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Review

Cannabidiol (CBD) and its analogs: a review of their effects on inflammation

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ABSTRACT

First isolated from *Cannabis* in 1940 by Roger Adams, the structure of CBD was not completely elucidated until 1963. Subsequent studies resulted in the pronouncement that THC was the 'active' principle of *Cannabis* and research then focused primarily on it to the virtual exclusion of CBD. This was no doubt due to the belief that activity meant psychoactivity that was shown by THC and not by CBD. In retrospect this must be seen as unfortunate since a number of actions of CBD with potential therapeutic benefit were downplayed for many years. In this review, attention will be focused on the effects of CBD in the broad area of inflammation where such benefits seem likely to be developed. Topics covered in this review are; the medicinal chemistry of CBD, CBD receptor binding involved in controlling Inflammation, signaling events generated by CBD, downstream events affected by CBD (gene expression and transcription), functional effects reported for CBD and combined THC plus CBD treatment.

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Abbreviations: CBD, cannabidiol; CBCy, cannabicyclol; CBCR, cannabichromene; CBGA, cannabigerolic acid; CBGV, cannabigerovarin; CBN, cannabinol; CBG, cannabigerol; DMH, dimethylheptyl; LPS, lipopolysaccharide; NBMPR, *S*-(4-nitrobenzyl)-6-thioinosine; NFAT, nuclear factor of activated T-cells; PLA_2, phospholipase A_2; THC, Δ^9-tetrahydrocannabinol; THCV, tetrahydrocannabivarin; TNF-α, tumor necrosis factor-α; ROI, reactive oxygen intermediates; NAgly, *N*-arachidonoyl glycine; CIA, collagen-induced arthritis; FAAH, fatty acid amide hydrolase; OPC, oligodendrocyte progenitor cells.

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1. Introduction

Recent years have seen a dramatic increase in interest in the major phytocannabinoid, cannabidiol. For the period 2008 to the present, 1205 publications can be found in a PubMed search using the keyword cannabidiol. This compares with lists of 225 reports for the years 2003–2007 and 50 for 1999–2002.[1] First isolated from *Cannabis* in 1940,[2] the structure shown in Figure 1 was not reported until 1963.[3] Subsequent studies on *Cannabis* resulted in the pronouncement that THC was the 'active' principle and research then focused primarily on it to the virtual exclusion of CBD. This was no doubt due to the belief that activity meant psychoactivity that was shown by THC and not by CBD. In retrospect, this must be seen as unfortunate since a number of actions of CBD with potential therapeutic benefit were overlooked for many years. In this review, attention will be focused on the effects of CBD on the broad area of inflammation where such benefits seem likely to be realized.

2. Medicinal chemistry of CBD

2.1. Conformation

Although there is considerable structural overlap between CBD and THC (Fig. 1), the conformational structures shown in Figure 1A differ significantly.[4] Whereas THC exists in an essentially planar conformation, CBD adopts a conformation in which the two rings are more or less at right angles to each other (Fig. 1). A result of this is the observation that CBD does not bind to or activate the CB1 receptor an action that THC is capable of doing. This in turn leads to a complete lack of psychoactivity by CBD unlike THC, which is the psychoactive principle of *Cannabis*. The basis of this is a so-called 'region of steric interference'[5] on the CB1 receptor that allows THC to bind but interferes with CBD binding.

2.2. Natural homologs and synthetic analogs

There are four known side-chain homologs of CBD; methyl, *n*-propyl, *n*-butyl and *n*-pentyl groups.[6] Of these, until recently, only the pentyl homolog, CBD itself, has been extensively studied in terms of biological activity.[7] The syntheses of the CBD derivatives, (−)-11-hydroxy-CBD, (−)-CBD-11-oic acid and their

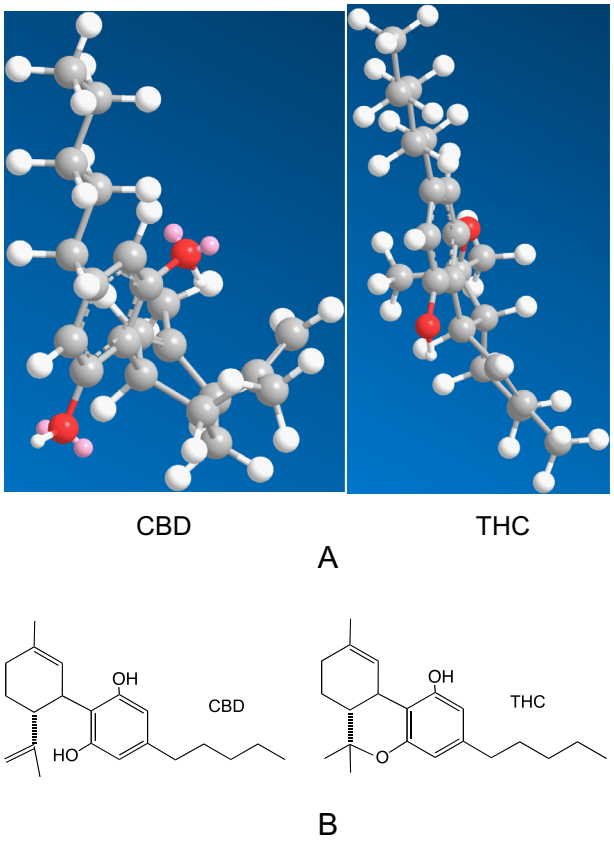

Figure 1. The minimal energy conformations of CBD and Δ^9-tetrahydrocannabinol (THC) are shown in 1A. THC has a fairly planar conformation whereas CBD has a bent conformation. This difference results in different pharmacological profiles even though there is considerable structural overlap of both when viewed in a two-dimensional as shown in 1B. CBD refers to (−)-CBD here and throughout this paper.

