Bioorganic & Medicinal Chemistry 23 (2015) 1377–1385

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Review

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

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ARTICLE INFO

Article history:
Received 24 December 2014
Revised 23 January 2015
Accepted 30 January 2015
Available online 7 February 2015

Keywords:
Cannabidiol
Δ^9-Tetrahydrocannabinol
Anti-inflammatory
CBD receptor binding
Signaling events
Downstream events
Functional effects

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.

Contents

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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http://dx.doi.org/10.1016/j.bmc.2015.01.059
0968-0896/

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

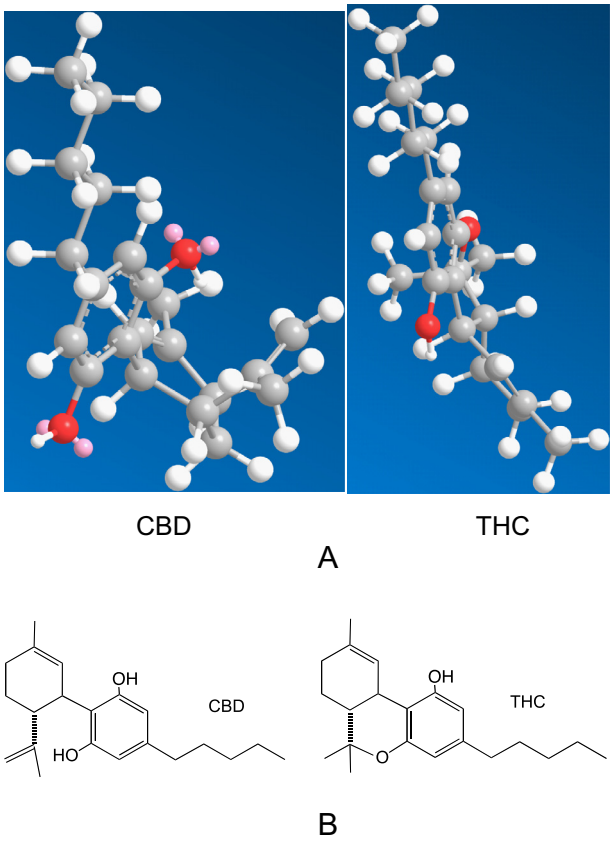

Figure 1. The minimal energy conformations of CBD and Δ^9-tetrahydrocannabinol (THC) are shown in 1A. THC has a fairly planar conformation whereas CBD has a bent conformation. This difference results in different pharmacological profiles even though there is considerable structural overlap of both when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

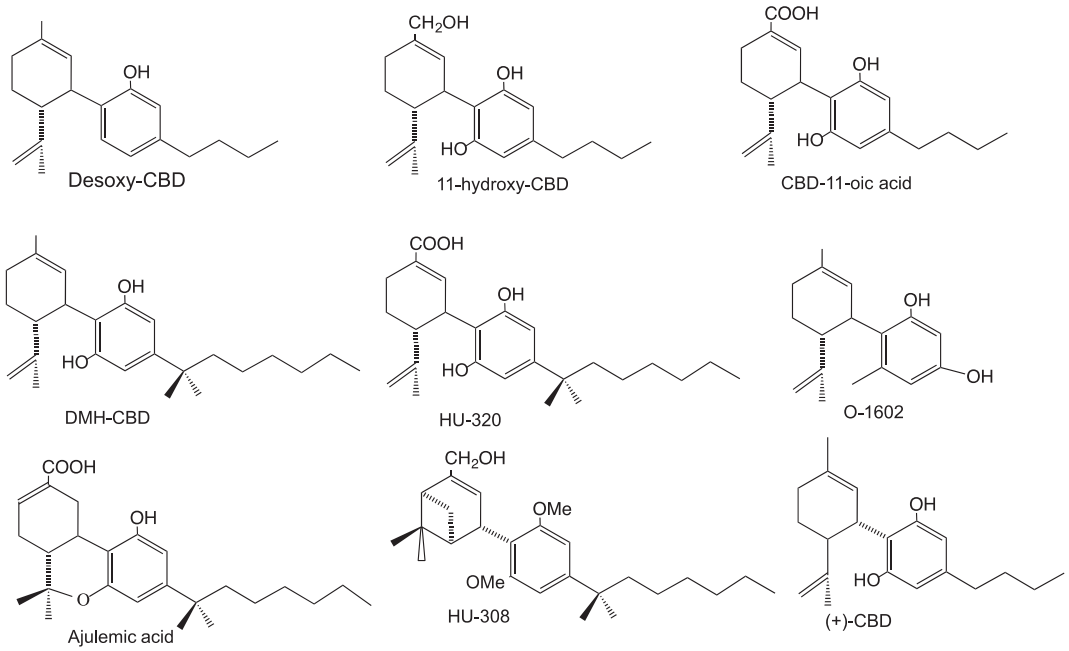

Figure 2. The structures of CBD analogs and related substances.

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]

Acknowledgement

The author thanks Dr. Akbar Ali for his assistance in preparing the graphical abstract and Figure 1.

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Published in final edited form as:
Free Radic Biol Med. 2011 September 1; 51(5): 1054–1061. doi:10.1016/j.freeradbiomed.2011.01.007.

