Cannabidiol modulation of oxidative stress and signalling

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Abstract
Cannabidiol (CBD), one of the primary non-euphoric components in the Cannabis sativa L. plant, has undergone clinical development over the last number of years as a therapeutic for patients with Lennox-Gastaut syndrome and Dravet syndromes. This phytocannabinoid demonstrates functional and pharmacological diversity, and research data indicate that CBD is a comparable antioxidant to common antioxidants. This review gathers the latest knowledge regarding the impact of CBD on oxidative signalling, with focus on the proclivity of CBD to regulate antioxidants and control the production of reactive oxygen species. CBD is considered an attractive therapeutic agent for neuroimmune disorders, and a body of literature indicates that CBD can regulate redox function at multiple levels, with a range of downstream effects on cells and tissues. However, pro-oxidant capacity of CBD has also been reported, and hence caution must be applied when considering CBD from a therapeutic standpoint. Such pro- and antioxidant functions of CBD may be cell- and model-dependent, and may also be influenced by CBD dose, the duration of CBD treatment and the underlying pathology.

Introduction
Production of reactive oxygen species (ROS) is commonly associated with oxidative stress and its pathological role in inflammatory diseases such as multiple sclerosis (MS), rheumatoid arthritis (RA), atherosclerosis and inflammatory bowel disease (IBD) [1-5]. During infection, immune cells produce ROS via the NADPH oxidase 2 (NOX2) complex as a mechanism to eradicate pathogens [6]. When NOX2-generated ROS production is dysregulated due to mutations in NOX2 complex proteins, this can result in defective phagocyte function characterized by severe and recurrent infections defined as chronic granulomatous disease (CGD) [7]. Moreover, ROS produced in the phagosome to activate proteolytic enzymes can escape the immune cell, thus damaging the surrounding tissue [8]. Despite this, it is clear that a deficiency in ROS production has the proclivity to aggravate disease processes [9]. In addition, leukocytes isolated from individuals with chronic MS produce less superoxide than those with a milder disease [10], and a similar scenario has been identified in Guillain-Barré syndrome (a subtype of acute inflammatory demyelinating polyneuropathy), where evidence suggests that leukocytes produce lower levels of oxygen radicals in the most severe cases of disease [11]. Taken together, these findings support a complex role of ROS in regulating inflammation in disease.

In recent years, cannabinoid molecules, such as cannabidiol (CBD) and Δ⁹-tetrahydrocannabinol (Δ⁹-THC), have drawn attention due to their anti-inflammatory, antioxidant and neuroprotective properties [12, 13]. The most well described targets for cannabinoids are their specific receptors, the cannabinoid receptors CB₁ and CB₂ [14, 15], but their pharmacological actions are not solely limited to these receptors. Indeed, cannabinoids are lipophilic and certain cannabinoids have also been shown to target a wide range of receptors, including the peroxisome proliferator-activated receptors...
(PPARs), the transient receptor potential cation channel subfamily V member 1 (TRPV1), G protein-coupled receptor 55 (GPR55), the 5-hydroxytryptamine receptor subtype 1A (5-HT1A), glycine α1 and α1β receptors, in addition to ion channels (Ca2+) and enzymes such as the adenosine membrane transporter phospholipase A2, lipoxygenase (LO) and cyclooxygenase-2 (COX-2) [16-22]. Depending both on the cannabinoid structure and cell/tissue targeted, the pharmacological effects of cannabinoids may vary.

Overall, cannabinoids have been shown to possess therapeutic efficacy in several inflammatory and neuronal diseases [23]. Given that the production of ROS is an intrinsic feature of neuroinflammation and peripheral immune responses, this review aims to gather the latest knowledge on the action of cannabinoids on oxidative signalling, with focus on the phytocannabinoid CBD. CBD is selected for review given recent advances in its therapeutic development [24-26].

**Oxidative signalling and stress**

The production and maintenance of controlled levels of intracellular ROS has a key role in several physiological functions, including the maintenance of redox homeostasis, cell cycle signalling and hormone production [27, 28]. When present, ROS can regulate several signalling pathways by reacting with transcription factors and genes, modifying their structure and thus their function. Hence, ROS can modulate gene expression patterns and signalling proteins related to the stress response and cell survival mechanisms [29]. It is when this homeostasis is impaired, by either an overproduction of ROS or inefficient ROS scavenging mechanisms, that oxidative stress ensues, promoting cellular damage, lipid peroxidation, DNA modifications and enzyme inactivation, and when persistent, can ultimately lead to cell death and tissue destruction [30-32]. This rationale has been the basis for the development of several anticancer drugs [33-35].

**ROS**

ROS represent a group of unstable oxygen radicals and molecules with strong oxidizing properties. Once formed, ROS are converted to other oxidative species or eliminated by the antioxidant mechanisms of the cell [29, 36]. The most common ROS are the superoxide anion (O2•−), hydrogen peroxide (H2O2) and the hydroxyl radical (HO•) [29]. There are three major cellular mechanisms of ROS production as described in Figure 1: (A) ROS are produced via the mitochondrial electron transport chain, in complex I and II; (B) production via the enzymatic reaction catalysed by the enzyme xanthine oxidase (XO); and (C) by NOX as a defence against pathogens [37-39]. To maintain the levels of ROS under control, the enzyme superoxide dismutase (SOD) converts O2•− into H2O2 (D), which is then converted to water, a reaction catalysed by catalase (CAT) and/or glutathione peroxidase (GPx) (E) [40, 41]. However, during periods of high ROS generation, H2O2 may cross cell membranes and react with O2•− and metal cations (Fe2+ or Cu+) to form HO• (Fenton and Haber-Weiss reactions) (F, G) [42]. Furthermore, oxygen radicals can also react with nitrogen species, namely nitric oxide (NO) (H). The reaction of O2•− with NO can generate the production of peroxynitrite (ONOO•) (H) [43], a particularly reactive radical that may promote generalized oxidative/nitrosative damage, including DNA fragmentation and lipid degradation.

**NOX2 production of ROS**

One of the cellular sources of ROS is the NOX2 complex, which belongs to a family of NADPH oxidases. NOX are transmembrane enzymes involved in the electron transport across biological membranes by oxidizing intracellular NADPH=NADH, while reducing molecular oxygen into O2•− anions [37]. Among the seven NOX members identified to date, NOX2 has the ability to produce an oxidative burst to eliminate pathogens [44]. The NOX2 complex consists in a transmembrane catalytic core, a heterodimer containing gp91phox and the protein p22phox. On the
cytosolic side of the membrane, the proteins p47phox, p67phox, p40phox and Rac (a small GTP-binding protein) regulate the complex activity [37, 45].

Antigen-presenting cells can produce ROS upon activation via the NOX2 complex [6, 46]. Interestingly, gp91phox is also expressed in bone marrow and thymus, contributing to the role of NOX2-derived ROS in B-/T-cell development and maturation [47, 48]. Although phagocytes express high levels of the NOX2 complex, components of this complex are also found in nonphagocytic cells, such as cardiomyocytes, endothelial cells and muscle cells [49-51]. In resting cells, the NOX2 complex remains inactive, and upon exposure to certain stimuli, the complex becomes primed or activated [52]. NOX2 priming prepares the complex to produce ROS, initiating a weak oxidative response without eliciting an oxidative burst. This can occur upon stimulation by pro-inflammatory cytokines (e.g. tumour necrosis factor-α (TNF-α) and interleukin (IL)-1β), toll-like receptor (TLR) agonists (e.g. lipopolysaccharide (LPS), flagellin), OONO- and proteases. The priming induces a partial phosphorylation of p47phox that alters its conformation, thus decreasing its autoinhibition and facilitating its translocation and interaction with the NOX2 enzymatic core. This mechanism exerts tight control over NOX2 activation to prevent unintended O2•− release [53, 54]. Full activation of the NOX2 complex requires additional stimuli, such as phorbol 12-myristate 13-acetate, a protein kinase C activator, and formyl-methionyl-leucyl phenylalanine, that acts via the formylpeptide receptor. Upon activation, NOX2 increases the production of superoxide [55], and therefore, mutations in any of the components of NOX2 dysregulate its activity and thus ROS signalling [7, 56].

Antioxidant mechanisms

Cellular antioxidant mechanisms regulate ROS signalling and furthermore prevent/reduce the oxidation of unintended molecules. Antioxidant mechanisms include enzymes such as CAT, SOD, GPx, thioredoxin and peroxiredoxin, in addition to the radical scavengers such as reduced glutathione (GSH) [40, 41, 57, 58]. As mentioned previously, CAT catalyses the conversion of H2O2 to water (Figure 1), and although this enzyme is highly expressed during inflammation, the GPx enzyme has even higher affinity to the H2O2 radical. GPx activity depends on the GSH ability to be oxidized to glutathione disulfide (GSSG) in the presence of NADPH, thus functioning as a proton donor. The GSH pool is afterwards replenished by both its regeneration from GSSG mediated by GSH reductase and de novo synthesis [59, 60]. Additionally, dietary antioxidant molecules are also useful at maintaining the level of ROS by directly scavenging ROS, namely tocopherols (vitamin E) and ascorbic acid (vitamin C) [61, 62].

More research is still needed to achieve a deeper understanding on the specific roles for each particular oxygen radical. Current methodologies are limited in their ability to measure isolated radicals but rather a group of radicals, making it difficult to differentiate amongst them. What is known today in the field is based on the use and development of various ROS scavengers, enzyme inhibitors, and antioxidant enzymes. Moreover, many in vitro studies employ the use of high levels of ROS and thus do not represent the physiological fine-tune modulatory effects of ROS in vivo.

In consideration of antioxidant mechanisms, much data indicate that nuclear factor erythroid 2-related factor-2 (Nrf2), a ubiquitously expressed redox-sensitive transcription factor, is important in initiating the transcription of antioxidant and cytoprotective genes [63]. In the cytoplasm, Nrf2 is bound to its inhibitor Kelch-like ECH-associated protein1 (Keap1), and in response to pro-oxidant stress, oxidative modification of Keap1 dissociates Keap1 from Nrf2, facilitating the nuclear translocation of Nrf2 [64]. Nrf2 binds to antioxidant response elements (AREs) to orchestrate the expression of antioxidant enzymes, including SOD [65], to promote a reduction in ROS. Antioxidants can act by promoting Nrf2 activation [66, 67], and deficiencies in Nrf2 factor have been associated with increased inflammation and carcinogenesis [68]. The enzyme heme oxygenase-1 (HO-1) is a key Nrf2 gene target, and a large body of data indicate that HO-1 possesses diverse antioxidant and anti-inflammatory capacity [69]. HO-1 is an enzyme that catalyses the degradation of heme and is induced under conditions of oxidative stress [70]. Importantly, HO-1 has to proclivity to
negate the production of ROS [71] and inflammatory mediators [72], making HO-1 inducers potential therapeutic targets in disease [73].

