizures (Engel, 1995). Epidemiological studies indicate that the incidence of epilepsy is of 1% in the world population (Ngugi et al., 2011); the incidence rate is 20 to 70 people in 100,000 in industrialized countries (Sander, 1997). An epileptic seizure is a clinical event presumed to result from an abnormal and excessive neuronal discharge. The clinical symptoms are paroxysmal and may include impaired consciousness and motor, sensory, autonomic, or psychic events perceived by the subject or an observer (Engel & Starkman, 1994). Although the availability of more than 20 different anti-seizures drugs for the treatment of epilepsy, more than 30% of patients have inadequate control of seizures with drugs, but the exact mechanism underlying this refractoriness to the treatment is unpredictable and still unknown (Kwan & Brodie, 2000). The approval in the last decades of new antiepileptic drugs, including several with new mechanisms of action, has not substantially reduced the proportion of patients with active disease (Brodie, Barry, Bamagous, Norrie, & Kwan, 2012). Many of these drugs are associated with side effects related to the CNS, compromising the quality of life of patients (Perucca & Gilliam, 2012). Furthermore, a proportion of patients suffering from epilepsy are unfortunately refractory to anticonvulsants available to date (Hirsch, 2015). So there is a real and immediate need for new treatments associated with fewer side effects able to control seizures. The ineffectiveness of the treatments available today, led, increasingly, patients and their families to seek alternative treatments. In this scenario, the treatment of seizures with *Cannabis sativa* has recently received prominent attention in the press and in social media reporting significant improvements in the control of seizures in adults and children with severe forms of epilepsy. To date there is growing scientific evidence that testifies the potential efficacy of cannabinoids for the treatment of different forms of epilepsy (Friedman & Devinsky, 2015; Katona, 2015; Rosenberg, Tsien, Whalley, & Devinsky, 2015). The mechanism of action of cannabinoids in controlling seizures has not been fully clarified, as well as the potential target through which the cannabinoids exert anticonvulsant effects. In particular, CBD showed anticonvulsant effects on both *in vitro* and *in vivo* experiments (Pertwee, 2008). In addition, neuroprotective action of CDB was also confirmed *in vitro*. Experiments conducted in rat cortical neuron cultures exposed to toxic levels of the excitatory neurotransmitter glutamate, revealed that CBD reduced the toxicity of glutamate, suggesting that it has potent antioxidant properties (Hampson et al., 1998). These data lead to hypothesize that CBD may be a potentially useful therapeutic agent for the treatment of oxidative neurological disorders. The absence of psychoactive action of CBD and its potential efficacy as an anticonvulsant has made it a very interesting molecule as a new potential therapeutic tool for patients with epilepsy. Despite these encouraging preclinical data and the presence of more anecdotal reports on the efficacy of cannabis in the treatment of different pathologies, a recent Cochrane review conducted by Gloss et al. concluded that no reliable conclusion

can be drawn regarding the efficacy of Cannabis sativa and in particular of CBD, because of lack of adequate data collected through randomized controlled trials testing CBD or any other cannabinoid in patients with epilepsy (Gloss & Vickrey, 2014). In an open-label trial, supported by GW Pharmaceuticals, it was evaluated whether the addition of CBD to existing anti-epileptic regimens would be safe, tolerated, and efficacious in children and young adults with treatment-resistant epilepsy (Devinsky et al., 2016). In this study, patients with severe, intractable, childhood-onset, treatment-resistant epilepsy, were enrolled. Oral CBD at 2–5 mg/kg per day was administered. The results of this clinical study showed that CBD reduces seizure frequency and could have a sufficient safety profile in children and adults with highly treatment-resistant epilepsy. Nevertheless, given the proprieties of CBD, that appears to be an excellent candidate among phytocannabinoids as an anti-epileptic drug, more randomized controlled trails are urgently needed and warranted to characterize its safety profile and true efficacy.