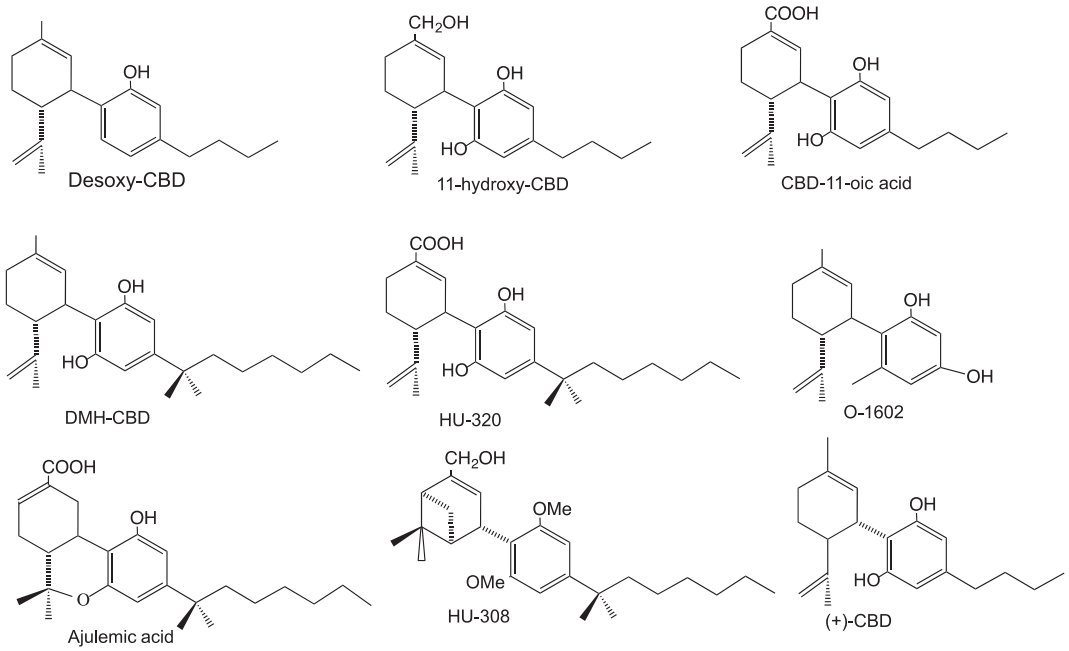

Figure 2. The structures of CBD analogs and related substances.

dimethylheptyl (DMH) analogs, as well as of the enantiomeric (+)-CBD series have been reported (Fig. 2).[8] The affinities of these compounds for both the CB1 and CB2 receptors were measured with unexpected results. Whereas the naturally occurring (−)-CBD series showed no affinity, the (+)-CBD series displayed affinities in the nano molar range. Regarding anti inflammatory action, (−)-DMH-CBD-11-oic acid showed anti inflammatory activity in a preclinical study (Section 6.2).[9,10]

Hydrogenation of both CBD and DMH-CBD (Fig. 2) yielded mixtures of dihydro and tetrathydro reduction products that were separated and structurally characterized.[11] Using murine macrophages, their effects on the production of reactive oxygen intermediates (ROI), nitric oxide (NO), and tumor necrosis factor (TNF-R) were determined. Unexpectedly, the reduced compounds showed affinities for CB1 in contrast to CBD and DMH-CBD that do not bind to this receptor.

As part of a study to characterize the CB1 receptor binding site, desoxy-CBD (Fig. 2), a CBD analog with only one hydroxy group was prepared.[4] Based primarily on computational studies, it was concluded that the analog would be able to occupy this site. Desoxy-CBD behaves as a partial agonist with an IC-50 of 30.9 nM in the mouse vas deferens assay. This type of activity is considered to be an indication of CB1 activation that would be predicted by the theoretical considerations. No direct measurement of receptor binding was reported.

3. Receptor binding involved in controlling inflammation

3.1. CB1 cannabinoid receptor

CBD itself has no affinity for CB1, however, several of its hydrogenated analogs bind with nano molar affinity. The most active analog was tetrahydro-DMH-CBD when tested using a synaptosomal membrane preparation derived from rat brain. It was reported to bind to this CNS cannabinoid receptor with a K_i of 17 nM.[11] The enantiomeric CBD derivatives, (+)-11-hydroxy-CBD, (+)-CBD-11-oic acid and their dimethylheptyl (DMH) analogs exhibit binding to CB1 in the low nano molar range.[8] These findings are difficult to reconcile with the earlier report on desoxy-CBD cited above in Section 2.2.[4] Arguments were presented that the non planar conformation of CBD prevents it from reaching the ligand binding site in CB1 since a planar structure is needed for this to occur. The analogs described here all contain two phenolic hydroxy groups that would prevent such a planar conformation.

3.2. CB2 cannabinoid receptor

A CBD analog with a modified terpene ring, HU-308 (Fig. 2) was reported to be a specific ligand for CB2 with low nano molar affinity ($K_i = 22.7 \pm 3.9$ nM).[12,13] It did not bind to CB1 (K_i >10 μM) and did not elicit CB1 mediated responses either in vitro or in vivo. However, forskolin stimulated cyclic AMP production in CB2 transfected cells was potently inhibited. An inflammatory effect, arachidonic acid-induced ear edema in mice, was inhibited, which was reversed by the CB2 antagonist SR144528 but not by the CB1 antagonist SR141716a.