**ROS and oxidative signalling**

While some studies suggest that oxidative stress plays a critical role in the progression of diseases including cancer, diabetes and CNS disorders [1-5, 74], many studies have failed to demonstrate that the therapeutic effects of antioxidants translate to a clinical setting [75]. Indeed, although antioxidants are believed to wield anti-inflammatory effects, their assessment as therapeutics in both human and rodent studies has provided contradictory findings [76-79]. On the contrary, the use of pro-oxidant molecules, such as phytol (NOX2 activator and vitamin E precursor), can improve, or even prevent, ongoing inflammation in animal models [80]. Considering this, the controlled production of ROS is recognised to exert crucial effects in regulating biological functions and cell signalling. This occurs via the rapid generation and removal of ROS within defined cell compartments, thus avoiding sustained signalling and/or oxidative stress [28]. Contrary to previous interpretation, ROS signalling may regulate the inflammatory response.

As previously described, to eliminate invading pathogens, phagocytic cells produce ROS into phagolysosomes following NOX2 activation, and this results in a pH increase within the vesicles, triggering the activation of proteolytic enzymes which in turn destroys the engulfed pathogens/debris [8, 81]. Radicals that escape the vesicle membrane into the cytoplasm are rapidly eliminated by the cell antioxidant mechanisms. It is important to note that there are some indications that ROS production can regulate the activation of the NLRP3 inflammasome during inflammation [82, 83]. The inflammasome consists in a protein complex that can activate caspase-1 to control the production of IL-1β, a classic pro-inflammatory cytokine [84]. Although ROS may regulate NLRP3 inflammasome activation, it appears to be independent of NOX2 activation. Other known NLRP3 inflammasome activators include innate stimuli (e.g. pathogens, environmental insults) and particulate adjuvants (e.g. alum, silica, urate crystals) [85].

As previously discussed, NO plays a role on oxidative signalling mechanisms [43], in addition to its important role in modulating physiological functions such as neurotransmission, vasodilatation and immunomodulation [86]. NO is synthesized on demand and is produced by one of the three known isoforms of nitric oxide synthase (NOS): neuronal NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS). The nNOS and eNOS isoforms are constitutively present in neuronal and endothelial cells, respectively [86], while iNOS expression is inducible and regulated at the transcriptional level, particularly in inflamed tissues [87]. As mentioned previously, the reaction of O$_2^-$ with NO can generate ONOO$^-$ [43] (Figure 1), which can contribute to generalized oxidative/nitrosative damage, including DNA fragmentation and lipid degradation.

Oxidative stress ensues when ROS are produced in high quantities without control, and ROS production plays a key role in neuroimmune disorders, resulting in several harmful nonspecific events such as DNA laddering and lipid peroxidation. While some radicals react rapidly and rarely escape the cell membrane (e.g. O$_2^-$), other more stable radicals can cross the cell membrane and diffuse to adjacent tissues (e.g. H$_2$O$_2$) [88]. Moreover, O$_2^-$ gives rise to HO$^\cdot$ and perhydroxyl (HOO$^\cdot$) radicals [29, 89], with data indicating that the HO$^\cdot$ radical can inactivate the mitochondrial enzyme pyruvate dehydrogenase [90], depolymerise gastrointestinal mucin [91] and inflict oxidative DNA damage [92]. The HOO$^\cdot$ is also highly deleterious, initiating lipid peroxidation [93], disturbing membrane permeability [94] and demonstrating toxicity [95]. Such events may be associated with mutagenesis in chronic intestinal inflammation [96] and in addition to intestinal inflammation, much data links the production of ROS to the pathology of a range of inflammatory diseases such as RA, atherosclerosis, MS and IBD [1-5]. The key role of ROS in physiological processes adds to the complexity of targeting ROS production therapeutically, and the balance between ROS elimination and generation may be key in terms of the management of disease.
Cannabinoids

The introduction of medicinal cannabis, and cannabis-based therapeutics, to mainstream medicine has proved a controversial topic, although medicinal benefits of the Cannabis sativa L. (C. sativa) plant have been recorded for centuries. Indeed, evidence exists for medicinal use of cannabis dating back to the 4th century when used in the treatment of a broad range of ailments [97]. Much public reticence with the introduction of cannabinoids to a medical setting are associated with the recreational use of cannabis, the euphoric effects of the drug and the evidence linking cannabis abuse with psychosis [98]. With the discovery and characterisation of many compounds in C. sativa, in addition to an increase in our understanding of cannabinoid pharmacology and toxicology, selective cannabinoid-based therapeutics continue to make advancement in pre-clinical and clinical studies.

To date, a large body of data suggest that cannabinoids have therapeutic properties, alleviating symptoms of several CNS disorders. Indeed, cannabinoids can mitigate inflammation, reduce CNS spasticity, alleviate neuropathic pain, and a body of evidence indicates that cannabinoids provide neuroprotection following injury or inflammation in the CNS [99, 100]. The most well-known cannabinoids are the phytocannabinoids synthesised by C. sativa, including THC, a euphoric component of the plant, in addition to CBD, cannabiol (CBN), cannabichromene (CBC) and cannabigerol (CGB), which are considered non-euphoric cannabinoids [101]. Cannabinoids also include a class of lipid messengers known as endogenous cannabinoids (eCBs), with N-arachidonylethanolamine (anandamide; AEA) and 2-arachidonoyl-glycerol (2-AG) representing the most actively studied eCBs [102]. Both AEA and 2-AG are synthesized on demand in the body to mediate diverse physiological functions via interaction with a range of receptors, particularly the G protein-coupled receptors (GPCRs), CB1 and CB2 [103]. The system of eCBs and cannabinoid receptors constitutes the eCB system (ECS). In addition, many synthetic agonists, antagonists and inverse agonists for the cannabinoid receptors have been developed and such classes of molecules are under investigation.

Phytocannabinoids

C. sativa is an annual dioecious plant containing a diverse repertoire of botanical cannabinoids commonly known as phytocannabinoids. To date over 150 phytocannabinoids have been characterised in the plant [104]. The primary phytocannabinoids are THC and CBD, which represent the most commonly studied phytocannabinoids in experimental and clinical settings, demonstrating a wide range of effects on physiological processes. Other phytocannabinoids currently under investigation include the less well characterised phytocannabinoids CBG and CBC. The biosynthetic pathways involved in the generation of the main classes of phytocannabinoids involve the formation of acidic cannabinoids including cannabigerolic acid (CBGA), the precursor of tetrahydrocannabinolic acid (THCA), cannabidiolic acid (CBD) and cannabichromenic acid (CBCA), which have poor oxidative stability. Following oxidation or decarboxylation, neutral forms of cannabinoids are generated by heating or naturally as the plant ages [105]. Other compounds isolated from C. sativa include a variety of terpenes [106]. Indeed, over 200 terpenes have been identified, including the monoterpenes (limonene, α-pine, linalool) and sesquiterpenes (β-caryophyllene), which share the same biological precursor with phytocannabinoids. Terpenes produced by the plant are responsible for the plant aroma [107], acting as botanical insecticides and attracting predatory mites [108].

Phytocannabinoids and terpenes accumulate in the secretory cavity of the glandular trichomes in C. Sativa [109, 110], and are present in the highest quantity on the female flower of the plant. Male plants produce lower levels of phytocannabinoids [111]. Glandular trichomes consist of a sac-like cavity packed with secretory vesicles known as glandular hairs. Glandular trichomes of C. sativa alter morphology and metabolite content during flower maturation, and phytocannabinoids/terpenes are found on the calyx and the underside of anthers of flowers, leaves and bracts [109]. Trichomes rupture due to environmental stress or damage (due to high temperatures and herbivorous
consumption), resulting in the release of phytocannabinoids and terpenes as a noxious, sticky liquid on the plant surface.

The ECS
The activity of phytocannabinoids was initially considered to result from their proclivity to fluidise membranes. However, in the early 1990’s the cannabinoid receptors CB₁ [14] and CB₂ [15], the receptors of the ECS, were identified. Both receptors are GPCRs and are responsible for many of the effects of cannabinoids on physiological systems. CB₂ is abundantly expressed in immune tissues [112] and is responsible for many immunomodulatory effects, while CB₁ expression is predominantly confined to the CNS, with low expression in peripheral tissues including the heart, reproductive organs and thymus [112]. Indeed, CB₁ is considered one of the most highly expressed GPCRs in the brain [113]. Under normal physiological conditions CB₂ exhibits low basal expression in the CNS, with evidence indicating that this receptor is expressed in the brain stem [114] and on hippocampal pyramidal neurons [115]. Importantly, in pathological conditions, data suggests that the expression of the CB₂ receptor is upregulated, particularly on microglial cells [116]. Cannabinoids vary in their affinity for the cannabinoid receptors. Indeed, THC is a partial agonist for both CB₁ and CB₂ [117], whereas CBD has low affinity for both CB₁ and CB₂ [117, 118].

The discovery of receptors that mediate the cellular action of components of C. sativa was followed by the subsequent identification of the eCBs, AEA [119] and 2-AG [120, 121], again in the early 1990’s. eCBs vary in their affinities for the cannabinoid receptors; AEA has relatively high affinity for CB₁ but little to no affinity for CB₂, while 2-AG is an agonist at both CB₁ and CB₂ [122]. AEA and 2-AG are non-charged, hydrophobic lipid molecules that can act to control neurotransmission [123] and are produced on demand in response to an increase in intracellular Ca²⁺ [124-126]. Basal concentrations of 2-AG in brain tissue are 170 times greater than AEA [127]. Furthermore, AEA is a member of the family of N-acylthanolamines, other members of which include the eCB-like compounds, palmitoylethanolamide (PEA) and oleoylethanolamide (OEA), both of which possess diverse physiological functions [128-130].

Although CB₁ and CB₂ are considered classic cannabinoid receptors, much evidence suggests the existence of further receptor targets for cannabinoids, including nuclear PPARs, GPR55 and the TRPV1. Although GPR55 shares a low amino acid sequence homology with both CB₁ and CB₂, data indicate that GPR55 is a receptor for cannabinoid ligands including AEA, PEA, THC and CBD [16, 17, 131-135]. TRPV1 is a cationic channel receptor dependent on intrinsic and extrinsic calcium concentrations, and evidence that TRPV1 is a target for CBD, 2-AG and AEA [18, 136, 137]. This receptor exists largely on sensory neurons and cannabinoid activity through this receptor has shown significant analgesic potential [138]. Several studies have also shown that CBD targets PPAR-γ [13, 19, 139, 140], and it should also be noted that PEA and OEA can exert their effects via PPARs [141-143]. In terms of CBD, further CB₁/₂-independent cellular targets include serotonin (5-HT) receptors [19, 20, 144, 145] and both µ- and δ-opioid receptors [146].

