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Cannabidiol affects the expression of genes involved in zinc homeostasis in BV-2 microglial cells

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ABSTRACT

Cannabidiol (CBD) has been shown to exhibit anti-inflammatory, antioxidant and neuroprotective properties. Unlike Δ^9-tetrahydrocannabinol (THC), CBD is devoid of psychotropic effects and has very low affinity for both cannabinoid receptors, CB_1 and CB_2. We have previously reported that CBD and THC have different effects on anti-inflammatory pathways in lipopolysaccharide-stimulated BV-2 microglial cells, in a CB_1/CB_2 independent manner. Moreover, CBD treatment of BV-2 cells, was found to induce a robust change in the expression of genes related to oxidative stress, glutathione deprivation and inflammation. Many of these genes were shown to be controlled by Nrf2 and ATF4 transcription factors.

Using the Illumina MouseRef-8 BeadChip platform, DAVID Bioinformatics and Ingenuity Pathway Analysis, we identified functional sets of genes and networks affected by CBD. A subset of genes was found to be regulated by the metal responsive element (MRE)-binding transcription factor-1 (MTF-1) and is shown to be related to zinc homeostasis. We found that CBD upregulates the expression of the mRNAs for *metallothionein 2* (*Mt2*), *N-myc-downstream regulated gene 1* and *matrix metalloproteinase 23* as well as of the zinc transporters *ZnT1/Slc30a1* and *Zip4/Slc39a4* but downregulates the expression of the mRNA for the zinc transporter *Zip10/Slc39a10* as well as for the *zinc finger protein 472*. Among these genes, ZnT1, Mt2 and the zinc transporters ZIPs are known to function together to control the intracellular zinc concentration.

These results show that CBD, but much less so THC, affects the expression of genes involved in zinc homeostasis and suggest that the regulation of zinc levels could have an important role through which CBD may exert its antioxidant and anti-inflammatory effects.

1. Introduction

1.1. Cannabinoids and immune cells

Preparations derived from *Cannabis sativa* (marijuana and hashish) have been used for centuries as recreational drugs as well as medicinal agents, due to their psychotropic and therapeutic properties (for reviews, see Earleywine (2002), Kogan and Mechoulam (2007), Murray et al. (2007) and Pertwee (2009)). To date, over 60 phytocannabinoids have been identified. The two most abundant phytocannabinoids in *Cannabis* preparations are Δ^9-tetrahydrocannabinol (THC), the major psychoactive constituent, and cannabidiol (CBD), which is not psychoactive.

Many cannabinoids were shown to posses immunosuppressive and anti-inflammatory properties and to modulate various activities of immune cells (Klein et al., 1998; McKallip et al., 2002; Cabral and Staab, 2005; Croxford and Yamamura, 2005; Klein and Cabral, 2006; Kozela et al., 2010, 2011; Rieder et al., 2010; Juknat et al., 2011). In addition, a large number of reports showed that several cannabinoids have proapoptotic, neuroprotective and anti-tumor properties (Galve-Roperh et al., 2000; van der Stelt and Di Marzo, 2005; Massi et al., 2006).

To date, two cannabinoid receptors have been characterized, the CB_1 and the CB_2 receptors. The CB_1 receptor is mostly localized in neural cells and mediates the psychoactive effects of THC, while the CB_2 receptor is highly expressed in immune cells and is involved in immunomodulation (for reviews, see Cabral et al. (2008), Cabral and Griffin-Thomas (2009) and Stella (2010)). THC is equally efficient at both of these receptors (Rhee et al., 1997) and has been reported to have effects on both the nervous and the immune systems (Cabral and Staab, 2005; Le Foll and Goldberg, 2005; Cabral et al., 2008; Woelkart et al., 2008; Cabral and Griffin-Thomas, 2009). CBD, unlike THC, has low

Abbreviations: CBD, cannabidiol; Mt, metallothionein; THC, Δ^9-tetrahydrocannabinol; Mmp23/CA-MMP, matrix metalloproteinase 23/cysteine array matrix metalloproteinase.

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affinity for both CB_1 and CB_2 receptors and as stated above, is devoid of the unwanted psychotropic effects characteristic of marijuana or THC (Pertwee, 2005; Mechoulam et al., 2007; Izzo et al., 2009). CBD displays a diversity of actions, including anticonvulsive, sedative, hypnotic, antipsychotic, anti-inflammatory and neuroprotective properties (Mechoulam et al., 2002, 2007; Scuderi et al., 2009; Liu et al., 2010; Kozela et al., 2010, 2011; Juknat et al., 2011). As CBD is not an efficient ligand of either CB_1 or CB_2, these effects are probably mediated through other receptors/targets (see below).

A wide range of literature reports the effects of phytocannabinoids on various populations of immune cells (Klein et al., 1998; Cabral and Staab, 2005; Croxford and Yamamura, 2005; Kozela et al., 2010, 2011; Juknat et al., 2011; Rimmerman et al., 2011). Both THC and CBD have been shown to decrease cytokine production in human immune cell lines (Srivastava et al., 1998; Kozela et al., 2010) and to suppress T cell proliferation and their effector functions (Nahas et al., 1974; Cabral and Dove Pettit, 1998; Kaplan et al., 2003; Klein et al., 2004; Jan et al., 2007; Kozela et al., 2011). However, the molecular mechanisms involved in these cannabinoid-mediated effects are not yet fully characterized. Altered adenosine signaling through inhibition of its uptake, has been reported as a potential non-cannabinoid receptor mechanism by which CBD, and less so THC, can decrease inflammation (Carrier et al., 2006). Other studies identified PPAR-γ as an intracellular target, which mediates the cannabinoid-associated immunosuppression in a manner that is independent of CB_1 and CB_2 receptors (O'Sullivan, 2007). Other targets including the G-protein-coupled receptors GPR55 and GPR18 as well as the transient receptor potential (TRP) channels were also suggested (Stella, 2010; De Petrocellis and Di Marzo, 2010; Pertwee et al., 2010).