The actions of CBD were studied in hypoxic–ischemic immature brain, forebrain slices from newborn mice.[14] At a concentration of μM, it produced significant reductions in IL-6 concentration, and TNF-α, COX-2, and iNOS expression. The use of selective antagonists for the CB2 and adenosine A2A receptors suggested their mediation in these actions. However, the high concentration of CBD needed makes the pharmacological relevance of these findings somewhat questionable. Functional heteromers composed of a mixture of A2A subunits with subunits from other unrelated G-protein coupled receptors have been found in the brain. In a subsequent report, using a hypoxic ischemic brain injury model in newborn pigs, CBD reduced IL-1 levels in lesioned animals; moreover, this effect was reduced when it was administered together with CB2 or 5HT1A receptor antagonists.[15] The CBD was given iv at 1 mg/kg and the levels of IL-1 were measured by Western blot analysis.

3.3. Adenosine A2A receptors

It has been suggested that A2A receptors can down regulate over-reactive immune cells, resulting in protection of tissues from

collateral inflammatory damage.[16] Also, it has been reported that CBD has the ability to enhance adenosine signaling through inhibition of uptake and provide a non cannabinoid receptor mechanism by which CBD can decrease inflammation.[17] They reported that in vivo treatment with a low dose of CBD (1 mg/kg, ip) decreases TNF-α production in LPS-treated mice; this effect was reversed by an A2A adenosine receptor antagonist and was abolished in A2A receptor knockout mice. The possible involvement of this receptor in CBD anti-inflammatory actions was also mentioned in the preceding section.[14] The A2A antagonist SCH58261 abolished the modulation by CBD of cytokine production and COX-2 induction, suggesting that A2A activation participates in the anti-inflammatory activity of CBD.

CBD has anti-inflammatory effects in a murine model of acute lung injury that appear to be mediated by the A2A receptor injury.[18] LPS-induced inflammation in mice was reduced by the administration of a single dose of 20 mg/kg of CBD. The effects included neutrophil migration into the lungs, albumin concentration in the bronchoalveolar lavage fluid, myeloperoxidase activity in the lung tissue, and production of TNF and IL-6 and chemokines (MCP-1 and MIP-2). The A2A antagonist ZM241385 inhibited all of these actions implicating this receptor in the anti-inflammatory effects of CBD.

One of the animal models for multiple sclerosis, Theiler's murine encephalomyelitis virus-induced demyelinating disease (TMEV), is accompanied by inflammation. In this model, CBD decreased leukocyte infiltration in the brains of TMEV-infected animals and it also significantly reduced microglial activation in the cerebral cortex.[19] In addition, the levels of the pro inflammatory cytokines TNF-α and IL-1β were reduced. These actions of CBD appear to be partially mediated by the A2A receptor based on inhibition of the effects by prior administration of the antagonist ZM241385. The authors concluded that CBD, 'can limit the harmful effects of an exacerbated inflammatory response, likely by increasing adenosine signaling, and prevent the development of secondary and irreversible damage'.

3.4. CB2/5HT(1A) heterodimerization

In an interesting recent study, evidence was found that CB2 and 5HT1A receptors may form hetero dimers in HEK-293T cells.[15] The study was focused on mechanisms of CBD neuroprotection (vide infra) in hypoxic-ischemic newborn pigs involving a possible role for 5HT(1A) and/or CB2 receptors. Bioluminescence resonance energy transfer assays were used to support the conclusion that CB2/5HT(1A) hetero dimerization is responsible for the observed actions of CBD in this model. Further evidence was provided by the cross-antagonism shown by the CB2 receptor antagonist (AM630) and a serotonin 5HT1A receptor antagonist (WAY100635). These findings have implications for receptor mediation in other actions of CBD and the actions of several other cannabinoids as well.

3.5. TRPV1 receptor

Injection of mice with the plant lectin Concanavalin A (Con A), results in polyclonal activation of T lymphocytes leading to a liver inflammatory response that can be reduced by the administration of 25 mg/kg of CBD.[20] Specifically, the levels of the pro-inflammatory cytokines IL-2, TNF-α, IFN-c, IL-6, IL-12 (p-40), IL-17, MCP-1 and eotaxin-1 (CCL11) were significantly decreased by CBD in Con A treated mice. By the use of vanilloid receptor knock-out mice, the authors showed that CBD induced suppression of inflammation in Con A-hepatitis was dependent on TRPV1. The data strongly support this conclusion, however, independent confirmation, possibly by the use of antagonists, is needed to firmly establish a role for TRPV1.

3.6. GPR55 Receptor

CBD has been reported to act as a functional antagonist to the GPR55 receptor.[21] The orphan receptor GPR55 was activated by the CBD analog O-1602 (Fig. 2) resulting in increased IL-12 and TNF-α production, and increased endocytic activity in LPS-activated monocytes. These effects of GPR55 were antagonized by CBD acting as a selective antagonist.

4. Signaling events generated by CBD

4.1. Eicosanoids

4.1.1. Arachidonic acid release

The initiating event in all eicosanoid biosynthesis is the release of free arachidonic acid from phospholipid storage sites where it exists in an esterified form. Thus, drugs affecting this process, presumably involving PLA_2, can have a profound effect on the physiological status of a variety of systems. Both CBD and THC produce a significant stimulation of arachidonic acid release in intact human platelets.[22] Interestingly, CBD is roughly 1.5 times more potent than THC suggesting that this action may not be involved in the psychotropic activity of THC. It was also found that a product shift from cyclooxygenase to lipoxygenase products occurs as a result of cannabinoid exposure. This probably involves action(s) on downstream events in the arachidonic acid cascade. Stimulated arachidonic acid release was also observed in neuroblastoma cells (NBA2). The arachidonic acid release effect was extended to a series of six primary phytocannabinoids to produce the following rank order of hydrolytic activity: CBD $\gg$ CBCy > THC = CBCR = CBN $\gg$ CBG.[23] The model used to obtain these data was the WI-38 human lung fibroblast that had been radiolabelled by equilibration with free arachidonic acid. Again, CBD was more active than THC in stimulating phospholipid hydrolysis. By way of comparison, the anti inflammatory actions of cannabinoid analogs such as NAgly[24] and ajulemic acid (Fig. 2)[25] have been attributed to their ability to promote the release of free arachidonic acid. In these examples, a result of this action was the elevation of pro resolving substances such as lipoxin A_4 and 15d-PGJ_2.[26] A similar mechanism may explain some of the anti inflammatory actions of CBD.

