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Cannabinoids: Novel Medicines for the Treatment of Huntington's Disease

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Abstract: Cannabinoid pharmacology has experienced a notable increase in the last 3 decades which is allowing the development of novel cannabinoid-based medicines for the treatment of different human pathologies, for example, Cesamet® (nabilone) or Marinol® (synthetic Δ^9 -tetrahydrocannabinol for oral administration) that were approved in 80s for the treatment of nausea and vomiting associated with chemotherapy treatment in cancer patients and in 90s for anorexia-cachexia associated with AIDS therapy. Recently, the british company GW Pharmaceuticals plc has developed an oromucosal spray called Sativex®, which is constituted by an equimolecular combination of Δ^9 -tetrahydrocannabinol- and cannabidiol-enriched botanical extracts. Sativex® has been approved for the treatment of specific symptoms (i.e. spasticity and pain) of multiple sclerosis patients in various countries (i.e. Canada, UK, Spain, New Zealand). However, this cannabis-based medicine has been also proposed to be useful in other neurological disorders given the analgesic, antitumoral, anti-inflammatory, and neuroprotective properties of their components demonstrated in preclinical models. Numerous clinical trials are presently being conducted to confirm this potential in patients. We are particularly interested in the case of Huntington's disease (HD), an autosomal-dominant inherited disorder caused by an excess of CAG repeats in the genomic allele resulting in a polyQ expansion in the encoded protein called huntingtin, and that affects primarily striatal and cortical neurons thus producing motor abnormalities (i.e. chorea) and dementia. Cannabinoids have been studied for alleviation of hyperkinetic symptoms, given their inhibitory effects on movement, and, in particular, as disease-modifying agents due to their anti-inflammatory, neuroprotective and neuroregenerative properties. This potential has been corroborated in different experimental models of HD and using different types of cannabinoid agonists, including the phytocannabinoids present in Sativex®, and we are close to initiate a clinical trial with this cannabis-based medicine to evaluate its capability as a disease-modifying agent in a population of HD patients. The present review will address all preclinical evidence supporting the potential of Sativex® for the treatment of disease progression in HD patients. The article presents some promising patents on the cannabinoids.

Keywords: Cannabinoids, Huntington's disease, neuroprotection, Sativex®.

CANNABINOIDS AND THE ENDOCANNABINOID SYSTEM

The use of cannabis preparations as a medicinal drug by different cultures and civilizations has an ancient history with the first evidence of its therapeutic properties about 5000 years ago [1, 2]. The interest for these compounds arrived to European and North-American societies in the early 19th century when animal and human studies showed that cannabis-based extracts were beneficial for the treatment of certain types of pain [3]. However, the investigation on this potential failed, on one hand, due to the urgency of developing new synthetic analgesics and, on other hand, because different international conventions on narcotic drugs held during the first part of the 20th century defined cannabis as a substance with high potential for abuse and no therapeutic properties [3, 4].

In the 60s, a few pioneer researchers were interested in studying the cannabis plant and in 1964 they identified and

isolated the main psychoactive component of this plant so-called Δ^9 -tetrahydrocannabinol (Δ^9 -THC) [5]. Other plant components, all having a particular tricyclic or bicyclic structure almost exclusively found in *Cannabis sativa*, were also identified and isolated (i.e. cannabinol, cannabidiol), although the pharmacological properties of some of them (i.e. cannabigerol, cannabichromene, Δ^9 -tetrahydrocannabivarin) have remained to be studied for long time. Recent studies have indicated that they have some interesting differences in their pharmacological properties compared to Δ^9 -THC [6].

Despite that numerous pharmacological effects (i.e. analgesia, hypomotility, catalepsy, ataxia) of Δ^9 -THC and, in part, of other classic cannabinoids were known during 60s and 70s, the elucidation of the molecular targets underlying these effects has remained elusive or unexplored for almost 20 years [7]. Indeed, the research on cannabinoids took an increasing interest in late 80s and early 90s with the discovery of two different types of cannabinoid receptors CB1 and CB2, both belonging to the superfamily of Gi/o protein-coupled membrane receptors, that are pharmacologically activated by Δ^9 -THC and other cannabinoids [8-10]. This was followed by the isolation and characterization of the