3.6. Neuroprotective efficacy of CBD in Huntington's disease

Huntington's disease, also known as Huntington's chorea, is an inherited neurodegenerative disorder that causes uncontrolled movements, emotional problems, and cognitive dysfunction. The primary cause of the disease is a mutation in the huntingtin gene consisting of an excess of CAG repeats in the genomic allele resulting in a polyglutamine expansion in the encoded protein called huntingtin. It becomes toxic for striatal and cortical neuronal subpopulations, causing motor abnormalities (i.e. chorea) and dementia (Fernández-Ruiz et al., 2013). At present, cannabinoids have been studied to alleviate hyperkinetic symptoms, given their inhibitory effects on movement, and due to their anti-inflammatory, neuroprotective and neurodegenerative properties. Combinations 1:1 of botanical extracts enriched in either Δ^9-THC or CBD, which are the main constituents of Sativex, reduced the striatal degeneration generated by 3NP intoxication in experimental models. This ability seems to be due to the antioxidant and cannabinoid receptor independent actions of these phytocannabinoids. Moreover, the neuroprotective effect of Δ^9-THC at the CB_1 and CB_2 receptors has been observed in other experimental models, for example, the transgenic mouse model, R6/2, in which the effects of Δ^9-THC are likely due to the activation of CB_1 receptors and possibly through the activation of CB_2 receptors too, preserving striatal neurons in this genetic model and also in malonate-lesioned rats, a model of striatal atrophy that involves mainly glial activation, inflammatory events and activation of apoptotic machinery (Iuvone, Esposito, De Filippis, Scuderi, & Steardo, 2009; Sagredo et al., 2011). Based on preliminary evidence, the neuroprotective efficacy of CBD has been investigated in 15 neuroleptic-free patients with Huntington's disease. The effects of oral CBD and placebo (sesame oil) were weekly verified for 6 weeks. CBD had no significant clinically important differences compared to placebo on chorea severity. Plasmatic levels of CBD did not differ significantly over the 6 weeks of CBD administration. Overall, collected results are too poor to support an efficacy for CBD in Huntington's disease treatment (Consroe et al., 1991).

3.7. Anti-tumor properties of CBD

The anti-tumor properties of cannabinoid agonists are well-established (Malfitano et al., 2011; Pisanti et al., 2009; Pisanti, Picardi, D'Alessandro, Laezza, & Bifulco, 2013; Salazar et al., 2009). Indeed, a lot of studies have demonstrated that cannabinoids exert anti-proliferative and anti-invasive actions in a large number of cancer types (Flygare & Sander, 2008; Guindon & Hohmann, 2011; McAllister et al., 2005; Velasco, Sánchez, & Guzmán, 2015). In particular these compounds are able to target different steps of tumor process as cancer cell migration, invasion and metastases formation and to stimulate autophagy-mediated apoptotic cancer cell death (Pisanti et al., 2013; Velasco et al., 2015; Velasco, Sánchez, & Guzmán, 2012).

Also in this field, the clinical use of Δ^9-THC and additional synthetic agonists is often limited by their undesired psychoactive side effects, and for this reason interest in non-psychoactive phytocannabinoids, such as CBD, has substantially enhanced in recent years. Several reports showed that CBD exhibits anti-proliferative, pro-apoptotic effects and inhibits cancer cell migration, adhesion, and invasion (Scott, Dalgleish, & Liu, 2014). In the first study of 1975, Munson et al. reported antiproliferative actions of CBD *in vitro* and *in vivo* on cells from Lewis lung adenocarcinoma. Since then, a lot of evidence has demonstrated that CBD is a potent inhibitor of both cancer growth and spread (Munson, Harris, Friedman, Dewey, & Carchman, 1975). The molecular mechanisms underlying the anti-tumoral properties of CBD are the same proposed above (Bifulco & Di Marzo, 2002). CBD increases ROS production that is responsible both of downregulation of Id-1, an inhibitor of some transcription factors and of upregulation of ERK phosphorylation, both molecules that are involved in promoting cell proliferation (Cho et al., 2009; Wirawan et al., 2010).