4.1.2. Cyclooxygenase and products

A group of six cannabinoids, including CBD and THC, were tested for their ability to inhibit both COX-1 (ram seminal vesicles) and COX-2 (sheep placental cotyledons) activity.[27] THC actually stimulated COX-1 whereas CBD had very little effect on its activity. In the case of COX-2, both THC and CBD stimulated activity with CBD being more than twice as potent. This agrees with the effects of these cannabinoids on the release of arachidonic acid mentioned above. Moreover, COX-2 likely mediates the synthesis of lipoxin A_4 and 15d-PGJ_2.

CBD was administered orally (5–40 mg/kg) once a day for 3 days following intraplantar injection of 0.1 ml carrageenan (1% w/v in saline) in the rat.[28] Measurements were made of prostaglandin E_2 (PGE_2) in plasma, cyclooxygenase (COX) activity, production of nitric oxide (NO; nitrite/nitrate content), and of other oxygen-derived free radicals (malondialdehyde) in inflamed paw tissues. All three markers, which were elevated by carrageenan treatment, were reduced in a dose-dependent fashion by CBD when compared to vehicle treated controls. In addition there was a dose related decrease in paw edema. These findings

strongly support the view that CBD has anti-inflammatory activity and may find a use in treating clinical inflammation.

The report cited above was subsequently extended using a different model of inflammation; complete Freund's adjuvant intraplantar injection in rats.[29] Again, CBD effected a reduction in the levels of several mediators, such as prostaglandin E_2, lipid peroxide and nitric oxide, and in the over-activity of glutathione-related enzymes. CBD's efficacy was not accompanied by any reduction in nuclear factor-kappa B activation and tumor necrosis factor alpha concentration. These latter two markers are common indicators for anti-inflammatory action suggesting that CBD may act by a novel mechanism.

4.1.3. Lipid storage diseases

The hydrolytic actions of CBD have been extended to the problem of the lipid storage diseases, for example, Niemann–Pick Disease.[30] Fibroblasts obtained from a Niemann-Pick patient were treated with 30 μM CBD and chromatographically analyzed for lecithin and sphingomyelin content. The former was decreased by 21% whereas the latter was reduced by 77%; excess sphingomyelin is a feature of Niemann–Pick Disease. A control experiment was done using fibroblasts from normal subjects that were treated in a comparable manner. Lecithin and sphingomyelin content in the control was reduced by 21% and 17% respectively suggesting a selective action of CBD on disease cells.

4.2. Cytokines

LPS-induced TNF-α production by RAW 264.7 mouse macrophage cells was completely inhibited by treatment with 8 μM CBD and its analog DMH-CBD (Fig. 2).[11] Surprisingly, the dihydro and tetrahydro derivatives of each cited in Section 2.1 showed very different effects on TNF-α synthesis; the reduced CBD analogs were inhibitory whereas the reduced DMH-CBD compounds were moderately stimulatory. There is no obvious explanation for this observation; however, full dose-response measurements may reveal biphasic responses for all of these substances accompanied by shifts in their potencies.

In a model of Alzheimer's disease-related neuroinflammation, where mice were inoculated with human Aβ (1–42) peptide, CBD reduced both iNOS and IL-1β protein expression, and also decreased related NO and IL-1β production.[31] A 50% reduction of each was found in hippocampal homogenates following treatment with 10 μg/kg of CBD. A smaller but significant effect was shown by treatment with 2.5 μg/kg of CBD. The authors suggested that CB2 may mediate these actions, however, no direct evidence was presented.

Endotoxin-induced uveitis induced by systemic or local injection of LPS in rats was used an in vivo model to study the effects of CBD on acute ocular inflammation.[32] The in vivo study was complemented by in vitro experiments using microglial cells that were isolated from the retinae of newborn rats. It was shown that LPS-induced release of TNF-α is inhibited almost entirely by the addition of 1 μM CBD. Data are also reported suggesting that the inhibition of p38 MAPK phosphorylation in responsible for this action. In vivo it was shown CBD at 5 mg/kg prevents retinal microglial activation or macrophage infiltration and inhibits serum and retinal TNF-α release in the LPS-treated rat. These findings provide compelling evidence for the use of CBD in the treatment of retinal inflammation and neuroprotection both in terms of its efficacy and safety.

The anti inflammatory action of CBD on cisplatin-induced inflammation, and tissue injury in the kidney was studied using an established mouse model of cisplatin-induced nephropathy.[33] CBD treatment (10 mg/kg/day ip) reduced mRNA expression of TNF-α and IL-1β in the kidneys 72 h after its administration to mice. Interestingly, several markers of nephrotoxicity were also reduced, however, little was offered by way of mechanism to explain these interesting findings.

It was reported that CBD, studied at 1, 5 and 10 μM, decreased the production and release of pro inflammatory cytokines such as interleukin-1β, interleukin-6, and interferon-β, from LPS-activated BV-2 microglial cells.[34] Neither CB1 or CB2 cannabinoid receptors, nor the abn-CBD-sensitive receptors, were involved in this action. In addition, CBD reduced the activity of the NF-κ B pathway and up-regulated the activation of the STAT3 transcription factor. Parallel experiments with THC revealed substantial differences in their actions.

