The Role of the Endocannabinoid System in Atherosclerosis

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The endocannabinoid system

The endocannabinoid system (ECS) comprises two distinct membrane receptors that have been identified by molecular cloning, CB₁ and CB₂, their endogenous ligands (named endocannabinoids) such as anandamide (AEA) and 2-arachidonoylglycerol (2-AG), as well as enzymes for ligand biosynthesis and inactivation (1). The receptor CB₁ is primarily localised in the central nervous system (CNS), but is also expressed in peripheral tissues and immune cells, whereas CB₂ is predominantly expressed peripherally, but is also present in the brain (2, 3). Therefore, activation of CB₁ and CB₂ receptors has several well known central and peripheral effects (2). In addition, there is emerging evidence suggesting that some cannabinoid effects are not mediated by either CB₁ or CB₂ receptors, implicating a role for additional receptors in these actions (4). In particular, AEA has been shown to activate transient receptor potential channels of type V1 (TRPV₁), also known as vanilloid VR₁ receptors, at an intracellular site (4, 5).

The most important pathway for AEA biosynthesis is mediated through the enzymatic hydrolysis of the precursor N-acyl-phosphatidylethanolamine (NAPE) which is catalysed by the NAPE-selective phospholipase D (PLD), whereas degradation occurs through fatty acid amide hydrolase (FAAH) (1). Conversely, 2-AG is mainly released from membrane lipids by the sn-1-specific diacylglycerol lipase (DAGL), and metabolised by FAAH as well as the catalytic action of a specific monoacylglycerol lipase (MAGL) (1). It is now well established that endocannabinoids are synthesised and released ‘on demand’ and that this process can be regulated both physiologically and under pathological conditions (1, 6).

Atherosclerosis is an inflammatory disease

Atherosclerosis is an inflammatory disease characterised by arterial lesions containing cholesterol, immune infiltrates, and connective-tissue elements (7, 8). It is responsible for major mortality causes, for example ischaemic heart disease and cerebrovascular disease...
Beyond genetic risk factors, hyperlipidaemia, diabetes, hypertension, obesity and smoking are the main cardiovascular risk factors, which enhance endothelial injury (7, 8). Increasing evidence suggests a crucial role for inflammatory processes in all phases of atherosclerosis from the fatty streak lesion to plaque rupture (9). Local and systemic soluble inflammatory mediators are pivotal players in regulating atherosclerotic plaque development (9). Cytokines, chemokines, growth factors and hormones orchestrate recruitment and activities of inflammatory cells in the plaque, with the subsequent induction of a systemic pro-inflammatory state, involving adipose tissue and liver (10). The acute complications of atherosclerosis are caused by the sudden thrombotic occlusion of an artery, as a consequence of physical disruption of the plaque (7, 8, 10). This process is mainly regulated by macrophages, T cells, mast cells and platelets, which produce cytokines and proteases, thus conferring to the plaque the susceptibility to rupture (10). In areas without enough collateral vessels (mainly heart and brain), thrombi arising from ruptured plaque can cause infarction of tissues, with devastating clinical consequences (7, 8, 10).

The link between atherosclerosis and metabolic syndrome

Obesity, characterised by an excess of adipose tissue mass, is closely associated with an increase in cardiovascular morbidity and mortality attributable to atherosclerosis (11). Obesity is a major underlying risk factor for atherosclerosis through other, well known risk factors, including the major risk factors (hypercholesterolaemia, hypertension and hyperglycaemia) and emerging risk factors (atherogenic dyslipidaemia, insulin resistance, pro-inflammatory state and pro-thrombotic state) (11). The clinical value of novel risk factors such as high-sensitivity C-reactive protein (CRP) are currently subject to ongoing discussions (12). This clustering of major and emerging risk factors which is found in most obese patients, is defined as ‘metabolic syndrome’ (11). It is well known that immune responses participate in all phases of atherosclerosis, from its initiation through to its complications, with prominent roles for both adaptive and innate immunity (9). Now it appears that obesity is associated with a low-grade inflammation of white adipose tissue resulting from chronic activation of the innate immune system that can subsequently lead to insulin resistance, impaired glucose tolerance and even diabetes (13). This might explain the close association between obesity and atherosclerosis; however, the precise underlying mechanisms of this low-grade inflammation in obesity remain to be clarified.