In line with this, the anti-proliferative effect of CBD was also reported on various glioma cell lines where it was shown that CBD actions were mediated by ROS production, release of cytochrome C and triggering autophagy and apoptosis cell death (Liu, Hu, Huang, Wey, & Jan, 2010; Scott, Shah, Dalgleish, & Liu, 2013). It has also been highlighted a beneficial effect of combined treatment of CBD with Δ^9-THC that enhances inhibitory effect on cell growth *in vitro* and *in vivo* models (Marcu et al., 2010; Scott et al., 2014; Torres et al., 2011).

Moreover, chemopreventive effects of CBD was demonstrated in other cancer cells as lung, colon and endocrine cells through CB receptor-dependent and -independent mechanisms (Aviello et al., 2012; Lee et al., 2008; Ramer, Merkord, Rohde, & Hinz, 2010). This appears particularly evident in tumours of immune origin that express high levels of CB2 as lymphomas and leukaemias (McKallip et al., 2006; McKallip, Lombard, Martin, Nagarkatti, & Nagarkatti, 2002).

In the following paragraphs, we will focus on the most recent evidence concerning the efficacy of CBD in the modulation of tumorigenesis in several types of cancer, such as breast, lung, colon, brain, and other tumours.

3.8. Breast cancer

CBD has been reported to inhibit the proliferation of both estrogen receptor-positive and estrogen receptor-negative human breast cancer cell lines and induce apoptosis in a concentration-dependent manner, whereas it shows little effect on non tumorigenic mammary cells. CBD causes autophagy-induced death in breast cancer cells by induction of endoplasmic reticulum (ER) stress in MDA MB-231 cells, followed by LC3-II accumulation, a classical marker of autophagy. CBD induces apoptosis in breast cancer cells by inhibiting AKT/mammalian target of rapamycin (mTOR) signaling and enhancing ROS generation in breast cancer. It mediates a complex balance between autophagy and mitochondria-mediated apoptosis in MDA-MB-231 breast cancer cells inducing translocation of beclin-1 to the mitochondria that is able to determine the mitochondrial release of cytocrome C into cytosol (Shrivastava, Kuzontkoski, Groopman, & Prasad, 2011). Autophagy and apoptosis-mediated cell death induced by CBD is independent from CB receptors (Rocha, Dos Santos Júnior, Stefano, & da Silveira, 2014).

Other evidence shows that CBD has an anti-tumorigenic activity in highly aggressive and metastatic breast cancer, especially in triple-negative breast cancer, by suppression of the activation of EGF/EGFR signaling transduction pathways. The inhibition of these pathways represses NF-kB activity by causing the arrest of breast cancer cell proliferation and breast cancer metastasis through multi-target effects. Furthermore, CBD inhibits actin stress fibers and focal adhesion formation and downregulates MMP2 and MMP9 secretion in triple negative cell lines and tumors, thus blocking breast cancer cell migration and invasion. CBD has been shown to inhibit the recruitment of total

macrophage and M2 macrophage populations within both primary tumors and metastatic sites, changing the secretion of cytokines from the cancer cells (less GM-CSF and CCL3) causing a reduced recruitment of macrophages to the tumor microenvironment that suppresses angiogenesis and inhibits the invasive potential of tumor cells (Elbaz et al., 2015). It has been reported that CBD reduces Id-1 gene expression in aggressive human breast cancer cells determining an inhibition of the proliferative and invasive phenotype of these cells. CBD up-regulates the active isoform of ERK causing the decrease of Id-1 gene expression and consequently the inhibition of cell growth and invasion. CBD produces a mitochondrial damage and an increase in the production of ROS. Indeed, the ROS scavenger, α-tocopherol, reverses the ability of CBD to inhibit Id-1 expression and the invasion of MDA-MB-231 cells (McAllister et al., 2011). Interestingly, CBD has been reported to induce TRPV2 overexpression in triple negative breast cancer cells enhancing the anti-tumor action of doxorubicin through its augmented uptake, reported also in glioblastoma (Elbaz et al., 2016). The antitumor effect of CBD was further confirmed in murine models of breast cancer metastatization to the lung, where CBD decreases the number of metastatic lung nodules (Elbaz et al., 2015; Ligresti et al., 2006).