The effect of CBD on LPS-induced TNF-α expression was examined in intestinal homogenates of LPS-treated mice.[35] Western blot analysis showed a 50% reduction in protein levels from CBD mice treated with 10 mg/kg given ip Similar results were obtained in ex vivo human derived colonic biopsies cultured for 24 h in the presence of LPS plus IFN-γ. Treatment of the cultures with a concentration of 1 μM CBD gave a >50% reduction in iNOS protein expression, nitrate levels and S100B protein expression. Evidence for possible PPAR-γ partial involvement was also reported. It was suggested that pharmacological control of glial cell activity represents a novel approach for the treatment of intestinal inflammatory pathologies.

Some data have been reported suggesting that CBD is a GPR55 antagonist.[36] In a more recent study, it was found that pretreatment of rat cerebellar granule cells (CGCs) with CBD inhibited LPS-induced cytokine mRNA expression.[37] RT-PCR analysis of cells that were treated with 50 μM CBD for 30 min, and then stimulated with LPS (3 μg/ml) for 4 h, showed reduced mRNA levels of IL-1β, IL-6, and TNF-α. The high concentration of CBD used reduces to some degree the significance of these findings.

CBD and its analog O-1602 showed anti-inflammatory activity in mice with cerulein-induced acute pancreatitis accompanied by an increased expression of GPR55 receptor in pancreatic tissues.[38]

4.3. Effects of CBD on intracellular Ca^{++} levels

Mast cells can contribute to chronic airway inflammatory responses, remodeling and symptomatology, involving the production of several of the eicosanoids and cytokines. Activation and degranulation of mast cells is triggered by an increase of $[Ca^{++}]i$. Using flow cytometry in a time-resolved mode, it was reported that CBD evoked, in a concentration dependent manner (1–10 μM), a persistent rise of [Ca++]i in RBL-2H3 cells.[39] The initiation of the arachidonic acid cascade is strongly dependent on $[Ca^{++}]i$. No evidence was presented for a specific receptor involvement, however, both cannabinoid receptors and the vanilloid receptor were excluded.

CBD stimulated TRPV3-mediated $[Ca^{2+}]i$ with high efficacy showing 50–70% of the effect of ionomycin and a potency of EC_{50} = 3.7 μM in TRPV3-mediated elevation in transfected HEK-293 cells.[40] CBD ranked high in efficacy when compared to a number *Cannabis* components including; THCV > CBD > carvacrol > THCVA > CBGV > CBC > CBG > THC > CBGA > CBDV > CBN > CBDA = THCA.

5. Downstream events affected by CBD: gene expression and transcription

5.1. Comparative microarray analysis

The transcriptional effects of CBD and THC were studied in BV-2 microglial cells in a comparative microarray analysis using the Illumina MouseRef-8 BeadChip platform Ingenuity Pathway Analysis

was performed to identify functional subsets of genes and networks regulated by CBD and/or THC.[41] It was reported that CBD affected the expression of many more genes, than those affected by THC. It was also found that CBD induced a robust response related to oxidative stress and GSH deprivation apparently controlled by Nrf2 and ATF4 transcription factors. The mechanism underlying the CBD actions involves depletion of intracellular GSH, activating the GCN2/eIF2a/p8/ATF4/ CHOP-TRIB3 pathway accompanied by generation of ROS via the (EpRE/ARE)-Nrf2/ATF4 system, and regulation of the Nrf2/Hmox1 axis. The anti-inflammatory effects of CBD were correlated with up-regulations of the expression of *Hmox1* and *IFNb1*, and down-regulation of the expression of *Ccl2*, via the IFN-b-STAT pathway.[41,42]

5.2. Expression of glial fibrillary acidic protein mRNA

The anti-inflammatory properties of CBD were demonstrated in a mouse model of Alzheimer's disease-related neuroinflammation.[31,43] Compared to vehicle controls, CBD (2.5 or 10 mg/kg, ip) dose-dependently inhibited glial fibrillary acidic protein mRNA and protein expression in beta-amyloid injected mice. In addition, under the same experimental conditions, CBD reduced iNOS and IL-1β protein expression, and NO and IL-1β release as well. The results of this study suggest that CBD can effectively inhibit beta-amyloid evoked neuro inflammatory reactions and may be effective in the treatment of Alzheimer's disease.

5.3. PPARγ involvement

An inhibitory effect of CBD on the release of inflammatory mediators by in vitro cultured astrocytes has been reported.[43] In this study, beta-amyloid challenged astrocytes (1 mg/ml) were treated with CBD (10^{-9} to 10^{-7} M) in the presence or absence of a PPAR-α antagonist (MK886, 3 μM) or a PPAR-γ antagonist (GW9662, 9 nM). After 24 h, NO production was determined by measuring nitrite (NO_2^-) accumulation in the culture medium, in addition, IL-1β, TNF-α, and S100B calcium binding protein release was determined by ELISA assay. The PPAR-γ antagonist was able to significantly reverse the CBD inhibitory effects on reactive gliosis, an important feature of many autoimmune inflammatory disorders, and, as a further result, on neuronal damage. It was concluded that CBD reduces beta-amyloid-induced neuroinflammation and promotes hippocampal neurogenesis through PPAR-γ involvement.

5.4. Production of reactive oxygen intermediates

The unusual receptor affinity of several CBD analogs was mentioned above in Section 3.1.[11] Cannabidiol (CBD) and cannabidiol dimethylheptyl (CBD-DMH) were hydrogenated to give four different epimers. These new derivatives were studied for their ability to modulate the production of reactive oxygen intermediates (ROI), nitric oxide (NO), and TNF-α by murine macrophages. Over a limited concentration range, variable effects were observed from inhibition to stimulation of the levels of these mediators of inflammation. It seems likely that biphasic responses would be seen if the compounds were tested at wider concentration ranges.

