The Endocannabinoid System and Heart Disease: The Role of Cannabinoid Receptor Type 2

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Abstract

Decades of research has provided evidence for the role of the endocannabinoid system in human health and disease. This versatile system, consisting of two receptors (CB1 and CB2), their endogenous ligands (endocannabinoids), and metabolic enzymes has been implicated in a wide variety of disease states, ranging from neurological disorders to cancer. CB2 has gained much interest for its beneficial immunomodulatory role that can be obtained without eliciting psychotropic effects through CB1. Recent studies have shed light on a protective role of CB2 in cardiovascular disease, an ailment which currently takes more lives each year in Western countries than any other disease or injury. By use of CB2 knockout mice and CB2-selective ligands, knowledge of how CB2 signaling affects atherosclerosis and ischemia has been acquired, providing a major stepping stone between basic science and translational clinical research. Here, we summarize the current understanding of the endocannabinoid system in human pathologies and provide a review of the results from preclinical studies examining its function in cardiovascular disease, with a particular emphasis on possible CB2-targeted therapeutic interventions to alleviate atherosclerosis.

Keywords

Cannabinoids; CB1; CB2; atherosclerosis; ischemia/reperfusion; 2-AG; AEA

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death worldwide, accounting for more than 15 million deaths in 2015. Currently, 92 million adults in the US suffer from some form of CVD, and it is estimated that CVD will account for greater than 800,000 deaths in the US in 2017, with coronary heart disease and its underlying cause, atherosclerosis, being responsible for ~45% of CVD-related deaths [1]. Antidyslipidemic agents and antiplatelet/

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CONFLICT OF INTEREST

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antithrombotic agents remain the two most prominent treatment modalities for CVD. However, ~25% of patients who have one myocardial infarction will have another within five years, despite regular statin therapy. It is believed that this is due to ongoing inflammation within the coronary arteries as inflammation is a driving force in atherosclerosis [2]. Fluctuating levels of endocannabinoid system (ECS) components have been seen in various aspects of cardiovascular disease, including atherosclerosis, myocardial infarction, and heart failure. And, over the past fifteen years, substantial evidence has accumulated demonstrating that the cannabinoid receptor type 2 (CB2) modulates several processes critical in the pathophysiology of atherosclerosis. In this review, we describe the components of the endocannabinoid system, focusing on the key findings and evidence indicating that targeting CB2 has therapeutic potential for the treatment of cardiovascular disease.

THE ENDOCANNABINOID SYSTEM

Ancient texts dating as far back as 15th century China describe the medicinal use of *Cannabis* for the treatment of a wide variety of human ailments, from headaches to sexually transmitted diseases to cancers [3, 4]. The medicinal properties of *Cannabis* are attributable to the unique aryl-substituted meroterpenoid compounds, called cannabinoids, produced in the *Cannabis* genera of plants [5]. Over 50 years ago, researchers at the Hebrew University of Jerusalem isolated and characterized the first cannabinoid, (−)delta-9-tetrahydrocannabinol (THC), the main psychoactive component of marijuana [6, 7]. Since then, more than 100 different cannabinoid compounds have been identified in *Cannabis*, however, only a few have been extensively studied [8]. For the next nearly thirty years, how THC (and other cannabinoids) produced specific biological effects was unknown, with most speculating that the hydrophobic nature of cannabinoids resulted in nonspecific interactions with cell membranes. However, in the late 1980s, evidence accumulated supporting a specific signaling mechanism for cannabinoids and when two membrane-bound cannabinoid receptors were identified, cloned and characterized in the early 1990s the mystery was solved [9, 10]. These cannabinoid receptors, and their later discovered endogenous ligands (endocannabinoids) and metabolic enzymes, collectively make up the ECS. Preclinical and clinical studies have shown that the ECS functions in a wide variety of physiological and pathological processes; with alterations in both receptor expression and endocannabinoid levels being associated with neurological conditions, metabolic disorders, cardiovascular diseases, as well as some cancers [11, 12].

Cannabinoid Receptors

The first cannabinoid receptor identified, and later termed as cannabinoid receptor type 1 (CB1), was independently cloned from rat and human brain cDNA libraries in 1990 [9, 13]. Shortly thereafter, another cannabinoid receptor, termed as CB2, was cloned from HL60 cells, a human promyelocytic leukemia cell line [10]. CB1 and CB2 are products of distinct intronless genes (*CNR1* and *CNR2*, respectively) with *CNR1* located on chromosome 6 in humans and chromosome 4 in mice, and *CNR2* located on chromosome 1 in humans and chromosome 4 in mice. Both are members of the G-protein coupled receptors (GPCR) superfamily of cell membrane receptors, containing seven transmembrane spanning domains

separated by three intracellular and three extracellular loops, a glycosylated extracellular N-terminus and an intracellular C-terminus. Overall, human CB1 and CB2 share 44% amino acid similarity, with 68% homology within their transmembrane domains [10]. Both receptors are relatively highly conserved among mammals, for example, CB2 sequence identity between humans and rodents is 80%.

CB1 is abundantly expressed by neurons in the brain, most notably in the cerebral cortex, basal ganglia, cerebellum, and hippocampus [9] where it is responsible for the psychotropic effects of THC. CB1 is also expressed at lower levels in parts of the peripheral and autonomic nervous system, as well as several other tissues, including heart, lung, thymus, spleen, and reproductive organs [14–16]. Expression of CB1 has also been noted in immune cells, but at levels up to 100 fold lower than CB2 [17]. Although CB1 has been shown to play a role in modulating nociception, anxiety, energy metabolism and lipogenesis, the potential for undesirable psychotropic effects has greatly hindered the development of CB1-targeted pharmacologic therapies. To date, only one CB1-selective therapy has ever gained approval for use in humans. Rimonabant, a CB1-selective antagonist developed by Sanofi-Aventis, was approved in Europe in 2006 as an anti-obesity drug [18–23]. In 2008, despite producing sustained weight loss and improving some cardiovascular risk factors (HDL-cholesterol, triglycerides and HbA1C) beyond that expected from weight loss alone, Rimonabant was withdrawn from use after reports of depression and suicidality, most likely arising from the effects on CB1 in the CNS. Concerns over serious adverse psychological side effects prevented the FDA from ever granting approval of Rimonabant for use in the United States.

CB2 is mainly expressed by cells of the peripheral immune system where it mediates the immunosuppressive effects of THC [24, 25]. Expression of CB2 by immune cells varies (B lymphocytes> natural killer cells >macrophages, monocytes neutrophils>T-cells) [10, 26, 27] and can be greatly affected by the differentiation and activation state of the cell. For example, macrophages activated by thioglycollate or interferon gamma (IFN-γ) express significantly more CB2 than unstimulated macrophages [28]. Generally, the expression of CB2 by non-immune cells is very low, but it has been observed in several other cell types including osteogenic cells [29], cardiomyocytes [30], fibroblasts [31], endothelial cells [32], and vascular smooth muscle cells (VSMCs) [33]. Expression of CB2 in the CNS is somewhat controversial, as some studies failed to detect CB2 mRNA in neurons by Northern blotting and *in situ* hybridization analysis [10], while others found very low levels using quantitative PCR analysis [34], although expression by hematopoietic-derived microglial cells, rather than neurons, could not be ruled out [34]. Furthermore, a number of immunostaining studies have reported low levels of CB2 in some regions of the brain [35–39], however, inconsistent results obtained with CB2 antibodies from different sources, as well as similar staining patterns observed in sections from CB2 knockout mice, make interpretation of these studies tenuous [24, 40]. Confirmation of CB2 expression in the CNS awaits better, more specific CB2 antibodies or other methods capable of specific CB2 detection.