Among the immune cells, microglia are considered to be the resident macrophage-like cells of the central nervous system (CNS). These cells are known to exert an important role in brain's innate immunity and in inflammatory neuropathologies (Hanisch and Kettenmann, 2007; Graeber and Streit, 2010). Microglia can be activated by injury or infection and have been suggested to be the first line of defense in the CNS (Streit, 2005). Microglial activation is associated with production and secretion of a variety of compounds such as cytokines, reactive oxygen species (ROS), reactive nitrogen species (RNS), matrix metalloproteinases and prostaglandins. Although acute microglial activation is a protective mechanism involved in regulating tissue repair and recovery, excessive or chronic activation can lead to harmful effects (Hanisch and Kettenmann, 2007). Interestingly, the mechanisms that give rise to the protective or the damaging microglial phenotypes are not fully elucidated. The possibilities of enhancing microglial-mediated innate immunity in the brain and of preventing the harmful effects associated with their chronic activation could offer new therapeutic approaches to the treatment of brain injury and neurodegenerative diseases.

Several groups including ours, have shown that microglial cells express CB_1 and CB_2 receptors (Pietr et al., 2009; for review see Stella (2010)). However, in addition to these receptors, cannabinoids were shown to affect microglial cells activation via various CB_1/CB_2 independent mechanisms (Walter and Stella, 2004; Stella, 2010). Recently, using the murine microglial BV-2 cell line, our group reported that the cannabinoids THC and CBD differentially inhibit the lipopolysaccharide (LPS)-activated NF-κB and IFN-β/STAT proinflammatory pathways in a CB_1/CB_2 independent manner (Kozela et al., 2010) and that CBD affects the expression of genes involved in oxidative stress, glutathione deprivation and inflammation (Juknat et al., 2011). Moreover, several of the genes affected seem to be related to zinc homeostasis.

1.2. Zinc homeostasis

Zinc is a critical factor in the regulation of a large number of genes via its role in the activation of transcription factors, as well as by promoting the link between zinc finger proteins and DNA. Cytosolic free zinc concentration is tightly controlled by zinc-binding proteins which include zinc sensors, zinc transporters and zinc buffering proteins (such as the metallothioneins; Mts). In mammalian cells, zinc transport from the cytoplasm toward the lumen of intracellular organelles or to the outside of the cell are mediated by the ZnT/SlcC30 family of transporters. There are at least 10 members of this ZnT family, most of them are ubiquitously expressed and their expression is tightly and dynamically coupled to changes in the intracellular levels of zinc.

Within the cytoplasm, zinc is bound to metal-free apo-metallothionein (apo-Mt) and to protonated glutathione to generate Zn–Mt and G-SZn, respectively. In mammalians there are four metallothionein (Mt) isoforms. Mt1 and Mt2 are ubiquitously expressed in virtually all mammalian cells, of which Mt2a is the major representative. Mt3 is expressed predominantly in the brain and Mt4 is limited to epithelial cells in skin and tongue (see Haq et al. (2003) for review). Mts belong to a family of low molecular weight, cysteine-rich, and high metal containing (as metal-thiolate complex clusters) stress response proteins, involved in a broad range of functions, including zinc and copper homeostasis, heavy metal (Hg, Cd, Ag, Cu) detoxification, scavenging of ROS (decreasing $OH^{\cdot}$ radicals), immune defense responses, regulation of Zn fingers and Zn-containing transcription factors, protein–protein and protein–nucleotide interactions, as well as cell survival and differentiation (for reviews, see Andrews (2000) and Haq et al. (2003)). Some of these actions seem to have physiological relevance in a range of chronic neurological disorders, in which inflammation and oxidative stress are central to the pathophysiology (Penkowa, 2006; Haase and Rink, 2009, and references therein). Many studies have by now demonstrated that the Mts are multipurpose factors important for host defense responses, immunoregulation, cell survival and brain repair. Moreover, it was reported that both Mt1 and Mt2 may reduce inflammation by interfering directly with immune cell–cell interactions, as both Mts were demonstrated to bind specifically to the membranes of activated macrophages, T and B cells, thus, inactivating them and decreasing the immune response (Penkowa, 2006, and references therein).

As described above, cannabinoids were shown to affect the immune system and to posses anti-inflammatory effects. However, no information has been yet reported regarding the effects of cannabinoids on the expression of zinc dependent genes. In this paper we describe the effects of CBD and THC on the expression of these genes.

2. Results

2.1. Effect of CBD on gene expression

mRNA samples were prepared from BV-2 cells treated for 6 h with CBD or THC (both at 10 μM) or with vehicle (ethanol 0.1%) as previously described (Juknat et al., 2011). Characterization of the transcriptional effects of CBD and THC were performed through comparative microarray analysis using the Illumina MouseRef-8 BeadChip platform. Differentially expressed genes were classified according to their gene ontology (GO), using DAVID Bioinformatics online tools (*D*atabase for *A*nnotation, *V*isualization and *I*ntegrated *D*iscovery; http://david.abcc.ncifcrf.gov/; Huang et al., 2009) and the *I*ngenuity *P*athway *A*nalysis (*IPA*; Ingenuity® Systems, http://www.ingenuity.com/). The *DAVID* tool uses the biological knowledge accumulated in public databases and provides a