# Cannabinoid Receptor and Inflammation

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**Abstract**

The eventual discovery of endogenous cannabinoid receptors CB1 and CB2 and their endogenous ligands has generated interest with regards to finally understanding the endocannabinoid system. Its role in the normal physiology of the body and its implication in pathological states such as cardiovascular diseases, neoplasm, depression and pain have been subjects of scientific interest. In this review the authors focus on the endogenous cannabinoid pathway, the critical role of cannabinoid receptors in signaling and mediation of neurodegeneration and other inflammatory responses as well as its potential as a drug target in the amelioration of some inflammatory conditions. Though the exact role of the endocannabinoid system is not fully understood, the evidence found leans heavily towards a great potential in exploiting both its central and peripheral pathways in disease management. Cannabinoid therapy has already shown great promise in several preclinical and clinical trials.

## 1.0 Introduction

Ethnopharmacological studies have shown the use of Cannabis sativa in traditional medicine for over a thousand years, with its widespread use promoted by its psychotropic effects (McCoy, 2016; Turcotte et al., 2016). The discovery of a receptor within human body, that is selectively activated by cannabinoids suggested the presence of at least one endogenous ligand for this receptor. This is confirmed by the discovery of two endogenously synthesized lipid mediators, 2-arachidonoyl-glycerol and arachidonoylethanolamide, which function as high-affinity ligands for a subfamily of cannabinoid receptors ubiquitously distributed in the central nervous system, known as the $CB_1$ receptors (Turcotte et al., 2016). A second cannabinoid receptor, the $CB_2$, has since been cloned, characterised and discovered to be primarily distributed in immune cells with some level of expression in the brain (Munro et al., 1993). $CB_2$ receptor is thus believed to be involved in immunomodulation following activation by cannabinoids and endocannabinoids (Galiegue et al., 1995). Collectively, endocannabinoids and their receptors, together with enzymes responsible for the synthesis and metabolism of endocannabinoids, constitute the endocannabinoid system (Zeng et al., 2019; Turcotte et al., 2016).

The endocannabinoid system plays crucial roles in critical processes involved in the normal physiology of the body, such as immune function. It also plays a role in pathological processes such as neuroinflammation, neoplastic diseases, cardiovascular diseases, ulcerative colitis, anxiety, depression and pain (Ligresti et al., 2009; McPartland et al., 2014). Activation of the $CB_2$ receptor has been reported to be associated with positive outcomes in atherosclerosis, ischemic reperfusion injury and multiple sclerosis (Wen et al., 2015; Zhao et al., 2010). Understanding the mechanisms involved in cannabinoid receptor-mediated modulation of homeostatic and defensive functions and the various signaling pathways associated, will aid in the development of ligands that can selectively modulate cannabinoid receptor activity in the management of inflammatory and immune disorders. Thus, in this review, we discuss the role of cannabinoid receptor activation in inflammation and immunobiology.

## 2.0 Endocannabinoid system

Endocannabinoid system is a neuromodulator system that consists of endogenous cannabinoids such as 2-arachidonoylglycerol and anandamide, their cognate receptors ($CB_1$ and $CB_2$), and the enzymes involved their synthesis (phospholipase C-β (PLC β) and 1,2-diacylglycerol lipase) and inactivation (monoglyceride lipase and fatty acid amide hydrolase) (Argenziano et al., 2019; Bie et al., 2018). This neuromodulatory system is important in maintaining immune system homeostasis, as well as inflammatory processes. Of the two cannabinoid receptors, $CB_2$, to a greater extent, is involved in the regulation of immune response compared to $CB_1$ receptors (Argenziano et al., 2019).

### 2.1 Endogenous cannabinoid ligands, their synthesis and inactivation

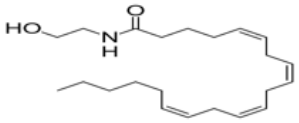
Endocannabinoids are N-acylethanolamines synthesized from membrane lipids in response to specific signals such as inflammatory signals (Barrie and Manolios, 2017; Katona and Freund, 2012). Of these endogenous signaling molecules, anandamide and 2-arachidonoylglycerol (2-AG) represent the two most investigated ligands (Devane et al., 1992; Mechoulam et al., 1995). Anandamide (arachidonoyl ethanolamine, AEA) was initially isolated from porcine brain, and so named due to its ability to increase motivation and pleasure (derived from Ānanda, a Sanskrit word for bliss) (Monteleone et al., 2015). It binds to both $CB_1$ and $CB_2$ receptors and is responsible for maintaining basal endocannabinoid signaling. This alludes to the capacity of anandamide to increase motivation and pleasure. On the other hand, 2-AG, initially isolated from canine intestines, binds to $CB_1$ and $CB_2$ with greater affinity than anandamide since it functions as a full agonist for these receptors (Sugiura et al., 2000; Argenziano et al., 2019). In addition to these ligands, a number of other biochemically similar endocannabinoids, including virodhamine, 2-AG ether and N-arachidonoyl dopamine have been identified. Knowledge on their exact biological functions is, however, yet to be fully elucidated (Argenziano et al., 2019). The chemical structures of most common endocannabinoids are presented in Figure 1. For illustration purposes, figure 1 also presents the chemical structures of synthetic analogs and phytocannabinoids.