Cannabinoid Receptor Signaling—CB1 and CB2 were initially characterized as GPCRs that couple primarily to pertussis toxin sensitive $G_{i/o}$ signal transduction proteins

and, when activated, inhibit adenylate cyclase to reduce intracellular cyclic AMP (cAMP) levels and modulate downstream cascades under the control of protein kinase A (PKA). CB1 was also found to inhibit N-type Ca^{2+} channels in neurons to reduce neurotransmitter release and L-type Ca^{2+} channels in VSMCs to induce relaxation and vasodilation [41, 42]. In addition to affecting cAMP/PKA and Ca^{2+} signaling, emerging evidence indicates that cannabinoid receptors, and CB2 in particular, modulate multiple diverse signaling networks in a variety of cell types, including the mitogen-activated protein kinases/extracellular signal–regulated kinases (MAPK/ERK1/2), phosphoinositide-3-kinase–protein kinase B/Akt (PI3K-PKB/Akt), phospholipase C, Janus kinase and Signal Transducer and Activator of Transcription (JAK/STAT) and nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) pathways [43–46]. These signaling pathways allow for CB2-mediated modulation of important cellular functions, most notably proliferation, migration, and survival of immune cells. The net result of activating CB2 signaling is an immunosuppression that may provide a therapeutically beneficial effect on diseases with an inflammatory component.

Endocannabinoids

The cloning of CB1 and CB2 led to the discovery of endogenous cannabinoids, or endocannabinoids, that act as ligands of CB1 and CB2, including N-arachidonoylethanolamide (anandamide, AEA) [47], 2-arachidonoylglycerol (2-AG) [48, 49], 2-arachidonyl glyceryl ether (2-AGE, noladin ether) [50], O-arachidonoyl-ethanolamine (OAE, virodhamine) [51] and N-arachidonoyl dopamine (NADA) [52]. Endocannabinoids are bioactive lipid mediators that share a common backbone structure resulting from their synthesis from membrane phospholipid precursors containing arachidonic acid. Of these, AEA and 2-AG are considered to be the primary endogenous activators of CB1 and CB2 and are the best studied, while the functions and physiological roles of the others are unclear and require further investigation. AEA is a high affinity partial agonist of CB1 [41, 53–55] and a weak partial agonist of CB2 [56]. 2-AG is a full agonist of both CB1 and CB2 although it has only moderate affinity [49, 56, 57]. Compared to other tissues, AEA and 2-AG levels are significantly higher in brain, however, detectable levels of both are found in other organ systems, including bone marrow, kidney, liver, spleen, plasma, adipose, and gut, with 2-AG being more abundant than AEA in most tissues [58].

Metabolism of Endocannabinoids—We summarized the synthesis and breakdown of the primary endocannabinoids, AEA and 2-AG, in Fig. (1). Generation of AEA and 2-AG has been shown for only a limited number of cell types, most notably in stimulated neurons, platelets and macrophages [59]. Both AEA and 2-AG are synthesized and released only on demand after an appropriate stimulation produces an elevation of intracellular Ca^{2+} concentrations, and in most cases, uptake and intracellular degradation rapidly terminates their activity. Synthesis of AEA mainly occurs as a two-step process, beginning with the formation of N-arachidonoyl-phosphatidylethanolamine (NAPE) from phosphatidylethanolamine by the action of a Ca^{2+}-dependent N-acyltransferase [60]. AEA is then directly generated by the hydrolysis of NAPE by the action of a Ca^{2+}-sensitive NAPE-specific phospholipase D (NAPE-PLD) [61, 62]. Studies conducted in NAPE-PLD deficient mice revealed multiple NAPE-PLD-independent pathways may also contribute to the

formation of AEA [63, 64]. The synthesis of 2-AG is seemingly less complex, involving a two-step process where sn-1-acyl-2-arachidonoylglycerols (DAG) are first generated by phospholipase C-mediated hydrolysis of phosphatidyl-inositols and then converted to 2-AG by the action of one of two Ca^{2+}-sensitive sn-2-selective DAG lipases (DAGLα and DAGLβ) [62, 65]. Deletion of one of the two DAG lipase genes (DAGLα) gene in mice resulted in similar body mass, metabolic and behavioral phenotypes as mice lacking CB1, further illustrating the prominent role 2-AG and CB1 play in the CNS to modulate food intake, energy balance and metabolic homeostasis [66].

Two enzymes are primarily responsible for the rapid turnover of endocannabinoids; fatty acid amide hydrolase (FAAH), an integral membrane enzyme that metabolizes AEA to free arachidonic acid and ethanolamine, and monoacylglycerol lipase (MAGL) which metabolizes 2-AG to free arachidonic acid and glycerol [67–71]. FAAH has also been reported to metabolize 2-AG, at least under some conditions [72] as well as other related fatty acid amides with diverse biological functions and mechanisms of action. Defects in FAAH can cause AEA to build up to levels many fold higher than normal and FAAH-deficient mice have elevated levels of AEA and other fatty acid amides, and display a CB1 receptor-mediated hypoalgesic phenotype [73, 74].

The general lack of overlap in the metabolism pathways of AEA and 2-AG suggests that their levels can be modulated independently from each other, thus allowing differential effects to be exerted in different systems and even within a specific tissue. In recent years, specific inhibitors of these pathways have been developed to evaluate if altering the endogenous levels of either AEA or 2-AG might produce therapeutic effects similar to cannabinoid receptor agonists and antagonists. These preclinical studies have primarily focused on small molecule inhibitors of the hydrolytic enzymes, DAGL, MAGL and FAAH (reviewed in [75, 76]). DAGL inhibitors, which reduce 2-AG synthesis, have shown promise in preclinical studies as potential ant obesity drugs [77]. FAAH inhibitors, which mainly prevent degradation of AEA, have demonstrated therapeutic potential in models of neuropsychiatric conditions (*e.g.*, anxiety, depression, pain) as well as conditions with prominent inflammatory components (*e.g.*, arthritis, colitis, multiple sclerosis, atherosclerosis) (reviewed in [78–83]). Similar to the effects of CB1 agonists, blockade of 2-AG degradation with selective MAGL inhibitors produces anti-nociceptive, anti-anxiety, and anti-nausea effects in rodents. MAGL inhibitors have also shown potential as anti-cancer drugs *via* endocannabinoid-dependent mechanisms and by reducing the production of arachidonic acid for eicosanoid synthesis. (reviewed in [84]). Blockade of MAGL and FAAH have been reported to not cause robust cannabinoid behaviors in rodents [85], giving hope that the clinical development of these inhibitors may avoid the adverse psychiatric side effects that limited the therapeutic success of CB1 receptor antagonists. Lastly, the transport mechanisms for the uptake and reuptake of AEA and 2-AG are not completely understood, however, evidence indicates that facilitated diffusion by a protein transporter is likely to occur as are intracellular transport mechanisms involving fatty acid binding proteins [62, 86–88].