Cannabidiol as an Emergent Therapeutic Strategy for Lessening the Impact of Inflammation on Oxidative Stress

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Abstract

Oxidative stress with reactive oxygen species generation is a key weapon in the arsenal of the immune system for fighting invading pathogens and to initiate tissue repair. If excessive or unresolved, however, immune-related oxidative stress can initiate further increasing levels of oxidative stress that cause organ damage and dysfunction. Targeting oxidative stress in these various diseases therapeutically has proven more problematic than first anticipated given the complexities and perversity of both the underlying disease and the immune response. However, growing evidence suggests that the endocannabinoid system, which includes the CB_1 and CB_2 G protein-coupled receptors and their endogenous lipid ligands, may be an area that is ripe for therapeutic exploitation. In this context, the related nonpsychotropic cannabinoid cannabidiol, which may interact with the endocannabinoid system, but has actions that are distinct, offers promise as a prototype for anti-inflammatory drug development. This review discusses recent studies suggesting that cannabidiol may have utility in treating a number of human diseases and disorders now known to involve activation of the immune system and associated oxidative stress, as a contributor to their etiology and progression. These include rheumatoid arthritis, types I and II diabetes, atherosclerosis, Alzheimer's disease, hypertension, the metabolic syndrome, ischemia-reperfusion injury, depression, and neuropathic pain.

Keywords

Inflammation; Oxidative Stress; Immune System; Metabolic Syndrome; Endocannabinoid

Introduction

(−)-Cannabidiol (CBD) is the major nonpsychotropic cannabinoid compound derived from the plant *Cannabis sativa*, commonly known as marijuana. CBD was first isolated in 1940 and its structure and stereochemistry determined in 1963 [1,2]. Interest in exploiting CBD therapeutically was initially focused on its interactions with the primary psychotropic ingredient of *Cannabis*, Δ^9-THC and its sedative and antiepileptic effects, and later its antipsychotic and anxiolytic actions and utility in treating movement disorders [3]. As

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chronicled elsewhere [3], the last several years have seen a renewed interest in CBD due to the discovery of its antioxidative, anti-inflammatory, and neuroprotective effects, actions that occur for the most part independently of the canonical cannabinoid CB_1 and CB_2 receptors [1,4]. CBD may prove to have therapeutic utility in a number of conditions involving both inflammation and oxidative stress, including Parkinson's disease, diabetes, rheumatoid arthritis, Alzheimer's disease, and ischemia-reperfusion injury.

The contribution of the endocannabinoid system to inflammation and regulation of the immune system is an area of intense study that is beyond the scope of this article, and the reader is referred to several recent excellent reviews [5–8]. However, a brief overview of the system is helpful in discussing CBD. The endocannabinoid system comprises the following: (1) the G protein-coupled cannabinoid receptors CB_1 and CB_2, which are located in both the central nervous system and periphery; (2) their arachidonate-based lipid ligands, e.g., 2-arachidonoylglycerol (2-AG) and anandamide (N-arachidonoylethanolamine, AEA) and (3) the enzymes that synthesize and degrade these ligands. The endocannabinoid system plays a role in a variety of physiological processes including appetite, pain sensation, and mood. Evidence indicates that both CB_1 and CB_2 are expressed by cells of the immune system and are upregulated in the activation state. Levels of CB_2 appear to be higher than those of CB_1 with decreasing amounts of CB_2 in human B cells, NK cells, monocytes, polymorphonuclear neutrophils and T cells [6]. Macrophages and related cells, microglia and osteoclasts, express both cannabinoid receptors. CB_2 activation of immune cells is associated with changes in cytokine release and migration [6].

Biochemistry of Cannabidiol

CBD (Fig. 1) is a resorcinol-based compound that was shown to have direct, potent antioxidant properties by cyclic voltammetery and a spectrophotometric assay of oxidation in a Fenton reaction [9]. In an *in vitro* glutamate neuronal toxicity model, CBD was shown to be more protective than either α-tocopherol or vitamin C and comparable to butylated hydroxytoluene (BHT); although as noted by the authors, CBD unlike BHT does not seem to promote tumors [9]. CBD was also reported to act as an antioxidant at submicromolar concentrations in preventing serum-deprived cell death of cultured human B lymphoblastoid and mouse fibroblasts cells [10]. The antioxidant chemistry of CBD may have utility *in vivo* as well. The protective effects of CBD in a rat binge ethanol-induced brain injury model [11] and a rat model of Parkinson's disease [12] were ascribed to its antioxidant properties. As will become clear from this review, however, the anti-oxidant actions ascribed to CBD in various *in vivo* models of human diseases likely exceed those attributable to its chemistry alone. Rather, the therapeutic anti-oxidant properties of CBD would seem to result in no small measure from its modulation of cell signaling events that underlie the self-sustaining cycle of inflammation and oxidative stress.

Mechanisms of Action

Several interactions with relevance to the immune system and oxidative stress are discussed here. First, despite having low affinity for CB_1 and CB_2 receptors, CBD has been shown to antagonize the actions of cannabinoid CB_1/CB_2 receptor agonists in the low nanomolar range, consistent with non-competitive inhibition [13]. At 1–10 μM, CBD appears to function as an inverse agonist at both CB_1 and CB_2 receptors [13]. Second, CBD acts as an inhibitor (IC_{50} = 28 μM) of fatty acid amide hydrolase (FAAH), the major enzyme for endocannabinoid breakdown. Because FAAH activity correlates with gastrointestinal mobility, CBD may have utility in treating intestinal hypermotility associated with certain inflammatory diseases of the bowel [14].