Synthetic Cannabinoids
Multiple synthetic cannabinoid compounds have been developed with the aim to pharmacologically target specific aspects of the ECS and to facilitate the development of cannabinoid therapeutics [147]. Firstly, the compound WIN55,212-2, an aminoalkylindole derivative, with affinity for both cannabinoid receptors, but binding to CB₂ with higher affinity, represents one of the most actively studied synthetic cannabinoids [148, 149]. Indeed, research on this compound has shown that WIN55,212-2 is an analgesic in rodent models of neuropathic pain [150] and mechanical allodynia [151], while topical administration of WIN55,212-2 was shown to reduce intraocular pressure in patients with glaucoma [152]. HU-210 is another synthetic cannabinoid agonist that has been extensively researched. HU-210 is a high affinity CB₁/CB₂ receptor agonist [153, 154] with agonist activity also at GPR55 [16]. This cannabinoid has been shown to be protective in models of
experimental colitis [155] and photoreceptor degeneration [156]. The cannabinoid agonist CP55,940 shares biochemical properties with THC, and demonstrates high affinity for CB1 and CB2 [157, 158] and is also a GPR55 agonist [16]. CP55,940 has been shown to mitigate tumour-evoked hyperalgesia in murine models in a dose-dependent manner [159], and also possesses antioxidant capacity in neutrophils [160]. The JWH family of synthetic cannabinoids have also been shown to be effective agonists of the cannabinoid receptors. Indeed, evidence indicates that JWH-133, a selective CB2 agonist [161], ameliorates spasticity in murine MS [162] and prevents microglial cell activation and inflammation following exposure to β-amyloid [163]. Finally, ACEA is a well described synthetic CB1 agonist and analogue of AEA [164]. ACEA has the proclivity to protect ischemic neurons from oxygen-glucose deprivation/reoxygenation and middle cerebral artery occlusion [165].

Overall, there is a large array of synthetic cannabinoid ligands currently employed in cannabinoid pharmacological research. Such synthetic cannabinoid ligands are useful pharmacological tools that may be used to identify therapeutic avenues in the ECS.

Antioxidant and anti-inflammatory effects of cannabinoids: Focus on CBD

The antioxidative and anti-inflammatory properties of cannabinoids across a variety of tissue types and cellular models have been studied for several decades and are well described [166]. A summary of the antioxidant capacity of CBD is presented in Figure 2. In particular, much evidence has highlighted that CBD, the major non-euphoric phytocannabinoid in C. sativa, has an array of anti-inflammatory effects, with proclivity to modulate oxidative processes in neuropathic and inflammatory models [167]. As discussed previously, CBD can act through cannabinoid receptor-dependent and -independent mechanisms, and demonstrates minimal agonist activity (and very low affinity) for both CB1 and CB2 [117, 118]. Furthermore, CB1/2-independent mechanisms of action for CBD have also been identified, including PPAR-γ [13, 19, 139, 140], TRPV1 receptor [18], GPR55 [16], 5-HT receptors [19, 20, 144, 145] and μ/δ-opioid receptors [146]. Much data suggest that CBD has antioxidant capacity, and CBD has been shown to reduce oxidative metabolism in polymorphonuclear leukocytes [168] and H2O2-treated nucleus pulposus cells [169], and furthermore reduces oxidative stress parameters in aged pancreatic cells [170]. In addition, CBD has the proclivity to improve cell viability following H2O2 treatment [169]. In addition, data from Barichello and colleagues (2012) indicate that CBD reduces host immune responses and exerts an anti-inflammatory effect (reduced cortical TNF-α) in the CNS of rats exposed to intrathecal S. pneumoniae [171]. This is significant given the key role played by TNF-α in the CNS, particularly in events associated with neuroinflammation, neurodegeneration and neurogenesis [172-174]. Broadly, CBD has an inhibitory effect on TNF-α expression in an array of inflammatory models, which has been recently reviewed by Nicols and Kaplan (2020) [175]. Interestingly, CBD attenuates neural production of ROS following cadmium chloride treatment, in a manner similar to vitamin E (α-tocopherol acetate) [176], and evidence also suggests that it is more neuroprotective than ascorbate and α-tocopherol against glutamate toxicity [177]. Owing to its functional and pharmacological diversity, and evidence of is comparable antioxidant capacity to known antioxidants, CBD is an attractive agent for therapeutic immunomodulation and has been studied in conditions including intractable epilepsy [26], Huntington’s disease [178] and schizophrenia [179], amongst others.

Antioxidant mechanisms of action of CBD

Regulation of antioxidants

A body of research evidence indicates that CBD modifies redox balance by altering the level and activity of antioxidant molecules (Figure 2). Indeed, CBD has been shown to target the regulation of redox-sensitive transcription factors such as Nrf2 in microglia [180], keratinocytes [181] and endothelia [182], which is important given the key role of Nrf2 in initiating the transcription of antioxidant and cytoprotective genes [63]. Indeed, recent data has shown that CBD can target the expression of Keap1 and Nrf2 in a pulmonary artery smooth muscles cells, which may contribute to
its antioxidant effect in a model of pulmonary arterial hypertension [183]. CBD has also been shown to regulate the expression of the inducible antioxidant enzyme HO-1 in keratinocytes [184], adipose tissue-derived mesenchymal stem cells [185], neuroblastoma cells [186] and smooth muscle [187], which may impact the proclivity of this phytocannabinoid to regulate cellular ROS levels. Indeed, CBD significantly increases HO-1 mRNA and protein expression in human umbilical artery smooth muscle cells in a time- and concentration-dependent manner independent of CB receptors, and this effect was reversed via glutathione precursor N-Acetyl Cysteine (NAC), indicating the participation of ROS signalling in this process [187]. Similar effects of CBD were determined in human umbilical vein endothelial cells [182]. Data elsewhere indicates that CBD can also regulate the activity of SOD and the enzymatic activity of Cu-, Zn- and Mn-SOD, which metabolise superoxide radicals [181, 188]. The vasorelaxant effects of CBD are reduced by a SOD inhibitor, suggesting that CBD acts via SOD to promote its vascular actions [189]. CBD also attenuates hippocampal oxidative damage post-oxygen–glucose-deprivation/reperfusion injury, upregulating GSH levels and simultaneously increasing SOD1 and GPx activity following injury [190]. In support of this, in vivo administration of CBD attenuates the reduction in reduced/oxidised glutathione ratio (GSH/GSSG) in myocardial tissue of diabetic mice [188], and furthermore prevents GSH depletion in cardiac tissue following doxorubicin cardiotoxicity [191].

**Free radical scavenging capacity**

A body of data indicates that CBD demonstrates intrinsic free radical scavenging capacity (Figure 2). Indeed, CBD has been shown to ameliorate LPS-induced ROS production in microglia [192]. Furthermore, CBD inhibits mitochondrial superoxide generation in high glucose-stimulated human coronary endothelial cells [193] and reduces mitochondrial ROS generation following hippocampal oxidative damage post-oxygen–glucose-deprivation/reperfusion injury [190]. Neuroprotective effects of CBD have been shown in models of retinal neurotoxicity whereby CBD directly mitigates N-methyl-D-aspartate (NMDA)-mediated oxidative stress by potentially targeting the production of nitrotyrosine, a product of tyrosine nitration [194]. Similarly, oligodendrocyte progenitor cells and keratinocytes are protected from H₂O₂-induced cell death by CBD, with this phytocannabinoid demonstrating ROS scavenging properties against H₂O₂-induced ROS in the progenitor cells and keratinocytes [195, 196]. CBD was recently shown to exert a similar effect on H₂O₂-induced ROS in intestinal cell monolayers [197]. Furthermore, data from Branca and colleagues (2019) indicate that CBD attenuates neural ROS production following cadmium chloride exposure in a manner similar to α-tocopheryl acetate [176], and CBD also dose-dependently reduces β-amyloid-induced ROS production in neurons [198]. In parallel, CBD has been shown to ameliorate cisplatin-induced production of renal nitrotyrosine in a model of nephrotoxicity [199], and has also been shown to dose-dependently reduce tert-Butyl hydroperoxide-induced ROS production in keratinocytes [184]. In support of this, CBD reduces chemotactic peptide-induced ROS production in polymorphonuclear leukocytes [168] and furthermore in vivo administration of CBD ameliorates the production of ROS, in addition to lipid peroxides, in myocardial tissue of diabetic mice [188]. Finally, recent data from Baeeri and colleagues (2020) indicates that CBD attenuates age-related increases in ROS production in pancreatic islets [170], overall indicating the proclivity of CBD to exert free radical scavenging capacity in response to a multitude of stimuli.

**Redox balance**

CBD principally affects redox balance via intrinsic mechanisms. Indeed, evidence indicates that CBD interrupts free radical chain reactions, transforming free radicals into more inert molecules via the electrophilic aromatic molecular region and hydroxyl groups of its phenol ring [200]. Using cyclic voltammetry, Hampson et al., (1998) demonstrated that CBD donated electrons at a similar potential to known antioxidants, and using the iron-catalysed ROS production system (Fenton reaction), CBD was shown to prevent hydroperoxide-induced oxidative damage in neurons [177]. Again, using
cyclic voltammetry, Hamelink and colleagues (2005) demonstrated that CBD is a comparable antioxidant to the common antioxidants tocopherol and butylated hydroxytoluene [201], with data indicating that CBD can reduce ROS production by chelating transition metal ions involved in the Fenton reaction [202]. Neuroprotective effects of CBD have also been shown following anti-Yo-associated paraneoplastic cerebellar degeneration, where data indicate that CBD minimises the downgrading of the mitochondrial membrane potential induced by anti-Yo in a manner similar to the ROS scavenger butylated hydroxytoluene, although simultaneously potentiated Yo-induced ROS production [203]. CBD has also been shown to protect hippocampal neurons from energy stress via modulation of glucose consumption by activation of the pentose-phosphate pathway in an oxygen–glucose-deprivation/reperfusion injury model [190].

Indirect antioxidant actions on XO and NOX

In addition to its intrinsic antioxidant function, CBD alters oxidative metabolism through more indirect mechanisms to modulate downstream mediators of oxidative stress (Figure 2). Indeed, Atalay and colleagues (2020) recently demonstrated that CBD attenuates XO activity in keratinocytes exposed to UVB irradiation and H_{2}O_{2} [204]. CBD has been shown to reduce the expression of the superoxide generators RENOX (NOX4) and NOX1 in a mouse model of cisplatin-induced nephrotoxicity [199], and in a comprehensive study, Rajesh et al., (2010) identified the proclivity of CBD to attenuate diabetes-induced mRNA expression of NADPH oxidase subunits (p22phox, p67phox and gp91phox) in myocardial tissue using a model of diabetic cardiomyopathy [188]. Elsewhere, CBD has been shown to attenuate LPS-induced intracellular NADPH synthesis in microglia [192], and similarly, in vivo administration of CBD reduces the hepatic expression of the NADPH NOX2 isoforms p67phox/gp91phox and nitrosative stress in ethanol-fed mice, while reducing oxidative burst in neutrophils isolated from the livers of ethanol-fed mice [205]. CBD has also been shown to reduce nitrosative stress in cardiac tissue following doxorubicin-induced cardiac injury in rats, promoting a reduction in nuclear factor-κB (NF-κB), Fas ligand, caspase-3 and TNF-α in cardiac tissue following doxorubicin administration [191]. Similarly, CBD attenuates H_{2}O_{2}-induced COX2 and iNOS in nucleus pulposus cells [169], attenuates iNOS expression in the myocardium of diabetic mice [188] and in rats following doxorubicin cardiotoxicity [191], and furthermore attenuates NO expression in paw tissue following sciatic nerve injury and intraplantar injection of complete Freund’s adjuvant [167]. In parallel, Rajesh and colleagues (2007) demonstrated that CBD blunts iNOS expression in high glucose-stimulated human coronary endothelial cells [193], and data elsewhere indicates that CBD inhibits cisplatin-induced renal iNOS in a mouse model of nephrotoxicity [199]. CBD has also been shown to ameliorate plasma levels of prostaglandin E_{2} (PGE_{2}) in animal models of neuropathic and inflammatory pain, using sciatic nerve constriction and intraplantar injection of complete Freund’s adjuvant, respectively [167]. By lowering ROS levels, CBD therefore protects non-enzymatic antioxidants, preventing their oxidation. Recent evidence also suggests that CBD can ameliorate H_{2}O_{2}-induced IL-1β expression and the expression of other NLRP3 inflammasome-related genes, and this area warrants full investigation [196].