Non-CB1/CB2 Receptors for Cannabinoids

Both endocannabinoids and some synthetic cannabinoids have been found to interact with non-CB1/CB2 GPCRs, deorphanized receptors, peroxisome proliferator-activated nuclear receptors (PPARs), as well as some ion channels. GRP55 was first identified as an orphan GPCR present in the brain in 1999 [89]. Despite a low sequence identity with CB receptors (13.5% to CB1, 14.4% to CB2), GRP55 reportedly binds AEA with potencies similar to CB1 and CB2, resulting in oscillations in calcium concentration in HEK293 cells [90, 91]. Similarly, 2-AG binds GRP55 but without effect on calcium mobilization [90]. THC is also a potent agonist of GRP55, producing a moderate increase in intracellular calcium in HEK293 cells and in mouse dorsal root ganglia [90]. In contrast, other studies found that neither AEA or THC activate GRP55, as evidenced by the use of internalization assays to study the relationship between GRP55 and CB ligands [92].

The transient receptor potential vanilloid-1 (TRPV1) ion channel, the first receptor cloned for capsaicin [93], was shown to bind to AEA, and to a much less common endocannabinoid, N-arachidonoyl dopamine [94, 95]. Later, 2-AG and other monoacylglycerols were also found to be endogenous activators of TRPV1 [96] and several synthetic cannabinoids, JWH-015 [97] and WIN55,212-2, were shown to activate TRPV1 *in vivo* [98]. Activation of TRPV1 produces a variety of responses (fluctuation in calcium levels, neuronal cation current induction, release of peptides from sensory nervous tissue) depending on the location, cell type and functional assay used [99]. PPARs are a family of ligand-activated transcription factors, some of which can act as general lipid sensors and are not activated by a single endogenous ligand [100]. *In vitro* experiments have provided evidence that some cannabinoids are agonists for at least one of the three PPAR subtypes ($\alpha,\beta/\delta,\gamma$) including AEA [101, 102], 2-AG [102–104], and THC [101, 105], albeit with potencies that are much lower than for CB1 and CB2. The number of relevant studies of non-CB1/CB2 receptors is limited in humans and animals, but the potential for these receptors to modulate CB signaling represents new therapeutic opportunities worthy of further investigation. For comprehensive reviews of these non-CB1/CB2 receptors see DePetrocellis et al [106] and Pertwee et al [99].

CB1/CB2 as Pharmacologic Targets

Initially, CB1 was the main target for developing cannabinoid-based therapeutic interventions, due mainly to its prominent role in neuromodulation of pain, food intake and energy metabolism. Several agonists of CB1, as either medicinal cannabis extracts like sativex, pharmaceutical formulations of THC like dronabinol, or synthetic derivatives of THC like nabilone and nabiximols, proved therapeutically useful in the treatment of emesis due to cancer chemotherapy, stimulation of appetite in AIDS or tumor cachexia, as well as in the management of neuropathic pain and symptoms of multiple sclerosis (see review [107]). A few CB1 antagonists (Rimonabant and Taranabant) also emerged as potential therapies, most notably for the treatment of obesity and associated metabolic dysregulation [108–110]. However, as discussed earlier, the therapeutic application of CB1 antagonism was accompanied by increased risks for major psychiatric side effects (depression and suicidality) and their clinical development was halted. Currently, further understanding of

the ECS is needed to develop new CB1-targeted therapeutic approaches that may avoid adverse psychiatric side-effects.

More recently, the prospect for CB2-selective ligands to produce beneficial effects while avoiding deleterious CB1-mediated psychotropic effects spawned numerous investigations of the role of CB2 in human pathologies. An exhaustive summary of these studies and the therapeutic potential of CB2 in human diseases is beyond the scope of the current review and several excellent reviews have been published [111–114]. The remainder of this review focuses on the accumulated evidence indicating a therapeutic potential for targeting CB2 to alter the progression of atherosclerosis, reduce acute ischemic events, and lessen the impact of cardiovascular disease.

CB2-deficient Mouse Models

Two different strains of CB2 knockout mice, genetically engineered to lack CB2 expression, were developed in the early 2000's for use as *in vivo* tools to access the function of CB2 in normal development and physiology, as well as in various disease states. The first CB2 knockout mouse (Cnr2^{tmZim}) was generated by Buckley *et al.* in chimeras of 129 (H-2b) and C57BL/6 (H2-b) mice using homologous recombination to eliminate part of the intracellular loop 3, transmembrane domains 6 and 7, and the carboxyl terminus to inactivate the *CNR2* gene [24]. Cnr2^{tmZim} mice have normal CB1 expression and function and display no morphological differences between wild type counterparts. At a cellular level, one study found that Cnr2^{tmZim} mice present with abnormal development of some subsets of T and B cells [115], however, spontaneous development of any observable immune disorder has never been reported in these mice. These mice were subsequently crossed for more than 10 generations to C57BL/6J mice to generate a congenic strain on the C57 background.

A second CB2 knockout mouse was generated by Deltagen (Cnr2^{tm1Dgen}) and is available from Jackson Labs (Bar Harbor, ME). Inactivation of *CNR2* was achieved by homologous recombination in E14 stem cells from129P2/OlaHsd mice followed by at least 5 generations of backcrossing with a mixed C57BL/6J;C57BL/6N background. However, it should be noted that strain-specific genetic effects can still be observed even after extensive backcrossing and in one study comparing Cnr2^{tmZim} and Cnr2^{tm1Dgen}mice, it was found that GRP55 signaling was impaired only in Cnr2^{tmZim} mice [116]. Thus, the unique genetic composition of these two CB2 knockout strains lines makes it somewhat precarious to compare similar studies performed using the different CB2 knockout models. Additionally, since Cnr2^{tmZim} mice have the ability to generate a truncated CB2 message due to the presence of an intact *CNR2* promotor and N terminus, it is possible that GRP55 signaling is affected by a dysfunctional CB2 message or signal [24,116]. Despite this caveat, both CB2 knockout models are widely used in numerous disease models and both strains have been instrumental in defining the role of CB2 in cardiovascular disease.

Synthetic Ligands of CB2

The use of synthetic ligands has also been an important tool in advancing our knowledge in how CB2 signaling affects a variety of disease states. For a comprehensive review of the

chemical properties of these compounds, see [117, 118]. A list of the CB2-specific ligands discussed in this review can be found in Table 1.

IMPLICATIONS OF CB2 IN INFLAMMATORY DISORDERS

The potential for the ECS to have an immunomodulatory role through the targeting of CB2 has gained much interest in several inflammatory conditions. Several *in vitro* and *in vivo* studies have provided evidence to support an anti-inflammatory role for CB2 in several inflammatory conditions. Administration of CB2-specific ligands exerts anti-inflammatory effects on various immune cells by downregulating cytokine release [127–129] and reducing production of reactive oxygen species [130]. The beneficial immunomodulatory role for CB2 has been examined in several acute inflammatory conditions, including dinitrofluorobenzene-induced hypersensitivity [131], LPS-induced interstitial cystitis [132], sepsis [133], traumatic brain injury [134], and experimental autoimmune encephalomyelitis [135]. In these studies, mice lacking a functional CB2 receptor developed a worsened inflammatory state, characterized by increased leukocyte infiltration and pro-inflammatory cytokine release [131, 136]. Conversely, activation of CB2 by administration of an exogenous agonist reduced the production of pro-inflammatory cytokines and migration of immune cells in animal models of acute inflammation [132–135].