Although anandamide and 2-AG are both lipid molecules synthesized from the breakdown of arachidonic acid (AA) liberated from the cell membrane, their biosynthetic pathways are largely dissimilar (Argenziano et al., 2019). N-acyltransferase catalyses the transfer of AA from the sn-1 position of a donor phospholipid to the primary amine phosphatidylethanolamine, resulting in the formation of$N$ -arachidonoylphosphatidylethanolamine (NAPE) (Sharke and Wiley, 2016). NAPE is then hydrolysed by$N$ -acylphosphatidylethanolamine-hydrolyzing phospholipase D to yield anandamide (Di Marzo et al., 1994). Other biosynthetic pathways for the production of anandamide such as sequential O-deacylation of$N$ -arachidonoylphosphatidylethanolamine by the lyso (N-acyl phosphatidylethanolamine)-lipase $\alpha$-$\beta$ hydrolase 4 and cleavage of the phosphodiester bond by the glycerophosphodiesterase GDE1 (Simon and Cravatt, 2006; 2008), direct liberation by N-acyl phosphatidylethanolamine-selective phospholipase D enzyme (Okamoto et al., 2004), among others, have been suggested and also reviewed extensively by Blankman and Cravatt, 2013. During the synthesis of 2-AG, phosphoinositol PLC β is first activated leading to hydrolyses of arachidonoyl phosphatidylinositol 4,5-bisphosphate (PIP2) species at the sn-2 position with subsequent production of diacylglycerol (Hashimotodani et al., 2005). Diacylglycerol is then hydrolysed by sn-1-selective diacylglycerol lipases-α and -β (DAGLα and -β) resulting in the production of 2-AG (Ledent et al., 1999). The biosynthetic pathways for endocannabinoids are summarized in figure 1.

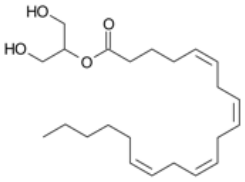
Unlike most hormones and other neurotransmitters, endocannabinoids are believed not to be transported into vesicles for storage following synthesis due to their hydrophobicity (Katona and Freund, 2012). Rather, they are thought to be mobilized in a process referred to as "on demand" biogenesis, where endocannabinoids are liberated from membrane phospholipid precursors and/or storage sites in an activity-dependent manner (Min et al., 2010; Alger and Kim, 2011). Upon release, these endocannabinoids diffuse, and regulate the release of multiple presynaptic messengers by acting locally as retrograde messengers (Barrie and Manolios, 2017). Soon after cellular uptake from the synaptic space, these endogenous molecules are inactivated by specific enzymes within the intracellular environment. For instance, in the nervous system, 2-AG and anandamide are inactivated primarily by the serine hydrolase enzymes monoacylglycerol lipase (MAGL) and fatty acid amide hydrolase (FAAH), respectively (Burstein and Zurier, 2009; Blankman and Cravatt,

2013). Although 2-AG is mostly inactivated by MAGL, α/β hydrolase domain-containing protein-6 and -12 are known to account for about 15% of the enzyme degradation (Blankman et al., 2007). In addition to the enzymatic transformation, cyclooxygenase-2 (COX2) also oxygenates 2-AG to form biologically active prostaglandin glyceryl esters, involved in the regulation of inflammation (Hermanson et al., 2014; Alhouayek and Muccioli, 2014). Enzymatic inactivation of anandamide yields arachidonic acid and ethanolamine while the degradation of 2-AG by respective enzyme yields glycerol as shown in figure 2 (Wang and Ueda, 2009).

**Endocannabinoids**

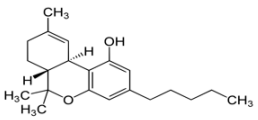


Anandamide

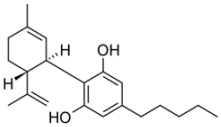


2-Arachidonoyl glycerol (2-AG)

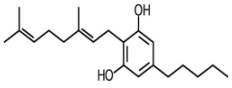
**Phytocannabinoids**



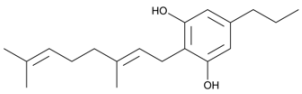
9Δ-Tetrahydrocannabinol



Cannabidiol

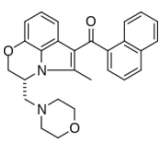


Cannabigerol

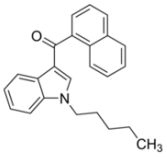


Cannabigerovarin

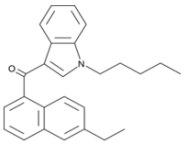
**Synthetic cannabinoids**



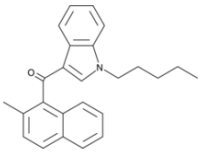
WIN 55, 212-2



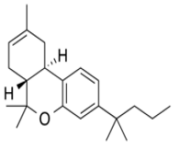
JWH-018



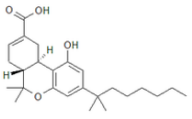
JWH-210



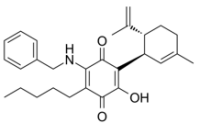
JWH-122



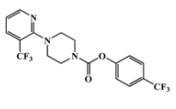
JWH-133



Ajulemic acid



VCE 004.8



OMDM198

Figure 1. Chemical structures of most common endocannabinoids, phytocannabinoids and synthetic cannabinoids