3.9. Lung cancer

Ramer and coworkers investigated the effect of CBD in human lung cancer cell lines, primary tumor cells and *in vivo* models of lung cancer (Haustein et al., 2014; Ramer, Merkord, et al., 2010; Ramer, Rohde, Merkord, Rohde, & Hinz, 2010; Ramer et al., 2012; Ramer, Fischer, Haustein, Manda, & Hinz, 2014; Ramer et al., 2013). CBD induces apoptosis associated with upregulation of both COX-2 and PPAR-γ expression and COX-2 and PPAR-γ inhibitors or siRNA reverted this effect. Indeed, CBD increases prostaglandine levels that induce PPAR-γ accumulation in the nucleus with activation of PPAR-γ-dependent apoptosis (Ramer et al., 2013). Furthermore, CBD decreases cancer cell invasiveness inducing both the expression of ICAM-1 and the levels of tissue inhibitor of metalloproteinases (TIMP1) in several lung cancer cells, in metastatic cells of a lung cancer patient as well as in athymic nude mice xenografted with A549 cells. The decreased invasiveness TIMP1-mediated has been associated with p38 and p42/44 MAPK phosphorylation and these events were reverted by CB_1, CB_2 or TRPV1 antagonists (Ramer et al., 2012). In another work, it was reported that exogenously added TIMP1, used to mimic the CBD-induced TIMP1 release, leads to inhibition of endothelial cell migration, tube and sprout formation, with sparing effects on cellular viability, suggesting a pivotal role of the anti-angiogenic factor TIMP1 in intercellular tumor-endothelial cell communication (Ramer et al., 2014). CBD-induced ICAM-1 upregulation enhanced the susceptibility of lung cancer cells to LAK cell-mediated cytolysis. Moreover, since CBD induced ICAM-1 expression in lung cancer and only marginally in non-tumor bronchial epithelial cells, authors speculated that these events occur specifically in tumor cells (Haustein et al., 2014). CBD was found to decrease invasiveness of human lung cancer cells trough a mechanism also mediated by a decrease of plasminogen activator inhibitor 1 (PAI-1) expression and release, without affecting levels of urokinase-type Plasminogen Activator (uPA) and its receptor uPAR. CBD anti-invasive effect and PAI-1 reduction were reverted using CB_1, CB_2 and TRPV1 antagonists. Moreover, the functional role of PAI-1 in invasiveness was demonstrated using recombinant PAI-1 and PAI-1 siRNA with consequent pro-invasive and anti-invasive effect respectively (Ramer, Rohde, et al., 2010).

3.10. Colon cancer

CBD showed potential antiproliferative effects also in colorectal cancer, a major cause of morbidity and mortality in Western countries.

Several studies have highlighted the effects of CBD in the gut. Among others capabilities, CBD is able to reduce intestinal inflammation, process directly linked to colorectal cancer initiation. Specifically, in Caco-2 cell line, CBD exerts antioxidant action through a reduction of ROS production and lipid peroxidation. These effects seem to be due to down-regulation of iNOS expression, but not of COX-2, and modulation of IL-1β and IL-10 (Borrelli et al., 2009).