Pro-oxidant effects of CBD

Despite data indicating that CBD promotes antioxidative metabolism in various cells/systems, conflicting data exists regarding the influence of CBD on redox status. The effects of CBD on cell viability [170, 181, 195, 206-210] and proliferation [187, 211] are dose-dependent, and this may also be the case with respect to its antioxidant function. Indeed, in terms of ROS production, CBD has been shown to disrupt mitochondrial integrity and also induce ROS production and apoptosis in human CD14^{+} monocytes in a time-dependent manner [206]. Studies also indicate that CBD elevates intracellular ROS production in several cell systems, including human THP-1 monocytes [207], mouse macrophages [212], breast cancer cells [213] and human glioma cells [214]. Similarly, CBD
promotes ROS production to promote apoptosis in leukaemia cells [208], thymocytes [211] and splenocytes [209], and data from Panja and colleagues (2019) indicate that CBD potentiates Yo-induced ROS production in a model of postparaneoplastic cerebellar degeneration treatment [203]. CBD has also been shown to overcome oxaliplatin resistance in human colorectal cancer cells by inducing mitochondrial dysfunction by increasing intracellular ROS and decreasing SOD2 [215]. Data from Gonzalez-Garcia et al., (2017) indicate that CBD induces apoptosis of encephalitogenic cells through oxidative stress induction (increase in ROS) in murine MS [210]. CBD has also been shown to increase the oxygen consumption rate and enhance mitochondrial bioenergetics [190], and at high concentrations can promote COX-2 expression in LPS-treated macrophages [216]. In further support of the pro-oxidant capacity of CBD, it is also important to note that CBD has been shown to increase the expression of the NOX4 and p22phox NAP(P)H oxidases [208], and also enhances the production of the p47 subunit of pro-oxidative NADPH oxidase in endotoxin-treated neutrophils from healthy subjects [217]. Finally, CBD promotes a reduction in GSH in splenocytes [209], and has been shown to deplete GSH in human gliomas [214].

Overall, CBD modulates redox function at multiple levels and a variety of downstream effects are presented in the literature. These pro- and antioxidant functions may be cell- and model-dependent, and may also to be influenced by the dose of CBD delivered, the duration of CBD treatment, and the underlying pathology.

Cannabis-based therapeutics
Cannabinoid-based therapeutics are currently approved for a range of symptoms in various disorders; anorexia associated with loss of appetite in acquired immunodeficiency syndrome (AIDS), chemotherapy-associated nausea, spasticity in people with (pw)MS and the treatment of seizures in Lennox-Gastaut and Dravet syndromes. The therapeutics include Epidiolex®, Sativex®, Marinol® and Cesamet®, and such cannabinoid-based therapeutics are used as treatment strategies in those patients where conventional treatments are ineffective.

Of particular relevance to this review, the CBD-based therapeutic in the form of Epidiolex®, has recently entered the clinic in the management of epileptic seizures. Epidiolex®, a purified solution of CBD oil formulated for oral administration [24], is a medication approved by the US Food and Drug administration (FDA) and the European Medicines Agency (EMA) for patients with Lennox-Gastaut Syndrome and Dravet syndromes. CBD demonstrates efficacy in reducing convulsive seizure frequency in double-blind placebo-controlled trials [218], and in a prospective open-label study assessing the efficacy of Epidiolex® in patients with treatment resistant epilepsy, Epidiolex® improved the severity and frequency of seizures, and this was sustained for up to 48 weeks of treatment [219]. Devinsky and colleagues (2018) also demonstrated that 10 mg/kg and 20 mg/kg doses of Epidiolex® (administered as two doses per day over a 14 week period) ameliorated drop seizures in patients with Lennox-Gastaut syndrome, when compared to placebo control cases [25].

In a second cannabis-based therapeutic, CBD is combined with THC as an oromucosal spray (Sativex®), developed to manage spasticity in pwMS [220]. Sativex® can be used as an add-on therapy to ameliorate MS symptoms, with each 100μl Sativex® actuation delivering 2.5 mg CBD and 2.7 mg THC, in addition to other constituents [221, 222]. In terms of effectiveness in ameliorating spasticity, data indicate that the majority of pwMS that administer Sativex® experience an improvement in spasticity score within a 4 week period [223].

Finally, both Marinol® (a pharmaceutical formulation of synthetic THC) and Cesamet® (a synthetic analogue of THC), are FDA approved cannabinoid therapeutics that can be administered orally in tablet form for the management of chemotherapy-induced nausea/emesis in patients who are refractory to other antiemetics [224, 225]. Marinol® is also indicated for anorexia associated with loss of appetite in AIDS patients [225], and data suggests that Cesamet® has efficacy as an analgesic in chronic non-cancer pain [226].
It should be noted that given that adverse effects may be associated with cannabinoids, certain cannabis-based therapeutics are contraindicated in patients with a history of psychotic illness, schizophrenia, substance abuse and also in pregnancy and patients who are breast feeding. Certain cannabis-based therapeutics are also not recommended for use in certain age groups.

Conclusions
It is clear that cannabinoid use in the clinical setting has a wide variety of uses in terms of ameliorating symptoms of neurological disease, such as seizure disorders and MS, and further cannabis-based therapeutics are in the pipeline for a range of disorders. Epidiolex® a purified solution of CBD, is in the clinic for the management of epileptic seizures. Given the recent advancements in the clinical development of CBD, this review has focussed on the signalling targets for this phytocannabinoid, and in doing so, aimed to outline both the complex role played by ROS in disease processes, in addition to the key role of ROS in regulating biological functions. It is clear that ROS are integral players in neuroinflammation, peripheral immune responses and physiological processes, and herein we have summarised evidence that CBD readily targets oxidative signalling and ROS production. Further research is required to fully understand the interplay of this phytocannabinoid with oxidative signalling. The balance between the ability of CBD to regulate both the generation and elimination of ROS may govern its effectiveness in impacting the symptoms of disease.

Completing interests
The authors declare no conflict of interest.

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Abbreviations
2-AG, 2-arachidonoyl-glycerol; 5-HT1A, 5-hydroxytryptamine receptor subtype 1A; AEA, anandamide; AIDS, acquired immunodeficiency syndrome; ARE, antioxidant response element; CAT, catalase; CBC, cannabichromene; CBCA, cannabichromenic acid; CBD, cannabidiol; CBDA, cannabidiolic acid; CBG, cannabigerol; CBGA, cannabigerolic acid; CBN, cannabionol; CGD, chronic granulomatous disease; COX-2, cyclooxygenase-2; eCB, endocannabinoid; ECS, eCB system; EMA, European Medicines Agency; eNOS, endothelial NOS; FDA, US Food and Drug administration; GPCR, G protein-coupled receptor; GPR55, G protein-coupled receptor 55; GPx, glutathione peroxidase; GSH, glutathione; GSSG, glutathione disulfide; HO-1, heme oxygenase-1; IBD, inflammatory bowel disease; IFN, interferon; IL, interleukin; iNOS, inducible NOS; Keap1, kelch-like ECH-associated protein1; LO, lipoygenase; LPS, lipopolysaccharide; MS, multiple sclerosis; NAC, N-Acetyl Cysteine; NF-κB, nuclear factor-κB; NMDA, N-methyl-D-aspartate; nNOS, neuronal NOS; NO, nitric oxide; NOS, nitric oxide synthase; NOX2, NADPH oxidase 2; Nrf2, nuclear factor erythroid 2-related factor-2; OEA, oleoylethanolamide; PEA, palmitoylethanolamide; PGE2, prostaglandin E2; PPAR, peroxisome proliferator-activated receptor; pwMS, people with MS; RA, rheumatoid arthritis; ROS, reactive oxygen species; SOD, superoxide dismutase; THC, tetrahydrocannabinol; THCA, tetrahydrocannabinolic acid; TLR, toll-like receptor; TNF-α, tumour necrosis factor-α; TRPV1, transient receptor potential cation channel subfamily V member 1; XO, xanthine oxidase.