Third, CBD is a competitive inhibitor with an IC_{50} in the nanomolar range of adenosine uptake by the equilibrative nucleoside transporter 1 (ENT1) of macrophages and microglial cells, the resident macrophage-like immune cells of the brain. By increasing exogenous adenosine, which in turn activates the A_{2A} adenosine receptor, CBD exerts immunosuppressive actions on macrophages and microglial cells as evidenced by decreased TNFα production after treatment with lipopolysaccharide (LPS) [15,16]. CBD may thus be of benefit in treating neueodegenerative diseases associated with hyperactivation of microglial, as well as retinal neuroinflammation seen in such conditions as uveitis, diabetic retinopathy, age-related macular degeneration, and glaucoma. Note, however, that adenosine activates other receptors besides A_{2A} that often have opposing consequences on immune regulation and inflammation [17,18]. In several *in vivo* models of neurodegeneration or inflammation, moreover, the beneficial effects of CBD were demonstrated not to involve adenosine receptors.

Fourth, CBD has been shown to have potent actions in attenuating oxidative and nitrosative stress in several human disease models, although the exact mechanism is unclear. For instance, CBD pretreatment was found to attenuate high glucose-induced mitochondrial superoxide generation and NF-κB activation in human coronary artery endothelial cells, along with nitrotyrosine formation and expression of inducible nitric oxide synthase (iNOS) and adhesion molecules ICAM-1 and VCAM-1 [19]. Notably, high glucose-induced transendothelial migration of monocytes, monocyte-endothelial adhesion, and barrier disruption were attenuated as well. These findings lend support to the conclusion that that CBD may have therapeutic utility in treating diabetic complications and atherosclerosis. In another study, CBD was reported to reduce expression of reactive oxygen species (ROS) generating NADPH oxidases, as well as iNOS and nitrotyrosine generation in a cisplatin nephropathy model *in vivo*, consequently lessening cell death in the kidney and improving renal function [20]. From these studies, it is tempting to speculate that CBD may act directly at the level of the mitochondrion or nucleus to oppose oxidative/nitrosative stress.

Fifth, at low micromolar concentrations, CBD was found to inhibit indoleamine-2,3-dioxygenase activity thereby suppressing tryptophan degradation by mitogen-stimulated peripheral blood mononuclear cells and LPS-stimulated myelomonocytic THP-1 cells *in vitro* [21]. Based on this finding, CBD might be useful therapeutically to counter the increased risk of depression in diseases associated with immune activation and inflammation, which often lead to decreased tryptophan, the precursor of serotonin. Finally, CBD has been shown to act as an antagonist at G protein-coupled receptor 55 (GPR55) and as an antagonist or agonist at several transient receptor potential (TRP) channels; however, these observations are controversial and the pharmacophysiological significance of these interactions is not known [1,4].

Actions on Immune Cells

CBD has been shown to modulate the function of the immune system. Overall these actions may be nuanced and concentration-dependent, but in general include suppression of both cell-mediated and humoral immunity and involve inhibition of proliferation, maturation, and migration of immune cells, antigen presentation, and humoral response [1,13]. Key aspects are discussed here. In most *in vivo* models of inflammation, CBD attenuates inflammatory cell migration/infiltration (e.g. neutrophils) [22]. During neuroinflammation, activated microglial cells migrate towards the site of injury where they release pro-inflammatory cytokines and cytotoxic agents, including ROS. Although important in removal of cellular debris and fighting infection, activated microglial cells often exacerbate local cell damage. CBD was shown to inhibit activated microglial cell migration by antagonizing the abnormal-cannabidiol (Abn-CBD)-sensitive receptor at concentrations < 1 μM [23]. Evidence that the

Abn-CBD receptor is the orphan G protein-coupled receptor GPR18 was recently reported [24]. CBD was also shown to block endotoxin-induced oxidative stress resulting from retinal microglial cell activation in uveitis [25]. CBD blocked the immediate activation of NADPH oxidase as well as a second wave of ROS formation and the associated TNFα secretion and p38 MAPK activation. The direct antioxidant property of CBD is unlikely to be the entire explanation for these actions as they occurred at a concentration of 1 μM. Inhibition of adenosine uptake as discussed previously may have been involved. However, a complete understanding of the anti-inflammatory actions of CBD on microglial cells is not yet available. Recently, through an unidentified mechanism, CBD was reported to suppress LPS-induced pro-inflammatory signaling in cultured microglial cells, including NF-κB and STAT1 activation, while enhancing STAT3-related anti-inflammatory signaling [26].

CBD induces apoptosis of monocytes and certain normal and transformed lymphocytes, including thymocytes and splenocytes, through oxidative stress and increased ROS levels [27–31]. The basis for this action appears to be glutathione depletion due to adduct formation with the reactive metabolite of CBD, cannabidiol hydroxyquinone, thereby triggering cell death through caspase 8 activation and/or the intrinsic apoptotic pathway. Increased ROS from the upregulation of NADPH oxidases via an undefined mechanism may contribute to cell death as well [31]. A recent study assessed the impact of repeated administration of relatively low levels of CBD to adult male Wistar rats on peripheral blood lymphocyte subset distribution [32]. At 2.5 mg/kg/day for 14 days, CBD did not produce lymphopenia, but increased the total number of natural killer T (NKT) cells and percentage numbers of NKT and natural killer (NK) cells. A dose of 5 mg/kg/day did have a lymphopenic effect, but by reducing B, T, Tc and Th lymphocytes. Thus, CBD would appear to suppress specific immunity, while enhancing nonspecific antitumor and antiviral immune response. As discussed by the authors [32], the lymphopenic effect of CBD was observed at a concentration shown to be efficacious in a number of animal models of neurodegenerative and inflammatory diseases, including blocking the progression of collagen-induced arthritis in a murine model of rheumatoid arthritis, decreasing damage to pancreatic islets in the NOD mouse model of type 1 diabetes, lessening hyperalgesia in rat models of neuropathic and inflammatory pain, and preventing cerebral ischemia in gerbils.

