JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 2014, 65, 2, 183-191
www.jpp.krakow.pl

Review article

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A NEW FACE OF ENDOCANNABINOIDS IN PHARMACOTHERAPY PART II. ROLE OF ENDOCANNABINOIDS IN INFLAMMATION-DERIVED CARDIOVASCULAR DISEASES

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Endocannabinoids play an important role in cardiovascular diseases caused by inflammatory disorders. Endocannabinoids are endogenous bioactive lipids that activate cannabinoid receptors and together with enzymes responsible for their synthesis and degradation constitute endocannabinoid system. The results obtained to date suggest the involvement of endocannabinoids in the pathology of many cardiovascular diseases associated with inflammation, such as atherosclerosis, restenosis, chemotherapy-induced myocardial injury, diabetic and hepatic cirrhosis cardiomyopathy. Our better understanding of cannabinoid system may result in the development of new strategies for the treatment of such disorders.

Key words: *endocannabinoid system, cardiovascular system, atherosclerosis, restenosis, diabetic cardiomyopathy*

INTRODUCTION

Endocannabinoids (ECs), anandamide (AEA) and 2-arachidonoylglycerol (2-AG) are arachidonic acid-derived endogenous bioactive lipids. Their production from cell membrane lipid precursors is activity-dependent and their actions are terminated by specific lipases, fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL), respectively. ECs are ligands of two main cannabinoid receptors, CB1 and CB2. The ECs, their receptors and enzymes responsible for their degradation constitute the endocannabinoid system (ECS) (1).

Increasing research evidence suggests a broad therapeutic potential of cannabnoids for a variety of conditions. Several reports demonstrated an implication of the ECS in various inflammatory conditions. Deregulation of this system was observed in the pathogenesis of several cardiovascular diseases in which increased plasma level of circulating inflammatory cells was observed (2). This problem was also addressed in a comprehensive review paper by Malinowska *et al.* (3). Activation of CB2 but not CB1 receptors is known to limit inflammatory responses (*Fig. 1*).

EFFECTS OF ENDOCANNABINOIDS IN VARIOUS PATHOLOGICAL CONDITIONS RELATED TO INFLAMMATION-DERIVED CARDIOVASCULAR SYSTEM PATHOLOGIES

Atherosclerosis

Ischemic heart disease (IHD) is mainly caused by atherosclerosis and its complications (4, 5). The contemporary views concerning the pathogenesis of atherosclerosis emphasize the role of endothelial dysfunction as a factor promoting the development of atherosclerotic plaque. The crucial role in the development of atherosclerosis is played by a chronic inflammatory-immune disease affecting the arterial wall (*Fig. 2*), which may be induced either by infection or other factors (endothelial damage, nicotinism, diabetes, obesity) (2, 6).

Obesity, one of the characteristics of the „metabolic syndrome", is the major underlying risk factor for atherosclerosis (7). It has been established that hyperactivity of the ECS leads to obesity (8). On the other hand, obesity may increase the activity of the ECS (feedback mechanism). ECs can stimulate food intake (increase the appetite) *via* central and peripheral pathways, by activation of CB1 receptors in vagal nerve terminals. The expression of CB1 receptor in the gastrointestinal tract has been observed to decrease after a meal and to increase in fasting subjects (9).

ECs also control signals sent from the periphery by adiponectin, leptin and ghrelin to hypothalamic neurons (10). The concentration of 2-AG in human blood has been demonstrated to correlate positively with abdominal obesity, levels of insulin and triglycerides after overnight fasting, and negatively with levels of HDL cholesterol and adiponectin (with antiatherogenic and antidiabetogenic properties) (11). Low level of these hormones is associated with disturbances of carbohydrate and fat metabolism and accompanies the cardiovascular complications of atherosclerosis (12, 13).

