
Is the cardiovascular system a therapeutic target for Cannabidiol?

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Introduction

Cannabidiol (CBD) is an abundant, non psychoactive, plant derived cannabinoid (phytocannabinoid) whose stereochemistry was first described in 1963 by Mechoulam and colleagues [1]. Isolation of the chemical structure of CBD revealed it to be a classical cannabinoid closely related to cannabinol and Δ^9 -tetrahydrocannabinol (THC). Since its isolation, a range of synthetic analogues have been synthesized based on the classic cannabinoid dibenzopyran structure, including abnormal CBD (Abn-CBD), O-1918 and O-1602 [2, 3]. CBD is reported to have a diverse pharmacology which is reviewed in depth elsewhere [4]. In brief, CBD shows antagonism of the classical cannabinoid 1 (CB₁) and cannabinoid 2 (CB₂) receptors in the low nanomolar range, yet has agonist/inverse agonist actions at micromolar concentrations. Other receptor sites implicated in the action of CBD include the orphan G protein coupled receptor GPR55, the putative Abn-CBD receptor, the transient receptor potential vanilloid 1 (TRPV1) receptor, α_1 -adrenoreceptors, μ opioid receptors and 5HT_{1A} receptors [4]. It has also been shown that CBD activates and has physiological responses mediated by peroxisome proliferator activated receptor γ (PPAR γ) [5–7]. As well as a rich pharmacology, CBD is suggested to have therapeutic potential in a vast range of disorders including inflammation, oxidative stress, cancer, diabetes, gastrointestinal disturbances, neurodegenerative disorders and nociception [8–12]. Evidence is also now accumulating that there are positive effects of CBD in the vasculature. It is the aim of this review to examine this evidence and establish whether or not the cardiovascular system is a potential therapeutic target for CBD. A recent review of the safety and side effects of CBD concluded that CBD appears to be well tolerated at high doses and with chronic use in humans [13], and thus has the potential to be taken safely into the clinic.

Direct vascular effects of CBD

Work to date investigating the vascular effects of cannabinoids has primarily concentrated on the response to endocannabinoids, THC and synthetic ligands, with only limited studies conducted using CBD. However, the effects of the CBD analogue, Abn-CBD, have been characterized. Jarai *et al.* [25] showed that Abn-Cbd caused hypotension in both CB₁^{+/+}/CB₂^{+/+} and CB₁^{-/-}/CB₂^{-/-} mice. The effects of Abn-CBD were inhibited by high concentrations of SR141716A, endothelium denudation and CBD. In this paper, CBD was shown to antagonize the vasorelaxant effects of Abn-CBD and AEA. Begg *et al.* [26] showed in human umbilical vein endothelial cells (HUVECs) that Abn-CBD causes hyperpolarization through PTX-sensitive activation of large conductance calcium activated potassium channels (BK_{Ca}). Similarly, in rat isolated mesenteric arteries, Abn-CBD causes vasorelaxation that is dependent on the endothelium, SR141716A sensitive pathways and potassium channel hyperpolarization through large, intermediate and small conductance calcium activated potassium (BK_{Ca}/IK_{Ca}/SK_{Ca}) channels [27]. Interestingly, the previous work reported an endothelial-independent pathway that involved Abn-CBD modulation of the Ca²⁺ channels. The findings of endothelial dependent and independent components of Abn-CBD-induced vasorelaxation is in agreement with a similar study of the same year showing that in rat mesenteric arteries, vasorelaxation to Abn-CBD was inhibited by PTX incubation and incubation with another CBD analogue, O-1918, and indeed this was dependent on the endothelium [28]. More recently it has been shown that Abn-CBD causes vasorelaxation in the human pulmonary artery through similar mechanisms [29]. Taken together, these findings offer support to the presence of an endothelial bound G_{i/o} protein coupled receptor that causes vasorelaxation through hyperpolarization that is activated by Abn-CBD.

Fewer studies have investigated the vascular effects of CBD. Jarai and colleagues [25] found no effect of perfusing 10 μ M CBD on vascular tone in phenylephrine-constricted rat mesenteric vascular bed. However, in arterial segments taken from the rat mesenteric vascular bed that have been mounted onto a Mulvany-Halpern myograph and constricted with phenylephrine, CBD causes a concentration-dependent

near-maximal vasorelaxation [28]. Unfortunately, this study did not probe the mechanisms underlying this vasorelaxant effect of CBD in rat mesenteric arteries.