REFERENCES
1. Firuzi, O., et al., Antioxidant therapy: current status and future prospects. Curr Med Chem, 2011. 18(25): p. 3871-88.
2. Drevet, S., et al., Reactive oxygen species and NADPH oxidase 4 involvement in osteoarthritis. Exp Gerontol, 2018. 111: p. 107-117.
3. Negre-Salayre, A., et al., Role of reactive oxygen species in atherosclerosis: Lessons from murine genetic models. Free Radic Biol Med, 2020. 149: p. 8-22.
4. TavassoliFar, M.J., et al., The Influence of Reactive Oxygen Species in the Immune System and Pathogenesis of Multiple Sclerosis. Autoimmune Dis, 2020. 2020: p. 5793817.
5. Patlevic, P., et al., Reactive oxygen species and antioxidant defense in human gastrointestinal diseases. Integr Med Res, 2016. 5(4): p. 250-258.
6. Kim, S.Y., et al., Pro-inflammatory hepatic macrophages generate ROS through NADPH oxidase 2 via endocytosis of monomeric TLR4-MD2 complex. Nat Commun, 2017. 8(1): p. 2247.
7. O'Neill, S., et al., Genetic disorders coupled to ROS deficiency. Redox Biol, 2015. 6: p. 135-156.
8. Paiva, C.N. and M.T. Bozza, Are reactive oxygen species always detrimental to pathogens? Antioxid Redox Signal, 2014. 20(6): p. 1000-37.
9. Dogan, S.A., et al., Perturbed Redox Signaling Exacerbates a Mitochondrial Myopathy. Cell Metab, 2018. 28(5): p. 764-775 e5.
10. Mossberg, N., et al., Oxygen radical production in leukocytes and disease severity in multiple sclerosis. J Neuroimmunol, 2009. 213(1-2): p. 131-4.
11. Mossberg, N., et al., Oxygen radical production and severity of the Guillain--Barre syndrome. J Neuroimmunol, 2007. 192(1-2): p. 186-91.
12. Downer, E.J., Cannabinoids and innate immunity: taking a toll on neuroinflammation. ScientificWorldJournal, 2011. 11: p. 855-65.
13. Esposito, G., et al., Cannabidiol reduces Abeta-induced neuroinflammation and promotes hippocampal neurogenesis through PPARgamma involvement. PLoS One, 2011. 6(12): p. e28668.
14. Matsuda, L.A., et al., Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature, 1990. 346(6284): p. 561-4.
15. Munro, S., K.L. Thomas, and M. Abu-Shaar, Molecular characterization of a peripheral receptor for cannabinoids. Nature, 1993. 365(6441): p. 61-5.
16. Ryberg, E., et al., The orphan receptor GPR55 is a novel cannabinoid receptor. Br J Pharmacol, 2007. 152(7): p. 1092-101.
17. Pertwee, R.G., GPR55: a new member of the cannabinoid receptor clan? Br J Pharmacol, 2007. 152(7): p. 984-6.
18. De Petrocellis, L., et al., Effects of cannabinoids and cannabinoid-enriched Cannabis extracts on TRP channels and endocannabinoid metabolic enzymes. Br J Pharmacol, 2011. 163(7): p. 1479-94.
19. Hind, W.H., T.J. England, and S.E. O'Sullivan, Cannabidiol protects an in vitro model of the blood-brain barrier from oxygen-glucose deprivation via PPARgamma and 5-HT1A receptors. Br J Pharmacol, 2016. 173(5): p. 815-25.
20. Russo, E.B., et al., Agonistic properties of cannabidiol at 5-HT1a receptors. Neurochem Res, 2005. 30(8): p. 1037-43.
21. Pellati, F., et al., Cannabis sativa L. and Nonpsychoactive Cannabinoids: Their Chemistry and Role against Oxidative Stress, Inflammation, and Cancer. Biomed Res Int, 2018. 2018: p. 1691428.
22. Massi, P., et al., 5-Lipoxygenase and anandamide hydrolase (FAAH) mediate the antitumor activity of cannabidiol, a non-psychoactive cannabinoid. J Neurochem, 2008. 104(4): p. 1091-100.
23. Schrot, R.J. and J.R. Hubbard, Cannabinoids: Medical implications. Ann Med, 2016. 48(3): p. 128-41.
24. Ali, S., I.E. Scheffer, and L.G. Sadleir, Efficacy of cannabinoids in paediatric epilepsy. Dev Med Child Neurol, 2019. 61(1): p. 13-18.
25. Devinsky, O., et al., Effect of Cannabidiol on Drop Seizures in the Lennox-Gastaut Syndrome. N Engl J Med, 2018. 378(20): p. 1888-1897.
26. Devinsky, O., et al., Trial of Cannabidiol for Drug-Resistant Seizures in the Dravet Syndrome. N Engl J Med, 2017. 376(21): p. 2011-2020.
27. Sena, L.A. and N.S. Chandel, Physiological roles of mitochondrial reactive oxygen species. Mol Cell, 2012. 48(2): p. 158-67.
28. Zhou, Z., et al., Dancing with reactive oxygen species generation and elimination in nanotheranostics for disease treatment. Adv Drug Deliv Rev, 2020. 158: p. 73-90.
29. Zorov, D.B., M. Juhaszova, and S.J. Sollott, Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release. Physiol Rev, 2014. 94(3): p. 909-50.
30. An, X., et al., Increasing the TRPM2 Channel Expression in Human Neuroblastoma SH-SY5Y Cells Augments the Susceptibility to ROS-Induced Cell Death. Cells, 2019. 8(1).
31. Hong, S.W., et al., p34 (SEI-1) inhibits ROS-induced cell death through suppression of ASK1. Cancer Biol Ther, 2011. 12(5): p. 421-6.
32. Schumacker, P.T., Reactive oxygen species in cancer: a dance with the devil. Cancer Cell, 2015. 27(2): p. 156-7.
33. Fan, X., et al., Daphnetin triggers ROS-induced cell death and induces cytoprotective autophagy by modulating the AMPK/Akt/mTOR pathway in ovarian cancer. Phytomedicine, 2021. 82: p. 153465.
34. Liang, H.H., et al., Heat shock protein 27 influences the anti-cancer effect of curcumin in colon cancer cells through ROS production and autophagy activation. Life Sci, 2018. 209: p. 43-51.
35. Zou, P., et al., ROS generation mediates the anti-cancer effects of WZ35 via activating JNK and ER stress apoptotic pathways in gastric cancer. Oncotarget, 2015. 6(8): p. 5860-76.
36. Sareila, O., et al., NOX2 complex-derived ROS as immune regulators. Antioxid Redox Signal, 2011. 15(8): p. 2197-208.
37. Brandes, R.P., N. Weissmann, and K. Schroder, Nox family NADPH oxidases: Molecular mechanisms of activation. Free Radic Biol Med, 2014. 76: p. 208-26.
38. Quinlan, C.L., et al., Native rates of superoxide production from multiple sites in isolated mitochondria measured using endogenous reporters. Free Radic Biol Med, 2012. 53(9): p. 1807-17.
39. Vergeade, A., et al., Xanthine oxidase contributes to mitochondrial ROS generation in an experimental model of cocaine-induced diastolic dysfunction. J Cardiovasc Pharmacol, 2012. 60(6): p. 538-43.
40. Espinosa-Diez, C., et al., Antioxidant responses and cellular adjustments to oxidative stress. Redox Biol, 2015. 6: p. 183-197.
41. Murphy, M.P., How mitochondria produce reactive oxygen species. Biochem J, 2009. 417(1): p. 1-13.
42. Sharpe, M.A., S.J. Robb, and J.B. Clark, Nitric oxide and Fenton/Haber-Weiss chemistry: nitric oxide is a potent antioxidant at physiological concentrations. J Neurochem, 2003. 87(2): p. 386-94.
43. Radi, R., Oxygen radicals, nitric oxide, and peroxynitrite: Redox pathways in molecular medicine. Proc Natl Acad Sci U S A, 2018. 115(23): p. 5839-5848.
44. Quinn, M.T., M.C. Ammons, and F.R. Deleo, The expanding role of NADPH oxidases in health and disease: no longer just agents of death and destruction. Clin Sci (Lond), 2006. 111(1): p. 1-20.
45. Bedard, K. and K.H. Krause, The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. Physiol Rev, 2007. 87(1): p. 245-313.
46. Paardekooper, L.M., et al., Human Monocyte-Derived Dendritic Cells Produce Millimolar Concentrations of ROS in Phagosomes Per Second. Front Immunol, 2019. 10: p. 1216.
47. Richards, S.M. and E.A. Clark, BCR-induced superoxide negatively regulates B-cell proliferation and T-cell-independent type 2 Ab responses. Eur J Immunol, 2009. 39(12): p. 3395-403.
48. Belikov, A.V., B. Schraven, and L. Simeoni, T cells and reactive oxygen species. J Biomed Sci, 2015. 22: p. 85.
49. Krijnen, P.A., et al., Increased Nox2 expression in human cardiomyocytes after acute myocardial infarction. J Clin Pathol, 2003. 56(3): p. 194-9.
50. Petry, A., et al., NOX2 and NOX4 mediate proliferative response in endothelial cells. Antioxid Redox Signal, 2006. 8(9-10): p. 1473-84.
51. Henriquez-Olguin, C., et al., Cytosolic ROS production by NADPH oxidase 2 regulates muscle glucose uptake during exercise. Nat Commun, 2019. 10(1): p. 4623.
52. Grauwers Wiktoria, H., et al., NOX2-Derived Reactive Oxygen Species in Cancer. Oxid Med Cell Longev, 2020. 2020. p. 7095902.
53. Panday, A., et al., NADPH oxidases: an overview from structure to innate immunity-associated pathologies. Cell Mol Immunol, 2015. 12(1): p. 5-23.
54. Rastogi, R., et al., NOX Activation by Subunit Interaction and Underlying Mechanisms in Disease. Front Cell Neurosci, 2016. 10: p. 301.
55. Judkins, C.P., et al., Direct evidence of a role for Nox2 in superoxide production, reduced nitric oxide bioavailability, and early atherosclerotic plaque formation in ApoE−/− mice. Am J Physiol Heart Circ Physiol, 2010. 298(1): p. H24-32.
56. Giardino, G., et al., NADPH Oxidase Deficiency: A Multisystem Approach. Oxid Med Cell Longev, 2017. 2017: p. 4590127.
57. Hunyadi, A., The mechanism(s) of action of antioxidants: From scavenging reactive oxygen/nitrogen species to redox signaling and the generation of bioactive secondary metabolites. Med Res Rev, 2019. 39(6): p. 2505-2533.
58. Beyrath, J., et al., KH176 Safeguards Mitochondrial Diseased Cells from Redox Stress-Induced Cell Death by Interacting with the Thioredoxin System/Peroxiredoxin Enzyme Machinery. Sci Rep, 2018. 8(1): p. 6577.
59. Lubos, E., J. Loscalzo, and D.E. Handy, Glutathione peroxidase-1 in health and disease: from molecular mechanisms to therapeutic opportunities. Antioxid Redox Signal, 2011. 15(7): p. 1957-97.
60. Lian, G., et al., Glutathione de novo synthesis but not recycling process coordinates with glutamine catabolism to control redox homeostasis and directs murine T cell differentiation. Elife, 2018. 7.
61. Niki, E., Role of vitamin E as a lipid-soluble peroxyl radical scavenger: in vitro and in vivo evidence. Free Radic Biol Med, 2014. 66: p. 3-12.
62. Carcamo, J.M., et al., Vitamin C is a kinase inhibitor: dehydroascorbic acid inhibits IkappaBalpha kinase beta. Mol Cell Biol, 2004. 24(15): p. 6645-52.
63. Vomund, S., et al., Nrf2, the Master Regulator of Anti-Oxidative Responses. Int J Mol Sci, 2017. 18(12).
64. He, X. and Q. Ma, NRF2 cysteine residues are critical for oxidant/electrophile-sensing, Kelch-like ECH-associated protein-1-dependent ubiquitination-proteasomal degradation, and transcription activation. Mol Pharmacol, 2009. 76(6): p. 1265-78.
65. Dong, J., K.K. Sulik, and S.Y. Chen, Nrf2-mediated transcriptional induction of antioxidant response in mouse embryos exposed to ethanol in vivo: implications for the prevention of fetal alcohol spectrum disorders. Antioxid Redox Signal, 2008. 10(12): p. 2023-33.
66. Furue, M., et al., Antioxidants for Healthy Skin: The Emerging Role of Aryl Hydrocarbon Receptors and Nuclear Factor-Erythroid 2-Related Factor-2. Nutrients, 2017. 9(3).
67. Hashimoto-Hachiya, A., G. Tsuji, and M. Furue, Antioxidants cinnamaldehyde and Galactomyces fermentation filtrate downregulate senescence marker CDKN2A/p16INK4A via NRF2 activation in keratinocytes. J Dermatol Sci, 2019. 96(1): p. 53-56.
68. Cheung, K.L., et al., Nrf2 knockout enhances intestinal tumorigenesis in Apc(min+)/ mouse due to attenuation of anti-oxidative stress pathway while potentiates inflammation. Mol Carcinog, 2014. 53(1): p. 77-84.
69. Gazzellino, R., V. Jeney, and M.P. Soares, Mechanisms of cell protection by heme oxygenase-1. Annu Rev Pharmacol Toxicol, 2010. 50: p. 323-54.
70. Keyse, S.M. and R.M. Tyrrell, Heme oxygenase is the major 32-kDa stress protein induced in human skin fibroblasts by UVA radiation, hydrogen peroxide, and sodium arsenite. Proc Natl Acad Sci U S A, 1989. 86(1): p. 99-103.
71. Seiwert, N., et al., Heme oxygenase 1 protects human colonocytes against ROS formation, oxidative DNA damage and cytotoxicity induced by heme iron, but not inorganic iron. Cell Death Dis, 2020. 11(9): p. 787.