The activity of ECS in human atherosclerotic diseases was also investigated. Patients with coronary diseases had higher serum levels of AEA and 2-AG compared to unaffected subjects (14). In addition, serum AEA was higher in coronary

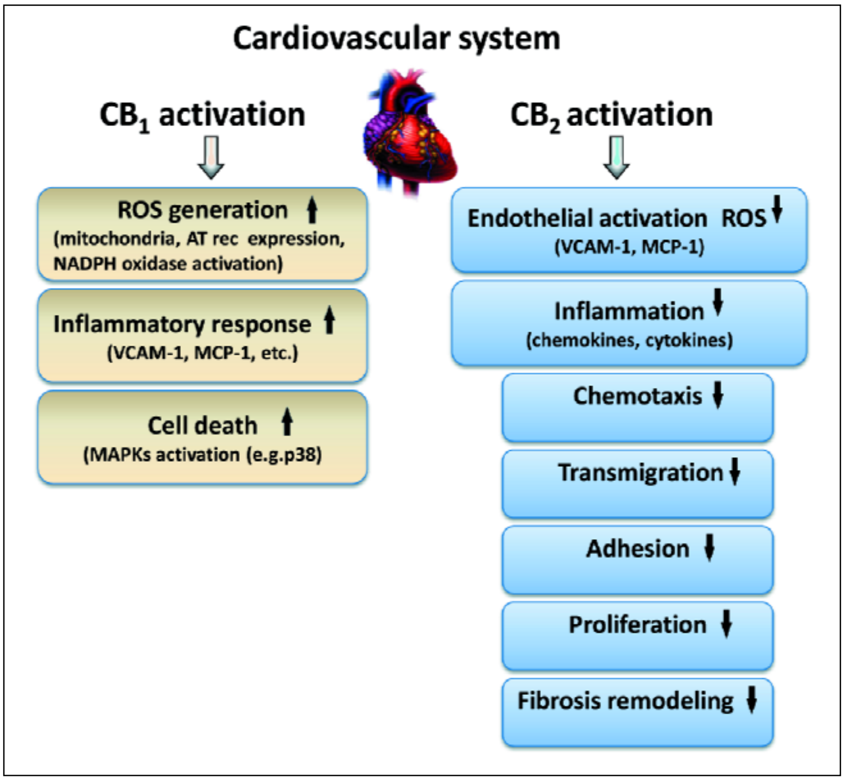

Fig. 1. Effects of CB1 and CB2 receptors activation on the cardiovascular system. CB1: promotion of cardiac dysfunction and cell death in cardiomyocytes and endothelial cells; CB2: attenuation of inflammation/injury.

culprit lesions than in the systemic bloodstream in patients with acute myocardial infarction (15). These studies suggest that patients with unstable coronary disease have increased levels of circulating ECs, released from ruptured plaques, capable of local activation of CB1. Increased levels of AEA and FAAH are associated with the development of smaller atherosclerotic plaques with high neutrophil content, accompanied by an increased proinflammatory immune response (16). In general, blockade of CB1 receptors with rimonabant (RIO) in peripheral tissues reduces atherosclerotic inflammation and plaque formation (17, 18). In contrast, activation of CB2 inhibits atherosclerotic plaque progression in mice, mainly by inhibiting macrophage recruitment (19). ECs released from endothelial cells, macrophages or platelets, reduce hypertension in rodents, a major risk factor for atherosclerosis. In addition, AEA inhibits expression of inflammatory genes, decreases generation of reactive oxygen species (ROS) in endothelial cells and monocyte adhesion (20). Conversely, ECs might also mediate pro-atherosclerotic effects by inducing platelet activation (12, 21). It has been emphasized that platelets themselves may induce the development of atherosclerotic lesions both, under physiological conditions and in response to an inflammatory process (22).