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endogenous compounds that are synthesized in the body to activate these receptors, so-called endocannabinoids [11]. The major characteristic is that they are lipids, mainly, the N-ethanolamine of the arachidonic acid, so-called anandamide (AEA) [12], the glycerol ester of this fatty acid, so-called 2-arachidonoyl-glycerol (2-AG) [13], and other minor and less-studied fatty acid derivatives. However, novel endogenous ligands active at the cannabinoid receptors but having a peptide structure have also been recently identified [14]. It was also demonstrated how these endocannabinoids are synthesized and the enzymes involved in these processes [15], as well as that their action was terminated by an endogenous mechanism of inactivation which involves a carrier-mediated system, still pending of complete molecular characterization [16], and at least two-degrading enzymes, one specific for 2-AG, so-called monoacyl-glycerol lipase (MAGL) [17], and other acting preferentially on AEA and other N-acyl ethanolamines but also on 2-AG, so-called fatty acid amide hydrolase (FAAH) [18]. All these discoveries allowed to demonstrate the existence of a new intercellular communication system called endocannabinoid signaling system that is active in the brain and the periphery playing modulatory and homeostatic functions [19, 20].

Although most of the pharmacological actions induced by cannabinoid compounds are generally due to their interaction with the two classic cannabinoid receptors, CB1 and CB2, which are distributed mainly in the Central Nervous System (CNS) (mainly the CB1 receptor; see [21]) and in the immune system (mainly the CB2 receptor; see [22]), other types of receptors have been also related to the endocannabinoid signals. This is the case of the transient receptor potential vanilloid type 1 (TRPV1) cation channel [23], the GTP-binding protein-coupled receptor GPR55 [24], and the abnormal-CBD receptor [25]. Moreover, recent evidence has indicated that certain cannabinoids are also able to bind and activate the nuclear receptors of the peroxisome-proliferator-activated receptor (PPAR) family [26], thus inducing the actions (i.e. control of inflammatory responses) in which these receptors are involved. Therefore, the evidence indicates that cannabinoid effects are not restricted only to the mediation of well-known cannabinoid CB1 and CB2 receptors and it is hoped that the list of cannabinoid targets will increase in coming years

NEUROPROTECTIVE EFFECTS OF CANNABINOIDS

The description of the endocannabinoid signaling system was in parallel to other research activities aimed at investigating the biological functions in which this modulatory system is involved [27, 28]. Although the system is also active in the periphery, major emphasis has been paid in its modulatory activity in the brain and, particularly, in its function as a retrograde signaling system at different synapses, in particular at glutamate and GABA synapses [29]. Thus, the endocannabinoid system has been involved in pain control, regulation of motor activity, learning and memory processes, control of food intake, emesis, regulation of body temperature, neuroendocrine processes, and others (see [27-31] that have exhaustively reviewed the function of the endocannabinoid system in the CNS).

More recent evidence indicates that the endocannabinoid signaling system also plays a role in the control of neuronal homeostasis and survival, a phenomenon that is possibly sustaining the well-known neuroprotective properties exhibited by certain cannabinoid compounds in various acute [32, 33] and chronic [34-36] neurodegenerative diseases. It is well-known that, in various pathological situations, there is an endogenous generation of endocannabinoids in response to brain damage [37-39], and this is possibly paralleled by up-regulation of specific cannabinoid receptor types, i.e. CB2 receptors [35, 36, 40]. Furthermore, the activation of cannabinoid receptors with either synthetic or natural agonists, including endocannabinoids, is associated with modifications in intracellular signals (i.e. PI3K/Akt) involved in cell responses of survival, homeostasis and repair [41], thus enabling neurons and glial cells to limit the extent of various cytotoxic processes (i.e. excitotoxicity, oxidative stress and inflammation) that operate in neurodegenerative disorders [34-36]. The neuroprotective and neurorepair properties of these compounds are likely based on these capabilities which, in the case of cannabinoids, show the rare characteristic of being all present in single molecules [34-36, 42]. We assume that this is consequence of the location of key elements of the endocannabinoid signaling in cellular and molecular substrates that are crucial for neuronal survival. For example, as mentioned above, CB1 receptors are located in glutamatergic synapses, both at the presynaptic or at the postsynaptic levels, then enabling the control of an excess of glutamate release and the overactivation of glutamatergic receptors, as well as by limiting the calcium influx and the activation of calcium-dependent destructive pathways [34-36, 43-45]. It has been also demonstrated that CB1 receptors are also located in GABAergic neurons, but, whereas the receptors located in glutamatergic neurons are activated under excitotoxic conditions, those present in GABA neurons become silenced [46, 47]. Therefore, those cannabinoids able to activate CB1 receptors may be effective in improving glutamate homeostasis. However, they may also activate CB2 receptors and these receptors due to their preferential location in glial elements, i.e. astrocytes and reactive microglia, may play a role in enhancing the protective effects or limiting the destructive actions of these cells [34-36]. For example, the activation of CB2 receptors can reduce the release of cytotoxic factors like proinflammatory cytokines (i.e. TNF- α , IL-1 β) and also reduce the generation of reactive oxygen species and nitric oxide by activated microglial cells then reducing the damage [35,48,49], in parallel to an increase in the production of prosurvival molecules such a neurotrophic factors or antiinflammatory cytokines [35,36,50]. The problem with CB2 receptors is the lack of selective tools (i.e. antibodies) for the study of this receptor type, in particular in the case of the human brain, so that most of the literature accumulated so far has been almost exclusively obtained in animal studies, and this remains as a major challenge for the future.