More recently, it has been demonstrated a chemopreventive effect of CBD in an animal model of colorectal cancer (azoxymethane-treated mice, that was associated with aberrant crypt foci, polyps and tumor formation). In this model, the protective effect of CBD was accompanied by the up-regulation of active caspase-3 and down-regulation of phosphatidylinositol-3-kinase (PI3K)/Akt survival signaling cascade. Interestingly, in Caco-2 cells and in highly metastatic colon cancer cell line HCT116, CBD was unable to induce DNA damage, but its protective effects were exerted through protection against hydrogen peroxide induced DNA damage, strongly implicated in tumor etiology (Aviello et al., 2012). Other authors reported CBD anti-proliferative effects in SW480 cell line, another *in vitro* model of colon cancer. Here CBD was able to induce poly (ADP ribose) polymerase (PARP) and caspase-3 cleavage in phosphatase-dependent and CB receptors-independent manner. Indeed, it was reported that CBD induces mRNA expression levels of several phosphatase, enzymes responsible of dephosphorylation, and thus inactivation, of many kinases involved in cancer progression, such as p42/44 MAPK, Akt, STAT3, c-Jun N-terminal kinase (JNK), ERBB2 and p38 MAPK (Sreevalsan, Joseph, Jutooru, Chadalapaka, & Safe, 2011).

In a recent work it was reported that in a colon carcinoma model CBD was able to reduce liver metastases *in vivo*, antagonizing G Protein Coupled Receptor-55 (GPR55), a well-known receptor involved in metastasis occurrence, cell migration and adhesion (Kargl et al., 2016).

Finally, as others natural cannabinoids, CBD represents a good lead compound in treatment of colon cancer. Many synthetic cannabinoids were obtained by optimization of natural lead compounds. For example, CBD-derived quinone HU331 showed a marked anticancer activity in HT29 xenografted mice. This effect was ascribable to HU331-mediated specific inhibition of Topoisomerase II (Kogan et al., 2004, 2007). Substantially, these findings indicate a wide effect of CBD in tumor biology suggesting its potential utility in clinical application for cancer therapy.

3.11. Brain cancer

The finding of the expression of different receptors in the brain that bind the phytocannabinoids led to the discovery that these molecules inhibit the viability of different cancer cells *in vitro* and in *in vivo*, resulting in an increased interest in the study of these active principles (Guzmán, 2003; Parolaro, Massi, Rubino, & Monti, 2002).

In particular, the main part of studies are focusing on glioblastoma multiforme, the most aggressive primary tumor of the CNS that is responsible of one-third of all brain tumor diagnoses (Kleihues et al., 2002). In the last years numerous studies have been conducted to clarify the potential therapeutic use of CBD for this type of tumor.

In 2004, Massi et al. have evaluated *in vitro* the antiproliferative effect of CBD on U87 and U373 human glioma cell lines (Massi et al., 2004). They observed that the addition of CBD to the culture medium determines a dramatic drop of mitochondrial oxidative metabolism and viability of glioma cells significantly inhibiting the growth of subcutaneously implanted U87 human glioma cells in mice. The antiproliferative effect of CBD was partially prevented by SR144528 (a CB_2 receptor antagonist) and tocopherol, whereas the CB_1 receptor antagonist (SR141716) was not able to revert the CBD anti proliferative effect. In 2005, Vaccani et al. demonstrated that CBD inhibits the migration of U87 human glioma cells *in vitro* in a concentration dependent manner (0.01 up to 9 μM) without affecting cell viability (Massi et al., 2004; Vaccani, Massi, Colombo, Rubino, & Parolaro, 2005). They suggested that the mechanism of action of CBD is independent from CB and vanilloid receptors, since the antagonists of CB_1, CB_2 and TRPV1

(capsazepine) did not revert the effect of CBD, but the mechanism of action has not been still clarified.

As regard the possible molecular mechanism by which CBD induces apoptosis in glioma cells it was demonstrated that CBD triggers apoptosis of human glioma cells by an early production of ROS and a contemporary decrease of glutathione (GSH) leading to caspase-8 and -9 activation (Massi et al., 2008). CBD was able to decrease the activity of 5-lipoxygenase and of leukotriene B4 but not the activity of COX-2 and of prostaglandin E2. Furthermore, CBD also enhanced the activity of FAAH, leading to a reduction of anandamide (AEA). From these data appear that one possible antitumor mechanism of CBD is the capability to modulate the lypoxigenase pathway and the endocannabinoid system. In 2010, Marcu et al., after the observation of antiproliferative effect of Δ^9-THC on glioblastoma tumors, suggested the hypothesis that CBD can modulate and enhance the anti-neoplastic effect of Δ^9-THC (Marcu et al., 2010).