Leptin, a cytokine-like hormone encoded by the ob gene, is expressed most abundantly in adipocytes and its circulating concentrations rise with increasing adiposity (14). Leptin is primarily involved in the regulation of food intake and energy homoeostasis (15). In addition, several studies have described direct leptin effects on immune cells, including the promotion of the T lymphocyte type 1 helper (Th1) response, which suggests leptin is involved in atherosclerosis (16). Various studies have reported a protective role for leptin in atherosclerotic mice, as demonstrated in the low-density lipoprotein receptor knockout (ldlr−/−) or Apoe−/− background (17–19); however, these mice have severe hypercholesterolemia, high triglyceride levels, and insulin resistance. In a recent study, atherosclerotic lesion development in leptin-deficient ldlr−/− mice was compared to ldlr−/− mice with similar cholesterol levels (20). Surprisingly, leptin-deficiency induced a strong (2.2- to 6-fold) reduction in atherosclerotic lesion development. The authors further suggest a role for leptin in the modulation of the regulatory immune response in this experimental model.

Another important protein that plays a role in metabolic syndrome and atherosclerosis is adipocyte fatty-acid-binding protein (aP2), which is expressed in adipocytes and macrophages (21, 22). Recent evidence shows that aP2 is not only involved in regulating systemic insulin resistance and lipid metabolism, but also in inflammatory responses, thus representing an attractive therapeutic target for atherosclerosis (23, 24).

In conclusion, mounting evidence suggests a close association between inflammatory processes in obesity/metabolic syndrome and atherosclerosis; however, the precise underlying mechanisms of the low-grade inflammation in obesity remain to be clarified.

Metabolic and cardiovascular effects of CB1 receptor antagonism

The ECS is known to play a crucial role in energy balance and substrate metabolism, in particular through central hypothalamic and leptin-regulated pathways (25). Overactivity of the ECS promotes excessive food intake and fat accumulation in animal models and humans (25, 26). In rodents, pharmacological blockade or genetic ablation of CB1 receptors reduces appetite and weight and prevents obesity and insulin resistance (25). In addition to central effects, the ECS also regulates food intake and metabolic factors through peripheral CB1 receptors located at multiple sites throughout the body (25, 27). Thus, CB1 blockade in rodents and humans acts on adipocytes to increase adiponectin expression, on hepatocytes to decrease de novo lipogenesis and increase fatty acid oxidation, and acts in the gastrointestinal tract to increase satiety (27). Rimonabant is the first CB1 antagonist studied and approved as an anti-obesity drug in Europe (28–31). In addition to reducing weight and abdominal adiposity, rimonabant improves multiple cardiometabolic parameters, as shown in large randomised trials, including increased levels of high density lipoprotein (HDL)-cholesterol and reduced triglycerides, as well as improved glycemic control in pre-diabetic patients and in type-2 diabetic patients (28–31). Downregulation of the ECS with rimonabant offers a promising strategy to reduce cardiovascular risk factors; however, long-term clinical trials are warranted to determine if the improvement in metabolic parameters translates into improved morbidity and mortality associated with cardiovascular disease.

The role of CB2 in atherosclerosis

Cannabinoids such as Δ9-tetrahydrocannabinol (THC) modulate immune functions and therefore have a therapeutic potential for the treatment of inflammatory diseases. It is thought that the immunomodulatory effects of cannabinoids are mediated by CB2.
receptors expressed on immune cells (2). We have recently provided the first experimental evidence for a possible role of CB₂ receptors in atherosclerosis progression (32). Using an experimental mouse model of atherosclerosis, oral administration of low-dose THC resulted in the significant inhibition of plaque development, an effect that could be reversed by the CB₂ antagonist SR144528. The anti-atherosclerotic effect was associated with reduced macrophage infiltration into atherosclerotic lesions.

The recruitment of inflammatory cells (mainly monocytes and T lymphocytes) in the intima is an essential step in the development and progression of atherosclerosis (33). Tethering, rolling, adhesion and trans-endothelial migration of leukocytes are triggered by local production of chemokines and chemokine receptors, as well as adhesion molecules (33, 34). Both synthetic and endogenous cannabinoids have been shown to modulate the migration of a variety of cell types, including immune cells via activation of CB₂ receptors (35). Although the results obtained with THC in a mouse model of atherosclerosis are promising, they are in conflict with the known health risks of marijuana smoking, as THC binds to and activates both cannabinoid receptors, CB₁ and CB₂ (36). This problem might be solved by using cannabinoids that selectively activate CB₂ receptors; however, additional experiments are warranted to better understand the complex actions of pharmacological modulation of the cannabinoid system.