In vitro studies have shown that the CB2 receptor modulates several macrophage processes associated with ongoing atherosclerosis, including migration and proliferation (19, 23, 24) as well as the susceptibility to oxidized low-density lipoproteins (OxLDL)/oxysterol-induced apoptosis (25). Macrophage apoptosis is an important process in the pathophysiology of atherosclerosis, during which macrophages in the vascular intima ingest atherogenic lipoproteins, such as modified LDLs, and transform them into cholesteryl ester-laden foam cells (26). OxLDL are a major component of lesions and potently induce macrophage apoptosis. As macrophage apoptosis in advanced lesions is considered a proatherogenic process occurring within the vascular wall, strategies for inhibiting CB2-dependent apoptotic pathways might be useful to retard lesion progression and prevent rupture. Steffens *et al.* (19) demonstrated that administration of cannabinoids reduced progression of established lesions in ApoE knockout mice *via* a presumably CB2-dependent mechanism. Zhao *et al.* (27) concluded that WIN55212-2 (administered by daily i.p. injection for 8 weeks before analysis) reduced atherosclerotic plaque formation, lesional macrophage content and mRNA levels of inflammatory markers IL-6, TNF-α and CCL2, as well as NF-κB activation in apolipoprotein E (ApoE) knockout mice fed 16 weeks on high-cholesterol diet. These protective effects were completely blocked by the highly selective CB2 receptor antagonist AM630 (27, 28).

It was thus suggested that selective activation of CB2 receptor could decrease atherosclerosis. In particular, treatment with the CB2 agonist JWH-015 reduced expression of chemokine receptors CCR-1 and CCR-2 (23). Stimulation of CB2 receptor also reduced *in vitro* pro-atherosclerotic TNF-α mediated endothelial cell activation, thereby possibly attenuating adhesion and transendothelial migration of monocytes *via* a direct protective effect on the endothelium (29).

In macrophages and/or endothelial cells, CB1 receptor activation triggers intracellular MAPK signaling pathway. This influences the release of atherosclerotic mediators that interfere with physiological vasodilatation (30-32) and induce the release of ROS which might favor not only endothelial dysfunction (31) but also arterial vasodilation (33).

In two experimental models of atherosclerosis, ApoE deficient mice and mice lacking the low density lipoprotein (LDL) receptor, responded to pharmacological treatment with CB1 antagonist, RIO, with reduced atherosclerotic plaque formation, and a significant reduction in circulating inflammatory cytokines and improved endothelial function, respectively (34-36). ApoE and FAAH deficient mice had