72. Zhang, F.H., et al., Protective effects of heme oxygenase-1 against severe acute pancreatitis via inhibition of tumor necrosis factor-alpha and augmentation of interleukin-10. BMC Gastroenterol, 2017. 17(1): p. 100.

73. Campbell, N.K., H.K. Fitzgerald, and A. Dunne, Regulation of inflammation by the antioxidant haem oxygenase 1. Nat Rev Immunol, 2021.

74. Egea, J., et al., European contribution to the study of ROS: A summary of the findings and prospects for the future from the COST action BM1203 (EU-ROS). Redox Biol, 2017. 13: p. 94-162.

75. Ohlow, M.J., et al., Why Have Clinical Trials of Antioxidants to Prevent Neurodegeneration Failed? - A Cellular Investigation of Novel Phenothiazine-Type Antioxidants Reveals Competing Objectives for Pharmaceutical Neuroprotection. Pharm Res, 2017. 34(2): p. 378-393.

76. Thal, L.J., et al., Idebenone treatment fails to slow cognitive decline in Alzheimer's disease. Neurology, 2003. 61(11): p. 1498-502.

77. Sakellariou, G.K., et al., Long-term administration of the mitochondria-targeted antioxidant mitoquinone mesylate fails to attenuate age-related oxidative damage or rescue the loss of muscle mass and function associated with aging of skeletal muscle. FASEB J, 2016. 30(11): p. 3771-3785.

78. Fiebig, S.M., et al., The antioxidant idebenone fails to prevent or attenuate chronic experimental autoimmune encephalomyelitis in the mouse. J Neuroimmunol, 2013. 262(1-2): p. 66-71.

79. Pemp, B., K. Kircher, and A. Reitner, Visual function in chronic Leber's hereditary optic neuropathy during idebenone treatment initiated 5 to 50 years after onset. Graefes Arch Clin Exp Ophthalmol, 2019. 257(12): p. 2751-2757.

80. Carvalho, A.M.S., et al., Phytol, a Chlorophyll Component, Produces Antihyperalgesic, Anti-inflammatory, and Antiarthritic Effects: Possible NFkappaB Pathway Involvement and Reduced Levels of the Proinflammatory Cytokines TNF-alpha and IL-6. J Nat Prod, 2020. 83(4): p. 1107-1117.

81. Joshi, G.N., A.M. Goetjen, and D.A. Knecht, Silica particles cause NADPH oxidase-independent ROS generation and transient phagolysosomal leakage. Mol Biol Cell, 2015. 26(18): p. 3150-64.

82. Martinon, F., Signaling by ROS drives inflammasome activation. Eur J Immunol, 2010. 40(3): p. 616-9.

83. Minutoli, L., et al., ROS-Mediated NLRP3 Inflammasome Activation in Brain, Heart, Kidney, and Testis Ischemia/Reperfusion Injury. Oxid Med Cell Longev, 2016. 2016: p. 2183026.

84. Rathinam, V.A. and K.A. Fitzgerald, Inflammasome Complexes: Emerging Mechanisms and Effector Functions. Cell, 2016. 165(4): p. 792-800.

85. He, Y., H. Hara, and G. Nunez, Mechanism and Regulation of NLRP3 Inflammasome Activation. Trends Biochem Sci, 2016. 41(12): p. 1012-1021.

86. Esplugues, J.V., NO as a signalling molecule in the nervous system. Br J Pharmacol, 2002. 135(5): p. 1079-95.

87. Nakazawa, H., et al., iNOS as a Driver of Inflammation and Apoptosis in Mouse Skeletal Muscle after Burn Injury: Possible Involvement of Sirt1 S-Nitrosylation-Mediated Acetylation of p65 NF-kappaB and p53. PLoS One, 2017. 12(1): p. e0170391.

88. Bienert, G.P., J.K. Schjoerring, and T.P. Jahn, Membrane transport of hydrogen peroxide. Biochim Biophys Acta, 2006. 1758(8): p. 994-1003.

89. De Grey, A.D., HO2*: the forgotten radical. DNA Cell Biol, 2002. 21(4): p. 251-7.

90. Bogaert, Y.E., R.E. Rosenthal, and G. Fiskum, Postischemic inhibition of cerebral cortex pyruvate dehydrogenase. Free Radic Biol Med, 1994. 16(6): p. 811-20.

91. Grisham, M.B., et al., Interaction between oxygen radicals and gastric mucin. Am J Physiol, 1987. 253(1 Pt 1): p. G93-6.
92. Xu, D., et al., Mechanism of unprecedented hydroxyl radical production and site-specific oxidative DNA damage by photoactivation of the classic arylhydroxamic acid carcinogens. Carcinogenesis, 2019.
93. Aikens, J. and T.A. Dix, Perhydroxyl radical (HOO.) initiated lipid peroxidation. The role of fatty acid hydroperoxides. J Biol Chem, 1991. 266(23): p. 15091-8.
94. Landi, L., et al., Injury of rat thymocytes caused by exogenous peroxyl radicals in vitro. Biochim Biophys Acta, 1995. 1239(2): p. 207-12.
95. Chistine, R.C., et al., Carotenoids are effective inhibitors of in vitro hemolysis of human erythrocytes, as determined by a practical and optimized cellular antioxidant assay. J Food Sci, 2014. 79(9): p. H1841-7.
96. Pereira, C., et al., Oxidative Stress and DNA Damage: Implications in Inflammatory Bowel Disease. Inflamm Bowel Dis, 2015. 21(10): p. 2403-17.
97. Zias, J., et al., Early medical use of cannabis. Nature, 1993. 363(6426): p. 215.
98. Radhakrishnan, R., S.T. Wilkinson, and D.C. D'Souza, Gone to Pot - A Review of the Association between Cannabis and Psychosis. Front Psychiatry, 2014. 5: p. 54.
99. Fitzpatrick, J.K. and E.J. Downer, Toll-like receptor signalling as a cannabinoid target in Multiple Sclerosis. Neuropharmacology, 2017. 113(Pt B): p. 618-626.
100. Pereira, S.R., et al., Recent advances in the understanding of the aetiology and therapeutic strategies in burning mouth syndrome: Focus on the actions of cannabinoids. Eur J Neurosci, 2020.
101. Bonini, S.A., et al., Cannabis sativa: A comprehensive ethnopharmacological review of a medicinal plant with a long history. J Ethnopharmacol, 2018. 227: p. 300-315.
102. Joshi, N. and E.S. Onaivi, Endocannabinoid System Components: Overview and Tissue Distribution. Adv Exp Med Biol, 2019. 1162: p. 1-12.
103. Di Marzo, V., The endocannabinoid system: its general strategy of action, tools for its pharmacological manipulation and potential therapeutic exploitation. Pharmacol Res, 2009. 60(2): p. 77-84.
104. Shahbazi, F., et al., Cannabinoids and Cannabinoid Receptors: The Story so Far. iScience, 2020. 23(7): p. 101301.
105. Sirikantaramas, S., et al., Tetrahydrocannabinolic acid synthase, the enzyme controlling marijuana psychoactivity, is secreted into the storage cavity of the glandular trichomes. Plant Cell Physiol, 2005. 46(9): p. 1578-82.
106. Downer, E.J., Anti-inflammatory Potential of Terpenes Present in Cannabis sativa L. ACS Chem Neurosci, 2020. 11(5): p. 659-662.
107. Russo, E.B., Taming THC: potential cannabis synergy and phytocannabinoid-terpenoid entourage effects. Br J Pharmacol, 2011. 163(7): p. 1344-64.
108. de Boer, J.G., M.A. Posthumus, and M. Dicke, Identification of volatiles that are used in discrimination between plants infested with prey or nonprey herbivores by a predatory mite. J Chem Ecol, 2004. 30(11): p. 2215-30.
109. Hoppanya, N., et al., Analysis of cannabinoids in laser-microdissected trichomes of medicinal Cannabis sativa using LCMS and cryogenic NMR. Phytochemistry, 2013. 87: p. 51-9.
110. Taura, F., et al., Phytocannabinoids in Cannabis sativa: recent studies on biosynthetic enzymes. Chem Biodivers, 2007. 4(8): p. 1649-63.
111. Chandra, S., et al., Cannabis cultivation: Methodological issues for obtaining medical-grade product. Epilepsy Behav, 2017. 70(Pt B): p. 302-312.
112. Gallegue, S., et al., Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. Eur J Biochem, 1995. 232(1): p. 54-61.
113. Busquets-Garcia, A., J. Bains, and G. Marsicano, CB(1) Receptor Signaling in the Brain: Extracting Specificity from Ubiquity. Neuropsychopharmacology, 2018. 43(1): p. 4-20.
114. Van Sickle, M.D., et al., Identification and functional characterization of brainstem cannabinoid CB2 receptors. Science, 2005. 310(5746): p. 329-32.
115. Stempel, A.V., et al., Cannabinoid Type 2 Receptors Mediate a Cell Type-Specific Plasticity in the Hippocampus. Neuron, 2016. 90(4): p. 795-809.
116. Ashton, J.C., et al., Cerebral hypoxia-ischemia and middle cerebral artery occlusion induce expression of the cannabinoid CB2 receptor in the brain. Neurosci Lett, 2007. 412(2): p. 114-7.

117. Pertwee, R.G., The diverse CB1 and CB2 receptor pharmacology of three plant cannabinoids: delta9-tetrahydrocannabinol, cannabidiol and delta9-tetrahydrocannabivarin. Br J Pharmacol, 2008. 153(2): p. 199-215.

118. Ibeas Bih, C., et al., Molecular Targets of Cannabidiol in Neurological Disorders. Neurotherapeutics, 2015. 12(4): p. 699-730.

119. Devane, W.A., et al., Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science, 1992. 258(5090): p. 1946-9.

120. Mechoulam, R., et al., Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. Biochem Pharmacol, 1995. 50(1): p. 83-90.

121. Sugiyama, T., et al., 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. Biochem Biophys Res Commun, 1995. 215(1): p. 89-97.

122. Bedse, G., M.N. Hill, and S. Patel, 2-Arachidonoylglycerol Modulation of Anxiety and Stress Adaptation: From Grass Roots to Novel Therapeutics. Biol Psychiatry, 2020. 88(7): p. 520-530.

123. Alger, B.E., Endocannabinoids at the synapse a decade after the dies mirabilis (29 March 2001): what we still do not know. J Physiol, 2012. 590(10): p. 2203-12.

124. Kano, M., et al., Endocannabinoid-mediated control of synaptic transmission. Physiol Rev, 2009. 89(1): p. 309-80.

125. Castillo, P.E., et al., Endocannabinoid signaling and synaptic function. Neuron, 2012. 76(1): p. 70-81.

126. Katona, I. and T.F. Freund, Endocannabinoid signaling as a synaptic circuit breaker in neurological disease. Nat Med, 2008. 14(9): p. 923-30.

127. Stella, N., P. Schweitzer, and D. Piomelli, A second endogenous cannabinoid that modulates long-term potentiation. Nature, 1997. 388(6644): p. 773-8.