The potential for CB2 modulation in inflammatory conditions extends beyond acute inflammatory disorders. CB2 also exerts beneficial effects in animal models of chronic inflammatory illnesses, such as rheumatoid arthritis [137, 138], collagen-induced arthritis [139], inflammatory bowel disease [140–145], amyotrophic lateral sclerosis ([146, 147]), and atherosclerosis (as reviewed here).

CB1/CB2 IN CARDIOVASCULAR DISEASE

Atherosclerosis

Atherosclerosis is a chronic inflammatory disease characterized by the presence of plaques, composed of lipids and other cellular debris, within arterial walls. The most common way to study atherosclerosis *in vivo* is by use of mice that have genetic modifications resulting in hypercholesterolemia. While almost ten different murine models of atherosclerosis exist, the most widely used are knockout mice that are deficient in either the low-density lipoprotein (LDL) receptor (Ldlr$^{-/-}$) or apolipoprotein-E (ApoE$^{-/-}$), the first gene-knockout mice shown to develop atherosclerosis coupled with severe hypercholesterolemia. The major differences between these two strains are the induction of atherosclerotic disease and the pace of progression, as reviewed by Garcia et al [148]. Briefly, ApoE$^{-/-}$ mice are severely hypercholesterolemic from birth while Ldlr$^{-/-}$ mice only have mildly elevated circulating cholesterol levels. On a standard chow diet, ApoE$^{-/-}$ mice develop quantifiable lesions within six weeks, and lesion progression can be exacerbated by the addition of a high-fat cholesterol-rich atherogenic diet, which is a common practice. On the other hand, Ldlr$^{-/-}$ mice will not develop quantifiable lesions for at least six months on a standard diet, as the extent of disease is mostly correlated with the degree of hypercholesterolemia. Most studies involving Ldlr$^{-/-}$ mice are conducted using an atherogenic diet to induce the formation of atherosclerotic lesions. A common atherogenic diet used for both of these models is the

"Western" diet, which is typically formulated to contain 21% butterfat and 0.2% cholesterol, although cholesterol levels may vary [149].

Atherosclerotic plaque formation begins when LDL particles become retained in the subendothelial space of arteries, leading to the activation of overlying endothelial cells. Upregulation of endothelial adhesion molecules, especially Vascular Cell Adhesion Molecule 1 (VCAM-1) and Intercellular Adhesion Molecule 1 (ICAM-1), allows for increased recruitment and transendothelial migration of blood monocytes into the intimal layer [150] (Fig. 2). This process begins a cycle of inflammation, recruitment of immune cells and other cellular components, and phenotypic changes of resident cells, ultimately leading to the formation of a fibrous, fatty plaque over the course of decades [151–154].

Clinical and experimental data emerged indicating that local and systemic elevations of endocannabinoid levels were strongly associated with atherosclerosis, suggesting that endocannabinoids might be risk factors for, or represent biomarkers of, ongoing vascular disease. Increased systemic EC levels are present in patients with coronary artery disease [155] and Montecucco *et al.* observed EC to present along with CB1 and CB2 in human carotid plaque specimens [156]. The first experimental evidence indicating a role for CB2 in atherosclerosis came from a study by Steffens *et al.* in 2005, demonstrating that ApoE$^{-/-}$ mice given a low oral dose of THC developed significantly smaller plaques with fewer macrophages than untreated control mice [57]. They further showed that the size and macrophage content of plaques reverted back to that of untreated controls when the mice were co-administered a CB2-selective antagonist, SR144528, along with THC, indicating that the anti-atherosclerotic effects of THC were likely mediated by CB2. Importantly, the observed anti-atherosclerotic effects were achieved with doses of THC below that known to elicit undesirable psychotropic side effects, a major concern for the development of any cannabinoid-based therapy. Since the pioneering study by Steffens et al., approximately one dozen *in vivo* studies have investigated the effects of either CB2 gene deletion or pharmacologically targeting of CB2 in murine models of atherosclerosis (Table 2). Although there were some contradictory conclusions among the early studies of the effects of CB2 gene deletion on atherogenesis [157–160], most likely arising from differences in the genetic backgrounds of the CB2 knockout mice strains (Cnr2^{tmZim} vs Cnr2^{tm1Dgen}), the atherosclerosis-prone murine models (ApoE$^{-/-}$ vs Ldlr$^{-/-}$), and the methods of analysis used in the studies, the accumulating evidence from *in vivo* and *in vitro* studies has coalesced into a consensus interpretation that CB2 signaling modulates the formation and progression of atherosclerotic plaque.

CB2 expression was found to be upregulated during macrophage activation [28, 33] and to be present in atherosclerotic plaques of humans and mice but not in adjacent, non-diseased areas of the same vessels [57]. The synthetic cannabinoid, WIN55,212-2, a mixed CB1/CB2 agonist, was shown to produce anti-atherosclerotic effects in mice similar to THC. WIN55,212-2 treatment not only reduced plaque size and macrophage content in ApoE$^{-/-}$ mice but also reduced expression of VCAM-1, ICAM-1, and P-selectin. WIN55,212-2 also reduced the expression of proinflammatory genes and NF-κB activation within aortas of ApoE$^{-/-}$ mice and attenuated the proinflammatory response of isolated peritoneal macrophages to treatment with oxLDL. All of these effects were abated by treatment with a

CB2-selective antagonist, AM630, suggesting that the anti-atherosclerotic effects of WIN55,212-2 were mediated by CB2 [161, 162]. In a similar study conducted using a different murine model of atherosclerosis, Ldlr$^{-/-}$ mice, Netherland Van Dyke et al. showed that intraperitoneal injections of WIN55,212-2 reduced plasma triglycerides and decreased macrophage accumulation in aortic root plaques. The observation that these beneficial effects of WIN55,212-2 were absent in CB2$^{-/-}$Ldlr$^{-/-}$ mice verified they were mediated through CB2-dependent mechanisms [163]. These results are consistent with a previous study showing that systemic CB2-deletion resulted in increased macrophage infiltration in hyperlipidemic Ldlr$^{-/-}$ mice [157]. An *ex vivo* study by Chiurchiu et al. provided further evidence of a role for CB2 in modulating human atherosclerosis [164] by showing that human-derived foam cells treated with JWH-015 produced less TNF-α, IL-10, and IL-12 after stimulation with LPS compared to untreated cells. They further demonstrated that JWH-015 treatment of human derived foam cells lowered the expression of CD36, the scavenger receptor largely responsible for the uptake of oxLDL during foam cell formation. When the treatments were performed in the presence of a CB2-specific antagonist, SR144528, these antiinflammatory and anti-atherogenic effects of JWH-015 on human derived foam cells were prevented, indicating they result from CB2-dependent mechanisms. Together, these studies demonstrate that CB2 activation reduces macrophage infiltration, accumulation, and progression of foam cell formation in the setting of atherogenesis, while also dampening the inflammatory response.

VCAM-1 is a necessary component of transendothelial migration of leukocytes and a reduction in its expression on the endothelium hinders plaque progression by limiting the rate at which monocytes migrate into the subendothelial space [165, 166]. Several *in vitro* studies have confirmed the inhibitory effect of CB2 activation on macrophage migration and expression of cell adhesion molecules. Treatment of human coronary artery endothelial cells (HCAECs) with highly selective CB2 agonists, HU-308 and JWH-133, blunted TNFα-induced expression of VCAM-1 and ICAM-1 [167]. Similar results were seen in human umbilical vein endothelial cells (HUVECs) cultured in the presence of WIN55,212-2 [162]. In both studies, a role for CB2 in mediating the reduction of monocyte/endothelial cell adhesion in response to the synthetic cannabinoid was confirmed by the ability of a CB2-selective antagonist to block the effects. Monocyte chemoattractant protein 1 (MCP1) is a key chemokine that is secreted in response to proinflammatory cytokines and plays a strong role in selectively regulating migration and infiltration of monocytes/macrophages into atherosclerotic plaques [168, 169]. Steffens *et al.* found that THC inhibited MCP1-stimulated migration of isolated macrophages and that the effect was blocked by SR144528 and absent in macrophages isolated from CB2-deficient mice [57].