Pain

Neuropathic pain is associated with microglia activation in the spinal cord and brain and their subsequent release of pro-inflammatory cytokines, such as interleukin-6 (IL-6), interleukin-1β (IL-1β), and tumor necrosis factor-α (TNFα) [33]. The etiology of neuropathic pain, which is common in cancer, diabetes, multiple sclerosis, and peripheral nerve injury, is poorly understood, but recent evidence indicates that increased ROS generation by microglial cells is the critical initiating factor [34]. The drug Sativex, which consists of Δ^9-THC and CBD, is approved in several countries for treatment of central and peripheral neuropathic pain and for spasticity associated with multiple sclerosis [35]. In a mouse model of type I diabetic peripheral neuropathic pain, intranasal or intraperitoneal administration of a moderate-high dose of CBD attenuated tactile allodynia and thermal hypersensitivity without affecting the diabetic state [36]. The antinociceptive effects of CBD were associated with less of an increase in microglial density and p38 MAPK activity in the dorsal spinal cord. Finally, the anti-inflammatory and immunosuppressive actions of CBD may be of use in treating rheumatoid arthritis and the associated pain [37,38].

Diabetes and Diabetic Complications

CBD was shown to reduce either the initiation of diabetes or the development of overt or latent diabetes in non-obese diabetes-prone (NOD) mice by reducing insulitis [39,40]. This

action was accompanied by a shift in the immune response from a dominant Th1 pattern with pro-inflammatory cytokines to a Th2 pattern with increased levels of the anti-inflammatory cytokine IL-10. Major effectors of β-cell death in type 1 diabetes are various free radicals and oxidant species, including NO, and infiltrating macrophages are one source of high concentrations of NO and inflammatory cytokines that further enhance NO and ROS formation [41]. CBD was also shown to be effective in blocking ROS-induced up-regulation of surface adhesion molecules on endothelial cells due to high glucose and in preserving endothelial barrier function [19,42]. Adhesion of monocytes followed by their transmigration into the subendothelial space is an early event in atherosclerosis, the most common macrovascular complication of diabetes, and may contribute as well to diabetic retinopathy [19,42,43]. The anti-inflammatory actions of CBD may also protect retinal neurons in diabetes by attenuating activation and ROS generation by Müller glia, thus preventing tyrosine nitration and inhibition of Müller cell glutamine synthetase and the consequent accumulation of glutamate, which in turn leads to oxidative stress-induced death of retinal neuronal cells [44].

In a mouse model of type I diabetic cardiomyopathy, both pre- and post-treatment with CBD attenuated cardiac fibrosis and cell death, myocardial dysfunction, inflammation, oxidative/nitrosative stress, and the activation of related signaling pathways [45]. CBD attenuated diabetes-induced activation in the heart of the key pro-inflammatory transcription factor, NF-κB and its consequences, e.g. expression of ICAM-1, iNOS, VCAM-1, and TNFα. These observations underscore the point that CBD likely attenuates inflammation far beyond its antioxidant properties *per se*. CBD also reduced high glucose-induced increases in both cytosolic and mitochondrial reactive oxygen and nitrogen species generation in primary human cardiac myocytes, which was accompanied by reduced NF-κB activation and cell death. These findings indicate that CBD may have great therapeutic potential in alleviating cardiac complications of diabetes.

Hypertension

Although CBD has not been considered for treating hypertension, a parallel between the role of microglia in diabetes and hypertension deserves mention. Activation of microglia within the paraventricular nucleus (PVN) was recently shown to contribute to neurogenic hypertension resulting from chronic angiotensin II infusion in the rat [46]. Microglia activation was associated with enhanced expression of pro-inflammatory cytokines, the acute administration of which into the left ventricle or PVN resulted in increased blood pressure. The hypertensive action of angiotensin II infusion could be blocked by overexpression of IL-10 in the PVN or intracerebroventricular infusion of minocycline, supporting the involvement of ROS.

The immune system contributes as well to systemic endothelial dysfunction observed in hypertension [47]. Local production of angiotensin II by activated leukocytes within the vessel wall is thought to reduce endothelial function and NO production, leading to attenuated vasodilation and increased blood pressure, through the production of inflammatory cytokines and ROS [48,49]. Interestingly, recent evidence has shown that the initial stimulus for peripheral leukocyte activation in angiogensin II-induced hypertension is the increase in blood pressure that results from stimulation of cells within the anteroventral third ventricle (AV3V) of the brain by angiotensin II [50].

Ischemia Reperfusion Injury

Redox stress and ROS produced by ischemia-reperfusion of organs activates the immune system, which aids in repair by removing debris and stimulating remodeling. An excessive or prolonged inflammatory response, however, may prove detrimental to organ function by

exacerbating ROS production and causing death of the parenchyma. Several hours after ischemia-reperfusion in the heart, a model of myocardial infarction, neutrophils accumulate in the myocardium [51]. Several lines of evidence suggest that this accumulation of neutrophils worsens injury to the myocardium [51]. In rats, treatment with CBD for 7 days following a 30 minute occlusion of the left anterior descending coronary artery markedly reduced infarct size, myocardial inflammation and IL-6 levels and preserved cardiac function [52]. In addition, the number of leukocytes infiltrating the border of the infarcted area was dramatically reduced. CBD has been shown to inhibit stimulated migration of neutrophils [22]. CBD treatment was also recently shown to reduce neutrophil migration in a rat model of periodontitis [53]. Hyperactive neutrophils exacerbate periodontal tissue injury and lead to tooth loss in part by excessive ROS formation in individuals with refractory periodontitis [54]. Finally, pre- or post-ischemic treatment with CBD was shown to have a prolonged and potent protective action in cerebral ischemia. The neuroprotective actions of CBD were attributed to reduced neutrophil accumulation and myeloperoxidase activity [55], as well as decreased high mobility group box 1 (HMGB1) expression by microglia [56].