Together with these key neuroprotective effects played by CB1 or CB2 receptors, it is also possible to find effects that are independent of both, for example, those developed by cannabinoid compounds with very low affinity (only at the micromolar range) for classic cannabinoid receptors, i.e. CBD [51], HU-211 [52]. CBD is a particular and interesting

cannabinoid, as it will be detailed below. It is also a natural compound of the plant and the interest on this compound as neuroprotectant lies on its powerful antioxidant properties derived from its particular chemical structure [53]. It is even more potent than other natural (i.e. Δ^9 -THC) or synthetic (i.e. HU-211, AM404) cannabinoids that also have antioxidant effects derived from the presence of phenolic groups in their chemical structure [54], but it is devoid of relevant side-effects (i.e. no development of tolerance, no psychoactive effects as it poorly binds CB1 receptors [51]). CBD has been reported to be highly effective as neuroprotectant in different models of neurodegenerative diseases including Parkinson's disease [55-57], Huntington's chorea (see [58] and below), ischemia [59] and Alzheimer's disease [60, 61]. Nowadays, the exact neuroprotectant mechanisms of CBD are still unknown, although they would be likely related to its ability to act as a scavenger of free radicals but also to its ability to regulate intracellular signals involved in the release of calcium by the mitochondria [62] or in the activation of endogenous antioxidant mechanisms (i.e. nrf-2/ARE signaling) [36].

NEUROPROTECTION WITH CANNABINOIDS IN HUNTINGTON'S DISEASE: PRECLINICAL STUDIES

Huntington's disease is an autosomal dominant neurodegenerative disease caused by a mutation in the gene encoding a protein called huntingtin (htt). The mutation consists in an excess of repeats in the CAG triplet within the coding region of the IT15 gene encoding htt, resulting in a polyQ tract near the N-terminus of this protein [63]. Normal subjects have no more than 35 CAG repeats, whereas gene variants with more than 39 CAG repeats define a HD allele encoding a pathogenic htt. A high number of repeats correlates with an earlier age of onset as well as with a more severe disease [64, 65]. The identification of the HD gene mutation in 1993 was assumed as a key step in the elucidation of the pathogenic mechanisms operating in HD and a great promise for the development of specific HD therapies. However, almost 20 years later, there are important questions that remain to be clarified, among them, the exact function(s) of normal htt in the body and the reasons for the extremely-selective toxicity of the mutated protein. Htt is ubiquitously expressed throughout the body and widely expressed within the CNS [66]. It has been involved in various cellular processes, i.e. BDNF transcription [67] and vesicular transport [68], regulation of apoptosis [69], neurogenesis [70] and mitochondrial energy metabolism [71]. The polyQ expansion present in the mutant htt disrupts these functions. For some authors and for a few processes, this occurs by a loss-of-function in mutated htt, although most of researchers support that the polyQ expansion confers a gain of function to the mutated protein that becomes toxic for processes related to transcriptional regulation, intracellular signaling, mitochondrial function and axonal transport [72, 73]. Despite mutant htt in patients is largely expressed in the body, the pathology occurs in a brain-specific manner affecting very restricted brain areas, i.e. striatum [74,75] and cerebral cortex [76, 77], and, within these structures, only to specific neuronal subpopulations, i.e. striatal projection GABAergic neurons and cortical glutamatergic neurons, respectively. The reasons for this selec-

tivity, particularly in the case of striatal projection neurons, have not been completely elucidated but several theories, including BDNF dependence, enhanced excitotoxic susceptibility and greater metabolic and mitochondrial activities, have been proposed as key factors [78].