128. Karwad, M.A., et al., Oleylethanolamine and palmitolethanolamine modulate intestinal permeability in vitro via TRPV1 and PPARalpha. FASEB J, 2017. 31(2): p. 469-481.

129. Okine, B.N., et al., N-palmitolethanolamide in the anterior cingulate cortex attenuates inflammatory pain behaviour indirectly via a CB1 receptor-mediated mechanism. Pain, 2016. 157(12): p. 2687-2696.

130. Vaia, M., et al., Palmitolethanolamide reduces inflammation and itch in a mouse model of contact allergic dermatitis. Eur J Pharmacol, 2016. 791: p. 669-674.

131. Kaplan, J.S., et al., Cannabidiol attenuates seizures and social deficits in a mouse model of Dravet syndrome. Proc Natl Acad Sci U S A, 2017. 114(42): p. 11229-11234.

132. Cruz, S.L., et al., Anandamide inhibits FcepsilonRI-dependent degranulation and cytokine synthesis in mast cells through CB2 and GPR55 receptor activation. Possible involvement of CB2-GPR55 heteromers. Int Immunopharmacol, 2018. 64: p. 298-307.

133. Kollberg, M.R., et al., THC Reduces Ki67-Immunoreactive Cells Derived from Human Primary Glioblastoma in a GPR55-Dependent Manner. Cancers (Basel), 2021. 13(5).

134. Sawdzardo, M., et al., Identification and cloning of three novel human G protein-coupled receptor genes GPR52, PsiGPR53 and GPR55: GPR55 is extensively expressed in human brain. Brain Res Mol Brain Res, 1999. 64(2): p. 193-8.

135. Kramar, C., et al., Palmitolethanolamide Modulates GPR55 Receptor Signaling in the Ventral Hippocampus to Regulate Mesolimbic Dopamine Activity, Social Interaction, and Memory Processing. Cannabis Cannabinoid Res, 2017. 2(1): p. 8-20.

136. Petroso, S., et al., The anti-inflammatory mediator palmitolethanolamide enhances the levels of 2-arachidonoyl-glycerol and potentiates its actions at TRPV1 cation channels. Br J Pharmacol, 2016. 173(7): p. 1154-62.

137. Fonseca, B.M., G. Correira-da-Silva, and N.A. Teixeira, Cannabinoid-induced cell death in endometrial cancer cells: involvement of TRPV1 receptors in apoptosis. J Physiol Biochem, 2018. 74(2): p. 261-272.

138. Starkus, J., et al., Diverse TRPV1 responses to cannabinoids. Channels (Austin), 2019. 13(1): p. 172-191.
139. Ramer, R., et al., COX-2 and PPAR-gamma confer cannabidiol-induced apoptosis of human lung cancer cells. Mol Cancer Ther, 2013. 12(1): p. 69-82.
140. Scuderi, C., L. Steardo, and G. Esposito, Cannabidiol promotes amyloid precursor protein ubiquitination and reduction of beta amyloid expression in SHSY5YAPP+ cells through PPARgamma involvement. Phytother Res, 2014. 28(7): p. 1007-13.
141. Lo Verme, J., et al., The nuclear receptor peroxisome proliferator-activated receptor-alpha mediates the anti-inflammatory actions of palmitoylethanolamide. Mol Pharmacol, 2005. 67(1): p. 15-9.
142. Fu, J., et al., Oleylethanolamide regulates feeding and body weight through activation of the nuclear receptor PPAR-alpha. Nature, 2003. 425(6953): p. 90-3.
143. LoVerme, J., et al., The search for the palmitoylethanolamide receptor. Life Sci, 2005. 77(14): p. 1685-98.
144. Alharris, E., et al., Role of miRNA in the regulation of cannabidiol-mediated apoptosis in neuroblastoma cells. Oncotarget, 2019. 10(1): p. 45-59.
145. Ledgerwood, C.J., et al., Cannabidiol inhibits synaptic transmission in rat hippocampal cultures and slices via multiple receptor pathways. Br J Pharmacol, 2011. 162(1): p. 286-94.
146. Kathmann, M., et al., Cannabidiol is an allosteric modulator at mu- and delta- opioid receptors. Naunyn Schmiedebergs Arch Pharmacol, 2006. 372(5): p. 354-61.
147. Chung, E.Y., et al., Pharmacology and adverse effects of new psychoactive substances: synthetic cannabinoid receptor agonists. Arch Pharm Res, 2021.
148. Showalter, V.M., et al., Evaluation of binding in a transfected cell line expressing a peripheral cannabinoid receptor (CB2): identification of cannabinoid receptor subtype selective ligands. J Pharmacol Exp Ther, 1996. 278(3): p. 989-99.
149. Felder, C.C., et al., Comparison of the pharmacology and signal transduction of the human cannabinoid CB1 and CB2 receptors. Mol Pharmacol, 1995. 48(3): p. 443-50.
150. Meng, I.D., et al., An analgesia circuit activated by cannabinoids. Nature, 1998. 395(6700): p. 381-3.
151. Ulugol, A., et al., The effect of WIN 55,212-2, a cannabinoid agonist, on tactile allodynia in diabetic rats. Neurosci Lett, 2004. 371(2-3): p. 167-70.
152. Porcella, A., et al., The synthetic cannabinoid WIN55212-2 decreases the intraocular pressure in human glaucoma resistant to conventional therapies. Eur J Neurosci, 2001. 13(2): p. 409-12.
153. Slipetz, D.M., et al., Activation of the human peripheral cannabinoid receptor results in inhibition of adenylyl cyclase. Mol Pharmacol, 1995. 48(2): p. 352-61.
154. Howlett, A.C., et al., Stereochemical effects of 11-OH-delta 8-tetrahydrocannabinol-dimethylheptyl to inhibit adenylyl cyclase and bind to the cannabinoid receptor. Neuropharmacology, 1990. 29(2): p. 161-5.
155. Lin, S., et al., The Anti-Inflammatory Effect and Intestinal Barrier Protection of HU210 Differentially Depend on TLR4 Signaling in Dextran Sulfate Sodium-Induced Murine Colitis. Dig Dis Sci, 2017. 62(2): p. 372-386.
156. Lax, P., et al., Neuroprotective effects of the cannabinoid agonist HU210 on retinal degeneration. Exp Eye Res, 2014. 120: p. 175-85.
157. Wiley, J.L., et al., Discriminative stimulus effects of CP 55,940 and structurally dissimilar cannabinoids in rats. Neuropharmacology, 1995. 34(6): p. 669-76.
158. Griffin, G., et al., Evaluation of cannabinoid receptor agonists and antagonists using the guanosine-5'-O-(3-[35S]thio)-triphosphate binding assay in rat cerebellar membranes. J Pharmacol Exp Ther, 1998. 285(2): p. 553-60.
159. Hamamoto, D.T., S. Giridharagopalan, and D.A. Simone, Acute and chronic administration of the cannabinoid receptor agonist CP 55,940 attenuates tumor-evoked hyperalgesia. Eur J Pharmacol, 2007. 558(1-3): p. 73-87.
160. Kraft, B., W. Wintersberger, and H.G. Kress, Cannabinoid receptor-independent suppression of the superoxide generation of human neutrophils (PMN) by CP55 940, but not by anandamide. Life Sci, 2004. 75(8): p. 969-77.
161. Correa, F., et al., Activation of cannabinoid CB2 receptor negatively regulates IL-12p40 production in murine macrophages: role of IL-10 and ERK1/2 kinase signaling. Br J Pharmacol, 2005. 145(4): p. 441-8.

162. Baker, D., et al., Cannabinoids control spasticity and tremor in a multiple sclerosis model. Nature, 2000. 404(6773): p. 84-7.

163. Ramirez, B.G., et al., Prevention of Alzheimer's disease pathology by cannabinoids: neuroprotection mediated by blockade of microglial activation. J Neurosci, 2005. 25(8): p. 1904-13.

164. Hillard, C.J., et al., Synthesis and characterization of potent and selective agonists of the neuronal cannabinoid receptor (CB1). J Pharmacol Exp Ther, 1999. 289(3): p. 1427-33.

165. Yang, S., et al., Cannabinoid CB1 receptor agonist ACEA alleviates brain ischemia/reperfusion injury via CB1-Drp1 pathway. Cell Death Discov, 2020. 6: p. 102.

166. Chiu, P., et al., The influence of delta9-tetrahydrocannabinol, cannabidiol and cannabidiol on tissue oxygen consumption. Res Commun Chem Pathol Pharmacol, 1975. 12(2): p. 267-86.

167. Costa, B., et al., The non-psychoactive cannabis constituent cannabidiol is an orally effective therapeutic agent in rat chronic inflammatory and neuropathic pain. Eur J Pharmacol, 2007. 556(1-3): p. 75-83.

168. Mabou Tagne, A., et al., A Novel Standardized Cannabis sativa L. Extract and Its Constituent Cannabidiol Inhibit Human Polymorphonuclear Leukocyte Functions. Int J Mol Sci, 2019. 20(8).

169. Chen, J., et al., Protective effect of cannabidiol on hydrogen peroxide-induced apoptosis, inflammation and oxidative stress in nucleus pulposus cells. Mol Med Rep, 2016. 14(3): p. 2321-7.

170. Baer, M., et al., Cannabinoids as anti-ROS in aged pancreatic islet cells. Life Sci, 2020. 256: p. 117969.

171. Barichello, T., et al., Cannabidiol reduces host immune response and prevents cognitive impairments in Wistar rats submitted to pneumococcal meningitis. Eur J Pharmacol, 2012. 697(1-3): p. 158-64.

172. Frankola, K.A., et al., Targeting TNF-alpha to elucidate and ameliorate neuroinflammation in neurodegenerative diseases. CNS Neurol Disord Drug Targets, 2011. 10(3): p. 391-403.

173. McCoy, M.K., et al., TNF: a key neuroinflammatory mediator of neurotoxicity and neurodegeneration in models of Parkinson's disease. Adv Exp Med Biol, 2011. 691: p. 539-40.

174. Guarnieri, G., et al., Tumor Necrosis Factor alpha Influences Phenotypic Plasticity and Promotes Epigenetic Changes in Human Basal Forebrain Cholinergic Neuroblasts. Int J Mol Sci, 2020. 21(17).

175. Nichols, J.M. and B.L.F. Kaplan, Immune Responses Regulated by Cannabidiol. Cannabis Cannabinoid Res, 2020. 5(1): p. 12-31.

176. Branca, J.J.V., et al., Cannabidiol Protects Dopaminergic Neuronal Cells from Cadmium. Int J Environ Res Public Health, 2019. 16(22).

177. Hampson, A.J., et al., Cannabidiol and (-)Delta9-tetrahydrocannabinol are neuroprotective antioxidants. Proc Natl Acad Sci U S A, 1998. 95(14): p. 8268-73.

178. Consroe, P., et al., Controlled clinical trial of cannabidiol in Huntington's disease. Pharmacol Biochem Behav, 1991. 40(3): p. 701-8.

179. Leweke, F.M., et al., Cannabidiol enhances anandamide signaling and alleviates psychotic symptoms of schizophrenia. Transl Psychiatry, 2012. 2: p. e94.