VSMC proliferation and migration are understood to play a vital role in establishing a stable plaque phenotype. Several studies provide evidence that these key events are influenced by CB2 activation. In the first study examining the consequences of CB2 gene deletion on atherosclerosis in a murine model, Netherland *et al.* observed that systemic CB2 deficiency increased SMC content within atherosclerotic plaques of Ldlr$^{-/-}$ mice [157]. TNF-α is a proinflammatory cytokine known to play a vital role in atherosclerosis by stimulating monocyte recruitment as well as SMC proliferation and migration [170–172]. Rajesh *et al.* demonstrated that CB2-selective agonists JWH-133 or HU-308 reduced TNFα-stimulated

proliferation and migration of human coronary artery smooth muscle cells, *in vitro*, by diminishing the activation of the MAPK pathway [33]. This effect was blocked by co-administration with CB2 antagonists, AM630 and SR144528, but was unchanged in the presence of a CB1-specific antagonist. These studies suggest that targeting CB2 may offer a novel approach for altering VSMC proliferation and migration to affect phenotypic changes within established atherosclerotic plaques.

Components of the extracellular matrix (ECM), particularly collagen, play a very important role in maintaining the structural integrity of vessels and plaques [173]. Degradation of the ECM by matrix metalloproteinases (MMPs) leads to the breakdown of collagen fibers, leaving atherosclerotic plaques weakened and more prone to rupture [174]. The most common MMPs implicated in rupture-prone regions of atherosclerotic plaques are MMP-1, MMP-3, and MMP-9 [175, 176] and an inverse correlation exists between the levels of CB2 and MMP-9 content in atherogenic vessels from humans [156] and mice[157]. Further, Netherland *et al.* found that systemic CB2 deficiency decreased collagen content and increased elastin fragmentation in plaques of Ldlr$^{-/-}$ mice after 12 weeks of atherogenic diet, in addition to significantly increasing MMP-9 activity in isolated peritoneal macrophages [157]. Other studies have discovered that MMP-9 activity in immune cells is suppressed by CB2 agonists and induced by CB2 antagonists/inverse agonist [157, 177, 178]. *In vitro* studies have shown that pre-incubation of human primary neutrophils with JWH-133 results in a reduction in TNFα-induced MMP-9 release by a mechanism that is sensitive to CB2 antagonism [156]. It is possible that CB2-mediated increases in MMP-9 are, at least in part, responsible for subsequent increased infiltration of immune cells into the plaque area due to the breakdown of structural components within the luminal endothelium. Interestingly, CB2 deficiency had no effect on collagen content, elastin fragmentation, or MMP-9 activity in plaques of Ldlr$^{-/-}$ mice after 8 weeks of atherogenic feeding [157] suggesting that CB2-dependent regulation of ECM degradation may not come into play in initial stages of plaque development but play a protective role in more advanced lesions.

Table 2. Summarizes studies of the effects of CB2 in murine models of atherosclerosis and *in vitro* studies.

Modulating Endocannabinoid Metabolism—Due to their rapid catabolism, the administration of EC in murine models to evaluate the effects of chronically elevated endocannabinoid levels on atherosclerosis is not feasible. To overcome this limitation, mice lacking either FAAH or MAGL, the enzymes primarily responsible for catabolism of AEA and 2-AG, respectively, were developed. Mice lacking FAAH cannot efficiently degrade AEA and therefore accumulate substantially higher AEA levels in brain and peripheral tissues [73]. FAAH-deficient mice have exaggerated CB1-dependent behavioral responses to AEA, including hypomotility, analgesia, catalepsy, and hypothermia, and also display an anti-inflammatory phenotype [179, 180]. Lenglet *et al.* created FAAH$^{-/-}$ApoE$^{-/-}$ mice to evaluate atherosclerosis in the setting of enhanced AEA levels due to FAAH deficiency [181]. Compared to ApoE$^{-/-}$ mice, FAAH$^{-/-}$ ApoE$^{-/-}$ mice have significantly elevated plasma AEA levels and develop smaller plaques with characteristics strongly associated with increased vulnerability to rupture, including elevated neutrophils, increased MMP-9 expression and lower SMC content. Pharmacological inhibition of FAAH activity in ApoE

−/− mice produced very similar results [182]. The role of cannabinoid receptors in the observed plaque destabilizing effects of FAAH deficiency was not evaluated in these studies. The fact that AEA is a weak partial CB1 agonist that is nearly inactive as a CB2 agonist [48, 56, 183], makes it likely that elevated AEA levels promote the formation of less stable plaque *via* CB1-mediated mechanisms. Conclusive evidence of the role of CB1/CB2 in promoting the vulnerable plaque phenotype resulting from impaired FAAH activity will require additional studies of the effects of either genetic ablation, or pharmacological antagonism, of CB1 and CB2 on the plaque phenotype in FAAH$^{−/−}$ApoE$^{−/−}$ mice and/or ApoE$^{−/−}$ mice treated with a FAAH inhibitor.

In 2011, Taschler *et al.* generated MAGL knockout mice to investigate the pathophysiological consequences of systemic elevation of 2-AG. These mice exhibit substantially less MAGL activity with concomitant increases in 2-AG levels in all tissues examined (white adipose, liver and brain [184]. Vujic *et al.* determined the effects of impaired 2-AG metabolism on atherosclerosis by creating MAGL$^{−/−}$ApoE$^{−/−}$ mice [185]. When fed an atherogenic diet, MAGL$^{−/−}$ApoE$^{−/−}$ mice develop significantly elevated 2-AG levels in plasma and in aortic tissue compared to ApoE$^{−/−}$ mice. *In vitro*, macrophages isolated from MAGL$^{−/−}$ApoE$^{−/−}$ mice displayed an impaired capacity to form foam cells as evidenced by significantly reduced lipid loading and expression of CD36. Somewhat surprisingly, the plaques in MAGL$^{−/−}$ApoE$^{−/−}$ mice were larger than in ApoE$^{−/−}$ mice, even though macrophage and lipid content were slightly reduced. This apparent discrepancy was explained by the observation that collagen and SMC content were both substantially increased in MAGL$^{−/−}$ApoE$^{−/−}$ plaques, resulting in thicker fibrous caps and a more stable plaque phenotype. Thin fibrous caps are strongly associated with atherosclerotic plaques that are susceptible to rupture and thrombus formation [186]. Notably, when MAGL$^{−/−}$ApoE$^{−/−}$ mice were given the CB2-selective inverse agonist/antagonist SR144528, plaques increased in size and became less stable resembling those in ApoE$^{−/−}$ mice, indicating that plaque stabilization in the setting of MAGL deficiency likely results from 2-AG activation of CB2-dependent mechanisms.