**The implication of endocannabinoids in atherosclerosis**

Synthetic and endogenous cannabinoids are known to exhibit complex cardiovascular actions by activating vascular and myocardial CB₁ receptors (37, 38). Both increases and decreases in blood pressure in response to cannabinoid treatment have been reported, depending on the experimental model, species and/or concentration used (39). The acute administration of cannabinoids in humans is typically associated with increased pulse rate and acute rise in blood pressure, but also sudden falls in blood pressure in orthostatic state (36). Conversely, endocannabinoid AEA and 2-AG release from endothelial cells, macrophages or platelets, results in hypotension in rodents (40–42). In spontaneously hypertensive rats, prevention of endocannabinoid AEA degradation by an inhibitor of fatty acid amidase hydrolase (FAAH) was shown to lower blood pressure and heart rate through reductions in both cardiac contractility and vascular resistance (43). These effects were prevented by CB₁ antagonists. FAAH⁻/⁻ mice, however, have normal blood pressure and cardiac function, indicating that under normal conditions anandamide does not play a major role in cardiovascular regulation (44). These findings suggest that the endocannabinoid system represents a therapeutic target for the treatment of hypertension, which is a major risk factor for atherosclerosis (38).

A more recent study investigated the age-associated decline of cardiac function and changes in inflammatory gene expression, nitrative stress and apoptosis in FAAH⁻/⁻ mice compared with wild-type mice (45). Enhanced anandamide levels in the FAAH⁻/⁻ animals were protective, which further supports the protective role of endocannabinoids in inflammatory disorders such as atherosclerosis. Moreover, anandamide dose-dependently attenuated the tumour necrosis factor α (TNFα)-induced intercellular adhesion molecule-1 (ICAM-1) and vascular cellular adhesion molecule-1 (VCAM-1) expression in human coronary artery endothelial cells (HCAECs), and the adhesion

![Diagram](image)

**Fig. 1.** By activating CB₂ cannabinoid receptors on adipocytes and vascular cells, endocannabinoids might exhibit pleiotropic effects on atherogenesis. It is not known yet whether atherogenesis is associated with increased endocannabinoid signalling (by enhanced endocannabinoid levels and/or upregulation of receptor expression) and what might be the overall impact on disease progression. It is conceivable that enhanced endocannabinoid (AEA, anandamide; 2-AG, 2-arachidonoylglycerol) levels might increase adipose tissue inflammation and induce platelet activation. Conversely, anandamide has been described to inhibit inflammatory gene expression in endothelial cells (EC) (45). Furthermore, activation of CB₂ receptors might inhibit monocyte recruitment by inhibiting chemokine receptor and adhesion molecule expression, as recently reported for synthetic ligands (51). CB₂ expression and downstream signalling following 2-AG activation has also been described on activated human T lymphocytes (52). In addition, activation of CB₂ receptors has been recently described to inhibit smooth muscle cell (SMC) migration and proliferation (53).
of THP-1 monocytes to HCAECs in a CB₁ and CB₂-dependent manner [45].

In contrast to the potentially beneficial effects in cardiovascular disease, endocannabinoids might exhibit pro-thrombotic effects. Indeed, both AEA and 2-AG have been described to activate rodent and human platelets [42, 46, 47]. Platelets are anucleated cellular fragments that circulate in the blood. In addition to their well-recognised role in hemostasis and acute thrombus formation, platelets are also thought to have pro-inflammatory and growth-regulatory properties that contribute to progression of atherosclerosis (48–50). Endothelial cells, macrophages or platelets themselves might increase their endocannabinoid synthesis during atherosclerosis, thus triggering platelet activation. Alternatively, these cells are also able to metabolise AEA and 2-AG, which might counter-balance enhanced endocannabinoids levels. Thus, the precise role of endocannabinoid signalling during atherosclerosis is poorly understood (Fig. 1). Whether an upregulation by increased endocannabinoid levels and/or receptor expression occurs during atherosclerosis remains to be determined.

Conclusions

Given the increasing prevalence of cardiovascular risk factors worldwide, there is an urgent need for a better understanding of the molecular mechanisms underlying atherosclerosis. Mounting evidence suggests a close association between obesity/metabolic syndrome and atherosclerosis. The finding that endocannabinoid signalling is crucially involved in pathogenic processes in obesity indicates that the endocannabinoid system might also affect underlying pathways of atherosclerosis (Fig. 1). The recent finding that low doses of THC reduce atherosclerosis progression in mice in a CB₂-dependent manner supports this hypothesis. Moreover, an increasing number of studies reported the modulation of endocannabinoid levels, receptors and related enzymes of biosynthesis and degradation in different inflammatory conditions. This knowledge evolved the concept of blocking endocannabinoid signalling for therapeutic use. However, the precise role of endocannabinoid signalling during atherosclerosis remains to be determined.

Conflicts of interest

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