The disease is characterized by the occurrence of two major symptoms, i.e. motor abnormalities and cognitive impairment [79]. The neurodegenerative process is progressive but, in terms of motor symptoms, has a biphasic profile. Thus, the early stages of the disease are characterized by involuntary movements termed "chorea" that correspond to a primary affectation of striatal GABA neurons that project to the globus pallidus [80-82]. Cortical glutamatergic neurons that project to the striatum also degenerate in these early stages and this could explain the cognitive impairments and psychological disturbances also appearing in HD patients [83]. Once the disease has advanced and the neuronal death is massive and also affects other striatal subpopulations, patients show a parkinsonian-like symptomatology with bradykinesia and rigidity episodes [84]. Although the primary cause of neurodegenerative process occurring in HD is the toxicity of the mutant htt, several additional processes, most of them common to other neurodegenerative disorders, i.e. protein misfolding, abnormal proteolysis, protein aggregation and deposition, transcriptional dysregulation, mitochondrial dysfunction, excitotoxic and oxidative events, and glial activation and local inflammatory events, have been also involved in neuronal death in HD [83, 85].

Unfortunately, HD has still no cure and patients die approximately 10-20 years after diagnosed. They only have relief therapy to alleviate some symptomatic features associated to the disease (i.e. antidopaminergic drugs to alleviate the hyperkinesia observed in the first stages [86]). There was also some attempts with antiglutamatergic agents to reduce excitotoxicity but the efficacy was very limited [87]. In the last years, various groups of novel compounds, i.e. unsaturated fatty acids, minocycline, coenzyme Q10, inhibitors of histone deacetylases, have been studied in preclinical models and, even, some of them have entered in the clinical evaluation phase as potential novel disease-modifying agents in HD [83, 88, 89]. Promising expectatives have been also obtained from cannabinoid compounds in cellular and animal models given their well-known neuroprotective effects (see above), even, some specific cannabinoid-related compounds have been proposed for attenuating hyperkinetic involuntary movements in HD [90, 91], although this possibility will not be addressed in this review article, which concentrates in their disease-modifying effects.

When speaking about neuroprotective effects with cannabinoid compounds in experimental HD, it is important to identify the pharmacological targets within the endocannabinoid system that are available for these compounds, as well as the type of cytotoxic processes operating in HD that can be controlled with the activation of these targets. This is an important issue for cannabinoids as they have the rare property to may afford neuroprotection by the combination of distinct but complementary effects (see above). A first target is the CB1 receptor. This receptor experiences a notable down-regulation in HD (demonstrated in HD patients using

in vivo imaging techniques [92] and postmortem brains [93], and also in experimental models [94, 95]) that is observed very early, even in presymptomatic phases, which might indicate that this down-regulation would play a causal role in the pathogenesis (i.e. losses of CB1 receptors are compatible with enhanced excitotoxicity; see Fig. (1)). We have recently demonstrated that this is true as the losses of CB1 receptors are a direct effect of the mutant *htt* exerted through the same intracellular signals (i.e. REST factor) that control BDNF expression [96]. Accordingly, the pharmacological correction of this deficit with compounds targeting this receptor reduced and/or delayed the progression of striatal degeneration as demonstrated in various studies (see [96-98] and Fig. (1)). However, the issue is far to be completely clarified as other authors showed no changes in CB1 receptors and no influence of CB1 receptor agonists in disease progression, using also a transgenic mouse model of this disease [99].

A second endocannabinoid target in HD is the CB2 receptor. This receptor is poorly abundant in the striatal parenchyma in healthy conditions, but it is overexpressed in parallel to the degenerative events occurring in HD (also demonstrated in postmortem human brains [100] and in experimental models including R6/2 mice [100] and malonate-lesioned rats [101]). The problem with this receptor is the lack of specific tools that may be useful when using human brains, as has been outlined before. This up-regulatory response occurs in glial cells, including astrocytes but it was particularly evident in reactive microglial cells that are recruited at the lesion sites [100,101], and has been also found in patients or animal models of other neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, multiple sclerosis, neuropathic pain, ischemia and others (reviewed in [35,36,40]). In HD (and also in the other disorders), the activation of CB2 receptors exerts beneficial effects by preserving striatal neurons from inflammatory insults. This has been seen in R6/2 mice [100] and also in some neurotoxin-based models, i.e. malonate-lesioned rats [101] and quinolinate-lesioned mice [100], that may be used as models of acute striatal injury. In all cases, the positive effects were related

mainly to a reduction in microgliosis and in the toxicity (i.e. generation of inflammatory cytokines) exerted by these cells (see [36,100] and Fig. (1)). However, it is also possible that targeting CB2 receptors may be associated with an increase in prosurvival molecules (i.e. neurotrophins, anti-inflammatory cytokines, metabolic substrates) produced in part by astrocytes which also contain these receptors [35, 36]. The possible application of selective CB2 receptor agonists in Huntington's disease received patent protection in 2007 [102].