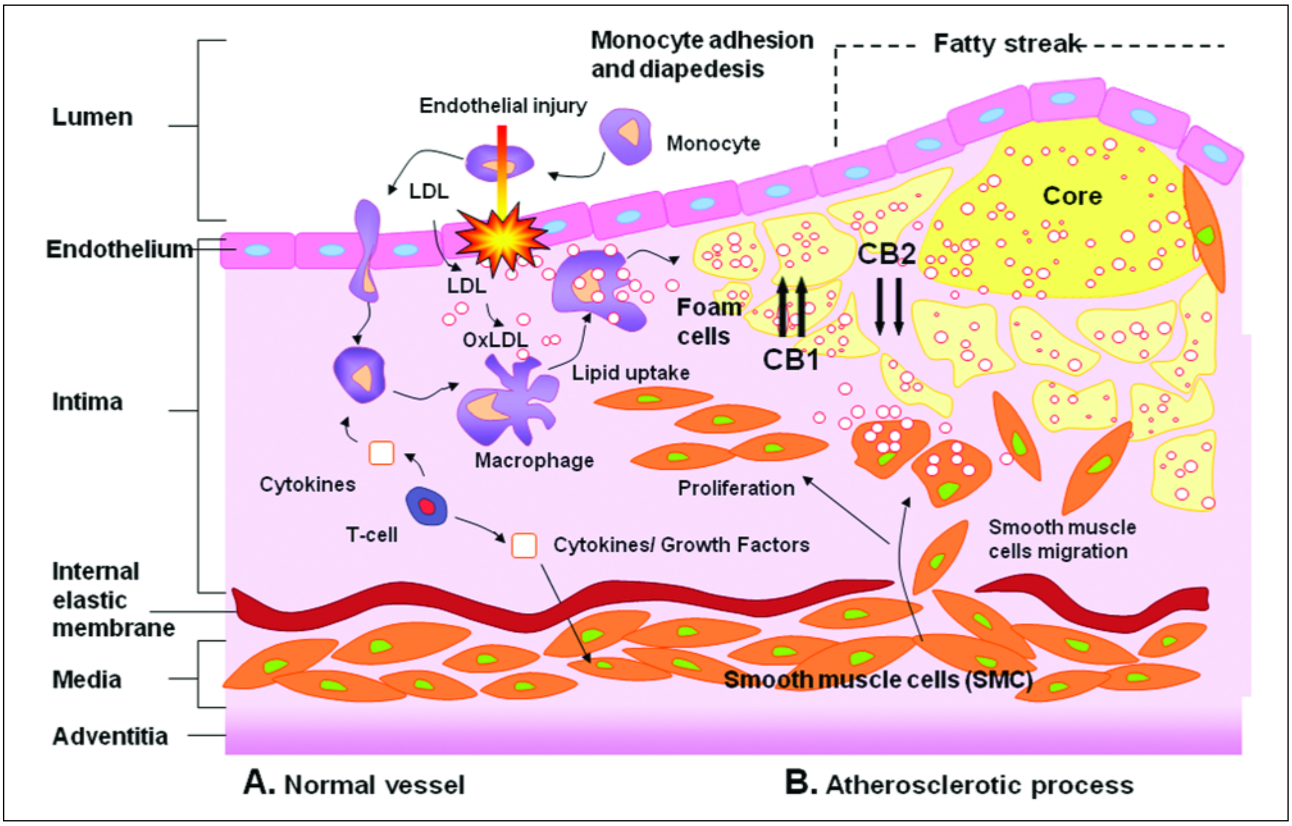

Fig. 2. The atherosclerotic process and role of the ECS in atherogenesis. (*A*): A normal artery viewed from inside consists of a one cell thick layer of endothelium, muscular membrane (tunica media) and adventitial membrane (adventitia). (*B*): The atherosclerotic process involves focal accumulation of: (1) inflammatory cells (mainly macrophages formed by transformation of monocytes); (2) low-density lipoproteins (LDL) and (3) transformed smooth muscle cells producing elements of connective tissue, between the endothelium and the muscular layer of large arteries (tunica intima). Macrophages phagocyting LDL (foam cells) die, and their lipid content accumulates in the extracellular space forming the lipid nucleus of the plaque. Connective tissue surrounding the lipid nucleus is the predominant component of the atherosclerotic plaque-coating layer. Treatments producing selective CB1 blockade or CB2 activation might improve atherogenesis by reducing the inflammation of atherosclerosis plaques and decrease the risk of cardiovascular events by enhancing plaque stability. CB1 up-regulation, CB2 down-regulation.

smaller plaques with significantly lower content of smooth muscle cells, increased matrix metalloproteinase-9 expression, and neutrophil content (16).

The crucial role in the process of atherosclerotic plaque destabilization is attributed to macrophages and proteolytic enzymes, matrix metalloproteinases (MMP), responsible for degradation of fibrous elements of the plaque and vascular remodeling (16, 37-40). It was observed that CB2 receptor levels were inversely correlated with MMP-9 (39) content and positively with collagen, indicating a protective role of CB2 in plaque vulnerability due to carotid artery ballooning (41). Accordingly, MMP-9 up-regulation was observed in double LDL/CB2 receptor knockout mice in the absence of any net effect on atherosclerotic plaque formation (42).

Therefore, pharmacological blockade of CB1 receptors might reduce the atherosclerosis risk factors not only in obesity, but also in prevention of cardiovascular diseases. Bojanowska *et al.* (43) investigated the effect of such blockade on inhibition of appetite in rat and found that it may act synergistically with the concomitant stimulation of glucagon-like peptide-1 (GLP-1) receptor. Selective CB2 receptor activation within atherosclerotic plagues might represent a very promising strategy for reducing atherosclerotic inflammation and therefore is a target for the development of drugs to treat inflammatory disease (20, 28, 41, 44, 45).

Restenosis

Restenosis is an inflammatory process in response to arterial injury, leading to secretion of cytokines and growth factors, recruitment of inflammatory cells as well as increased migratory, proliferative and secretory responses of vascular smooth muscle cells (46, 47).