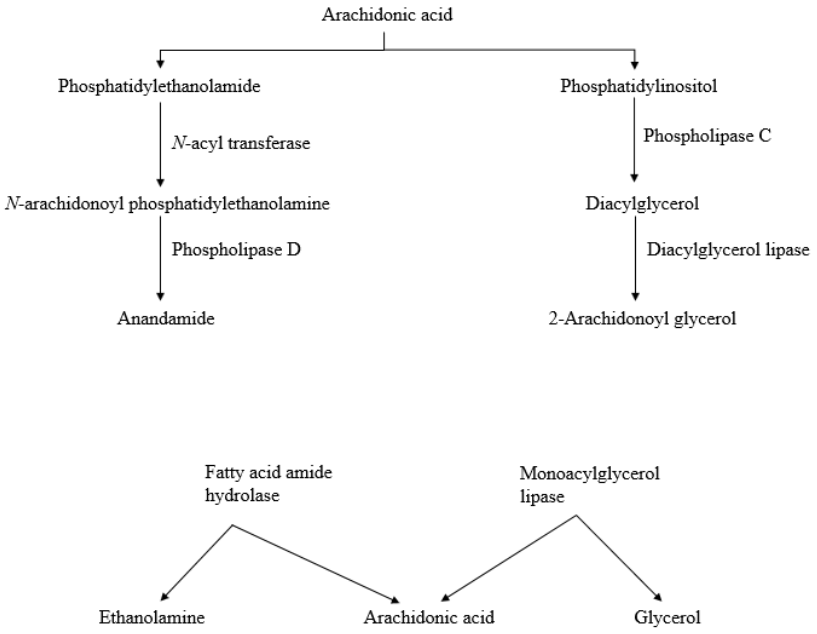



Figure 2. Most prominent pathways for the biosynthesis and enzymatic inactivation of the most investigated endocannabinoid ligands: anandamide and 2-AG.

### 2.2 Cannabinoid receptor subtypes

Endocannabinoids signal via two main cell surface receptors, $CB_1$ and $CB_2$, belonging to the superfamily of G-protein-coupled receptors. $CB_1$ is the principal receptor present in the central nervous system (CNS), and is one of the most abundant G-protein–coupled receptors in the brain (Sharke and Wiley, 2016). Within the CNS, $CB_1$ receptors are densely expressed in several brain regions such as the cortex, dorsal root and basal ganglia, thalamus, hippocampus, and periaqueductal gray, and supraspinal regions where they regulate a wide variety of neurotransmission processes including pain transmission and neuroinflammation (Martin et al., 1995; Barrie and Manolios, 2017). $CB_1$ receptors are also densely expressed on the epithelium of the gastrointestinal (GI) tract and on all classes of enteric neurons except inhibitory motor neurons (Trautmann and Sharkey, 2015). Their widespread distribution explains the wide array of biological effects produced by cannabinoid ligands, both endogenously expressed and exogenously supplied. With the widespread distribution, it is not surprising that therapies targeting $CB_1$ receptors are sometimes limited by challenges associated with central and peripheral nervous systems effects (Barrie and Manolios, 2017).

$CB_2$ receptors are expressed by immune cells and also by enteric neurons and the epithelial cells in the GI tract (Trautmann and Sharkey, 2015; Wright et al., 2008). Their wide distribution in the periphery on immune cells makes them targets for modulating inflammatory processes such as inflammatory pain processing (Barrie and Manolios, 2017). This is supported by the discovery that HU-308, a selective $CB_2$ agonist, significantly decreases nociceptive behaviour in formalin-induced rodent inflammatory pain model. $CB_2$ receptor activation suppressed local secretion of pro-inflammatory factors by non-neural cells, thus inhibited sensitization of neighbouring nociceptive neuronal terminals (Hanus et al., 1999; Barrie and Manolios, 2017). It has therefore been proposed that, in the settings of neuropathic pain or inflammatory hyperalgesia, activation of peripheral $CB_2$ receptors mediate anti-nociceptive responses by acting locally on immune cells in the periphery and microglia in the CNS (Ibrahim et al., 2003).

### 3.0 Cannabinoid receptor signaling and acute inflammation

In keeping inflammatory responses to tissue injury in check and preventing it from going awry, activated immune cells produce and release anti-inflammatory mediators in addition to the pro-inflammatory cytokines produced in response to tissue damage. A classic example is the release of the anti-inflammatory, IL-10, which is, in part, regulated by the endocannabinoid system (Donvito et al., 2018; Klein, 2005). Endogenous cannabinoid receptor ligands are able to regulate cytokines production and release them at different stages during the inflammatory response (Cabral and Griffin-Thomas, 2009). For instance, anandamide and $\Delta^9$-tetrahydrocannabinol ($\Delta^{9}$-THC), the primary active constituent in *Cannabis sativa* , suppress pro-inflammatory cytokines and enhance anti-inflammatory cytokines in both innate and adaptive immune responses (Cabral and Griffin-Thomas, 2009). The amide conjugate of arachidonic acid and ethanolamine, anandamide, directly inhibits tumor necrosis factor α (TNF-α) activation of the transcription nuclear factor kappa B (NFκB) via direct inhibition of the IκB kinase (Sancho et al., 2003), and also inhibits microglial nitric oxide (NO) production through the mitogen-activated protein kinase (MAPK) pathway (Eljaschewitsch et al., 2006).

Cannabinoids, however, depending on the type of inflammatory model employed, dose of cannabinoid used and drug probe, may also enhance the synthesis of pro-inflammatory cytokines (Klein, 2005). This is supported by rimonabant- and SR144528-mediated reversal of the suppression of IL-12 and interferon (IFN)-γ. Rimonabant produces selective antagonism of $CB_1$ receptors while SR144528 selectively antagonises $CB_2$ receptors (Klein et al., 1985). THC's effect on cytokine synthesis *in vitro* is biphasic. In nanomolar concentrations, it inhibits pro-inflammatory cytokine synthesis, while at micromolar concentrations, THC stimulates pro-inflammatory cytokine synthesis (Berdyshev et al., 1997). Moreover, both anandamide and THC inhibit lipopolysaccharide-induced IL-6 production and NO release from macrophages *in vitro* (Chang et al., 2001). In modulating inflammation, cannabinoid receptor activation by endogenous ligands supresses Th1 pro-inflammatory activity and promotes Th2 anti-inflammatory activity by shifting the balance of $CD4^+$ 'Helper' T cells (Yuan et al., 2002).