In human mesenteric arteries, we have very recently shown that CBD causes vasorelaxation of U46619 and endothelin-1 pre-constricted arterial segments (Stanley & O'Sullivan, 2012, under review). In human mesenteric arteries, CBD-induced vasorelaxation has a pEC_{50} in the mid-micromolar range which is similar to that observed in rat mesenteric arteries. However, CBD-induced vasorelaxation in human arteries has a maximal response of ~40% reduction of pre-imposed tone. We went on to show that CBD-induced vasorelaxation in human mesenteric arteries is endothelium-dependent, involves CB₁ receptor activation and TRPV channel activation, nitric oxide release and potassium hyperpolarization

Direct vascular effects of CBD measured in isolated arteries. TRPV, transient receptor potential vanilloid; NO, nitric oxide; CB₁, cannabinoid receptor 1; PPAR γ , peroxisome proliferator activated receptor gamma; SOD, superoxide dismutase

It is interesting to note that Ruiz-Valdepenas *et al.* [34] recently showed that CBD inhibited lipopolysaccharide (LPS)-induced arteriolar and venular vasodilation. LPS has been suggested to cause hypotension through activation of a novel as yet unidentified cannabinoid receptor which could be inhibited by SR141716A but not AM251 [35]. Since CBD is suggested to be an antagonist of this receptor [25], this could explain how CBD inhibits LPS-induced vasodilation.

Time-dependent Vasorelaxant Effects of CBD (and PPAR γ agonism)

PPAR γ agonists have been shown to have positive cardiovascular effects, which include increased availability of nitric oxide, reductions in blood pressure and attenuation of atherosclerosis [36, 37]. Some of the beneficial effects of PPAR γ ligands are brought about by anti-inflammatory actions, including inhibition of pro-inflammatory cytokines, increasing anti-inflammatory cytokines and inhibition of inducible nitric oxide synthase (iNOS) expression (for review see [38]). Increasing evidence has indicated that cannabinoids are capable of binding to, activating and causing PPAR-mediated responses [39]. We have shown that the major active ingredient of cannabis, THC, activates PPAR γ , and that THC causes time-dependent, endothelium-dependent, PPAR γ -mediated vasorelaxation of the rat isolated aorta [40, 41]. Subsequently, we tested whether CBD might also activate PPAR γ and that this might mediate some of the pharmacological effects of cannabidiol. In these experiments we showed that CBD is a weak/partial agonist at the PPAR γ receptor which increases PPAR γ transcriptional activity in PPAR γ overexpressing HEK293 cells, and CBD binds to the PPAR γ ligand binding domain with an $IC_{50} \approx 5 \mu M$ [5]. Like THC, CBD (at concentrations above 100 nM) was also found to cause a time-dependent vasorelaxation of rat aortae. This time-dependent vasorelaxation was inhibited using the PPAR γ antagonist GW9662 or the superoxide dismutase (SOD) inhibitor diethyldithiocarbamate (DETCA). Increased SOD activity promotes vasorelaxation through reductions in reactive oxygen species, and our data are in agreement with other work showing PPAR γ ligands cause the induction of Cu/Zn-SOD [42]. However, it should be noted that recent work has suggested the use of TZDs may lead to decreases in cardiovascular function and could prompt incidents such as acute myocardial infarction, heart failure and stroke [43–45]. Side effects associated with PPAR γ ligands include weight gain, oedema and increased plasma lipoproteins [46]. However, weak/partial agonists at the PPAR γ receptor may be void of these detrimental side effects [46]. CBD may prove to have therapeutic utility as a low affinity agonist of PPAR γ .

Haemodynamic Effects of CBD

Few studies to date have examined the haemodynamic responses to CBD. One study has shown that in pentobarbitone anaesthetized rats, that CBD ($50 \mu g kg^{-1}$ i.v. but not $10 \mu g kg^{-1}$) causes a significant but transient 16 mmHg fall in mean arterial blood pressure without affecting heart rate [47]. However, other studies do not report any acute effects of *in vivo* treatment with CBD on baseline heart rate or blood pressure in animal studies [48, 49]. In a recent review, Bergamaschi *et al.* [13] concluded that CBD treatment in humans did not result in changes in blood pressure or heart rate. Thus, the majority of evidence suggests there is no effect of CBD on haemodynamics. However, as has been observed with other cannabinoid compounds, the potential hypotensive effects of CBD may need to be revealed in models of raised blood pressure. Additionally, any change in haemodynamics that might occur may be rapid [47] and therefore not observed in chronic treatment studies.