Depression

CBD is reported to have anti-depressive actions, the basis for which is not established although activation of 5-HT_{1A} receptors may be involved at least at higher concentrations [13,57,58]. Growing evidence in recent years has implicated pro-inflammatory cytokines, free radical species, and oxidants in the etiology of depression [59,60]. One explanation is that the resultant oxidative stress adversely affects glial cell function and leads to neuron damage in the brain.

Neurodegenerative Diseases

Microglial hyperactivation is a common feature of a number of neurodegenerative diseases, including Parkinson's, Alzheimer's, Huntington's, amyotrophic lateral sclerosis (ALS) and multiple sclerosis(MS) [61,62]. Activated microglia produce a number of pro- and anti-inflammatory cytokines, chemokines, glutamate, neurotrophic factors, prostanoids, and a variety of free radicals that together create a state of oxidative stress. Alzheimer's disease, which is the most common form of dementia, is characterized by the deposition of "senile" plaques that are sites of microglia activation and inflammation. The resultant oxidative stress is a critical factor in the pathophysiology of Alzheimer's [63]. The plaques are composed of insoluble aggregates of the beta-amyloid peptide (Aβ), which self-assembles as monomers, oligomers and finally fibrils. Recent evidence shows that the oligomeric form of beta-amyloid is the most neurotoxic species and is most effective as a chemotactic agent for microglia and stimulator of microglial oxidative stress [61,64]. Activated microglia are a major contributor of inflammatory factors in Alzheimer's and secret a number of pro-inflammatory cytokines, which ironically further enhance Aβ production by neuronal cells [65]. In addition, an inflammatory state was shown to block the ability of microglia to phagocytize fibrillar Aβ [66]. Aging was also shown to negatively impact on the ability of microglia to internalize Aβ [67]. Microglia from aged mice were also shown to be less responsive to stimulation and to secrete greater amounts of IL-6 and TNFα compared to microglia of younger mice. Aged microglia also had lower levels of glutathione, suggesting an increased susceptibility to the harmful effects of oxidative stress. Finally, although controversial, evidence has been put forward suggesting that bone marrow-derived monocytic cells may somehow gain access to the diseased brain in Alzheimer's and be better at phagocytosing amalyoid plaques than resident microglia [65,68].

Based on rather scant evidence, some have proposed that CBD might have utility in treating neurodegenerative diseases [1,3,69–71]. CBD was shown to have a protective effect on

cultured rat pheocromocytoma PC12 cells exposed to Aβ [72,73]. In a concentration-dependent manner, CBD increased cell survival, while decreasing ROS and nitrite production, lipid peroxidation, and iNOS protein expression. CBD was shown to have anti-inflammatory actions *in vivo* in a mouse model of Alzheimer's neuroinflammation induced by injection of human Aβ into the hippocampus. CBD dose-dependently attenuated Aβ-induced glial fibrillary acidic protein (GFAP) mRNA, iNOS and IL-1β protein expression, and NO and IL-1β release [74]. In a recent study, CBD was found to protect against amphetamine-induced oxidative protein damage in a rat model of mania and to increase brain-derived neurotrophic factor (BDNF) expression levels in the reversal protocol [75]. Results of these preclinical studies are persuasive and support the need for double-blind placebo controlled trials to assess the therapeutic utility of CBD in patients with neurodegenerative diseases.

Obesity and the Metabolic Syndrome

Metabolic syndrome is a combination of medical disturbances including central obesity, glucose intolerance, hypertension, and dyslipidemia that increases the risk for developing cardiovascular diseases and type 2 diabetes. Adipocyte dysfunction leading to a low-grade chronic inflammatory state is thought to underpin the etiology of the metabolic syndrome [76]. Metabolic overload of adipocytes causes production of ROS, pro-inflammatory cytokines, and adipokines that activate inflammatory genes and stress kinases and interfere with insulin signaling [76,77]. Saturated fatty acids also activate toll-like receptors on adipocytes and macrophages, components of the innate immune system, to induce production of proinflammatory cytokines and chemokines. Enhanced mitochondrial flux together with relative hypoxia due to adipocyte tissue hypertrophy, endothelial cell apoptosis, and inflammation-impaired angiogenesis further enhances ROS generation. Enhanced rupture of adipocytes due to excessive hypertrophy attracts and activates macrophages that further exacerbate the inflammatory state through the production of inflammatory cytokines and ROS. The chronic inflammatory state compromises the ability of adipose tissue to absorb incoming fat leading to fat build up in other organs, including liver, heart, and skeletal muscle, and creating a local inflammatory state that progresses to insulin resistance in those organs as well. Increased ROS levels are thought to be the major contributing factor to insulin resistance [78,79].

Macrophages, both resident and to a greater extent bone marrow-derived, play a critical role in initiating adipose tissue dysregulation and inflammation in the metabolic syndrome and together with adipocytes constitute a paracrine loop that sustains the chronic inflammatory state [80]. Macrophages secrete TNFα which acts on hypertrophied adipocytes to downregulate adiponectin and induce pro-inflammatory cytokines and lipolysis. The released free fatty acids act in turn on the toll-like receptor 4 (TLR4) of macrophages to induce production of pro-inflammatory cytokines, including TNFα. Both macrophages and adipocytes secrete monocyte chemotactic protein 1 (MCP1), which serves to recruit more macrophages to the adipose tissue.