6. Functional effects reported for CBD

6.1. Anti-arthritic effect in CIA

In collagen-induced arthritis (CIA), pro-inflammatory cytokines, such as TNF-α and IL-1β, are highly expressed in the arthritic joints of mice with CIA, and inhibition of the levels of these molecules can result in a reduction of clinical symptoms. Experimental evidence that CBD given at 25 mg/kg per day orally in murine collagen-induced arthritis was efficacious in achieving such a response.[9] A modest reduction in TNF-α production by synovial cells from CBD treated mice was observed, however, a more robust reduction was reported in the LPS-induced rise in serum TNF-α. The authors concluded that the 'data show that CBD, through its combined immunosuppressive and anti-inflammatory actions, has a potent anti-arthritic effect in CIA'.

6.2. Anti-inflammatory clinical effects of HU-320 (Fig. 2)

Modifications of the structure of CBD, namely the introduction of a carboxy group and replacement of the *n*-pentyl side-chain with a 1,1-dimethylheptyl group, resulted in an anti-inflammatory agent called HU-320 (Fig. 2).[10] An earlier publication[44] where the same changes were made on Δ^8-THC also produced a molecule with potent anti-inflammatory actions named ajulemic acid (HU-239) (Fig. 2) that in some preclinical studies showed apparent CB1 activity.[45] However, it was recently reported that a carefully executed synthesis of ajulemic acid resulted in a product that was essentially free of CB1 activity but still retained anti-inflammatory action.[46] In vivo, HU-320 like HU-239 did not exhibit a cannabimimetic profile but did produce anti-inflammatory clinical effects in a murine, collagen-induced arthritis model. In vitro, it inhibited production of TNF-α by mouse macrophages and of ROIs from RAW 264.7 cells and, in addition, suppressed the rise in serum TNF-α levels following an LPS challenge.

6.3. Edema and hyperalgesia

The anti-inflammatory and anti-hyperalgesic effects of CBD, administered orally (5–40 mg/kg) once a day for 3 days after the onset of acute inflammation induced by intraplantar injection of 0.1 ml carrageenan (1% w/v in saline) in the rat were reported.[28] Prostaglandin E_2 (PGE_2) was assayed in the plasma, and cyclooxygenase (COX) activity, production of nitric oxide (NO; nitrite/nitrate content), and other oxygen-derived free radicals (malondialdehyde) in inflamed paw tissues were significantly increased following carrageenan paw injection. CBD treatment produced decreases in PGE_2 plasma levels, tissue COX activity, production of oxygen-derived free radicals, and NO after three successive doses of CBD. Thus, oral CBD exhibited a beneficial action on two symptoms of inflammation: edema and hyperalgesia.

6.4. Arachidonic acid-induced ear inflammation

The CBD metabolite CBD-11-oic acid (Fig. 2) and its synthetic analog CBD-dimethylheptyl-11-oic acid (HU-320) (Fig. 2) were reported to exhibit anti-inflammatory activity in a model of arachidonic acid-induced ear inflammation in the mouse.[47] The latter gave a potent response at a dose of 0.1 mg/kg given ip, which was comparable to that shown by indomethacin. A major metabolite of CBD is CBD-11-oic acid[48] suggesting the possibility that this in vivo bioconversion can enhance and may even be required for anti- inflammatory activity. A similar argument has been made for THC-11-oic acid, a major metabolite of THC.[49]

6.5. Inflammatory bowel disease

A review of the possible use of CBD to treat inflammatory bowel diseases has recently been published.[50] CBD selectively decreases croton oil-induced hypermotility in mice, a model for inflammatory bowel disease, in vivo.[51] Surprisingly, it was observed that the effect appeared to involve CB1 since it is

believed that CBD does not bind to the CB1 receptor. It was also reported that CBD did not reduce motility in mice treated with the FAAH inhibitor *N*-arachidonoyl-5-hydroxytryptamine. It was suggested that CBD might indirectly activate (via FAAH inhibition) enteric CB1 receptors and thus reduce motility. Inhibition of FAAH would elevate levels of anandamide a well-documented CB1 ligand.

6.6. Chemically induced colitis

In a murine model in mice, colitis was induced by intracolonic administration of trinitrobenzene sulfonic acid (TNB).[52] In the inflamed colon, the effects of CBD on COX-2 and inducible nitric oxide synthase (iNOS) were measured by Western blot; changes in interleukin-1β and interleukin-10 were assayed using ELISA, and endocannabinoids determined by isotope dilution liquid chromatography-mass spectrometry. Human colon adenocarcinoma (Caco-2) cells were used to study the effect of CBD on oxidative stress. CBD was reported to reduce colon injury, inducible iNOS (but not COX-2) expression, and IL-1β, interleukin-10, and endocannabinoid changes associated with TNB administration. CBD also reduced reactive oxygen species production and lipid peroxidation in Caco-2 cells.

The route of administration of CBD was studied in chemically induced colitis.[53] In this study, the efficacy of CBD administered either orally (20 mg/kg) or rectally (20 mg/kg) in the TNB mouse model of colitis was determined with a view toward possible clinical use in humans. These were compared with mice that received CBD (10 mg/kg) given intraperitoneally. The extent of colitis was evaluated by macroscopic scoring, histopathology and the myeloperoxidase (MPO) assay. Oral administration was not effective, however, both rectal and intraperitoneal treatment reduced the extent of colitis in this model.