In addition to modulation of cytokine production, pharmacological agents targeting various components of the endocannabinoid system also exert anti-inflammatory effects via the inhibition of inflammatory cell proliferation and migration, and induction apoptosis (Nagarkatti et al., 2009). CP55,940, a cannabinoid receptor agonist on both $CB_1$ and $CB_2$, decreased the migration of rat macrophages in *in vivo* and *in vitro* assays (Sacerdote et al., 2000). Cannabinoids have also been demonstrated to inhibit cell-specific proliferation of B and T lymphocytes (Cabral and Griffin-Thomas, 2009; Klein and Cabral, 2006). THC inhibits the proliferation of human T cells stimulated with antigen-primed dendritic cells (Yuan et al., 2002), mouse splenic T cells stimulated by concanavalin A, and B cells stimulated by lipopolysaccharide (Klein et al., 1985). THC can also induce apoptosis of mouse T and B cells, and macrophages in primary thymic and splenic cultures (McKallip et al., 2002). In a study by Zheng et al. (2019), $CB_2$stimulation by JWH133, an agonist at the $CB_2$ receptor, protected mice against lung ischemic reperfusion injury by dampening inflammation in a process that was inhibited by pre-treatment with a PI3K-inhibitor. This therefore suggests the involvement of the PI3K/Akt pathway in the protective effect of $CB_2$ receptor activation in lung inflammation.

### 4.0 Cannabinoid receptor signaling and neurodegeneration

Glial cells are resident immune cells of the CNS and represent over 70% of the total cell population of the CNS (Bie et al., 2019). They function as the first line of defence against tissue insults such as inflammation. Glial cells include brain parenchymal-resident microglia, perivascular microglia, oligodendrocytes and astrocytes (Romero-Sandoval et al., 2008; Villacampa and Heneka, 2018). Depending on the type of stimuli received by microglia, they may assume either a neuroprotective phenotype or neurotoxic one (Schwartz et al., 2006). For instance, microglia are neuroprotective and their activities are central during the healing response in nerve transection models of glutamate injury (Schwartz et al., 2003). On the other hand, when primed with IFN-γ and then administered with LPS, microglia adopt a phenotype suited for defensive immunity, and hence become neurotoxic (Ashton and Glass, 2007). However, it has also been proposed that microglia may make a transition from neuroprotective to neurotoxic phenotype depending on the intensity of the neuronal

insult and also duration of it (Zipp and Aktas, 2006).

Under normal physiological conditions, microglia modify synaptic structure and the local environment of neurons, thus play an essential role in induction and maintenance of synaptic plasticity, through processes such as phagocytosis (Tremblay and Majewska, 2011; Schafer and Stevens, 2013). As such, the intensity and nature of microglial-mediated synaptic pruning is central to the maintenance of synaptic structure or the destruction of the synapse and the onset of neurodegeneration (Paolicelli et al., 2011; Wake et al., 2013; Kettenmann et al., 2013). A wide array of noxious signals is capable of priming microglial cells, with subsequent transition of microglial phenotype from protective, anti-inflammatory to the rogue pro-inflammatory phenotype (Lan et al., 2017). The latter expresses several receptors such as purinergic P2X4 receptors and Toll-like receptors (TLRs) (Naguib et al., 2012). Activation of these receptors leads to downstream events that release several pro-inflammatory cytokines and chemokines with subsequent neuronal damage (Bie et al., 2018). Microglial activation and neuroinflammation is thus implicated in the pathogenesis of neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), Huntington's disease (HD), multiple sclerosis, neuropathic pain and immunodeficiency virus-induced encephalitis (Ramirez et al., 2005; Benito et al., 2005; Bie et al., 2018).

It is a known fact that cannabinoids, such as 2-AG, stimulate neurogenesis in the adult brain (Ashton and Glass, 2007). This is supported by evidence from numerous studies, including a study by Jin et al. (2004) where defects associated with the process of neurogenesis in $CB_1$-knockout mice were reported. Moreover, $CB_2$ receptor stimulation is associated with upregulation of neurogenesis in neurological disease, leading to the production of new neurons in the hippocampus and the subventricular zone-olfactory bulb system (Ashton and Glass, 2007). In terms of microglial polarisation, changes in the levels of expression of $CB_2$, but not $CB_1$, closely correlate (Cabral and Marciano-Cabral, 2005). In a study by Cabral and Cabral (2005), the authors argued that $CB_2$ expression is closely related to the multi-step activation of microglia.

In addition to stimulating proliferation and migration of the benign phenotype microglia, activation of $CB_2$ receptors in microglia also blocks microglial differentiation to a neurotoxic phenotype. Thus, activation of the $CB_2$ receptor inhibits microglial-mediated neuronal damage in a number of neurodegenerative disease models, including in β-amyloid and CD40L-induced oxidative damage, lipopolysaccharide-induced neuroinflammation and NMDA-injury models (Ehrhart et al., 2005). As proof of concept, the expression of markers of microglial activation following β-amyloid injections was inhibited by $CB_2$ receptor stimulation with WIN 55,212-2, in an *in vivo* murine model of AD (Ramirez et al., 2005). In addition, *in vitro* $CB_2$ receptor stimulation inhibits the release of pro-inflammatory mediators, including TNF-α, IL-1 and NO, from previously primed cytotoxic microglia phenotype. It, however, remains to be determined if this effect is as a result of reversal of microglia phenotype, from the cytotoxic pro-inflammatory to a neuroprotective anti-inflammatory phenotype (Ashton and Glass, 2007). Moreover, in a rodent model of multiple sclerosis, Rossi et al. (2013) demonstrated that genetic ablation of $CB_1$ worsens neuronal loss in experimental autoimmune encephalomyelitis. In their study, AAT trinucleotide short tandem repeat polymorphism of gene that encodes $CB_1$ receptor (located on chromosome 6) was associated with increased degeneration of gray matter in response to inflammatory lesions of the white matter, especially in areas crucial for cognitive function.