Since the confirmed expression in glioblastoma cells of TRPV2, a CBD target, CBD was evaluated in combination with the drugs currently used in glioblastoma treatment, as temozolide, carmustine and doxorubicin (Nabissi et al., 2015). CBD increased TRPV2 expression and activity. Activation of these channels by Ca^{2+} influx was able to increase drug uptake and synergize with cytotoxic agents to induce apoptosis of glioma cells, whereas no effects were observed in normal human astrocytes.

Administration of CBD reduced proliferation and invasiveness of glioblastoma cells and caused a decrease of proteins specifically involved in growth, invasion and angiogenesis. In particular, CBD caused down-regulation of survival signaling pathways as ERK and Akt. Furthermore, CBD was also able to decrease the hypoxia inducible factor 1-α (HIF-1-α). These data provide new insights on molecular mechanisms of action of CBD as novel potential anti neoplastic treatment (Solinas et al., 2013).

CBD has been also reported to modulate Id-1 transcriptional factor, a pathway with a critical role in modulating the invasiveness of GBM cell lines and primary GBM cells. Furthermore, other studies suggest that there is a positive correlation with Id-1 expression and invasion and self-renewal, as measured by an *ex vivo* invasion assay and neurosphere growth assay, respectively. The down-regulation of Id-1 induces a significant inhibition of cell invasion but only a modest reduction in cell growth. Moreover, CBD reduced the expression of markers associated with epithelial mesenchymal transition (EMT) (vimentin and snail) and invasion (membrane type 1-matrix metalloproteinase 1 (MT1-MMP), matrix metalloproteinase-2 (MMP-2), and focal adhesion kinase (FAK)) (Soroceanu et al., 2013). The study confirmed this hypothesis *in vivo* using an intracranial U251 glioma xenograft mouse model. The treatment of mice with CBD reduced the level of Id-1 within the tumor tissue, suggesting a new mechanism of action of CBD to inhibit tumor invasiveness.

One of the main problems to employ phytocannabinoids to treat different types of cancer is the drug carrier system, due to high lipophilicity exhibited by these compounds. Hernàn Pérez de la Ossa et al., 2013 faced the problem and tried to encapsulate Δ^9-THC and CBD into biodegradable microspheres, in order to overcome the obstacle of drug carrier system (Hernàn Pérez de la Ossa et al., 2012). They attempted the alternative delivery system in a murine xenograft model of glioma. They observed that coadministration of a mixture of Δ^9-THC- and CBD-loaded in biodegradable polymeric microparticles (containing approximately 3.075 mg of Δ^9-THC and 3.75 mg of CBD per administration) enhances apoptosis, inhibits proliferation of cancer cells and tumor angiogenesis in glioma xenograft model. Furthermore, the authors observed that the combined administration of Δ^9-THC or Δ^9-THC + CBD with temozolomide synergistically reduces the growth of glioma xenografts (Torres et al., 2011).