There exist additional mechanisms, independent of CB1 and CB2 receptors, that have been involved in the neuroprotective effects exerted by certain cannabinoids in experimental models of HD. This is the case of those cannabinoids with antioxidant properties like the phytocannabinoids Δ^9 -THC and CBD. They were highly effective in an experimental model of HD in which oxidative injury is a key cytotoxic mechanisms (see [58] and Fig. (1)). As mentioned above, these effects have been associated with an scavenger action of reactive oxygen species facilitated by the particular phenolic structures of these phytocannabinoids, although there are some proposals in the sense that these compounds may be acting through the regulation of those intracellular signals that control the expression of antioxidant enzymes of phase II (i.e. *nrf-2*/ARE signaling) [36].

NEUROPROTECTION WITH CANNABINOIDS IN HUNTINGTON'S DISEASE: CLINICAL STUDIES

The data described in the above section provide solid evidence for going to a clinical evaluation of a cannabis-based medicine in HD patients. Indeed, previous studies have already tried to determine whether specific cannabinoids are efficacious in HD patients, although these studies have concentrated on specific symptoms (i.e. chorea, behavioral abnormalities) rather than on disease progression. For example, nabilone was assayed in two uncontrolled, single-patient studies [103,104]. The rationale was to enhance the reduced CB1 receptor signaling observed in HD patients

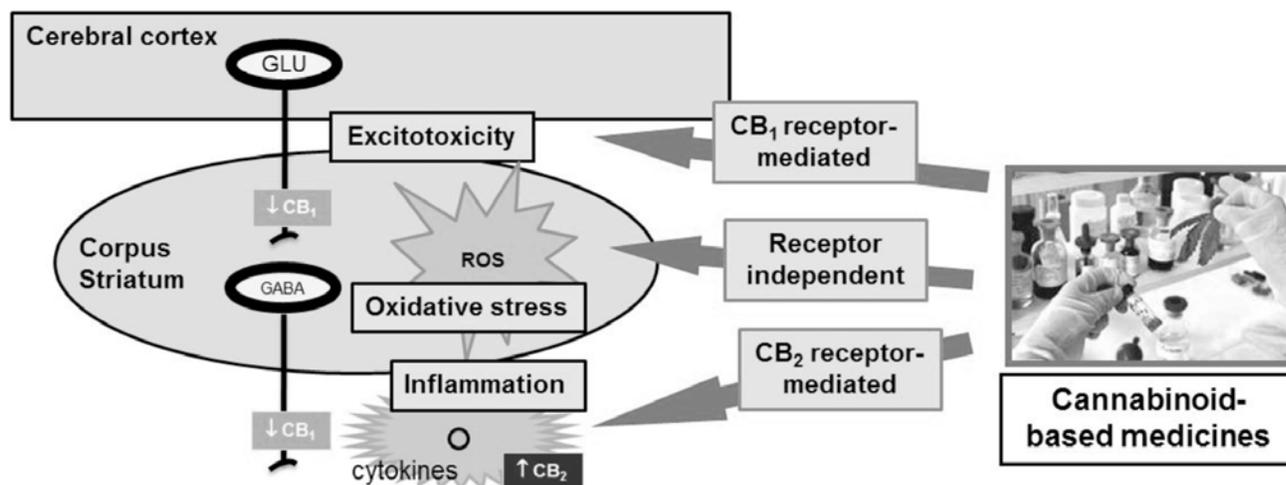


Fig. (1). Potential targets and cytotoxic processes (i.e. excitotoxicity, oxidative stress, inflammation) that may be susceptible of a disease-modifying treatment with cannabinoid-based medicines in HD patients. This is supported by preclinical studies showing CB1 receptor down-regulation/losses in the striatum associated with excitotoxicity, and CB2 receptor up-regulation in glial elements (reviewed in [35]).

with this Δ^9 -THC analog (available for clinical use as Cesamet®) that is highly effective at the CB1 receptors, trying to attenuate with this strategy the hyperkinetic movements and also some behavioral abnormalities. However, these studies yielded conflicting results. Thus, although nabilone induced signs of improvement in one of these studies [103], in the other study, it made symptoms worse [104]. More recently, nabilone was also used in a double-blind, placebo-controlled, cross-over study [105] in which it induced improvements in various motor and cognitive indices. Other cannabinoid compound, CBD, has been also studied in a controlled trial [106], but despite the compound was well-tolerated, it did not show any beneficial effect on chorea severity in 15 HD patients.