### 5.0 Cannabinoid activity and Gut inflammation

The GI tract is rich in components of the endocannabinoid system. In addition to the classical $CB_1$ and $CB_2$receptors, the gut also expresses G protein-coupled receptor 55 (GPR55), a cannabinoid-responsive non-$CB_1$/$CB_2$receptor, which upon activation leads to increased intracellular calcium concentration (Grill et al., 2018). This mechanism has been shown to involve $G_q$, $G_{12}$, actin, RhoA, phospholipase C and calcium released from inositol 1,4,5-triphosphate receptor (IP3R)-gated stores (Lauckner et al., 2008). Within the GI system, $CB_2$ is largely expressed on immune cells whereas $CB_1$ is largely expressed on cholinergic neurons (Sibaev et al., 2009; Schmole et al., 2015). Moreover, the gut also contains enzymes of the endocannabinoid system including diacylglycerol lipase and N-acyl phosphatidylethanolamine-specific phospholipase D, FAAH and MAGL (Grill et al., 2018).

By examining the role of endocannabinoids in maintaining gut integrity, it is most apparent that these endogenous ligands have multiple regulatory roles including, maintaining the integrity of the epithelial barrier, regulating gut microbiota and keeping the immune cells tolerant to commensals (Cani et al., 2016; Karwad et al., 2017). The latter is achieved through regulation of the expansion of the regulatory T cell (Treg) subset Tr1 and the presence of $CX3CR1^{hi}$, an immunosuppressive macrophage population (Acharya et al., 2017). Thus, it is unsurprising that changes in the levels of endocannabinoids and endocannabinoids-like lipids are implicated in inflammatory bowel disease (IBD) and colorectal cancer (Di Sabatino et al., 2011; Chen et al., 2015). It however remains to be determined if such variations correlate with disease progress (Grill et al., 2018).

Functional data from animal models shows alterations in the components of the endocannabinoid system during experimental intestinal inflammation (Massa et al., 2004; D'Argenio et al., 2006). It has been shown that, pharmacological activation of $CB_1$ and $CB_2$ attenuates experimental colitis (Kimball et al., 2006; Storr et al., 2009), while pharmacological antagonism or genetic ablation of these receptors worsens gut inflammation (Massa et al., 2004; Storr et al., 2008). This is evident from studies that demonstrated that increased levels of anandamide and 2-AG, following inhibition of FAAH or MAGL or genetic deficiency in genes that encodes these enzymes, is protective against experimental colitis such as dextran sodium sulfate (DSS)-induced colitis or trinitrobenzene sulfonic acid (TNBS)-induced colitis (Pagano et al., 2016; Shamran et al., 2017; Zhao et al., 2017).

Synthetic analogs of endocannabinoids as well as phytocannabinoids have been shown to ameliorate gut inflammation in animal models of intestinal inflammation (Grill et al., 2018). In a study by Jamontt et al. (2010), both the psychotropic $\Delta^9$-THC and the non-psychotropic cannabidiol reduced colonic injury in a rat model of TNBS-induced colitis. Cannabidiol, however, has very low affinity on $CB_1$ and $CB_2$, and is antagonistic on GPR55 (Ryberg et al., 2007). In addition, cannabidiol acts on PPAR$\gamma$ (De Filippis et al., 2010) and TRPV1 (De Petrocellis et al., 2011) expressed in the gut. It also inhibits the activity of FAAH and has been shown to ameliorate intestinal inflammation in dinitrobenzene sulfonic acid (DNBS)-induced colitis in mice (Borrelli et al., 2009). Significant reduction in intestinal inflammation by cannabidiol appears to be a combined effect with other compounds in *Cannabis sativa* rather than a single effect (Pagano et al., 2016). Moreover, O-1602, an analog of cannabidiol and a GPR55 agonist, has been shown to protect against intestinal damage in experimental colitis. This effect, however, was not mediated by GPR55, and by extrapolation, not by $CB_1$ or $CB_2$ since O-1602 lacks affinity for $CB_1$and $CB_2$ (Ryberg et al., 2007). Several other phytocannabinoids, endocannabinoids, endocannabinoid-like substances and synthetic endocannabinoid analogs have been demonstrated to inhibit intestinal inflammation in various models of experimental colitis. Table 1 summarizes these ligands and the mechanisms involved in the inhibition of inflammatory processes in the GIT.

By employing the use of chronic ileitis model, Leinwand et al. (2017) identified upregulation of $CB_2$ and anandamide in actively inflamed ileum of $TNF^{\Delta APE/+}$ mice compared with controls. In $TNF^{\Delta APE/+}$ mice, $CB_2$mRNA was relatively expressed 11-fold more on Tregs as compared to T effector cells, while in wild-type mice, there was a 2.4-fold increase in its expression on Tregs compared to T effector cells. The authors reported that GP-1a, a previously classified $CB_2$receptor agonist, acts at $CB_2$ as an inverse agonist, thus allows the receptor to resensitize. This finding is also supported by a previous study by Soethoudt et al. (2017), where GP-1a was shown to actively inhibit the reuptake of anandamide and also act as an inverse agonist at the $CB_2$ receptors *in vitro* . GP-1a however did not stimulate $CB_1$ receptors. The inverse agonism at $CB_2$ enhances the activation of Treg-expressed $CB_2$ receptors by endocannabinoids, with subsequent increase in Treg suppressive function and associated increase in IL-10 secretion (Leinward et al., 2017). As opposed to active inflammation, $CB_2$R was downregulated in both chronically inflamed $TNF^{\Delta APE/+}$ mice and in Crohn's disease patients. Activation of $CB_2$ receptors inhibited ileitis in mice, thus establishing the protective effect of the endocannabinoid system in intestinal inflammation (Leinward et al., 2017).