For many years, researchers have been fighting to prevent the restenosis after percutaneus coronary transluminal angioplasty (PCTA) and after coronary artery bypass grafting (CABG) procedures. The role of inflammation in atherogenesis, and, consequently, the progress of restenosis, is undeniable. In many studies conducted to date, it was attempted to identify the risk factors for restenosis. Angioplasty involving stent implantation has been demonstrated to induce acute local inflammatory response, which favors restenosis (48). The main cause of restenosis developing in the stent is neointimal growth in response to an inflammatory reaction due to the presence of a foreign body (49).

Moreover, late stent thrombosis due to the lack of complete endothelial repair has emerged as a major safety concern. Efforts to limit this constrictive vascular remodeling process have focused on inhibiting smooth muscle cell proliferation and migration, leading to the development of local stent-based delivery of antiproliferative agents (50). Molica *et al.* (41)

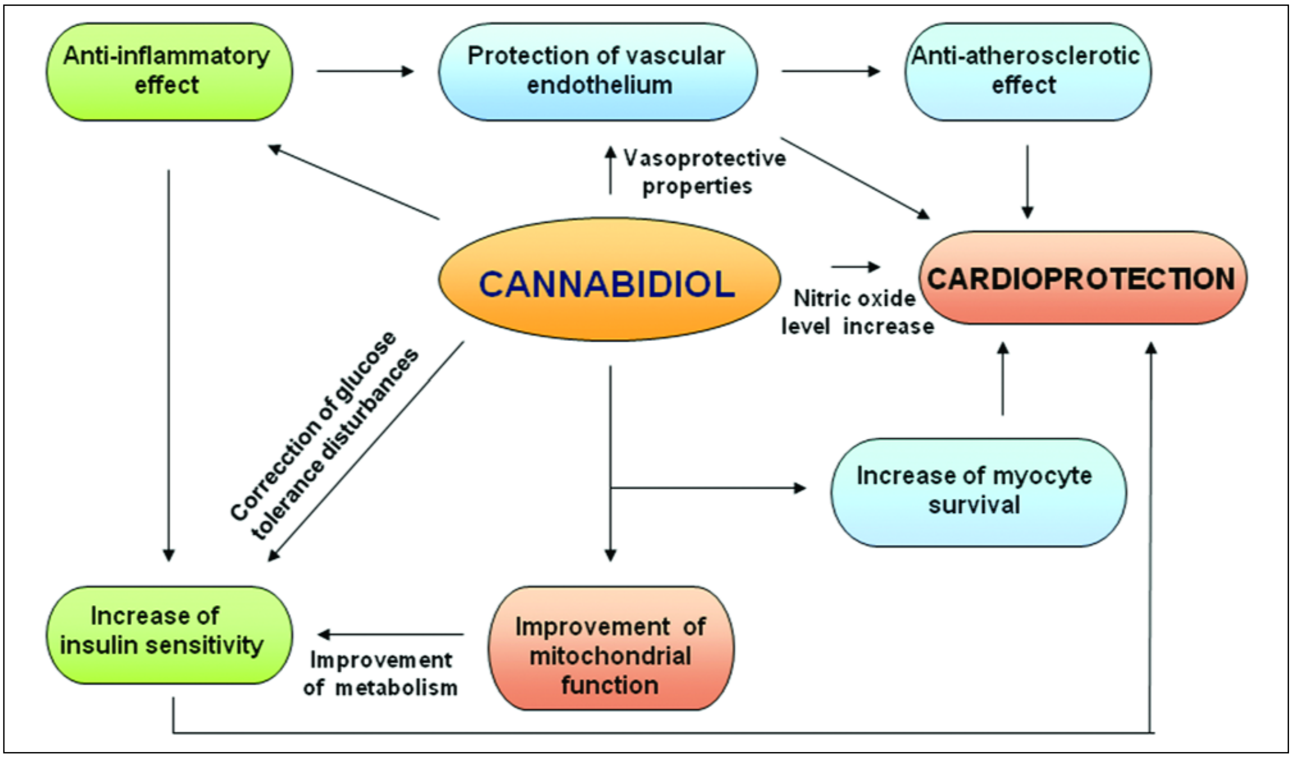

Fig. 3. Therapeutic potential targets of cannabidiol (CBD) in diabetes. CBD may exert beneficial effects against various diabetic complications by attenuating high glucose (inducing endothelial cell activation and inflammatory response), cardioprotection, increasing sensitivity to insulin, protection of vascular endothelium, improvement of metabolism, anti-inflammatory and anti-atherosclerotic effects.