Scott et al. verified for the first time the effect on glioblastoma multiforme of two forms of cannabinoids as botanical drug substance (BDS) and as pure form (Δ^9-THC and CBD) (Scott et al., 2014). The authors evaluated the effect of Δ^9-THC and CBD both alone and in combination with radiotherapy in T98G, U87MG and GL261 glioma cell lines. The results suggested a duration and dose-dependent reduction in cell viability with each cannabinoid and furthermore Δ^9-THC-BDS was more efficacious than Δ^9-THC-pure but not BDS-CBD, that was less incisive than CBD-pure. Moreover pretreating the cells with Δ^9-THC-pure and CBD-pure increased radiosensitivity of the cells. These results were extended and verified *in vivo*, in an orthotopic murine model of glioma in which the triple combination of CBD, Δ^9-THC and radio-irradiation significantly inhibited tumor progression. These results confirm the synergistic effects of the cannabinoids with the common available anti-tumor therapy. CBD has been reported to inhibit proliferation and clonogenic capability and further to induce autophagy through TRPV2 activation in glioma stem-like cells (GSCs), a cell subpopulation that seems to be involved in glioblastoma multiforme tumor onset and that is characterized by the acquisition of chemoresistance (Guertin & Sabatini, 2007). Acute myeloid leukemia (Aml-1), a transcription factor that plays a pivotal role in GBM proliferation and differentiation has been observed to bind TRPV2 and CBD upregulates Aml-1 expression, in a TRPV2 and PI3K/AKT dependent manner. With these hypotheses, the authors suggest a novel mechanism by which CBD induces autophagic process in GSCs differentiation, abrogating carmustine chemoresistance in GSCs (Nabissi et al., 2015). The hypothesis that CBD acts as an anticancer agent increasing intracellular levels of ROS was further evaluated in GSCs. It was reported that CBD induces a robust increase in ROS with consequent inhibition of cell survival, phosphorylated AKT and self-renewal. Furthermore, it was demonstrated that CBD can inhibit intracranial growth of primary GSC-derived tumors *in vivo* and this effect is mediated by an increase in ROS levels. Finally, the use of CBD in combination with inhibitors of antioxidant response genes can amplify the inhibition of GBM progression. These data were further confirmed in glioma cell lines where CBD induced an increase of heat shock proteins (HSP) as a consequence of up-regulation of ROS. The authors verified that co-administration of CBD and HSPs inhibitors enhanced the cytotoxic effect of CBD and also increased the radiosensitivity (Scott, Dennis, Dalgleish, & Liu, 2015). The studies here reported show that CBD can be considered a potential treatment solution for brain cancers, primarily due to ineffective therapeutic options for these types of cancer and for its synergistic effects with the currently available drugs and radiotherapy.

3.12. Other tumors

Since it has been reported that TRPV2 is activated by CBD, Yamada and colleagues used CBD as a selective agonist of TRPV2, in bladder cancer cell lines. In these models, CBD has been reported to induce apoptotic cell death via TRPV2 affecting calcium influx (Yamada et al., 2010).

In melanoma models, Δ^9-THC reduces cell viability and tumor xenograft growth, but when CBD was combined with lower doses of Δ^9-THC, the antitumor effect was increased *in vitro* and was as effective as higher doses of Δ^9-THC alone *in vivo*. Moreover, it has been suggested that CBD and Δ^9-THC induce different mechanisms that cooperate to promote cancer cell death (Armstrong et al., 2015). De Petrocellis et al. (2013) explored the anti-tumor effect of CBD in prostate cancer. They reported that CBD significantly inhibits cell viability and induces apoptosis in both androgen receptor (AR)-expressing and AR-non expressing prostate cancer cell lines. In LNCaP cells, the pro-apoptotic effect of CBD seems to be due to Transient Receptor Potential Melastatin type-8 (TRPM8) antagonism and was accompanied by p53 activation and ROS elevation (De Petrocellis et al., 2013). Moreover, treatment with Cannabis extract containing high CBD, inhibits spheroid formation in prostate cancer stem cells from LNCaP cells and down regulates CB_1, CB_2, vascular endothelial growth factor (VEGF), prostate specific antigen (PSA) and pro-inflammatory cytokines IL-6/IL-8 (Sharma, Hudson, Adomat, Guns, & Cox, 2014).

Interestingly, CBD was able to reduce tumor size in LNCaP xenografted mice and significantly enhanced the anti-cancer effect of

bicalutamide, but not of docetaxel. Conversely, in DU-145 xenografts, CBD was inactive when given alone, but potentiated the effect of docetaxel (De Petrocellis et al., 2013). The role of CBD as a chemotherapeutic sensitizing was supported by the finding that CBD and other cannabinoids are able to reverse the ATP-binding cassette sub-family G member 2 (ABCG2) mediated multidrug resistance (Holland, Lau, Allen, & Arnold, 2007).