Table 1. Other cannabinoid receptor ligands in murine models of intestinal inflammation

| Ligand | Animal model | Mechanism |
|---|---|---|
| Cannabigerol | DNBS-induced colitis | Decrease NO production in macrophages and ROS formation in intestinal epithelial cells (Borrelli et al., 2013) |
| Cannabichromene | DNBS-induced colitis | Inhibits NO production in macrophages (Romano et al., 2013) |
| WIN 55,212-2 | DSS-induced colitis | Partly inhibits of p38 MAPK (Feng et al., 2016) |
| HU210 | DSS-induced colitis and DNBS-induced colitis | Maintains intestinal barrier integrity independent of TLR4, but produces extraintestinal anti-inflammatory effects that via TLR4-mediated p38 MAPK activation (Lin et al., 2017, Massa et al., 2004) |
| Palmitoylethanolamide | DSS-induced colitis and DNBS-induced colitis | Inhibits intestinal inflammation and maintains barrier integrity via activation of $CB_2$, GPR55 and modulation of inflammation-associated VEGF signaling via the Akt/mTOR pathway in a selective PPARα-dependent manner (Borrelli et al., 2015; 49-52) |

CB, cannabinoid receptor; DSS, dextran sodium sulfate; DNBS, dinitrobenzenesulfonic acid; GPR55, G protein-coupled receptor 55; mTOR, mammalian target of rapamycin; MAPK, mitogen-activated protein kinase; NO, nitric oxide; PPARα, peroxisome proliferator-activated receptor alpha; ROS, reactive oxygen species; TLR, toll-like receptor; VEGF, vascular endothelial growth factor

**6.0 Cannabinoids and joint inflammation**

Inflammatory mediators such as neuropeptides, including substance P (SP), calcitonin gene-related peptide (CGRP) and neurokinin A, released during neurogenic inflammation by local afferent neurons act on adjacent mast cells with subsequent release of histamine (Barrie and Manolios, 2017). The histamine released then induces the release of additional SP from terminal varicosities of branches of neurons in the dorsal horn, and CGRP, thus creating a positive feedback loop which amplifies the inflammatory response (Rosa and Fantozzi, 2013). In the arthritic joint, subsequent release of these neuropeptides from peripheral terminals of Aδ and C-fibers result in the development of local neurogenic inflammation, and also neuropathic pain via increased TRPV1 expression (Bánvölgyi et al., 2004; Heppelmann and Pawlwak, 1997). The release of other mediators of inflammation such as prostaglandins, nerve growth factor and bradykinins, within the synovium also contributes to the development and sustenance of joint inflammation in rheumatoid arthritis (RA) by sensitizing TRPV1 (Raychaudhuri et al., 2011).

It has been shown that $CB_1$ and $CB_2$co-localize with TRPV1, and that an interplay between the endocannabinoid and endovanilloid systems may play a potential role in the modulation of inflammatory responses (Mlost et al., 2018). For instance, anandamide and some of its lipoxygenation products, also function as potent ligands on TRPV1 (Ahluwalia et al., 2003; Starowicz et al., 2013). Depending on whether or not cyclic adenosine monophosphate (cAMP) signaling pathway is activated, stimulation of $CB_1$ receptors may either inhibit or potentiate stimulation of TRPV1 by its ligands (Hermann et al., 2003). Because cAMP-dependent

protein kinase A can phosphorylate TRPV1 and enhance its sensitivity, it is possible that increased phosphorylation of TRPV1 during inflammation by protein kinase A can be inhibited by $CB_1$-mediated inhibition of adenylate cyclase (Maione et al., 2006).

In a rat monoiodoacetic acid model of osteoarthritis, the FAAH inhibitor and TRPV1 antagonist, OMDM198, inhibitor reversed some of the monoiodoacetic acid effects on the spinal cord. In that study, OMDM198 exclusively upregulated the expression of $CB_1$ on the ipsilateral side of the spinal cord, but did not affect $CB_2$ expression. Moreover, OMDM198 significantly inhibited the expression of *Mapk14* and *Prkcg* mRNA compared to non-diseased controls, in the ipsilateral side of the spinal cord (Mlost et al., 2018). Thus, dual regulation of endocannabinoid system and endovanilloid receptors may provide a very useful alternative in multi-drug therapy for osteoarthritis. Fig. 3 summarizes the hypothetical mechanisms of action underpinning the potential therapeutic relevance of OMDM198 in osteoarthritis.

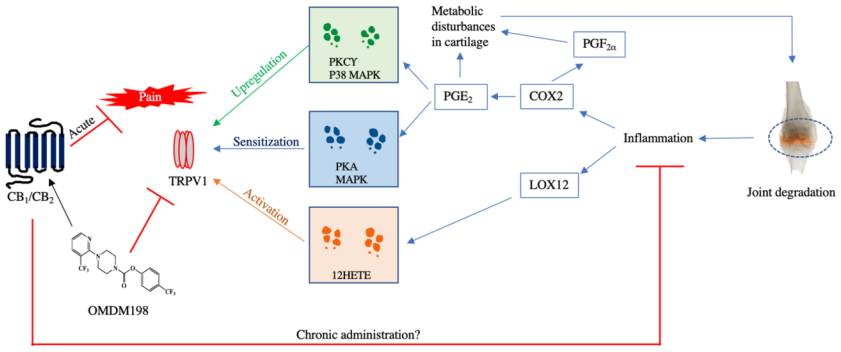



Figure 3. Schematic representation of the proposed molecular mechanism underlying the therapeutic potential of OMDM198 in the treatment of osteoarthritis

### 7.0 Cannabinoid signaling and skin inflammation

Inflammatory skin conditions such as acne, allergic contact dermatitis (ACD), dermatomyositis, psoriasis and scleroderma are associated with substantial systemic burden, and social and psychological effects. The negative psychological effects may impact greatly on the patient's quality of life, especially when pain and pruritus are present (Scheau et al., 2020). Fibrotic changes that occur in the course of these diseases, may result in permanent scarring, thus increasing the disease burden (Sanclemente et al., 2014; Chovatiya and Silverberg, 2019). Among the recently investigated therapeutic options for the management of inflammatory skin conditions are cannabinoids. As an advantage, transcutaneous administration of cannabinoids avoids first-pass metabolism, and produces a steady and prolonged drug infusion, with minimal adverse effects (Huestis, 2007).