investigated the effect of CB2 activation in a mouse model of balloon angioplasty. As reported in other models of organ injury or inflammation (51), balloon injury increased vascular CB2 expression in hypercholesterolemic ApoE knockout mice (41). Injured vessels of mice treated with CB2 agonist, JWH133, showed reduced intimal and medial thickening, associated with decreased proliferation and decreased amount of smooth muscle cells and macrophages. Reendothelialization was not inhibited by treatment with the CB2 agonist. Conversely, CB2 deficiency resulted in increased intima formation compared with wild-type mice. The underlying mechanisms involved increased mRNA levels of adhesion molecule ICAM-1, chemokine receptors CCR1 and CCR5, as well as the proinflammatory chemokine CCL2. Interestingly, Molica *et al.* (52) reported that CB1 receptor activation contributes to vascular smooth muscle cell (SMC) proliferation and neointima formation in response to arterial injury leading to restenosis and reendothelialization which was not inhibited by CB1 antagonist, AM251. Conversely, CB2 deficiency resulted in increased intima formation compared with wild-type mice, whereas JWH133 did not affect intimal formation in CB2 deficient mice. The underlying mechanisms involved increased mRNA levels of adhesion molecule ICAM-1, chemokine receptors CCR1 and CCR5, as well as the proinflammatory chemokine CCL2 (41).

In view of the above, the authors recommended regular monitoring of the levels of cytokines, inflammatory proteins and endothelial regeneration which would allow to detect an inflammatory process if it begins and to institute appropriate management to prevent restenosis (48, 53). Numerous reports published so far assess the effect of various substances administered systemically or locally (released from the stent) on inhibition of the inflammatory process and consequent occurrence of restenosis (54).

Statins display pleiotropic properties and exert their benefits partly through the inhibition of vascular smooth muscle cell (VSMC) proliferation. This effect is important for the prevention of restenosis after percutaneus coronary intervention (PCI). Atorvastatin does not impair endothelial cell wound healing but is capable of curtailing the production of inflammatory cytokines. The authors indicate that atorvastatin is safe to use after PCI as it will not delay endothelial cell recovery from injuries (55). Novel strategies should be targeted on restenosis prevention without impairing the arterial healing process (50).

Chemotherapy-induced myocardial injury

Besides the numerous papers indicating cardioprotective effect of cannabinoids discussed above, there are recent reports undermining these findings, concerning the studies of *in vivo* and *in vitro* effect of doxorubicin on the heart (56-58). Doxorubicin (DOX) which belongs to anthracyclines is a chemotherapeutic agent used in the anticancer therapy, but capable of inducing cardiotoxicity (58). Administration of DOX induces a series of dramatic effects such as apoptosis, cell necrosis, autophagy and senescence in cardiomyocytes, leading to collagen deposition and adverse cardiac remodeling (60). The major hypothesis regarding the pathophysiology of DOX-induced cardiotoxicity is that cardiac damage is caused by oxidative stress through the generation of ROS. Mitochondria are a primary target of DOX-induced cardiotoxicity mediated by the induction of ROS (61, 62).

In response to DOX, FAAH knockout mice exhibited elevated AEA levels in myocardium related to increased myocardial dysfunction, cardiomyocyte oxidative stress and increased mortality compared to controls (56, 58). The cardiotoxic effect of DOX was abolished by the pretreatment with CB1 receptor antagonists, RIO and AM 281. Besides prevention of cardiomyocyte apoptosis in mice, these compounds improved also the hemodynamic parameters of cardiac function and inhibited the DOX-induced increase of AEA levels in the heart. In contrast, such effect was not observed with CB1 and CB2 agonists nor with SR 144528, a CB2 antagonist *in vitro* (56).