6.7. Human neutrophil migration

The inhibition of human neutrophil chemotaxis by CBD and related molecules has been reported.[54] It was found that (−)-CBD (Fig. 1) is a partial agonist with an IC-50 value of 0.45 nM, being about 40 fold more potent than (+)-CBD (Fig. 2); abnormal-cannabidiol, an isomer of CBD, is a full agonist. In addition, it was observed that the abnormal-cannabidiol analog O-1602 (Fig. 2) inhibits migration with an IC-50 value of 33 nM. Moreover, (−)-CBD and related ligands showed potent inhibition of human neutrophil migration, and the data implicated a novel receptor that was distinct from cannabinoid CB1 and CB2 receptors. The endogenous lipoamino acid *N*-arachidonoyl-l-serine antagonized this receptor. The possibility that GPR55 is this novel receptor is discussed in the report.

6.8. Type I diabetic cardiomyopathy

Beneficial effects of CBD were reported in a study using a mouse model of type I diabetic cardiomyopathy and primary human cardiomyocytes exposed to high glucose.[55] CBD showed beneficial effects on myocardial dysfunction, cardiac fibrosis, oxidative/nitrosative stress, inflammation, cell death, and interrelated signaling pathways. Markers that were measured included NF-κB and MAPK (JNK and p-38, p38α), expression of adhesion molecules (ICAM-1, VCAM-1), TNF-α, markers of fibrosis (TGF-β, CTGF, fibronectin, collagen-1, MMP-2 and MMP-9), cell death (caspase 3/7 and PARP activity), chromatin fragmentation and Akt phosphorylation. This very comprehensive report provides yet another example of the anti-inflammatory actions of CBD.

A review paper on the therapeutic uses for CBD in inflammation, oxidative stress, the immune system, the metabolic syndrome and the endocannabinoids was recently published.[56] In the paper, recent studies reporting that CBD may have utility in treating several diseases and disorders believed to involve activation of the immune system and associated oxidative stress as a contributor to their etiology and progression are presented. Included are rheumatoid arthritis, types I and II diabetes, atherosclerosis, Alzheimer's disease, hypertension, the metabolic syndrome, ischemia-reperfusion injury, depression, and neuropathic pain. It is suggested that CBD's therapeutic actions are a result of the fact that inflammation and oxidative stress are intimately involved in many human diseases.

6.9. Elevation of cytokine production

CBD is generally anti-inflammatory and immuno-suppressive, however under certain conditions, it can elevate cytokine production.[57] Both THC and CBD suppressed or enhanced IFN-γ and IL-2 production by mouse splenocytes under optimal or suboptimal stimulation, respectively. It was reported that these two cannabinoids suppressed or enhanced HIVgp120-specific T cell responses. It was further demonstrated that THC and CBD differentially regulated NFAT nuclear translocation and cytokine production. In all cases, intracellular calcium was elevated regardless of the degree of cellular activation. These studies provide a possible explanation for the widely reported discrepancies regarding cannabinoid actions on immune responses.

In support of the previous report it was later found that CBD exacerbates LPS-induced pulmonary inflammation.[58] This effect of CBD in vivo likely involves the parent compound, metabolites, inhibition of certain metabolizing enzymes, and inhibition of NFAT activity. It was concluded that CBD should be considered an immune modulator, rather than only an immune suppressive agent.

6.10. Pneumococcal meningitis

CBD has anti-inflammatory effects in pneumococcal meningitis and reduces cognitive sequelae.[59] The intense inflammatory response generated is accompanied by a significant mortality rate and neurologic sequelae, such as, seizures, sensory-motor deficits and impairment of learning and memory. Male Wistar rats underwent a cisterna magna tap and received either 10 ml of sterile saline as a control or an equivalent volume of *Streptococcus pneumoniae* suspension. Rats subjected to meningitis were treated by intraperitoneal injection with CBD (2.5, 5, or 10 mg/kg once, or daily for 9 days after meningitis induction). Controls were sham operated and vehicle treated rats. The chronic administration of CBD at several doses reduced the TNF-α level in the frontal cortex. Prolonged treatment with CBD at 10 mg/kg, reduced memory impairment in rats with pneumococcal meningitis.

6.11. Treatment of demyelinating pathologies

The protective effect of CBD against damage to oligodendrocyte progenitor cells (OPCs) mediated by the immune system has been reported.[19,60] Treatment of cells with 1 μM CBD protects them from oxidative stress by decreasing the production of reactive oxygen species. CBD also protects OPCs from apoptosis induced by LPS/IFNγ through the decrease of caspase-3 induction by mechanisms not involving CB1, CB2, TRPV1 or PPAR-γ receptors. In addition, tunicamycin-induced cell death was reduced by CBD, suggesting a role for endoplasmic reticulum stress in the mode of action of CBD. This protection against endoplasmic reticulum stress-induced apoptosis was related to the reduced phosphorylation of eiF2α, one of the initiators of the endoplasmic reticulum

Table 1
Anti-inflammatory actions of CBD

Response	Model	Reference
Reduces immune response	Rats subjected to pneumococcal meningitis	59
Prevents experimental colitis	Murine model of colitis	52
Reduced iNOS and IL-1β expression	Mouse model of Alzheimer's disease	31,43
Reduces β-amyloid-induced neuroinflammation	Cultured astrocytes	43
TNF-α and IL-1β levels reduced	Murine collagen-induced arthritis	9
Decreases in PGE_2 plasma levels	Carrageenan paw injection in the rat	28
Reduced the extent of colitis	TNB mouse model of colitis	53
Inhibition of neutrophil chemotaxis	Human neutrophil migration	54
Effects on NF-κB, MAPK, ICAM-1, VCAM-1, TNF-α	Mouse model of type I diabetic cardiomyopathy	55
Enhanced IFN-γ and IL-2 production	Mouse splenocytes	57
Exacerbates LPS-induced pulmonary inflammation	Pulmonary inflammation in C57BL/6 mice	58
Reduced the TNF-α level in the frontal cortex	Pneumococcal meningitis in rats	59
Decreases hepatic ischemia-reperfusion (I/R) injury	Mouse model of hepatic I/R	61
Reduced LPS-induced increase in TNFα and COX-2	Mouse model of sepsis-related encephalitis	62
Reduced effects of autoimmune encephalomyelitis	Immunized C57BL/6 mice	34,63
Reduces inflammation in acute lung injury (ALI)	Mouse model of lipopolysaccharide-induced ALI	64,18

stress pathway. Moreover CBD diminished the phosphorylation of PKR and eiF2α induced by LPS/IFNγ. The data suggest that inhibition of the endoplasmic reticulum stress pathway is a factor in the 'oligoprotective' effects of CBD during inflammation. It was further suggested that CBD has therapeutic potential for the treatment of demyelinating pathologies.