As mentioned above, all these previous trials did not examine disease progression and were conducted with individual cannabinoid agonists, whereas the data obtained recently in animal models suggest, as mentioned above, that combinations of different cannabinoids or the use of a broad-spectrum cannabinoid, is to be recommended for clinical testing of neuroprotective effects given the diversity of targets and cytotoxic processes where cannabinoid compounds may afford positive results. This might explain the lack of positive effects found in some of these previous trials. In this context, a good choice may be the recently-licensed cannabis-based medicine Sativex®. This medicinal preparation is an equimolecular combination of Δ^9 -THC- and CBD-enriched botanical extracts [107-109]. Both Δ^9 -THC and CBD have been already investigated in animal models of HD with positive results [58,110] and the combination of both in the form of enriched botanical extracts, the same used in Sativex®, have been also recently evaluated in these animal models with similar effects [111] and subjected recently to patent protection [112]. The presence of Δ^9 -THC in Sativex® enables this medicine to activate both CB1 and CB2 receptors, two targets that have been involved in neuroprotective effects of different cannabinoid compounds, particularly against excitotoxicity and inflammation, respectively [96-98,100,101]. In addition, the presence of CBD strongly elevates the antioxidant potential of this medicine, a property that also has therapeutic value in HD [58] and that is also provided by Δ^9 -THC, as this potential is directly related to the type of chemical structure of both phytocannabinoids. The presence of CBD provides additional advantages as this cannabinoid exhibits a broad spectrum of biological effects *in vivo* and *in vitro*, and some of them may be relevant for neuroprotection (i.e. anti-inflammatory effects [51,113,114]). It is curious that, despite CBD is a compound with multiple beneficial effects demonstrated in numerous disorders, its specific mechanism(s) of action is(are) still pending of complete characterization, as has been reviewed recently [61,115,116]. Although there is certain debate about the possibility that CBD binds to a novel cannabinoid receptor or to a specific intracellular novel target within the endocannabinoid system, the truth is that it does not bind CB1 and CB2 receptors in the nanomolar range despite it may act at the CB2 at the micromolar range. It also blocks the GPR55 receptor but also at high concentrations, whereas it has certain activity at the mechanism inactivating endocannabinoid signals (i.e. endocannabinoid transport, FAAH en-

zyme), as well as in the activation of ionotropic (i.e. TRPV1) or nuclear (i.e. PPARs) receptors. Out of the endocannabinoid system, CBD has been related to adenosine uptake or serotonin receptors (reviewed in [61,115,116]). Lastly, despite the long-term effects derived from the chronic use of Sativex® in human disorders are not completely known and would possibly require additional experimentation, it is also important to note that CBD has a good tolerance and low toxicity by itself, and, interestingly, it is able to attenuate potential side-effects associated with the chronic use of Δ^9 -THC, whereas enhancing its therapeutic effects. This makes their combination in Sativex® as an interesting tool for developing novel therapies in HD and other neurological disorders.

CURRENT & FUTURE DEVELOPMENTS:

Therefore, the beneficial effects found with different cannabinoids in different experimental models of HD support: (i) that this type of compounds may be considered a novel disease-modifying therapy susceptible to be evaluated at the clinical level, and (ii) that the type of cannabinoid compound(s) that may be useful for a disease-modifying therapy in HD patients should be a multi-targeting cannabinoid or a combination of different selective compounds, given that different targets and compounds have been associated with the control of different cytotoxic mechanisms. We have discussed that an attractive possibility is the cannabis-based medicine Sativex®, which is a combination of botanical extracts enriched with Δ^9 -THC and CBD. We have recently demonstrated this Sativex®-like combination attenuated cytotoxic events (i.e. oxidative injury and local inflammatory events) and preserved striatal neurons in models of acute striatal injury reminiscent of HD, thus supporting the need to go to the clinical level, in a trial directed at assessing the efficacy of Sativex® as a disease-modifying agent in a population of early symptomatic HD patients.

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CONFLICT OF INTEREST

None.

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