Similar to cardiac cells knockout mice, CB1 activation was deleterious in human primary cardiomyocytes treated with DOX *in vitro*. The authors suggested that CB1 activation might amplify cardiomyocyte death *via* deregulation of reactive oxygen/nitrogen levels and increased peroxynitrite formation (56, 63).

Diabetic cardiomyopathy

In diabetic patients, cardiovascular complications represent the principal cause of morbidity and mortality (64, 65). Hyperglycemia is a major etiological factor in the development of diabetic cardiomyopathy. Most diabetic complications are associated with pathologic alterations in the vascular wall, leading to atherosclerosis, which increases the risk of myocardial infarction, stroke, and peripheral arterial disease (66).

Numerous studies indicate that the presence of diabetic cardiomyopathy is independent of arteriosclerosis, coronary artery disease and hypertension. Hemodynamic disorders in diabetic cardiomyopathy are characterized by hypertrophy of myocardial left ventricular (LV), cardiac dysfunction, first diastolic and later systolic, and eventually heart failure (67-69). On the basis of both clinical data and animal models, multifaceted metabolic, biochemical and microcirculatory disorders should be distinguished among the mechanisms responsible for the development of diabetic cardiomyopathy (67, 69, 70).

Increased levels of free fatty acids activate PPAR-α signaling, leading to up-regulation of many genes involved in fatty acid oxidation and increased production of ROS and reactive nitrogen species (RNS) (69-74). Diabetic cardiomyopathy is associated with activation of various downstream transcription factors responsible for many proinflammatory cytokine expression (75-78) and cell death signaling pathways (79, 80), as well as accumulation of advanced glycation end products (68, 81, 82). Among the biochemical abnormalities, impaired calcium homeostasis involve "overloading" myocytes with Ca^{2+} which results in excessive stimulation of calcium-dependent ATP-ases and decreased activity of sarcoplasmic/endoplasmic reticulum (83-85).

The association of the ECS with the pathogenesis of diabetes was further supported by the up-regulation of CB1 expression and increase in AEA levels in the myocardium of diabetic patients. Patients with type 2 diabetes had higher serum levels of both AEA and 2-AG than healthy individuals (86, 87).

CB1 receptor expressed in rat pancreatic islets have also been implicated in insulin secretion; while the presence of CB2 receptor is debated (88, 89). Pharmacological inhibition or genetic deletion of CB1 receptor attenuated the diabetes-induced cardiac dysfunction and the pathological alterations. Activation of CB1 by ECs may play an important role in the pathogenesis of diabetic cardiomyopathy by facilitating MAPK activation, oxidative/nitrative stress, inflammation, and fibrosis (14, 21, 58, 90, 91). CB1 receptor activation may directly or indirectly (*via* its metabolic consequences) enhance diabetes-associated inflammation and ROS generation, promoting tissue injury and the development of diabetic complications.

Conversely, CB1 receptor inhibition may be beneficial in the treatment of diabetic cardiovascular complications. Pharmacological inhibition with selective CB1 antagonist, RIO, or genetic deletion of CB1 receptor attenuates cardiac dysfunction, oxidative stress, and inflammation (91). The blockade of CB1, or its genetic deletion was alleviated by proteinuria and/or vasculitis and cell death in the experimental models of type 1 diabetic neuropathy (92) or retinopathy (93). CB2 receptor activation may exert beneficial effects against various diabetic complications by attenuating high glucose-induced endothelial cell activation and inflammatory response; chemotaxis, transmigration, adhesion, and activation of inflammatory cells. Subsequent proinflammatory responses and ROS generation also attenuates TNF-α-triggered activation of NF-κB up-regulation of adhesion molecules, and increased expression levels of monocyte chemoattractant protein-1 (MCP-1) in endothelial cells (51).