Recent evidence has revealed that most macrophages in obese adipose tissue are polarized towards the M1 or classically activated, pro-inflammatory state, as opposed to the M2 or alternatively activated, anti-inflammatory state [80,81]. Th1 cytokine interferon gamma (IFNγ), microbial byproducts (e.g., LPS), and free fatty acids from visceral adipose tissue promote polarization towards the M1 state, whereas Th2 cytokines IL-4 and IL-13 promote polarization towards the M2 phenotype. Ligand-dependent transcription factors peroxisome proliferator activated receptors (PPARs) play a key role in determining the M1/M2 phenotype [81,82]. Activation of PPARγ or PPARδ promotes differentiation towards the M2 phenotype, while PPARγ activation inhibits M2 to M1 phenotype switch and represses the

M1 pro-inflammatory gene expression profile. Of interest, CBD as well as some other cannabinoids, has been shown to activate PPARγ, possibly through direct binding [83,84]. Although tonic activation of CB_1 receptors by endocannabinoids is implicated in the development of abdominal obesity and CB_1 antagonists and inverse agonist reduce obesity, their clinical use is problematic due to serious neuropsychiatric effects [85]. Given its anti-inflammatory actions and PPARγ agonism, CBD might serve as the basis for design of a new anti-obesity drug [1]. In this regard, a cautionary note regarding the PPARγ agonism associated with CBD should be sounded, although this was observed only in very high concentrations and only *in vitro*, which is that several PPARγ agonists have been retracted because of various problems [86,87].

Atherosclerosis

Atherosclerosis is an inflammatory disease in which monocytes/macrophages play a critical role in the initiation and progression, as well as rapture, of the atherosclerotic plaque [88]. Plaques form in the arterial wall at areas of disturbed flow and endothelial dysfunction (Fig. 2). The initiating event is the transcytosis of low density lipoprotein (LDL) into the subendothelial space where it is trapped by binding to proteoglycans of the extracellular matrix [88,89]. LDL is oxidized by various cells including macrophages, first to minimally modified LDL (mmLDL) and then extensively oxidized LDL (oxLDL). The former activates endothelial cells to secrete various factors that attract monocytes and to express adhesion molecules that support the binding and transmigration of monocytes into the subendothelial space. Once there, monocytes differentiate into macrophages under the influence of cytokines and oxLDL. Macrophages take up oxLDL and differentiate into foam cells that secrete a number of cytokines and growth factors that sustain the inflammatory response and stimulate migration of smooth muscle and endothelial cells into the intima. Continued oxLDL uptake by foam cells combined with impaired cholesterol efflux results in their apoptosis and exposure of thrombogenic lipids [88,89]. A number of events in monocyte/macrophage physiology may be potential therapeutic targets for dealing with atherosclerosis and are discussed in detail elsewhere [88,89].

ROS play a pivotal role in atheroma development and macrophages are the major source for ROS with NADPH oxidase, cyclooxygenases (COX), lipooxygenases (LOX), iNOS, and myeloperoxidase contributing [88,89]. ROS participate in atherosclerosis in part by causing LDL oxidation, activating stress signaling pathways, inducing apoptosis, and facilitating plaque rupture [88]. Based on their ability to inhibit 15-LOX, CBD and its mono- and dimethylated derivatives have been proposed as potentially useful in treating atherosclerosis [90]; however, the question of whether 15-LOX has a detrimental or beneficial role in atherosclerosis is unsettled [91]. Nevertheless, a growing body of evidence supports the utility of targeting endocannabinoid signaling, particularly that of macrophages, in the treatment of atherosclerosis [92]. Differentiation of human monocytes, including that induced by oxLDL, results in a change in their CB_1 and CB_2 expression profile such that CB_1 becomes more prominent [93]. Activation of macrophage CB_1 receptor was shown to upregulate the CD36 scavenger receptor and cholesterol accumulation by macrophages/foam cells [94]. CB_1 receptor activation of human macrophages was linked to ROS generation via p38 MAPK activation, as well as production of TNFα and MCP1 [93]. In contrast, activation of the CB_2 receptor was shown to attenuate the pro-inflammatory actions of the CB_1 receptor through activation of the Ras family small G protein, Rap1 [93]. Consistent with these findings, a nonselective CB_1/CB_2 receptor agonist reduced oxLDL-induced ROS generation and TNFα secretion via the CB_2 receptor of murine macrophage, which in contrast to human macrophages do not express much CB_1 receptor [93,95]. Such tantalizing findings have fueled the idea that the endocannabinoid system may be a avenue for further

drug development in dealing with atherosclerosis, likely involving a role for CBD as well [7].

Opposing regulatory effects of CB_1 and CB_2 receptors on inflammation and oxidative/nitrative stress is a general theme that has significance in atherosclerosis, as well as other human maladies. CB_2 activation in endothelial cells, which play a key role in development of early atherosclerosis and any inflammatory response, decreases activation and the inflammatory response [96], while CB_1 activation in human coronary artery endothelial cells was reported to induce ROS-dependent and -independent MAPK activation and cell death [97]. CB_1 cannabinoid receptors promote oxidative stress and cell death in murine models of doxorubicin-induced cardiomyopathy and in human cardiac myocytes [98]. In contrast, CB_2 activation was found to reduce oxidative stress and neutrophil infiltration in the infracted mouse myocardium [99]. In nephropathy, CB_2 limits oxidative/nitrosative stress, inflammation, and cell death [100], while activation of CB_1 cannabinoid receptors promote oxidative/nitrosative stress, inflammation, and cell death [101].