180. Juknat, A., et al., Microarray and pathway analysis reveal distinct mechanisms underlying cannabinoid-mediated modulation of LPS-induced activation of BV-2 microglial cells. PLoS One, 2013. 8(4): p. e61462.

181. Jastrzab, A., A. Gegotek, and E. Skrzylowska, Cannabidiol Regulates the Expression of Keratinocyte Proteins Involved in the Inflammation Process through Transcriptional Regulation. Cells, 2019. 8(8).

182. Bockmann, S. and B. Hinz, Cannabidiol Promotes Endothelial Cell Survival by Heme Oxygenase-1-Mediated Autophagy. Cells, 2020. 9(7).
183. Lu, X., et al., Cannabidiol attenuates pulmonary arterial hypertension by improving vascular smooth muscle cells mitochondrial function. Theranostics, 2021. 11(11): p. 5267-5278.

184. Casares, L., et al., Cannabidiol induces antioxidant pathways in keratinocytes by targeting BACH1. Redox Biol, 2020. 28: p. 101321.

185. Bublitz, K., et al., Cannabinoid-Induced Autophagy and Heme Oxygenase-1 Determine the Fate of Adipose Tissue-Derived Mesenchymal Stem Cells under Stressful Conditions. Cells, 2020. 9(10).

186. Duivigneau, J.C., et al., Cannabidiol Protects Dopaminergic Neurons in Mesencephalic Cultures against the Complex I Inhibitor Rotenone Via Modulation of Heme Oxygenase Activity and Bilirubin. Antioxidants (Basel), 2020. 9(2).

187. Schwartz, M., S. Bookmann, and B. Hinz, Up-regulation of heme oxygenase-1 expression and inhibition of disease-associated features by cannabidiol in vascular smooth muscle cells. Oncotarget, 2018. 9(77): p. 34595-34616.

188. Rajesh, M., et al., Cannabidiol attenuates cardiac dysfunction, oxidative stress, fibrosis, and inflammatory and cell death signaling pathways in diabetic cardiomyopathy. J Am Coll Cardiol, 2010. 56(25): p. 2115-25.

189. O'Sullivan, S.E., et al., Time-dependent vascular actions of cannabidiol in the rat aorta. Eur J Pharmacol, 2009. 612(1-3): p. 61-8.

190. Sun, S., et al., Cannabidiol attenuates OGD/R-induced damage by enhancing mitochondrial bioenergetics and modulating glucose metabolism via pentose-phosphate pathway in hippocampal neurons. Redox Biol, 2017. 11: p. 577-585.

191. Foud, A.A., et al., Cardioprotective effect of cannabidiol in rats exposed to doxorubicin toxicity. Environ Toxicol Pharmacol, 2013. 36(2): p. 347-357.

192. Dos-Santos-Pereira, M., et al., Cannabidiol prevents LPS-induced microglial inflammation by inhibiting ROS/NF-kappaB-dependent signaling and glucose consumption. Glia, 2020. 68(3): p. 561-573.

193. Rajesh, M., et al., Cannabidiol attenuates high glucose-induced endothelial cell inflammatory response and barrier disruption. Am J Physiol Heart Circ Physiol, 2007. 293(1): p. H610-9.

194. El-Remessy, A.B., et al., Neuroprotective effect of (−)-Delta9-tetrahydrocannabinol and cannabidiol in N-methyl-D-aspartate-induced retinal neurotoxicity: involvement of peroxynitrite. Am J Pathol, 2003. 163(5): p. 1997-2008.

195. Mecha, M., et al., Cannabidiol protects oligodendrocyte progenitor cells from inflammation-induced apoptosis by attenuating endoplasmic reticulum stress. Cell Death Dis, 2012. 3: p. e331.

196. Liu, C., et al., Cannabidiol Protects Human Skin Keratinocytes from Hydrogen-Peroxide-Induced Oxidative Stress via Modulation of the Caspase-1-1L-1beta Axis. J Nat Prod, 2021.

197. Cocetta, V., et al., Cannabidiol Isolated From Cannabis sativa L. Protects Intestinal Barrier From In Vitro Inflammation and Oxidative Stress. Front Pharmacol, 2021. 12: p. 641210.

198. Iuvone, T., et al., Neuroprotective effect of cannabidiol, a non-psychoactive component from Cannabis sativa, on beta-amylloid-induced toxicity in PC12 cells. J Neurochem, 2004. 89(1): p. 134-41.

199. Pan, H., et al., Cannabidiol attenuates cisplatin-induced nephrotoxicity by decreasing oxidative/nitrosative stress, inflammation, and cell death. J Pharmacol Exp Ther, 2009. 328(3): p. 708-14.

200. Borges, R.S., et al., Understanding the molecular aspects of tetrahydrocannabinol and cannabidiol as antioxidants. Molecules, 2013. 18(10): p. 12663-74.

201. Hamelink, C., et al., Comparison of cannabidiol, antioxidants, and diuretics in reversing binge ethanol-induced neurotoxicity. J Pharmacol Exp Ther, 2005. 314(2): p. 780-8.

202. Atalay, S., I. Jarocka-Karpowicz, and E. Skrzydelwska, Antioxidative and Anti-Inflammatory Properties of Cannabidiol. Antioxidants (Basel), 2019. 9(1).

203. Panja, D., C.A. Vedeler, and M. Schubert, Paraneoplastic cerebellar degeneration: Yo antibody alters mitochondrial calcium buffering capacity. Neuropathol Appl Neurobiol, 2019. 45(2): p. 141-156.

204. Atalay, S., et al., Cannabidiol protects keratinocyte cell membranes following exposure to UVB and hydrogen peroxide. Redox Biol, 2020. 36: p. 101613.
205. Wang, Y., et al., Cannabidiol attenuates alcohol-induced liver steatosis, metabolic dysregulation, inflammation and neutrophil-mediated injury. Sci Rep, 2017. 7(1): p. 12064.

206. Wu, H.Y., et al., Cannabidiol induced apoptosis in human monocytes through mitochondrial permeability transition pore-mediated ROS production. Free Radic Biol Med, 2018. 124: p. 311-318.

207. Schultz, N., et al., Mitochondrial functions of THP-1 monocytes following the exposure to selected natural compounds. Toxicol, 2017. 377: p. 57-63.

208. McKallip, R.J., et al., Cannabidiol-induced apoptosis in human leukemia cells: A novel role of cannabidiol in the regulation of p22phox and Nox4 expression. Mol Pharmacol, 2006. 70(3): p. 897-908.

209. Wu, H.Y., et al., Cannabidiol-induced apoptosis in primary lymphocytes is associated with oxidative stress-dependent activation of caspase-8. Toxicol Appl Pharmacol, 2008. 226(3): p. 260-70.

210. Gonzalez-Garcia, C., et al., Mechanisms of action of cannabidiol in adoptively transferred experimental autoimmune encephalomyelitis. Exp Neurol, 2017. 298(Pt A): p. 57-67.

211. Lee, C.Y., et al., A comparative study on cannabidiol-induced apoptosis in murine thymocytes and EL-4 thymoma cells. Int Immunopharmacol, 2008. 8(5): p. 732-40.

212. Muthumalage, T. and I. Rahman, Cannabidiol differentially regulates basal and LPS-induced inflammatory responses in macrophages, lung epithelial cells, and fibroblasts. Toxicol Appl Pharmacol, 2019. 382: p. 114713.

213. de la Harpe, A., N. Beukes, and C.L. Frost, CBD activation of TRPV1 induces oxidative signaling and subsequent ER stress in breast cancer cell lines. Biotechnol Appl Biochem, 2021.

214. Massi, P., et al., The non-psychoactive cannabidiol triggers caspase activation and oxidative stress in human glioma cells. Cell Mol Life Sci, 2006. 63(17): p. 2057-66.

215. Jeong, S., et al., Cannabidiol Overcomes Oxaliplatin Resistance by Enhancing NOS3- and SOD2-Induced Autophagy in Human Colorectal Cancer Cells. Cancers (Basel), 2019. 11(6).

216. Yeisley, D.J., A.S. Arabiyat, and M.S. Hahn, Cannabidiol-Driven Alterations to Inflammatory Protein Landscape of Lipopolysaccharide-Activated Macrophages In Vitro May Be Mediated by Autophagy and Oxidative Stress. Cannabis Cannabinoid Res, 2021.

217. Wojcik, P., et al., Cannabidiol Modifies the Formation of NETs in Neutrophils of Psoriatic Patients. Int J Mol Sci, 2020. 21(18).

218. Devinsky, O., et al., Trial of Cannabidiol for Drug-Resistant Seizures in the Dravet Syndrome. The New England Journal of Medicine, 2017. 376: p. 2011-2020.

219. Szafiarski, J.P., et al., Cannabidiol improves frequency and severity of seizures and reduces adverse events in an open-label add-on prospective study. Epilepsy Behav, 2018. 87: p. 131-136.

220. Flachenecker, P., T. Henze, and U.K. Zettl, Nabiximols (THC/CBD oromucosal spray, Sativex®) in clinical practice--results of a multicenter, non-interventional study (MOVE 2) in patients with multiple sclerosis spasticity. Eur Neurol, 2014. 71(5-6): p. 271-9.

221. Stott, C.G., et al., A phase I study to assess the single and multiple dose pharmacokinetics of THC/CBD oromucosal spray. Eur J Clin Pharmacol, 2013. 69(5): p. 1135-47.

222. Feliu, A., Moreno-Martet, M., Mecha M. Carrillo-Salinas, F. J., Lago, E., Fernandez-Ruiz, J. and Guaza, C., A Sativex-like combination of phytocannabinoids as a disease-modifying therapy in a viral model of multiple sclerosis. British Journal of Pharmacology, 2015.

223. Patti, F., et al., Efficacy and safety of cannabionid oromucosal spray for multiple sclerosis spasticity. J Neurol Neurosurg Psychiatry, 2016. 87(9): p. 944-51.

224. Zurier, R.B. and S.H. Burstein, Cannabinoids, inflammation, and fibrosis. FASEB J, 2016. 30(11): p. 3682-3689.

225. Badowski, M.E. and P.K. Yanful, Dronabinol oral solution in the management of anorexia and weight loss in AIDS and cancer. Therapeutics and clinical risk management, 2018. 14: p. 643-651.

226. Berlach, D.M., Y. Shir, and M.A. Ware, Experience with the synthetic cannabinoid nabilone in chronic noncancer pain. Pain Med, 2006. 7(1): p. 25-9.
Figure 1. Modulation of cellular ROS. The majority of ROS originate from various sources within the cell. Production is via (A) the mitochondrial electron transport chain, (B) the enzymatic reaction catalysed by XO, and (C) by NOX. Endogenous antioxidant mechanisms are exerted via (D) SOD conversion of O$_2^•$- to H$_2$O$_2$, followed by conversion to water by (E) CAT and/or GPX. When ROS production exceeds the endogenous antioxidant mechanisms capacity, (F, G) H$_2$O$_2$ may react with O$_2^•$- to form HO$^•$ (Haber-Weiss and Fenton reactions). (H) Oxygen radicals can also react with NO to generate ONOO$^•$. 
Figure 2. Overview of the antioxidant propensity of CBD.