In contrast to the studies performed in the setting of reduced systemic MAGL-mediated degradation of 2-AG [182, 185], Jehle *et al.* employed adoptive transfer of bone marrow stem cells to evaluate atherosclerosis under conditions of myeloid-specific deficiency in DAG lipase α (DAGLα), a key enzyme in the biosynthesis of 2-AG [187]. Macrophages isolated from ApoE$^{−/−}$ mice reconstituted with bone marrow from mice lacking DAGLα had greatly reduced DAGLα activity and 2-AG levels compared to wild type macrophages; however, plasma levels of 2-AG were notably unchanged and only slightly reduced in aortic tissue, indicating that macrophage synthesis of 2-AG does not significantly contribute to circulating 2-AG levels in plasma or in aortic tissue. Consistent with the correlation established between decreased 2-AG degradation and enhanced plaque size in studies of MAGL deficiency, myeloid-specific deficiency in 2-AG synthesis correlated with decreased plaque size. Interestingly, systemic MAGL deficiency and myeloid-specific DAGLα deficiency, despite having opposite effects on 2-AG levels and plaque size, were both associated with substantially reduced immune cell infiltration. However, only 2-AG elevation resulting from MAGL-deficiency altered the SMC and collagen content of plaque. Together, these results indicate that macrophage 2-AG exerts a proinflammatory effect on

early lesion formation by accelerating the infiltration of immune cells into the vessel wall, and suggests that as plaques progress, nonmyeloid-derived cells become active to exert plaque stabilizing mechanisms in response to chronic elevation of 2-AG. Table 3 summarizes the effects of cannabinoid metabolism enzymes on atherosclerosis in murine models of atherosclerosis.

Although FAAH and MAGL have substrates in addition to AEA and 2-AG which may contribute to the observed effects on atherosclerosis, these studies provide compelling evidence that alteration of AEA and 2-AG homeostasis affects plaque phenotype. Mechanisms modulating plaque phenotype are clinically very important as they can affect plaque progression from stable to unstable disease conditions [171]. In this regard, the opposite effects on plaque stability imparted by systemic MAGL deficiency and systemic FAAH deficiency strongly suggest that the primary endocannabinoid substrates of these enzymes (2-AG and AEA) are modulators of plaque phenotype, with AEA activation of CB1-dependent mechanisms decreasing plaque stability and 2-AG activation of CB2-dependent mechanisms increasing stability. Alternatively, since AEA is only a weak partial agonist, but still capable of blunting 2-AG activation of CB2, it is possible that AEA acts indirectly on plaque phenotype by modulating 2-AG signaling. Further investigation of the ability of pharmacological activation of FAAH and inhibition of MAGL to reduce plaque vulnerability and thus the risk for an acute clinical event due to plaque rupture is certainly warranted. Another potential therapeutic approach maybe to reduce AEA levels and/or CB1 signaling while simultaneously enhancing 2-AG levels and/or CB2 signaling. As was found during the clinical development of CB1 antagonists, therapeutic targeting of endocannabinoid metabolizing enzymes to enhance endocannabinoid-mediated beneficial effects may also carry the risk for exacerbation of undesirable endocannabinoid-mediated adverse effects.

Taken together, these results support an anti-atherosclerotic role for CB2 activation, at least in part, through modulation of inflammatory processes and biochemical signaling pathways involved in atherosclerotic plaque formation and stability. Further studies could potentially result in a CB2-selective therapy that aims to reduce inflammation and related plaque vulnerability in atherosclerosis. Results from this basic research provide a promising strategy to reduce the burden of atherosclerosis, especially among populations in Western countries where atherosclerosis and associated complications account for the highest cause of death.

Ischemia-Reperfusion Injury

Ischemia-reperfusion (I/R) injury, characterized by inflammation and oxidative damage of tissues following a period of hypoxia, is a condition commonly seen in patients that suffer from a stroke or myocardial infarction (MI). Several preclinical studies have shown that activation of CB2 is cardioprotective after I/R injury. Pretreatment with JWH-133 before ischemic insult resulted in a reduction in infarct size in rats, an effect that was diminished with co-administration of CB2 antagonist AM630 [188]. Infarct area was also reduced in animals pretreated with WIN55,212-2 and cardioprotection was lost when AM630 was co-administered, while no effect was seen with co-administration of a CB1 antagonist [189].

HU-308 reduced infarct size, ROS, and TNFα [190]. Activation of CB2 following ischemic injury also results in a decrease in infarct area as well as reduced neutrophil recruitment [191]. The incidence of ventricular arrhythmias as a result of I/R were reduced in rats treated with non-selective CB agonist HU-210, and this was found to be CB2 dependent as CB2 antagonist SR144528 was able to abolish the antiarrhythmic effect while a CB1 antagonist had no effect [192].

Cardiac remodeling after MI can result in interstitial fibrosis, leading to eventual heart failure due to ventricular stiffening and diastolic/systolic dysfunction. CB2 activation with AM1241 after MI resulted in reduced cardiac fibrosis in the infarcted area in mice [193]. AM1241 administration following MI resulted in decreased synthesis of ECM collagen and increased degradation of ECM in infarcted areas [194], as well as increased activation of cardiac progenitor cells and proliferation of cardiomyocytes [193]. This reduction in cardiac fibrosis mediated by AM1241 led to improved cardiac function, characterized by improved left ventricular ejection fraction and fractional shortening [193]. A few *in vivo* and *ex vivo* studies aimed at determining the mechanism(s) involved in the cardioprotective effects of CB2 activation following I/R have found involvement of CB2-dependent inhibition of TNFα and ROS as well as activation of PI3K/Akt, p38/ERK1/2, and PKC signaling pathways within infarcted myocardium [188, 190, 191, 195]. Quick activation of these intracellular pathways following an ischemic event has shown to be important in cardiomyocyte rescue and thus CB2 may prove to be a beneficial direct target of cardiac cells.

Intra-cardiac remote preconditioning is characterized by brief episodes of ischemic events in one area of the vasculature that protects surrounding tissue from subsequent coronary occlusion and is a well-documented cardioprotective phenomenon [196]. Two major ways ischemic preconditioning is cardioprotective is through reduction of infarct size and the severity of subsequent arrhythmias. Hajrasouliha *et al.* found that CB2 contributes to the cardioprotective effects elicited by remote ischemic preconditioning, where administration of selective CB2 antagonist AM630 abolished the protective nature of preconditioning on infarct size and arrhythmias [197]. Another study found that the cardioprotective effects of heat-stress pre-conditioning were also abolished in the presence of SR144528, with no effect seen in the presence of a CB1-selective antagonist [198]. Lagneux *et al.* found similar results with SR144528 abolishing the cardioprotective actions of lipopolysaccharide pretreatment of rat hearts when administered before and during ischemia [199]. These studies support the need for further investigation of CB2 signaling in order to identify potential therapeutic approach to CB2-dependent cardioprotection during and following ischemia/reperfusion. Table 4 summarizes studies of the effects of CB2 in I/R.

Heart Failure

While many studies have identified a beneficial role for CB2 activation in atherosclerosis and ischemia-reperfusion injury, less is known regarding the function of CB2 in heart failure. Limited research has shown a relationship between the ECS and heart failure, characterized by elevated circulating endocannabinoids in the blood and increased CB2 expression in the left ventricular myocardium of patients with chronic heart failure [200]. Much work must be done in order to identify if CB2 signaling plays a protective or

detrimental role in heart failure and whether or not modulation of CB2 with specific ligands would provide relief for patients suffering with this disease.