CBD is known to be anxiolytic. CBD treatment reduces anxiety related to public speaking or fearful stimuli in humans [10]. A number of studies have now also shown that CBD reduces the cardiovascular response to anxiety or stressful situations. Resstel and colleagues have shown in Wistar rats that a single dose of CBD (10 or 20 mg kg⁻¹ i.p.) reduced the heart rate and blood pressure response to conditioned fear [49] or to acute restraint stress [48]. The inhibitory effect of CBD on the cardiovascular response to stress was shown to be inhibited by WAY100635, a 5HT_{1A} receptor antagonist. This effect appears to be mediated in the brain, as the same effect of CBD on cardiovascular responses could be mimicked when CBD was injected into the bed nucleus of the stria terminalis (a limbic structure) [50]. The potential ability of CBD treatment in humans to reduce the cardiovascular (as well as behavioural) response to stress could have significant effects on the development of atherosclerosis and hypertension, which are known to be accelerated by stress [51, 52].

Cardioprotective Effects of CBD

Several studies have shown that CBD is beneficial in preventing ischaemia-reperfusion damage in the liver [53, 54] and brain [55]. In 2007, Durst and colleagues first showed that *in vivo* treatment with CBD (5 mg kg⁻¹ i.p. pre-ischaemia and then for 7 days after) significantly reduced the infarct size of hearts where the left anterior descending (LAD) coronary artery had been ligated, and this was associated with a reduction in infiltrating leucocytes and circulating interleukin (IL)-6 concentrations. Furthermore, they showed that this cardioprotective effect of CBD could not be mimicked *in vitro*, and suggested that the cardioprotective effects of CBD are due to a systemic immunomodulatory effect rather than a direct effect on the heart [56]. Walsh *et al.* [47] subsequently showed that a single dose of CBD (50 µg kg⁻¹ i.v.) given 10 min pre-ischaemia or 10 min pre-reperfusion could significantly reduce infarct size after LAD coronary artery ligation. This was also associated with a reduction in ventricular ectopic beats, suggesting an additional anti-arrhythmic role for CBD. Rajesh *et al.* [57] showed that 11 weeks *in vivo* treatment with CBD (20 mg kg⁻¹ i.p.) significantly reduced cardiac dysfunction in diabetic mice, associated with decreased myocardial inflammation, oxidative stress, nitrative stress and fibrosis, mediated by reduced nuclear factor-κB activation (NFκB), reduced mitogen-activated protein kinase (MAPK) activation and reduced expression of adhesion molecules and tumour necrosis factor (TNF). Other studies have found that the anti-inflammatory effects of CBD via NFκB are not mediated by CB₁, CB₂ or Abn-CBD receptor activation [58].

Together, these data suggest that *in vivo* treatment with CBD has significant cardioprotective effects, which may be through a direct action on the heart or via a general anti-inflammatory, anti-oxidant mechanism.

Vasculoprotective Effects of CBD

There is a growing body of evidence that administration of CBD can ameliorate the negative effects of conditions associated with endothelial dysfunction. The high glucose conditions associated with diabetes have been reported as a causal factor in endothelial dysfunction. High glucose promotes inhibition/uncoupling of endothelial nitric oxide, increased superoxide production, increased actions of constrictor prostanoids, decreased actions of vasorelaxant prostanoids and increased reactive oxygen species [59]. Alongside these changes, high glucose is also reported to increase leucocyte adhesion and monocyte endothelial migration [60], which has been reported to be through NFκB activity [61].