6.12. Hepatic ischemia-reperfusion injury

Hepatic ischemia-reperfusion (I/R) injury is a major clinical problem believed to be responsible for liver failure following transplantation, hepatic surgery and circulatory shock. The beneficial effects of CBD treatment in a mouse model of hepatic I/R injury were described in a recent study.[61] Several markers of liver injury (serum trans aminases), hepatic oxidative/nitrative stress (4-hydroxy-2-nonenal, nitrotyrosine content/staining, gp91phox and inducible nitric oxide synthase mRNA), mitochondrial dysfunction (decreased complex I activity), inflammation (TNF-α), COX-2, macrophage inflammatory protein-1α/2, intercellular adhesion molecule mRNA levels, tissue neutrophil infiltration, nuclear factor kappa B (NF-κB) activation, stress signaling (p38MAPK and JNK) and cell death (DNA fragmentation, PARP activity, and TUNEL) were studied. The inhibitory effects of CBD were retained in CB2 knockout mice and were not reduced by CB1 or CB2 antagonists in vitro suggesting a novel mechanism of action.

6.13. Sepsis-related encephalitis

The effects of CBD in a mouse model of sepsis-related encephalitis induced by intravenous administration of lipopolysaccharide (LPS) have been described.[62] Intravital microscopy was used to measure vascular responses of pial vessels and inflammatory parameters were measured by qRT-PCR. It was seen that CBD prevented LPS-induced arteriolar and venular vasodilation as well as leukocyte margination. CBD also reduced LPS-induced increases in TNF-α and COX-2 expression as measured by quantitative real time PCR. In addition, the expression of inducible-nitric oxide synthase was reduced. These observations demonstrate both the anti-inflammatory and the vascular-stabilizing effects of CBD in endotoxic shock.

6.14. Autoimmune encephalomyelitis

CBD reduced the severity of the clinical signs of autoimmune encephalomyelitis (EAE) when administered to myelin oligodendrocyte glycoprotein-immunized C57BL/6 mice at the onset of the disease.[34,63] It also decreased axonal loss and reduced inflammation as shown by reductions in the infiltration of T cells and microglial activation. In addition, CBD inhibited myelin oligodendrocyte glycoprotein (MOG)-induced T-cell proliferation in vitro at both low and high concentrations of the myelin antigen and the effect was not mediated by either the CB1 or the CB2 receptors. Suppression of microglial activity and T-cell proliferation by CBD was suggested to contribute to these beneficial effects.

6.15. Inflammatory lung diseases

This report[64] is an extension of an earlier one where it was shown that prophylactic treatment with CBD reduces inflammation in a model of acute lung injury (ALI).[18] In the current publication, the effects of therapeutic treatment with CBD (20 and 80 mg/kg) in a mouse model of lipopolysaccharide-induced ALI on pulmonary mechanics and inflammation was reported. CBD decreased total lung resistance and elastance, leukocyte migration into the lungs, myeloperoxidase activity in the lung tissue, protein concentration and production of the pro-inflammatory cytokines (TNF and IL-6) and chemokines (MCP-1 and MIP-2) in the bronchoalveolar lavage supernatant. It was concluded that CBD could be efficacious in the treatment of inflammatory lung diseases.

7. Combined THC and CBD treatment

It has been suggested that the combination of THC and CBD has a better therapeutic profile in a variety of actions than each cannabinoid component alone.[65,66]

A example of such synergism in the area of inflammation has been reported in a mouse model of Alzheimer's disease.[67] They observed reduced astrogliosis, microgliosis, and inflammatory-related molecules in treated AβPP/PS1 mice that were more marked after treatment with THC + CBD than with either THC or CBD alone. It was suggested that the anti-inflammatory effects had a role in the positive cognitive effects that were seen as a result of cannabinoid treatment.

A combination of phytocannabinoids that is primarily composed of THC and CBD, is neuroprotective in malonate-lesioned rats, an inflammatory model of Huntington's disease.[68] Evidence was presented that suggested a role for both CB1 and CB2 receptors in the anti-inflammatory actions of the cannabinoid mixture.

8. Summary

Although it was discovered early on, CBD has become a major area of research only in recent years. In particular, its biological

actions are a topic of many interesting reports that suggest possible therapeutic applications. Included are its anti inflammatory actions in a variety of preclinical models (Table 1). Some examples are experimental colitis, collagen-induced arthritis, β-amyloid-induced neuroinflammation, neutrophil chemotaxis, hepatic ischemia-reperfusion (I/R) injury, autoimmune encephalomyelitis, acute lung injury (ALI), etc. These and others need to be pursued in human trials with a view toward clinical applications where CBD's absence of psychotropic effects and other adverse events offers a major advantage over other cannabinoids. Another area in need of new research is the discovery of synthetic analogs with greater potency than CBD that still retain a favorable therapeutic ratio. A review covering other areas of CBD actions has recently been published by Hill et al.[7]

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