Genetic Studies

Identifying associations between genetic polymorphisms and disease is useful in determining the relevance of animal disease models in human pathological conditions. A few single nucleotide polymorphisms (SNPs) in genes that code for ECS proteins have been associated with cardiometabolic risk factors, such as dysregulation in lipid homeostasis [201–205], providing evidence for a potential genetic link between EC signaling and risk of onset or severity of cardiovascular disease. Several SNPs have been identified in the gene encoding CB1, *CNR1*, and are associated with dyslipidemia, insulin resistance, and increased BMI [201, 203–205]. Several SNPs are associated solely with HDL-cholesterol levels, while others are associated with triglyceride and total cholesterol independent of BMI. Polymorphisms in the *FAAH* gene have also been associated with obesity and dyslipidemia [202, 206]. One SNP in particular (rs324420) is associated with a worsened cardiometabolic profile in the setting of diabetes mellitus, affecting weight, insulin resistance, and levels of TNF-α and adipocytokines [207]. Less is known regarding *CNR2* gene variation and association with cardiovascular or cardiometabolic risk. To date, analysis of SNPs in *CNR2* have shown no known association with myocardial infarction, obesity, diabetes mellitus, hypertension, or dyslipidemia [208]. Data collected from human genetics studies thus far suggests a role for the ECS and CB1 in lipid homeostasis and that variants in these genes could promote dyslipidemia, increasing the risk in affected individuals to developing atherosclerotic lesions.

LIMITATIONS OF CANNABINOID-BASED THERAPY

Careful consideration must also be taken when employing cannabinoid-based therapies, as other pathological conditions can be affected by both CB1 and CB2 signaling. For instance, it was previously found that SR144528 blocked acyl CoA: cholesterol acyl transferase activity and lipid loading in macrophages [209]. Increases in intracellular lipid accumulation in hepatocytes have also been observed in response to CB2-selective agonists [210]. Therefore, although CB2 activation is atheroprotective in terms of stabilizing lesions, it may be detrimental in modulating lipid metabolism. Further, cannabinoid receptor manipulation has also been shown to affect the pathophysiology of other diseases, such as rheumatoid arthritis, liver cirrhosis, gastrointestinal disorders, and neurodegenerative diseases [139, 211–213]. Studies will need to be thoroughly conducted in order to identify how CB2-specific compounds used in the treatment of atherosclerosis may affect the pathology of other diseases that could result in beneficial and/or adverse effects. The development of a tissue-specific drug intervention may be necessary to avoid any potential complications arising from systemic CB2 activation.

Several CB1/CB2 agonists are used clinically to treat maladies such as side effects produced by chemotherapy treatment or neuropathic pain. As of 2017, there are 29 states in the U.S. that have approved the use of cannabis for medical purposes, with each state governing its regulation independent of another [214]. The marijuana plant, *Cannabis sativa*, contains over

700 chemical compounds, 104 of which are considered to be unique cannabinoids [215, 216]. The U.S. Food and Drug Administration has not approved marijuana as a "safe or effective drug for any indication," but awareness is heightened regarding research interests in testing marijuana in the treatment of several medical conditions and much support exists for the medical research community studying the effects of cannabinoids. While marijuana appears to be beneficial in some disease states, increasing knowledge is being obtained about the adverse cardiovascular effects of the drug, such as myocardial infarction, cardiomyopathy, stroke, arrhythmias, and sudden cardiac arrest, all which are mediated through CB1 [217]. THC is a partial agonist at CB1 and therefore would not likely produce harmful cardiovascular effects at lower potencies, but over the past 10 years, the potency of THC in marijuana has increased 10-fold, paralleling a substantial rise in severe, and sometimes fatal, adverse cardiovascular effects. Therefore, it appears that the composition of the plant has a large effect on the likelihood of adverse events to occur. Further, poison outbreaks and deaths have been on the rise with the increased use of synthetic cannabinoids, consisting of several CB1 agonists that have an overall potency of up to 200-times greater than that of THC. These drugs, referred to as "designer drugs," have been manufactured to exhibit increased potency over the last 2 years, coinciding with an increase in fatalities [218–221]. Common names for these compounds are Spice, K2, Bombay Blue, and Black Mamba, and these designer drugs are typically composed of CB1 agonist variants such as cannabicyclohexanol, JWH-018, JWH-073, JWH-200, and XLR-11; however, it is estimated that several hundred unknown variants exist [217]. The rise in adverse effects associated with recreational cannabinoid use has made it difficult to expand the clinical indication for marijuana or marijuana-based products in the U.S.

CONCLUSION

Several studies provide evidence to support a role of CB2 signaling and the ECS in disease. Specifically, a large body of evidence exists to support a beneficial role for CB2 through modulation of inflammatory mechanisms. By further delineating the mechanisms involved, advancements in CB2-directed therapy can be made. Current limitations include off-target effects of synthetic compounds due to low receptor specificity as well as an incomplete understanding of the complexities of the ECS. Furthermore, the possibility of having differential effects on different tissues can create complications when targeting a single pathological condition. Because of this, use of CB2 ligands requires careful consideration and a wide scope of analysis. However, the anti-inflammatory and anti-atherogenic effects of CB2 signaling are promising and may make it possible to develop treatments to reduce the burden and mortality seen from complications of heart disease. A more in depth understanding of the cellular and molecular roles of CB2 signaling in heart disease is necessary to identify novel pharmacological targets to slow down or eliminate CVD and/or a broad range of pathologies.

Acknowledgments

LIST OF ABBREVIATIONS

2-AG	2-arachidonoylglycerol
AEA	Anandamide
ApoE	Apolipoprotein E
CB1	Cannabinoid receptor 1
CB2	Cannabinoid receptor 2
DAGL	Diacylglycerol lipase
EC	Endocannabinoids
ECM	Extracellular matrix
ECS	Endocannabinoid system
FAAH	Fatty acid amide hydrolase
ICAM	Intracellular cell adhesion molecule
I/R	Ischemia/reperfusion
LDL	Low-density lipoprotein
MAGL	Monoacylglycerol lipase
MI	Myocardial infarction
MMP	Matrix metalloproteinase
THC	(−) Delta-9-tetrahydrocannabinol
VCAM	Vascular cell adhesion molecule
VSMC	Vascular smooth muscle cell

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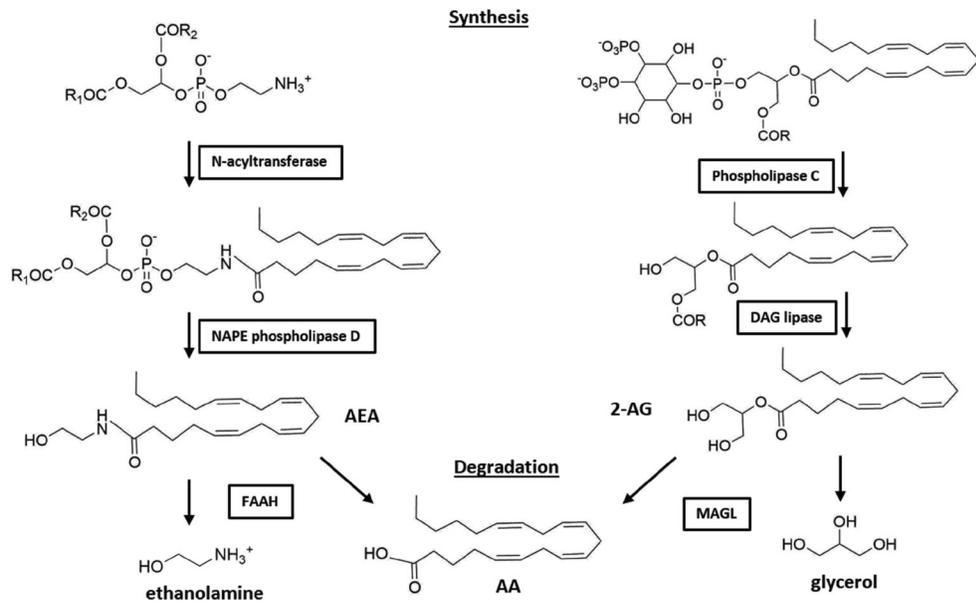
Fig (1).