Conclusions

Inflammation and oxidative stress are intimately involved in the genesis of many human diseases. Unraveling that relationship therapeutically has proven challenging, in part because inflammation and oxidative stress “feed off” each other. However, CBD would seem to be a promising starting point for further drug development given its anti-oxidant (although relatively modest) and anti-inflammatory actions on immune cells, such as macrophages and microglia. CBD also has the advantage of not having psychotropic side effects. Studies on models of human diseases support the idea that CBD attenuates inflammation far beyond its antioxidant properties, for example, by targeting inflammation-related intracellular signaling events. The details on how CBD targets inflammatory signaling remain to be defined. The therapeutic utility of CBD is a relatively new area of investigation that portends new discoveries on the interplay between inflammation and oxidative stress, a relationship that underlies tissue and organ damage in many human diseases.

Acknowledgments

This work was supported by grants from the National Heart, Lung, and Blood Institute (R01HL088101-04 and R01HL088101-02S1).

List of Abbreviations

2-AG	2-arachidonoylglycerol
5-HT$_{1A}$ receptor	5-hydroxytryptamine (serotonin) receptor subtype 1A
A_{2A}	adenosine A_{2A} receptor (ADORA2A)
Abn-CBD	abnormal-cannabidiol
AEA	N-arachidonoylethanolamine (anandamide)
ALS	amyotrophic lateral sclerosis
AV3V	anteroventral third ventricle
Aβ	beta-amyloid peptide
BDNF	brain-derived neurotrophic factor
BHT	butylated hydroxytoluene

CB_1	cannabinoid receptor type 1
CB_2	cannabinoid receptor type 2
CBD	cannabidiol
CD36	Cluster of Differentiation 36
COX	cyclooxygenases
ENT1	equilibrative nucleoside transporter 1
FAAH	fatty acid amide hydrolase
GFAP	glial fibrillary acidic protein
GPR55	G protein-coupled receptor 55
HMGB1	high mobility group box 1
ICAM-1	intercellular adhesion molecule 1
IFNγ	interferon gamma
IL	interleukin
iNOS (or NOS2)	inducible NOS
LDL	low density lipoprotein
LOX	lipooxygenases
LPS	lipopolysaccharide
MAPK	mitogen-activated protein kinase
MCP1	monocyte chemotactic protein 1
mmLDL	minimally modified LDL
MS	multiple sclerosis
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
NK	natural killer
NKT	natural killer T
NO	nitric oxide
NOD mouse	non-obese diabetic mouse
oxLDL	oxidized LDL
PPARs	peroxisome proliferator activated receptors
PVN	paraventricular nucleus
Rap1	ras-related protein 1
ROS	reactive oxygen species
STAT1/STAT3	single transducers and activators of transcription 1/3
Tc cell	cytotoxic T cell
Th cell	T helper cells
TLR4	toll-like receptor 4
TNFα	tumor necrosis factor-α

TRP	transient receptor potential
VCAM-1	vascular cell adhesion molecule 1
Δ^9-THC	delta9-tetrahydrocannabinol

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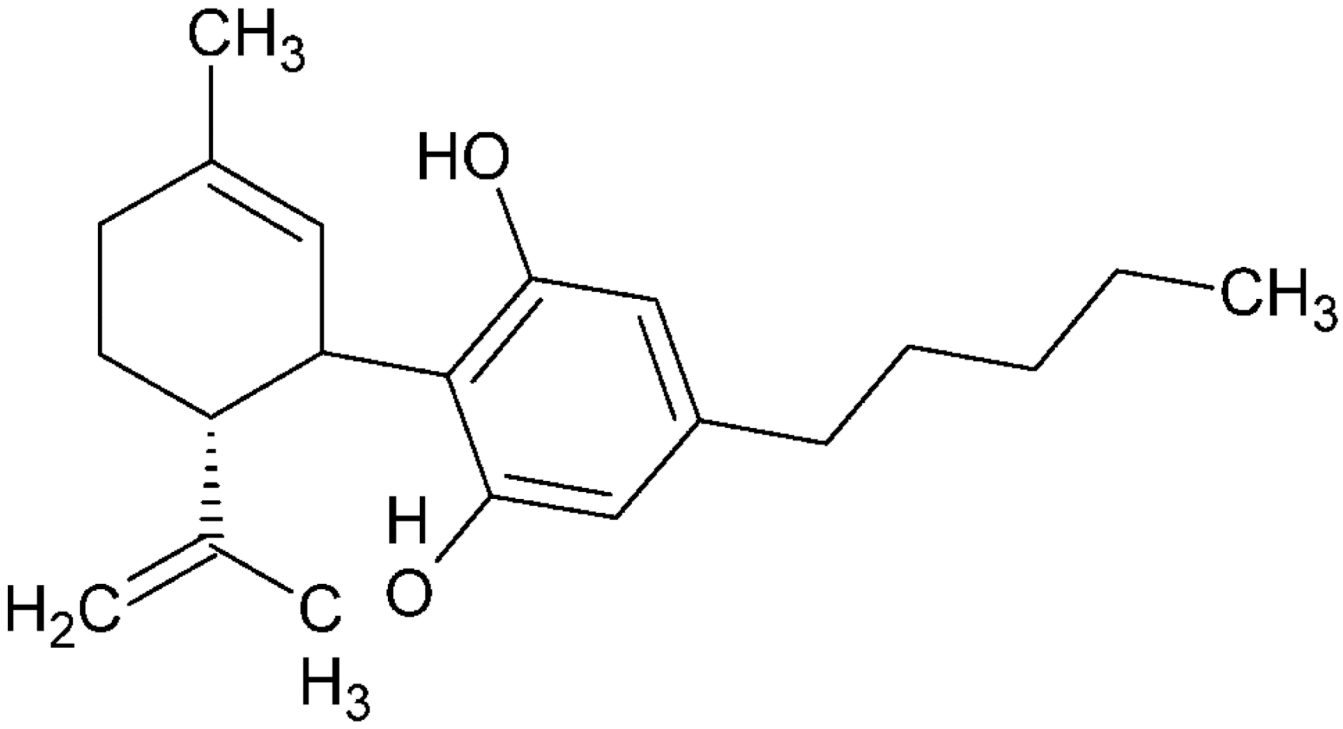