In human coronary artery endothelial cells, prolonged exposure to high glucose has been shown to cause increased levels of adhesion molecules (ICAM-1 and VCAM-1), disruption of the endothelial barrier, mitochondrial superoxide production, iNOS and NFκB expression [62]. These effects were all reduced when the cells were co-incubated with CBD compared with high glucose alone. CBD also decreased monocyte adhesion and trans-endothelial migration, which are key elements in the progression of atherosclerosis. Neither the CB₁ nor CB₂ receptors were responsible for mediating the effects of CBD [62]. Using an *in vivo* model of diabetic retinopathy, El-Remessy *et al.* [63] similarly found that CBD treatment (10 mg kg⁻¹, every 2 days, i.p.) prevented vascular hyperpermeability at the blood-retinal barrier (BRB), and protected the retina against oxidative damage, inflammation and an increase in adhesion molecules. Thus, CBD-mediated protection of the vasculature in a model of diabetes may lead to a reduction in complications such as retinopathy, although this could also be driven by the neuroprotective effects of CBD.

Sepsis-related encephalitis, modelled by parenteral injection of LPS in mice, induces profound arteriolar dilation, resulting in brain hyperaemia and blood-brain barrier (BBB) disruption [34]. Administration of CBD (3 mg kg⁻¹ i.v.) at the same time as LPS maintained BBB integrity, inhibited LPS-induced arteriolar and venular vasodilation, leucocyte margination, and suppressed excessive nitric oxide production. Although cerebral blood flow (CBF) was not measured directly, the results observed from various parameters led the authors to suggest that CBD had ameliorated the LPS-induced drop in CBF [34].

We have carried out some preliminary experiments examining the ability of CBD to modulate vasodilator responses. Using the Zucker diabetic rat model of type 2 diabetes, where endothelium-dependent vasorelaxation is known to be impaired, we showed that incubation of the aorta for 2 h with CBD (10 μM) significantly enhanced the vasorelaxant response to acetylcholine, an endothelium-dependent vasodilator [64]. We have similarly shown that incubation with CBD enhances the vasorelaxant response to acetylcholine in the spontaneously hypertensive rat (O'Sullivan, unpublished data).

Taken together these studies show that *in vitro* and *in vivo*, using cell culture, isolated tissue and animal models, CBD has been demonstrated to reduce the negative effects of high glucose, diabetes and inflammation on the vasculature and on vascular hyperpermeability. As yet, the receptor sites of action for CBD in some of these studies remain unclear, but a common theme is the reduction in inflammatory markers (see Table 1).

CBD in Models of Stroke

Administration of endogenous, synthetic or phytocannabinoids has been shown to provide neuroprotection using a variety of *in vivo* and *in vitro* disease models, including stroke [65]. The neuroprotective potential of CBD in ischaemic stroke was first explored by Hampson and colleagues [66], where they subjected rats to middle cerebral artery occlusion (MCAO) and demonstrated that CBD, given at onset of insult (5 mg kg⁻¹ i.v.) and 12 h after surgery (20 mg kg⁻¹ i.p.), reduced infarct size and neurological impairment by 50–60%. Similarly, post ischaemic administration of CBD (1.25 to 20 mg kg⁻¹ i.p.) protected against ischaemia-induced electroencephalographic flattening, hyperlocomotion and neuronal injury in gerbils after MCAO [67]. More recently, it has been shown that CBD (3 mg kg⁻¹ i.p.) reduced infarct volume following MCAO, independent of CB₁ receptor or TRPV1, but sensitive to the 5HT_{1A} receptor antagonist WAY100135 (10 mg kg⁻¹ i.p.) [68, 69]. Furthermore, CBD (3 mg kg⁻¹ i.p.) provided neuroprotection even when administered up to 2 h post reperfusion without the development of tolerance [70, 71].

CBF is reduced or completely abolished in certain areas of the brain during ischaemic stroke, thus, restoring CBF to provide adequate perfusion is of great importance. CBD has been shown to be successful in increasing CBF, as measured by laser-Doppler flowmetry, following MCAO and after reperfusion (3 mg kg⁻¹ i.p.) [69–71]. The increased CBF induced by CBD (3 mg kg⁻¹ i.p.) was partially decreased by 5HT_{1A} receptor antagonism, suggesting that CBD may exert these beneficial effects, at least in part, via the serotonergic 5HT_{1A} receptor [69]. Exposing newborn piglets to hypoxia-ischaemia, Alvarez and colleagues [72] also demonstrated the ability of CBD (0.1 mg kg⁻¹ i.v., post insult) to provide neuroprotection in a manner that included the preservation of cerebral circulation.