Metabolism of AEA and 2-AG. Synthesis of both 2-AG and AEA occurs as a two-step process, and degradation of both yields arachidonic acid.
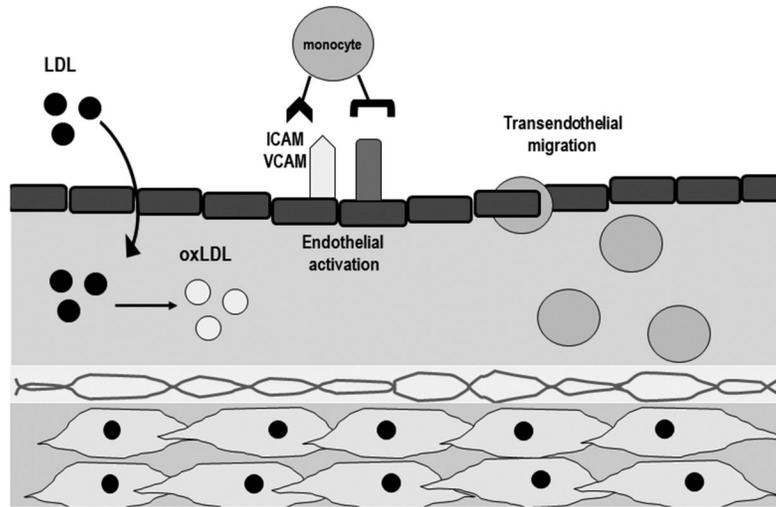
Fig. (2).

Initiation of atherosclerotic plaque formation. LDL is retained in the subendothelial space of the vessel wall where it is subject to modification, such as oxidation to form oxLDL. This process leads to upregulation of ICAM and VCAM on the surface of endothelial cells, leading to increased transendothelial migration of monocytes into the subendothelial space.

Table 1

Synthetic CB2 ligands.

Compound	Function	Human CB2 Ki (nM)	Selectivity over CB1	Refs.
WIN55,212-2	Agonist	3.3	19X	[119]
JWH-015	Agonist	13.8	28X	[120]
JWH-133	Agonist	3.4	200X	[121]
SR144528	Inverse agonist	0.6	>700X	[122]
HU-308	Agonist	22.7	>5000X	[123]
HU-210	Agonist	0.52	N/A	[124]
AM1241	Agonist	2	>100X	[125]
AM630	Inverse agonist	31.2	165X	[126]

Table 2

In vivo and *in vitro* models of CB2 in atherosclerosis.

Model	Treatment	Effect of Genotype and/or Treatment	Ref.
In vivo studies			
ApoE$^{-/-}$CB2$^{+/+}$	THC	↓ plaque size and macrophage content	[57]
Ldlr$^{-/-}$ CB2$^{-/-}$ Ldlr$^{-/-}$CB2$^{+/+}$		↑macrophages, SMCs, and MMP-9 ↓lesional apoptosis and collagen	[157]
ApoE$^{-/-}$ CB2$^{+/+}$	WIN55,212-2	↓expression of VCAM-1, ICAM-1, P-selectin ↓ plaque size, macrophage adhesion and infiltration, and NFκB activation	[162]
ApoE$^{-/-}$CB2$^{-/-}$ ApoE$^{-/-}$CB2$^{+/+}$	JWH-133	↓ plaque formation and ROS levels (CB2$^{+/+}$) Improved endothelial function (CB2$^{+/+}$)	[160]
Ldlr$^{-/-}$CB2$^{-/-}$ Ldlr$^{-/-}$ CB2$^{+/+}$	JWH-133	No effect on plaque development (CB2$^{+/+}$)	[158]
ApoE$^{-/-}$CB2$^{+/+}$	JWH-133	↓MMP9 in plaques	[156]
Ldlr$^{-/-}$ CB2$^{-/-}$ Ldlr$^{-/-}$CB2$^{+/+}$	WIN55,212-2	↓ triglycerides and SMCs (CB2$^{+/+}$ and CB2$^{-/-}$) ↓ macrophages and apoptosis (CB2$^{+/+}$ only) No effect on plaque size or collagen and elastin	[163]
In vitro studies			
HCASMC	JWH-133 HU-308	↓migration and proliferation	[33]
HCAEC	JWH-133 HU-308	↓ICAM-1 and VCAM-1 ↓Monocyte adhesion/migration	[167]
Peritoneal macrophages ApoE$^{-/-}$CB2$^{+/+}$	THC	↓migration	[57]
Human primary neutrophils	JWH-133	↓MMP-9 release	[156]
HUVEC	WIN55,212-2	↓VCAM-1, ICAM-1, p-selectin ↓Monocyte adhesion	[161]

Table 3

In vivo and *in vitro* models of EC metabolism enzymes in atherosclerosis.

Model	Treatment	Effect of genotype and/or treatment	Refs.
ApoE$^{-/-}$FAAH$^{-/-}$		↓plaque size, ↑neutrophil infiltration, ↑MMP9, ↓SMC, ↑vulnerability to rupture	[181]
ApoE$^{-/-}$	URB597	No effect on plaque size or macrophage content, ↓collagen, ↑MMP-9, ↑neutrophil infiltration	[182]
ApoE$^{-/-}$ MAGL$^{-/-}$		↑plaque size, ↓macrophages and lipids, ↑collagen, ↑SMCs, ↑stability ↓foam cell formation *in vitro*	[185]
ApoE$^{-/-}$ DAGLα$^{-/-}$ (myeloid-specific)	Adoptive transfer	↓plaque size, ↓immune cell infiltration, no effect on SMCs or collagen	[187]

Table 4

The role of CB2 in ischemia/reperfusion.

Treatment	Administration	Effect	Citation
JWH-133	Before ischemia	↓infarct size	[188]
WIN55,212-2	Before ischemia	↓infarct size	[189]
HU-210	Before ischemia	↓ventricular arrhythmia	[192]
JWH-133	After ischemia	↓infarct size, ↓neutrophil infiltration	[191]
AM1241	After ischemia	↓infarct size, ↑activation cardiac progenitor cells, ↑ cardiomyocyte proliferation, ↑cardiac function	[193]
AM1241	After ischemia	↓collagen deposition in infarct area	[194]
HU-308		↓infarct size, ↓ROS, ↓TNFα	[190]
AM630	Before preconditioning	Abolished protection of preconditioning (↑ infarct size, ↑arrhythmia)	[197]
SR144528	After precondition, before/during ischemia	Abolished protection of precondition (↑ infarct size)	[198]
SR144528	After precondition, before/during ischemia	Abolished protection of precondition (↑ infarct size)	[199]