In ACD, primary exposure to allergen results in absorption of the allergen through the stratum corneum and covalent binding of the allergen to keratinocytes in the stratum spinosum, subsequently initiating innate immune responses (Kaplan et al., 2012). Subsequent exposure to the same allergen or similar molecules triggers a delayed-type hypersensitivity reaction mediated by effector T cells and various cytokines and chemokines (Novak-Bilić et al., 2018). This action can be inhibited by cannabinoids. In a study on polyinosinic:polycytidylic acid-induced ACD in human keratinocyte cells, administration of cannabidiol reduced inflammation by inhibiting monocyte chemotactic protein-2, IL-6, IL-8 and TNF-α (Petrosino et al., 2018). *In vitro* , cannabidiol decreases T and B-cell-mediated effects including T-helper 17 responses, in splenocytes and also inhibits, in addition to previously mentioned pro-inflammatory cytokines, IL-17 and interferon (Kozela et al., 2015; Harvey et al., 2014). $CB_1$-mediated downregulation of mast cells, reduction in the production of chemokines such as CCL2, CCL8, and CXL10, and activation of PPARs are possible mechanisms underlying the inhibition of phorbol ester-induced acute inflammation by $CB_1$ agonists (Kim et al., 2018).

Non $CB_1/CB_2$-mediated anti-inflammatory effects of cannabinoids such as topical $\Delta^9$-THC in ACD have also been reported, especially in 2,4-dinitrofluorobenzene-induced ACD (Gaffal et al., 2013). Thus, cannabidiol can suppress inflammatory component of ACD with minimal or no cytotoxic effects.

Macrophages are important players in the inflammatory response to pathogens and tissue damage such as in necrotic tissues (Brancato et al., 2011). When exposed to inflammatory signals, macrophages polarisation is favoured toward the pro-inflammatory M1 phenotype, with subsequent release of numerous cytokines including IL-1, IL-6, IL-8, IL-12 and TNF-$\alpha$ (Duque and Descoteaux, 2014). Activation of $CB_2$ has been shown to inhibit M1 polarisation and also enhance polarisation toward the anti-inflammatory M2 phenotype (Luo et al., 2018). This argument, however, opens the door to questions on the role of $CB_2$ and M2 macrophages in fibrosis, since activation of $CB_2$ inhibits fibrosis (Li et al., 2016; Wang et al., 2016; Tang et al., 2018) whereas M2 activation promotes fibrosis (He et al., 2013; Duru et al., 2016). Additional studies in this area is thus needed to fully elucidate the relation between $CB_2$, M2 macrophages and the development of fibrosis. In a study to evaluate the protective effects of cannabinoids against neuroinflammation using a murine controlled cortical impact model of traumatic brain injury, Braun et al. (2018) observed a significant upregulation of $CB_2$ receptor within infiltrating myeloid cells after 72 hours. The team also observed that selective activation of $CB_2$ inhibited M1 polarisation and reduced cerebral oedema. Administration of a $CB_2$ receptor antagonist effectively reversed $CB_2$-mediated neuroprotection and worsened outcomes (Braun et al., 2018).

In psoriasis, the administration of $\Delta^9$-THC and CBD inhibit the proliferation of keratinocyte and modulate associated inflammation by promoting the conversion of Th1 lymphocytes to the anti-inflammatory Th2 phenotype (Sheriff et al., 2019). These effects, however, are independent of CB receptors, and appears to be mediated predominantly by PPAR$\gamma$ (Wilkinson and Williamson, 2007). Arachidonoyl-chloro-ethanolamide, a synthetic $CB_1$agonist, decreases both keratinocyte cell proliferation *in situ*in human skin cultures, and the expression of K6 and K16 in organ cultured human skin samples (Ramot et al., 2013). In a patent (20190060250) filed for psoriasis treatment with topical CBD and cannabigerol, it was showed that these phytocannabinoids dose-dependently inhibits the disease course via the restoration of balance between pro-inflammatory Th1 and anti-inflammatory Th2, and also through the inhibition of inflammatory cytokines and angiogenic growth factors (Changoer and Anastassov, 2019).

Inflammation in localised scleroderma, caused by excessive deposition of collagen, displays a particular profile of reduced Treg function, decreased Th17-derived cytokines such as IL-17, IL, 22 and IL-23, and increased chemokine (C-X-C motif) ligands 9 and 10 (Du et al., 2019). VCE-004.8, a synthetic cannabinoid, has been shown to reduce vascular collagen deposits and prevent macrophage infiltration and fibroblast migration in mouse models of scleroderma. Activation of $CB_2$ appears to mediate the anti-inflammatory component of macrophage infiltration and IL-$\beta$ secretion. Through interaction with Smads, PPAR$\gamma$ inhibits TGF-$\beta$ production, accounting for the antifibrotic effects of VCE-004.8 (del Rio et al., 2016). Another synthetic non-psychoactive $CB_2$ receptor agonist useful in the treatment of scleroderma is ajulemic acid (Burstein, 2018). Evidence from preclinical studies, and Phase 1 and 2 clinical trials shows that anabasum, a synthetic analog of $\Delta^8$-THC-11-oic acid, is a useful alternative in treating scleroderma (Man et al., 2017; Spiera et al., 2017;). Analysing skin biopsies from patients enrolled in these trials revealed that, ajulemic acid decreases inflammation-related genes and extracellular matrix-related genes important for fibrosis, and increases lipid-metabolising genes relevant for the production of pro-resolving eicosanoids (Martyanov et al., 2017).