As previously discussed, administration of CBD (3 mg kg⁻¹ i.v.) at the same time as LPS maintains BBB integrity [34]. Although CBF was not measured directly, the results observed from various parameters led the authors to suggest that CBD had ameliorated the LPS-induced drop in CBF [34]. BBB disruption is an important facet in the pathophysiology of ischaemic stroke [73]. Therefore, CBD-mediated preservation of this barrier, as demonstrated in other disease models could represent another mechanism of CBD-mediated protection in ischaemic stroke. Agonism of PPAR_γ may represent another mechanism of action for the beneficial effects of CBD in stroke. Several groups have found that synthetic PPAR_γ agonists, thiazolidinediones (TZDs), a class of drugs used to improve insulin sensitivity, reduced infarct size and improved functional recovery from stroke in rats [74–78]. Improvement is associated with reduced inflammation which is a probable mechanism of recovery, and, importantly, improvement is seen whether TZDs are administered before or after MCAO [75, 77]. Recently, *in vivo* CBD treatment has been shown to have neuroprotective effects in an Alzheimer's disease model which were inhibited with a PPAR_γ antagonist [6]. Similarly, we have shown in a cell culture model of the BBB that CBD restores the enhanced permeability induced by oxygen glucose deprivation, which could be inhibited by a PPAR_γ antagonist (Hind & O'Sullivan, unpublished observations).

In summary, CBD provides neuroprotection in animal and *in vitro* models of stroke. In addition to any direct neuroprotective effects of CBD, this is mediated by the ability of CBD to increase cerebral blood flow and reduce vascular hyperpermeability in the brain

Haematological Effects of CBD

In addition to the effects of CBD on the heart and vasculature, there is evidence that CBD also influences blood cell function. Early studies showed that CBD increases phospholipase A₂ expression and lipooxygenase products in platelets [79] and that CBD inhibits adenosine or epinephrine stimulated platelet aggregation [80], and more recently, collagen stimulated platelet aggregation [47]. 5-HT release from platelets has been shown to be decreased by CBD [81] or not affected by CBD [80].

In white blood cells, CBD induces apoptosis of fresh human monocytes [82] and human leukaemia cell lines [83, 84], which the later study showed was dependent on CB₂ activation, but not CB₁ or TRPV1. However, CBD can also prevent serum-deprived cell death of lymphoblastoid cells in serum-free medium by anti-oxidant mechanisms [85]. McHugh *et al.* [86] showed that CBD itself did not affect neutrophil migration, but that CBD inhibited formyl-Met-Leu-Phe-OH (fMLP)-stimulated neutrophil migration. CBD also inhibits monocyte adhesion and infiltration [62] and white blood cell margination in cerebral blood vessels after LPS treatment [34]. CBD significantly inhibits myeloperoxidase (which is expressed in neutrophils, monocytes and some populations of human macrophages) activity at 1 h and 20 h after reperfusion in mouse MCAO models [70, 87]. CBD also causes a dose-dependent suppression of lymphocyte proliferation in a murine collagen-induced arthritis model [88].

Together these studies show that CBD influences both the survival and death of white blood cells, white blood cell migration and platelet aggregation, which could underpin the ability of CBD to delay or prevent the development of cardiovascular disorders.

Conclusion

In summary, this review has presented evidence of the positive effects of CBD in the cardiovascular system, summarised in Table 1. In isolated arteries, direct application of CBD causes both acute and time-dependent vasorelaxation of precontracted arteries and enhances endothelium-dependent vasorelaxation in models of endothelial dysfunction. *In vivo*, CBD treatment does not appear to have any effect on resting blood pressure or heart rate, but does reduce the cardiovascular response to various types of stress. *In vivo*, CBD treatment has a protective role in reducing the effects of cardiac ischaemia and reperfusion, or in reducing cardiac dysfunction associated with diabetes. Similarly, CBD has a protective role in reducing the ischaemic damage in models of stroke, partly due to maintaining cerebral blood flow. In models of altered vascular permeability, CBD reduces the hyperpermeability of the BRB in diabetes and BBB hyperpermeability after LPS injection. Similarly, CBD ameliorates the negative effects of a high glucose environment on cell adhesion molecules and barrier function. Together, these data suggest that the cardiovascular system is indeed a valid therapeutic target for CBD. However, the target sites of action for CBD remain to be established for most of these responses. Whether these responses to CBD will translate into the human cardiovascular system also remains to be established.