3.13. CBD and angiogenesis

Angiogenesis is a fundamental aspect of tumor progression since, through the formation of new blood vessels promote the invasiveness of a tumor and its metastatization. Several studies have investigated the capacity of cannabinoids to inhibit migration (Blázquez et al., 2003, 2004) and proliferation of vascular endothelial cells that contribute to cannabinoids antiangiogenic effect (Blázquez et al., 2003; Pisanti et al., 2007; Velasco et al., 2015). However, in different tumor cells it was observed a cannabinoid-mediated inhibition of VEGF and other pro-angiogenic growth factors production (Blázquez et al., 2004; Casanova et al., 2003; Portella et al., 2003). A paper from Solinas' group reported the ability of CBD to modulate tumour angiogenesis by multiple mechanisms (Pisanti et al., 2011; Solinas et al., 2012). CBD anti-angiogenic properties were shown on HUVEC endothelial cells. CBD inhibited cell proliferation, migration and invasion, all steps of the angiogenic process *in vitro* and was also effective to block angiogenesis *in vivo* in the matrigel sponge model. Furthermore, CBD exhibits its effects also interfering with the protein expression of various modulators involved in the angiogenic process such as MMP2, MMP9, TIMP1, uPA and endothelin-1 (Massi et al., 2008; Solinas et al., 2012) but the molecular mechanism that underlie its anti-angiogenic action and the receptor and precise signalling involved remains to date unknown.

Taken together, these observations suggest a plethora of CBD effects on tumor biology and prompt its potential utility in clinical application for cancer therapy.

3.14. Concluding remarks

The scientific evidence collected in these years on CBD beneficial properties, that are particularly relevant in treatment-resistant epilepsy and other severe and invalidating pathologies (Fig. 1) as discussed above, has prompted an increased use of *Cannabis*-based medicinal products, containing Δ^9-THC and CBD in different ratios, by patients discouraged by the ineffectiveness of conventional therapies or desperate for the absence of any treatment. Also thanks to the intervention of social and press media, most governments around the world have introduced specific laws approving *Cannabis* medical use, instituting dispensaries where it is possible to buy medicinal *Cannabis* and related products such as marijuana-edibles, so that patients requests to have access to this kind of treatment have further increased becoming very common in clinical practice (Bifulco & Pisanti, 2015; Pisanti & Bifulco, 2017). On the other side, such greater popularity of medical *Cannabis* has not been accompanied by a parallel recognition of cannabinoids as medicines by national regulatory agencies, thus generating a paradoxical situation. Since CBD, differently from Δ^9-THC is not considered an abuse drug and is at the same time legal but not regulated, this has caused the development of a market of CBD-based products for medical purpose, such as CBD oil, tinctures and vapors that has rapidly expanded, flourishing in a no man's land with potential health dangers for patients and all end-users. Indeed, the lack of regulation of such products does not assure the patient about the quality of the product itself, the effective dosage of CBD that is fundamental for its therapeutic effectiveness, the purity and the absence of chemical or microbiological contaminations, thus raising critical public safety concerns. The Medications Health Care Products Regulation Agency (MHRA) has only recently addressed such issue at least in UK thanks to the final approval of CBD as a medicine. Medicinal products with CBD will be therefore considered like all the other medicines, requiring a product licensing that assures safety, efficacy and quality standards to safeguard public health and all the products already on the market without license are outlaw and have to be withdrawn. Such new regulation in UK follows the release of the results of GW Pharmaceuticals latest Phase III trial for its CBD treatment Epidiolex for seizures associated with Lennox-Gastaut syndrome (LGS), a rare and severe form of childhood-onset epilepsy and of other previous trials on treatment-resistant epilepsy (Devinsky et al., 2016). It is expected that other regulatory agencies around the world will soon deal with the review of CBD regulatory status, as more large and robust clinical trials will definitely establish its therapeutic relevance in several pathologies.

Fig. 1. The multifaceted pharmacological effects of CBD.

Disclosure of potential conflicts of interest

The authors declare no conflicts of interest.

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