Figure 1.
Chemical structure of cannabidiol (CBD).

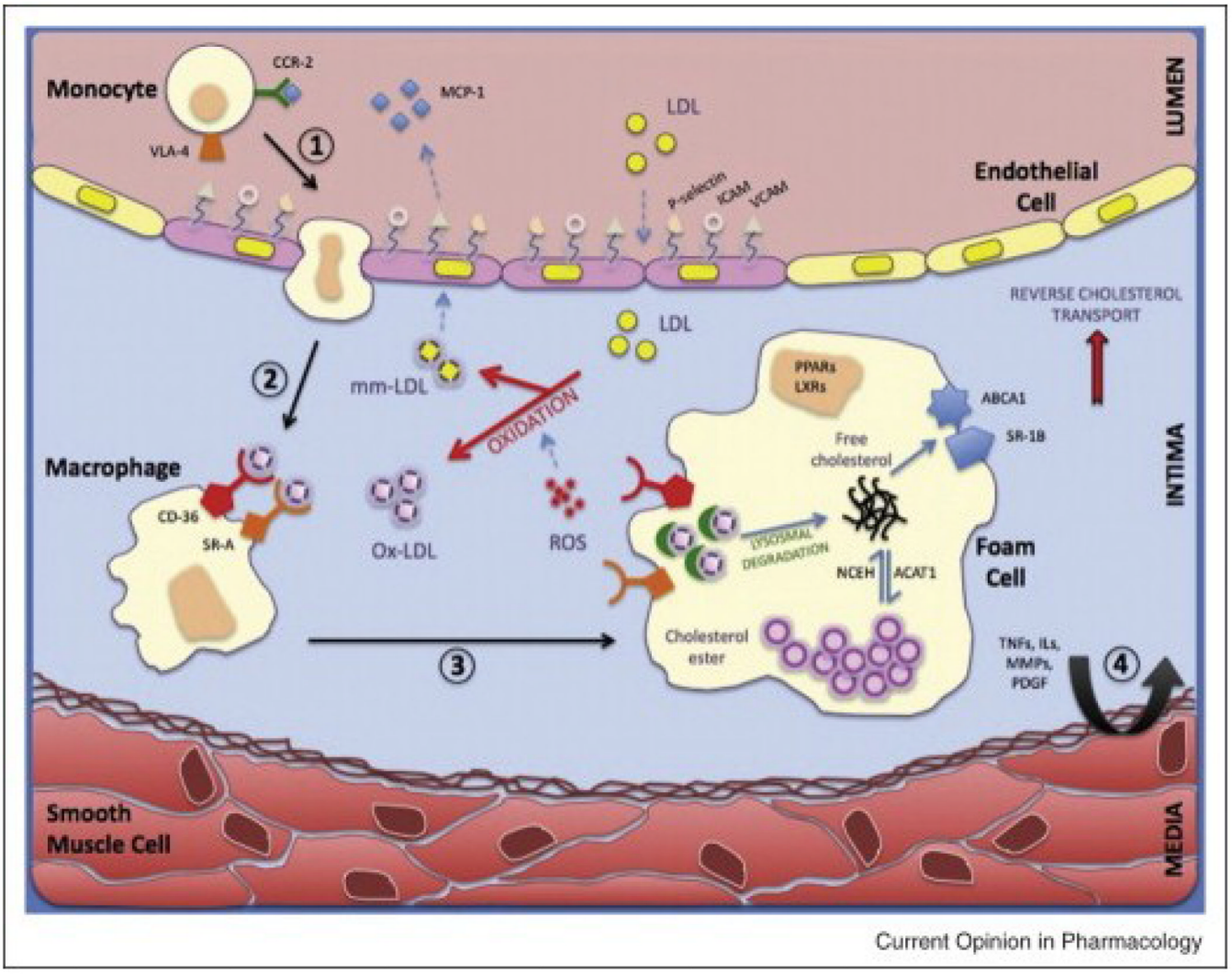

Figure 2.

Inflammation and oxidative stress in atherosclerotic plaque formation. Endothelial dysfunction causes monocyte activation and their binding to endothelial cells, via the production of MCP-1, its binding to CCR2 receptors, and the upregulation of adhesion molecules on endothelial cells (1). Monocytes cross the endothelium and differentiate into macrophages (2). Due to ROS, LDL that traverses the endothelium is converted to mmLDL and oxLDL. Macrophages accumulate oxLDL through scavenger receptors and are turned into foam cells (3). Along with T cells, foam cells produce inflammatory mediators that stimulate migration of smooth muscle and endothelial cells into the intima (4). Figure taken with permission from Reference 88.