Not only are topical phytocannabinoids effective in the management of psoriasis, they are effective in decreasing erythema and skin sebum as shown in pre-clinical mouse model of 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced erythema and human trials involving topical application for 12 weeks (Ali and Akhtar, 2015; Dobrosi et al., 2008). Phytocannabinoids safely inhibits the production of sebum and the proliferation of sebocytes and reduce the expression of pro-inflammatory cytokines (Oláh et al., 2016). As observed in psoriasis, the positive effects of cannabinoids appear to be independent of CB receptor activation. For instance, CBD decreases sebum secretion and the proliferation sebocytes through the activation of TRPV1, 3 and 4 activation (Oláh et al., 2014). The anti-inflammatory effects appear to be dependent on $A_{2A}$ adenosine

receptor activation, thus the inhibition of the p65 NF-ϰB pathway upon administration (De Petrocellis et al., 2012). Similar to CBD, cannabichromene and $\Delta^9$-tetrahydrocannabivarin, inhibits excessive sebaceous lipid production and decreases inflammation in SZ95 sebocytes. These non-psychotropic cannabinoids significantly inhibited arachidonic acid-induced acne-like lipogenesis, thus may show significant potential for use as novel agents in acne treatment. In contrast, CBG and cannabigerovarin increase sebaceous lipid synthesis, despite their ability to significantly inhibit inflammation. CBG and cannabigerovarin may therefore be useful in the treatment of dry-skin syndrome (Oláh et al., 2016).

Inflammation associated with dermatomyositis is driven by the activation of lymphocytes and dendritic cells which increases the production of IFNs and complement factors. This subsequently results in inflammatory myopathy, characterised by typical muscle tissue necrosis and regeneration, and may also lead to vasculopathy (Volc-Platzer, 2015). Administration of the $CB_2$ receptor agonists decreases pro-inflammatory cytokine release such as TNF-α, and also the production of IFN-α and IFN-β (Scheau et al., 2020). In a study by Chen et al. (2017), it was identified that lenabasum, a $CB_2$ receptor agonist, decreases CD4 cell populations and downregulates type 1 and 2 IFN activities in lesional dermatomyositis skin. In addition, $CB_2$ receptor agonists have been shown to be safe, tolerable, and efficient thus represents a useful alternate in the treatment of inflammatory skin conditions (Scheau et al., 2020).

Cannabinoid receptor signaling is also implicated in the complex role of inflammation in carcinogenesis. Both the endocannabinoid system and exogenous cannabinoids attenuate inflammation associated with certain skin cancers such as melanoma, squamous cell and basal carcinomas and Kaposi sarcoma (Scheau et al., 2020). In summary, cannabinoids inhibit inflammatory processes associated with skin cancers by inhibiting TGF-β-mediated immunosuppression and tumor growth, TNF-α-mediated cell survival and proliferation, matrix metalloproteinase-mediated enhancement of epithelial–mesenchymal transition and the release of several other mediators of inflammation capable of fuelling carcinogenesis (Candido and Hagemann, 2013; Scheau et al., 2019; Cioni et al., 2019).

**8.0 Conclusion and Future studies**

The cannabinoid system has been shown in several preclinical and clinical studies to be involved in the regulation of inflammatory and immunomodulatory responses. Even though the exact role of the endocannabinoid system in inflammation is not fully elucidated, a pool of evidence points to the potential pharmacological modulation of this system in the treatment or management of several inflammatory disease conditions, including both central and peripheral inflammatory disorders. Although individual variations in patient response to cannabinoid-inclusive therapy have been reported, findings from these studies have largely pointed to the potential successful translation of pre-clinical research to the clinic. Additional well-controlled randomized trials are however required to comprehensively evaluate the true clinical efficacy and long-term risks associated with cannabinoid therapy in inflammatory disorders.

$CB_2$ receptors are primarily expressed on immune cells, and pharmacological targeting of these receptors have been shown to produce selective immunomodulation without profound immunosuppression. Interestingly, $CB_2$ receptor activation is without psychotropic side effects, thus making it a suitable target for pharmacotherapy. Since the psychotropic effects of cannabinoids pose legal, social and therapeutic challenges, future studies may therefore focus on the synthesis or identification of novel molecules with increased affinities for the $CB_2$ receptor for use in inflammatory and autoimmune diseases. In addition, more studies are required to investigate the exact biological effects of the "not-so-popular" endocannabinoids virodhamine, 2-AG ether and N-arachidonoyl dopamine and their possible effects on co-stimulatory molecules, adhesion molecules, chemokines and cytokines, to ascertain their roles in the inflammatory and immunomodulatory process.

Owing to the complexity of the pathophysiology of inflammatory disorders, multi-target drug development and pharmacotherapy strategies may be advantageous compared to single-target therapy as partly evidenced by the effectiveness of OMDM198 over single-target small molecules such as the FAAH inhibitor URB597 and the TRPV1 receptor antagonist SB366791 in the management of osteoarthritis (Mlost et al., 2018).

It has been demonstrated that gut inflammation alters the expression of metabolizing enzymes of the en-

docannabinoid system, resulting in marked changes in the levels of these enzymes in the local environment (Sharkey and Wiley, 2016). Further studies aimed toward identifying genetic and/or epigenetic alterations that affect functioning of the endocannabinoid system, including the effect of glucocorticoid receptors on the regulation of the expression of cannabinoid receptors and*vice versa* , are needed to shed more light on the relation between inflammation and cannabinoid signaling. Notwithstanding, pharmacological targeting of the cannabinoid system demonstrates potential for safe and effective use in the treatment of inflammatory diseases.

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