Recently, several studies highlighted the important role of the ECS in the regulation of vascular inflammation, oxidative stress, and atherosclerosis, suggesting that the modulation of the EC levels or the administration of plant-derived cannabinoids with antioxidant and anti-inflammatory properties might be beneficial in the treatment of cardiovascular complications associated with diabetes (94-96). Recent research has been focused on two natural plant-derived constituents, cannabidiol (CBD) and Δ^9-tetrahydrocannabivarin, with negligible psychotropic effects and great therapeutic potential in inflammatory diseases, diabetes, and diabetic complications (80, 95-99). In particular, CBD (*Fig. 3*) was selectively effective against myocardial complications of diabetic cardiomyopathy, ameliorating cardiac function *via* a reduction of inflammation produced by the release of oxidants, cell death and fibrosis, by activating CB2 receptors. Moreover, it protects retinal neurons by preserving glutamine synthase activity in diabetes (100). THCV seems to be a promising therapeutic compound because it has been shown to behave as a CB1 receptor antagonist but at the same time it activates CB2 receptors, thereby decreasing inflammation and oxidative stress (101, 102) which are key processes in the development of diabetes and diabetic complications.

Hepatic cirrhosis cardiomyopathy

In patients with hepatic cirrhosis cardiomyopathy develops irrespectively of its etiology and affects primarily the diastolic, and to a lesser extent, the systolic function of the heart. The disease is usually subclinical, and the onset of symptoms is associated with the response to stress, due to cardiac dysfunction. In more severe cases, in addition to adverse cardiac remodeling, changes in contractility and hepatic fibrosis, prolonged QT segment and conductivity disturbances are observed (103, 104).

The results of *in vitro* studies indicate that AEA, whose concentration in the hearts of humans and animals with hepatic cirrhosis is increased, impairs myocardial contractility by activation of CB1 receptors, whereas normal contractility is restored by CB1 receptor antagonists (105, 106) and attenuates liver fibrosis in various animal models (107). Treatment of rats with the CB1 receptor antagonist, RIO, significantly increased systemic blood pressure by decreasing peripheral vasodilation and reduced mesenteric blood flow and portal pressure, all potential pathophysiological entities underling cirrhotic cardiomyopathy (108). In contrast, endogenous activation of CB2 receptor alleviates antifibrogenic effects and regulation of liver inflammation (109-110). Recently, it has been shown that blocking MAGL protects against inflammation and damage from hepatic I/R. MAGL modulates hepatic injury *via* EC and eicosanoid signaling. Blockade of this pathway by selective MAGL inhibitor, JZL 184, protects mice from liver injury (111). Thus, MAGL inhibitors might be developed to treat conditions that expose liver to oxidative stress and inflammatory damage. The studies discussed above suggest that cannabinoid receptor antagonists and MAGL inhibitors might be used in future in the treatment of cardiomyopathy associated with hepatic cirrhosis.

CONCLUSIONS

Mounting evidence points to an inflammation-heart disease connection. Inflammation contributes to the development of heart disease by narrowing the opening through which blood can flow. Cholesterol, a component of plaque, further narrows arteries by clogging them with gunk. Inhibition of inflammation processes can be achieved by activation of CB2 receptors and blockade of CB1 receptors. It can be expected that obtaining cannabinoid ligands retaining their medicinal properties but devoid of adverse psychoactive effects of cannabis will result in great progress in the treatment of cardiovascular disease.

Abbreviations: 2-AG: 2-arachidonoylglycerol; AEA: anandamide or N-arachidonoyl ethanolamide; CB1: cannabinoid receptor type 1; CB2: cannabinoid receptor type 2; CBD: cannabidiol; DOX: doxorubicin; ECs: endocannabinoids; ECS: endocannabinoid system; FAAH: fatty acid amide hydrolase; MAGL: monoacyl glycerol lipase; MAPK: mitogen-activated protein kinases; MMP: matrix metalloproteinase; NF-κB: nuclear factor-kappaB; PPARα: peroxisome proliferator-activated receptor α; RIO: rimonabant (SR141716A); ROS: reactive oxygen species; TNF-α: tumor necrosis factor-α

Conflict of interest: None declared.

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Received: July 25, 2013
Accepted: January 18, 2014

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